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Interventions for improving livestock productivity in developing countries

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Interventions for improving livestock productivity in developing countries

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Editorial: Interventions for improving livestock productivity in developing countries

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Editorial on the Research Topic
Interventions for improving livestock productivity in developing countries

Livestock systems are foundational to developing economies, contributing significantly to economic stability, food security, and rural livelihoods. An estimated one billion people in Africa, Asia and Latin America depend on livestock for livelihoods, with smallholder systems providing critical income, nutrition, and resilience against economic shocks in developing countries. Animal-derived foods contribute approximately 37% of the global protein supply, and their role is particularly significant in arid and semi-arid regions, where limited rainfall and poor soils constrain crop production, making livestock a critical source of food and income (Smith et al., 2024). In most of sub-Saharan Africa, livestock contributes 20–40% of agricultural GDP, with some countries reporting figures as high as 80% depending on the structure of their agricultural sector and economy (Erdaw, 2023). Beyond direct outputs, livestock in developing countries supports crop productivity through draft power-utilized on approximately 250 million hectares for soil tillage, transport, and post-harvest activities (Harrigan, 2022), and through manure, which supplies about 12–23% of nitrogen inputs in mixed crop-livestock systems, thereby reducing the need for synthetic fertilizers (FAO, 2018). These diverse contributions demonstrate how livestock not only generates food and income but also underpins sustainable crop production and rural livelihoods in developing regions.

Livestock productivity in developing countries remains constrained by interconnected challenges. Limited supplies of feed are exacerbated by seasonal variability and low-quality forage (Balehegn et al., 2020; Baltenweck et al., 2020). Disease prevalence, compounded by fragmented veterinary services and limited vaccine access cause a significant reduction in livestock productivity in developing countries, in some cases causing a GDP reduction of up to 4% (Countryman et al., 2024). Socioeconomic barriers-such as limited access to credit (Yang et al., 2022), low rates of technology adoption, and underinvestment in livestock research and development continue to constrain productivity growth in developing countries Rosegrant et al., 2022).

Given the complexity and context-dependent challenges in livestock systems, sustainable productivity improvements demand context-specific integration of technological, managerial, and policy interventions (Kazanski et al., 2025). In this

Research Topic, the 14 studies collectively advance actionable insights across three critical domains: (1) optimizing feed resources through circular, locally adaptable strategies, (2) enhancing genetic potential via participatory and genomic approaches, and (3) dismantling institutional barriers through gender-responsive policies and inclusive market systems.

1 Feed innovation and optimization

Chronic feed shortages and reliance on resource-intensive grains are tackled through circular and locally adaptable solutions. Chisoro et al. demonstrated that substituting 30–40% of conventional maize/soy with wild fruit byproducts (WFBP) in ruminant diets reduces methane emissions by 12–18% while maintaining productivity, offering a sustainable alternative for smallholders in arid regions. Similarly, Somparn et al. validated black soldier fly larvae oil (BSFLO) as a replacement for rice bran oil in broiler diets, preserving growth performance and reducing dependence on imported feed ingredients in Southeast Asia. In Ethiopia, Kebede et al. identified high-yielding forage crops like oats and vetch, which increase dry matter yields by 40–60% compared to traditional grasses, addressing feed scarcity in mixed crop-livestock systems. In an experiment by Mengistu et al. aimed at evaluating forage cultivars with potential for improving yields, Maralfalfa grass, tested in Ethiopia's highlands, showed optimal productivity at 50 cm intra-row spacing, enhancing forage availability for dairy systems. Ashagrie et al. further highlighted the economic viability of nutrient-rich feed interventions, boosting milk yields by 33% in smallholder dairy farms. Similarly, Sanfo et al. investigated the performance of improved food–feed maize and cowpea cultivars under monoculture and intercropping systems among smallholder crop–livestock farmers in southern Burkina Faso, and found out that intercropping improved maize (Barka) and cowpea (KVx745-11P) cultivars significantly increases both grain and fodder yields, enhancing land-use efficiency, and providing higher quality fodder, offering a practical solution to seasonal feed shortages and supporting sustainable crop–livestock integration in the region.

2 Genetic and breeding improvements

Mismatched breeding programs and low genetic potential are addressed through participatory and genomic approaches. Zorobouragui et al. documented farmer-led selection of heat-tolerant Gudali cattle in Benin, prioritizing coat color and milk yield, which improved lactation performance by 17% in semi-arid agroecologies. Chafai et al. quantified negative genetic correlations (−0.89) between milk yield and fertility traits in Moroccan Holsteins, advocating for balanced breeding strategies to reduce culling rates. Epigenetic research by Rodrigues et al. revealed that creep-feeding in crossbred beef cattle alters methylation patterns in genes like IGF2, enhancing post-weaning growth by 15% in resource-limited systems. Moreover, Samuel et al. investigated the effect of the DGAT1 K232A gene mutation and breed differences on milk yield and composition in five Ethiopian cattle populations. They found that the DGAT1 K232A marker significantly influences daily milk yield, fat, and lactose content, suggesting its potential use in marker-assisted selection to improve milk production and quality traits in Ethiopian cattle after further validation in larger populations.

3 Socio-institutional and policy interventions

Gender disparities, market inefficiencies, and fragmented extension services are mitigated through inclusive and integrated strategies. Njiru et al. investigates the gender dynamics influencing the adoption and commercialization of Brachiaria forage among smallholder dairy farmers in Kenya, revealing that while both men and women benefit from Brachiaria's high productivity and drought tolerance, women face greater barriers to adoption due to limited access to land, extension services, and cooperative membership. Ayantunde et al. mapped feed market dynamics in Burkina Faso, showing that transhumant pastoralists in the Sahel pay 40% more for crop residues during dry seasons, underscoring the role of decentralized feed networks. Hatew et al. demonstrated synergies in Ethiopia where combined feed, management training, and genetics increased milk yields by 25%, highlighting the importance of holistic interventions.

The studies in this Research Topic show that improving livestock productivity in developing countries depends on integrated, context-specific strategies that combine technical, genetic, and institutional innovations. Specific lessons include the use of alternative feeds and intercropping to boost yields and sustainability, the alignment of breeding programs with local environmental needs, and the removal of social and market barriers through inclusive policies. The significance of these results lies in demonstrating that only by blending practical, locally adapted solutions across multiple domains can livestock systems achieve lasting productivity gains, resilience, and equitable benefits for smallholders.

Key lessons from this Research Topic include the need to reinforce and expand context-specific interventions that address the diverse challenges facing livestock productivity in developing countries (Kazanski et al., 2025). Future efforts should prioritize bolstering feed improvement strategies, as inadequate and low-quality feed remains the most significant bottleneck for smallholder systems across regions (Tran et al., 2023; FAO, 2018). Integrating livestock productivity goals with climate change mitigation and adaptation is essential, not only to reduce emissions and build resilience but also to unlock climate finance opportunities that can support sustainable intensification and innovation (Bashiru and Oseni, 2025). Policy frameworks must encourage sustainable intensification and commercialization, which have been shown to stimulate investment and the adoption of improved inputs and technologies (Abay and Jensen, 2020). Finally, there is a need for focused research to close persistent gaps in feed resources, genetics, and socioeconomic constraints, and to ensure that new technologies and management practices are effectively transferred and adopted at scale. Only through a coordinated, evidence-based approach that combines technical, policy, and institutional innovations can livestock systems in developing countries achieve sustainable productivity, climate resilience, and inclusive growth.

Author contributions

MB: Writing – original draft, Writing – review & editing. AA: Writing – original draft, Writing – review & editing. GD: Writing – original draft, Writing – review & editing.

Conflict of interest

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Effects of replacing Brachiaria hay with either *Desmodium intortum* or dairy concentrate on animal performance and enteric methane emissions of low-yielding dairy cows

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In Africa, cattle are often fed low quality tropical roughages resulting in low-yielding animals with high methane (CH_4) emission intensity (EI, g CH_4/per unit of product). Supplementation with protein is known to improve the nutritive value of the otherwise low-quality diets. However, animal nutrition studies in East Africa that are accompanied by CH_4 emission measurements are lacking. Thus, an animal experiment was conducted to quantify the effect of supplementing cattle fed mainly on low-quality *Urochloa brizantha* hay (control diet; CON; crude protein (CP) = 7.4%) or supplemented with either a tannin-rich leguminous fodder, *Desmodium intortum* hay (DES) or a commercial dairy concentrate (CUBES) on voluntary dry matter intake (DMI), nutrient apparent total tract digestibility, nitrogen (N) retention, enteric CH_4 production and animal performance (milk and average daily gain). Twelve mid-lactating crossbred (Friesian × Boran) cows (initial liveweight = 335 kg) were used in a 3×3 (Period × Diet) Latin square design with each period running for four weeks. Compared to CON, DES decreased nutrient (DM, OM, CP) intake, apparent total tract digestibility and daily milk yield. In contrast, CUBES increased nutrient intake and animal performance compared to CON, while nutrients' apparent total tract digestibility was not different, except for CP digestibility that increased. Compared to CON, DES and CUBES improved overall N retention by the animals as a proportion of N intake. The DES diet compared with CON and CUBES, shifted the proportion of N excretion *via* urine to the fecal route, likely because of its tannin content. Both DES and CUBES, compared to CON, reduced methane yield (MY, g CH_4/kg DMI) by 15% and 9%, respectively. The DES diet reduced absolute enteric CH_4 emissions by 26% while CUBES increased emissions by 11% compared to CON. Based on the present findings, high supplementation levels (>50%) of *Desmodium intortum* hay is not recommended especially when the basal diet is low in CP content.

Supplementation with lower levels of better managed *Desmodium intortum* forage however, need to be investigated to establish optimal inclusion levels that will improve animal productivity and reduce environmental impact of livestock in smallholder tropical contexts.

KEYWORDS

protein supplementation, tannins, milk production, digestibility, low quality diet

Introduction

One of the main limiting factors to ruminant production in smallholder systems in developing countries is feeding of low-quality forages (Kebreab et al., 2005; Castro-Montoya et al., 2018). In Sub-Saharan Africa, tropical grasses and crop residues form the main diet component for cattle with low supplementation, if any. These high roughage diets are often found to be low in crude protein (CP; <7.5%) and high in fiber (>70%) (Leng, 1990) which compromises their utilization by animals resulting in low animal productivity. Also, such diets are thought to lead to higher enteric methane (CH_4) yield (MY; CH_4 emitted per unit feed intake (Goopy et al., 2020) and higher CH_4 emission intensities (EI; CH_4 per unit of product; (Opio et al., 2013; Ericksen and Crane, 2018) compared to animal diets that are of better nutritive value. Earlier studies have, however, shown that supplementing of such low-quality diets with protein concentrates could improve their nutritive value and hence animal production (Koster et al., 1996; Mathis et al., 2000; Shreck et al., 2021).

Protein supplementation is known to increase intake and apparent total tract digestibility of low-quality roughages by cattle (Bohnert et al., 2011) by improving microbial activity in the rumen. The degradation of supplementary protein in the rumen increases the ammonia concentration and this enhances microbial growth and activity because nitrogen is known to be limiting to microbial nourishment with low quality tropical grasses (Detmann et al., 2014). Commercial protein supplements such as soybean meal are commonly used in intensive cattle production, however, these are often inaccessible and/or unaffordable to many smallholder livestock farmers in the Global South (Bakrie et al., 1996; Franzel et al., 2005). Leguminous forage crops and shrubs that are produced on-farm at a lower cost have then over time been promoted as alternative protein sources to ruminants in smallholder systems even though adoption of these feed resources is still quite low (Kebede et al., 2016).

Leguminous fodder crops such as *Desmodium* spp. and lucerne inherently have higher CP content than most of the tropical grasses (Topps, 1992) and have been used as a supplement in tropical cattle production to improve the feeding value of low quality roughages (Tolera and Sundstol, 2000). Furthermore, most leguminous forages grown in tropical environments are rich in plant secondary compounds (PSC), such as tannins, saponins and essential oils (Topps, 1992). Presence of tannins, both condensed tannins (CT) or hydrolysable tannins (HT) in ruminant diets have been shown to decrease enteric MY and EI (Beauchemin et al., 2007; Williams et al., 2020) and reduced ammonia and nitrous oxide emitted from manure by binding dietary nitrogen (N) and shifting its excretion from the urinary to the less volatile fecal route (Grainger et al., 2009; Korir et al., 2016). With high affinity for protein (Makkar and Singh, 1991), moderate level of tannins in ruminant's diet has been shown to increase dietary bypass protein which improves protein utilization efficiency (Frutos et al., 2004). The effects of tannins depend on their source and concentration in the diet (Makkar, 2003; Pineiro-Vazquez et al., 2018). Feed intake and nutrient apparent digestibility (DMD) have been observed to increase or remain unaltered at low to moderate concentration of tannins in the diet (1-3% on DM basis; (Kariuki et al., 1999; Tolera and Sundstol, 2000; Mbuthia and Gachuiri, 2003; Pineiro-Vazquez et al., 2018). At high dietary content (>5% on DM basis) however, diet these parameters have often been shown to be reduced and this had been associated with reduced CP availability in the rumen and low palatability (Waghorn, 2008; Ahnert et al., 2015). Studies from East Africa quantifying the potential of locally available tannin-rich leguminous fodder crops on improving the feeding value of low-quality grass-based diets remain scanty and inconclusive, with no research quantifying their enteric CH_4 mitigation potential *in-vivo*. Animal studies with leguminous fodder elsewhere have shown that, in addition to increasing rumen passage rate that minimizes contact time with methanogens and reducing MY (Piñeiro-Vázquez et al., 2018), tannin rich fodder have also been shown to directly exert anti-methanogenic activity in the rumen by reducing methanogen numbers (Beauchemin et al., 2007; Aboagye et al., 2018).

The present experiment was conducted to test whether *Desmodium intortum*, a locally grown legume, could be a potential alternative supplement to commercial dairy concentrates in smallholder dairy production systems in East Africa. We hypothesized that: i) both *Desmodium intortum* hay and commercial dairy concentrate supplementation would increase voluntary feed intake, apparent total tract digestibility of nutrients and improve animal production (milk yield and ADG) compared to animals fed a low quality basal diet alone; ii) both *Desmodium intortum* hay and commercial dairy concentrate supplementation would reduce MY and EI (g CH_4/unit of product) compared to feeding a low quality basal diet alone to lactating cows, and iii) Desmodium hay but not dairy concentrate supplementation would reduce absolute enteric CH_4 emissions and shift N excretion from the urinary to the fecal route.

Materials and methods

The study was conducted at the Mazingira Centre of the International Livestock Research Institute (ILRI), Nairobi, Kenya. The experimental protocol was reviewed and approved by the Institutional Animal Care and Use Committee (IACUC, Reference number: IACUC 2019-09).

Experimental design and animals used

A 3×3 (Period × Dietary treatment) Latin square design was used. Each experimental period ran for 28 d consisting of 14 d of dietary adaptation, 7 d of separate collection of feces and urine and 7 d of measurements of enteric CH_4 emissions.

Twelve crossbred cows (Friesian × Boran) with initial live weights (LW) of 335 ± 14.2 kg (mean ± standard error of mean) in either parity 1 or 2 and in mid-lactation (days in milk; DIM: 119 ± 6.4 d) were included in the experiment. The animals were sourced from ILRI's Kapiti Research Station situated 60 km South-East of Nairobi, where the animals were maintained extensively on natural pastures in a rangeland ecosystem and were only supplemented with mineral licks. The animals were milked by hand once a day. Selection of the cows was done from a herd of 128 lactating animals. Calves were weaned and the cows moved to ILRI campus 8 d later. On arrival, the animals were quarantined for 21 d in an enclosed animal facility, where they were kept in two group pens (six cows per pen). While in quarantine, the animals were fed on Brachiaria hay alone for the first 3 d. From Day 4, a commercial dairy concentrate was introduced in the animals' diet to improve their milk production to a level that was considered sensitive to treatments' effect (> 4 L) before the start of the experiment and thus also minimize chances of the cows drying up midway through the experiment. The dairy concentrate, that was fed during milking that was done once per day in the morning by hand, was gradually introduced to the animals with 2 kg per animal offered in Day 4 and 5 and increased to 3 kg per day from Day 6. The concentrate was formulated from wheat bran, whole maize, maize bran, rice bran, soya bean meal, wheat pollard, sunflower seed cake, stock feed lime and molasses. Two animals that were already at ILRI campus and fitted in the selection criteria were added to this cohort to allow for replacement animals in case an animal would have to be removed from the experiment. The cows were producing on average 1.7 ± 0.48 kg of milk per day at the start of quarantine and gradually increased to a peak of 4.7 ± 0.49 kg by Day 11 of quarantine. From Day 6 of quarantine, a milking machine (Sezer PLS 2, Bursa, Turkey) was introduced and used subsequently instead of hand-milking. We anticipated that the dietary changes introduced during the pre-trial and at the onset of the experiment would have minimal confounding effects on milk production, if any, because the cows used were low yielding animals.

After completion of the 21-d quarantine period, the cows were moved to the experimental animal facilities and housed in individual pens (2 m × 3 m), with each animal fed singly and therefore served as an experimental unit. The pens had the roof covered with shade sails and a concrete floor covered with a rubber mat. The walls were partitioned with aluminum rails. The animals were allocated to one of the three experimental groups (each with four animals), balanced for milk yield, DIM, liveweight and parity. The three dietary treatments were then randomly allocated to the three animal groups for the first period of the experiment and rotated among the animal groups in subsequent periods (Period 2 and 3) such that all animals went through all the dietary treatments. Carryover effect was minimized by assigning two animals from one treatment group to the other treatments in subsequent periods instead of all the four animals being assigned to the same diet, as detailed in Table 1. The average (± SEM) liveweight, milk yield and DIM immediately before the experiment were 325 ± 18, 348 ± 16 and 332 ± 35 kg; 3.6 ± 0.5, 3.6 ± 0.6 and 3.6 ± 1.0 kg; and 118 ± 9, 120 ± 15 and 117 ± 12 d for the three animal groups.

Experimental diets

Three diets tested in the present study consisted of a low-quality control and two improved rations. The control diet (**CON**) consisted mainly of grass hay harvested in late bloom (*Urochloa brizantha* var. Xaraes, further on named Brachiaria hay) while the two improved diets consisted of CON and supplemented either with dairy cubes (commercial protein and energy supplement (Fugo Dairy meal; Unga Feeds Company, Kenya), subsequently named CUBES) or with *Desmodium intortum* hay (further on named DES). The nutrient density in the two supplemented diets was intended to be isonitrogenous and isocaloric to support the same level of animal production. Due to late harvesting and delay in baling of the Brachiaria hay used, it had only 5.5% CP (% of DM) which was lower than the expected 7-10% (Njarui et al., 2016). Thus, urea was added to the

TABLE 1 Animal groups (A, B, and C) allocation to three the dietary treatments (control: CON; control diet plus *Desmodium intortum* hay: DES and control diet plus dairy cubes: CUBES) over the three experimental periods.

Animal groups (n=2)	Period 1	Wash-out period (3 d)	Period 2	Wash-out period (3d)	Period 3
A1	CON		DES		CUBES
A2	CON		CUBES		DES
B3	CUBES		DES		CON
B4	CUBES		CON		DES
C5	DES		CUBES		CON
C6	DES		CON		CUBES

A, B, C, - Animal groups each with 4 crossbred lactating cows, resulting in n=12 animals per treatment.

Brachiaria hay to increase the CP content in CON to about 7.5%. The three diets were initially formulated to support a daily milk production of 4 L for the CON and 6 L for the supplemented diets, using an excel-based feed formulation model developed for crossbred animals by Moran (2005). Sugar cane molasses, an energy supplement, was included initially in the formulated diets at 20.0% of the total rations but was reduced to 12.2%, 6.4% and 8.5% for CON, DES and CUBES respectively, after the first 6 d of the experiment. The proportion of molasses included in the ration was reduced to the latter levels because milk yield by the animals peaked at a lower level than anticipated and that the 20% inclusion was already considered marginally high for a ruminant ration and could predispose the cows used to molasses toxicity. Despite these reductions, however, total energy intake by the animals was still sufficient to meet the target level of milk production because the animals' feed intake was higher than predicted. The proportions of the ingredients used in each experimental diet are shown in Table 2.

The molasses, urea and dairy cubes were purchased from local distributors each as a single batch. The grass hay, being the fifth cut since establishment, was harvested from a 2.5 ha Brachiaria plot at ILRI Nairobi campus. Desmodium hay was obtained from eight smallholder farmers in south Nyanza, western Kenya, within a radius of 20 km and similar soil type. *Desmodium intortum* is a leguminous herb commonly grown in strips in push-pull systems in maize fields in western Kenya to control stem borers and striga weed (Khan et al., 2008). Push-pull is biological strategy for controlling crop pests by intercropping repellant plants; often Desmodium, with a crop (e.g., maize or sorghum) vulnerable to a pest (e.g., stemborer), to keep the pests away from destroying the main crop. Around the perimeter of the production plot, a crop of lower economic value (e.g., grasses) is planted to attract (pull) and trap the pests (Cook et al., 2007). The Desmodium was harvested manually in late vegetative stage, sun dried for 3 d then baled using hand balers and transported to ILRI. Prior to the start of the experiment, all the Desmodium hay was chaffed to a length of about 3-7 cm using a forage chopper, homogenized and stored in plastic sacks. The Brachiaria hay was chaffed on daily basis during the experiment, just before the diets were formulated for the next day.

The diets were prepared daily in the evening by weighing the respective ingredients of each diet according to the proportions in Table 2. The chaffed hay was spread out on a canvas on a concrete floor. Urea was then dissolved in two parts of water and mixed with molasses before the mixture was sprinkled over the hay and thoroughly mixed by hand. The prepared feed was then stored overnight in covered plastic bins separately per treatment until when it was fed to individual animals.

Feeding and milking

The amounts of feed offered to each animal were determined daily as 105% of the previous day's intake to allow *ad libitum*

TABLE 2 Proportion (in % DM) of the different feed ingredients used in the formulation of the three experimental diets: control diet consisting mainly of *Urochloa brizantha* var. *Xaraes* hay (CON); control diet plus *Desmodium intortum* hay (DES) and control diet plus commercial dairy supplement (dairy cubes, CUBES).

Diet ingredients	CON (%)	DES (%)	CUBES (%)
Brachiaria hay	87.0	35.0	65.7
Desmodium intortum hay	_	57.7	_
Dairy meal	_	_	25.0
Urea	00.8	00.8	00.8
Molasses[1]	12.2	06.5	08.5

[1]The proportions of molasses indicated are after the adjustments done on Day 6 of the experiment.

feed intake with minimal allowance for selection. The ration for each animal was weighed into plastic bags in the morning and fed out in four tranches spread throughout the day to minimize spillages and soiling of the feed.

Throughout the experimental period, milking was done in the morning starting at 0700 h using a double cow milking machine while the animals were restrained in crushes. Milk output from each animal was then weighed on a digital platform scale (Model JS00-1, Shanghai Puchun Measure Instrument Co., Ltd. Shanghai, China, precision = 1 g) and the milk yields (in kg) were recorded.

Intake and liveweight measurements

Daily feed intake by the animals was determined as the difference between the total feed offered and the refusals collected the following morning over the 28 d in each period. The refusals from each animal were collected every morning, weights taken and pooled per treatment because these were too low for the selection differences from animal to animal to cause any meaningful differences in nutrients intake by the animals. A sub-sample (~300 g) from each treatment pool was then picked, stored in labeled paper bags and kept at -20°C. Every second day, a sample of the feed offered (~300 g as fed) from each dietary treatment was collected and handled as described for the refusals' samples. At the end of each period, the stored feed and refusal samples were then thawed and pooled within treatment per week (n=4 for rations and refusals separately) from which, subsample were transferred to pre-labeled paper bags and dried in an air-forced oven at 50°C for 96 h.

Liveweight was recorded twice a week before milking and morning feeding, except that during the enteric CH_4 and total urine and feces collection periods, animals were weighed only once a week. Liveweight was measured using a digital animal weighing bridge (Gallagher Weigh Scale W210, Australia, precision = 0.5 kg) with the figures obtained corrected for the weight of milk recorded from the respective animals.

Urine and fecal collection for N balance measurements

Total urine and feces were collected separately for seven consecutive d, while the cows were maintained in their individual pens and fed on their respective diets. Urine was collected using purpose-built urine collection devices that were attached to shaved areas around the vulva of the animals using adhesive glue (Ergo 5011, Kisling AG, Wetzikon, Switzerland). Silicon tubing was attached to the base of the devices to direct urine into 10 L barrels prefilled with 300 mL of 20% hydrochloric acid (HCl) to reduce the pH to 2-3. During collection, the animals were restrained in their pens using neck collars to minimize tampering of urine collection devices. The collection barrels were continuously monitored and replaced when about three-quarter full. Each morning, before feeding, the total volume of urine (plus acid) collected from each animal over the 24 h cycle was measured using a measuring cylinder. The collected urine was homogenized by stirring and a 1% aliquot was taken, filtered through a cotton gauze to remove gross dirt, homogenized again by shaking and a subsample of 100 mL dispensed into labelled plastic bottles and stored at -20°C until further processing. Total urine collection was not achieved every day on all animals resulting from either the urine collection bags peeling off from the animal or the tubing breaking. At least three complete collections were, however, achieved for each animal in each period with the incomplete collections discarded.

Feces excreted were collected from the floor every 3 h during the day when the animals were active and defecated more frequently and scooped once the following morning for the night excretions. Total feces collected over 24-h cycles (0900-0900h) were weighed and homogenized by hand mixing. A subsample (500 g) was then picked and oven dried (Genlab Oven, Genlab Ltd, UK.) at 50°C for 96 h. The dried fecal samples were packed in zip-lock polythene bags and stored at room temperature awaiting further processing. The total collection of feces and urine was conducted in weeks 3 and 4 of each experimental period with six animals (two animals per treatment) measured per week. When one cohort of six animals were going through total urine and fecal collections, enteric CH_4 emission measurements were carried out on the remaining animals concurrently.

Apparent total tract nutrient (DM, OM, CP, NDF and ADF) digestibility was determined as the difference in total nutrient intake and total faecal excretion. Nitrogen retention was calculated as the net difference between total N intake and N excreted in milk, urine and faeces.

Measurements of enteric methane emissions

Three open-circuit respiratory chambers (No Pollution Industries, Edinburgh, UK) described in detail in Goopy et al. (2020) were used to measure daily enteric CH_4 emissions from the experimental animals. The chambers had an internal volume of 8.90 m^3, each fitted with a separate environmental control unit. The internal chamber environment was set at 22°C and relative humidity at 50%, while internal air circulation and ventilation rate were set at 220 L/s and 18 L/s, respectively. A cavity ringdown laser absorption spectrometer (Picarro G2508 analyzer, Santa Clara, USA) was used to measure CH_4 and CO_2 concentration (ppm) both on the incoming and outgoing air streams with the instrument taking a sample from each chamber in rotation, every 12 minutes. Air flow rates (L/s) were measured using the venturi principle and daily enteric CH_4 emissions were calculated from the total volume of air flow through the chamber multiplied by the net mean CH_4 concentration over each measurement event.

On the measurement days, the total ration for the day was placed in the feed bins in the respective chambers after removal of the refusals from the previous day, and the chamber cleaned. The animals were milked then placed in the individual chambers and the doors closed until the following morning when the animals were taken out and another cohort brought in after cleaning the chambers again. Continuous access to clean water was also provided in the chambers using automatic waterers. The CH_4 measurements were conducted for ~22.5 h with an average of the last 3 h of measurements being assumed for the remaining 1.5 h to complete a 24-h data set. Each animal was measured three times in each period on three alternate days, with each measurement event carried out in a different respiration chamber. The three replicates were then averaged for statistical analysis.

The CH_4 recovery rate in each chamber system was measured at the start and at the end of each period by injecting pure CH_4 at a known mass flow into the respiration chamber using a gas phase titration unit (Environics 4020, Environics Inc., Tolland, USA) and measuring CH_4 concentration in the exhaust air at equilibrium. Recovery rates were on average 46 ± 2.6% during the experimental period due to a worn-out rotor seal in the 16-port distribution manifold feeding the analytical instrument and consequently, the enteric CH_4 data were corrected mathematically to adjust for the recovery losses. This was done by multiplying the calculated animal emission figure in each measurement event by the inverse of the recovery rates measured for the respective periods. Though the cause of the low recovery rates was detected early in the experiment, this could not be fixed immediately because appropriate spare part was not available locally and we had to source it from the Picarro company in the USA which took up to 3 months to be delivered. The final MY and Ym obtained agreed with those obtained from previous measurements with the same respiration chambers with animals fed almost similar diets.

Laboratory analysis

Dried samples of feed offered, refusals and feces were ground through a 1-mm sieve using a hammer mill (MF 10 basic, IKA, Werke GmbH & CO. KG, Staufen, Germany) and analysed for DM, OM, neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), ether extract (EE) and total extratable phenols (TEP). True DM was determined by drying the samples at 105°C for 24 h while ash was determined by combustion in a muffle furnace at 550°C for 6 h according to the methods of the Association of Official Analytical Chemists (AOAC, 1990 methods no. 924.05). Feed, refusal and fecal samples were analysed for NDF and ADF by the methods of Van Soest et al. (1991). Total N content in feed, feces, milk and urine was determined by micro-Kjeldhal procedure of AOAC (AOAC, 1990, method no. 988.05) using selenium catalyst. Acid detergent lignin content was analysed in an external laboratory (National Institute of Nutrition, Hyderbad, India) using near infrared spectroscopy. Ether extract in feed and refusal samples was determined in a commercial laboratory (Cropnut Ltd, Kenya), following AOAC procedures (AOAC, 1990, method no. 14.018). Total extratable phenols, total tannins (TT) and CT in feed samples were determined at the University of Hohenheim animal nutrition laboratories using the procedure according to Makkar et al. (1993) with TT, expressed as tannic acid equivalent, separated from TEP using an insoluble matrix of polyvinyl polypyrrolidone as described by Makkar et al. (1995). Metabolizable energy (ME; kJ) in all the three diets was calculated based on equation by AFIA (2014) as follows:

$$\mathrm{ME\ (kJ)} = (0.203 \times \%\mathrm{OMD} - 3.001)$$

Where %OMD is the organic matter digestibility (%) measured during the experiment. Because of the minimal differences in EE and MY among the three experiemental diets tested in the present study, these were assumed to have a neglible effect on the overall diet-to-diet differences in ME content and hence a similar equation was used for the three diets.

Condensed tannins were determined according to the method by Porter et al. (1986) using butanol-HCl reagent and expressed as leucocyanidin equivalent. Milk was analysed for fat, solid not fat (SNF), lactose, protein and total solids using an ultrasonic milk analyzer (Lactoscan S standard 1040, Milkotronic, Nova Zagora, Bulgaria). Fat and protein corrected milk (FPCM) was calculated according to the equation by Gerber et al. (2011):

$$\mathrm{FPCM\ (kg)} = \mathrm{raw\ milk\ (kg)} \times (0.337 + 0.116 \times \mathrm{fat\ content\ (\%)} + 0.06 \times \mathrm{protein\ content\ (\%)})$$

Statistical analysis

Data were analyzed using a linear mixed model in R 3.5.3 (R Development Core Team, USA). The dependent variables measured were first tested for normality by plotting normal quantile-quantile plots in R. The model for each variable was then fitted using the "Lme4" package (Bates et al., 2007) with treatment, period and treatment by period interaction included as fixed effects and animal identity as a random effect. Animal group (square) effect was not significant ($P > 0.05$) and was dropped from the model. ANOVA Type 3 analysis was used to test for significance of the fixed factors and the interaction was dropped in the final model used when $P > 0.05$. For the model fitted for mean daily milk production, the average milk yield of the three days prior to the start of each experimental period was included in the model fitted as a covariate to account for pre-experimental animal to animal differences in the initial milk yield. Least square means were calculated using the "lsmeans" package (Lenth and Lenth, 2018) and Tukey's built in "Multicompview" package (Graves et al., 2015) in R was used to separate the means per treatment. Level of significance was determined at $P < 0.05$.

Results

Composition of diet ingredients and experimental diets

The chemical composition of the feed ingredients and the experimental diets fed to the animals are shown in Table 3. The CON diet had a lower CP content compared to the two supplemented diets, with DES having a slightly lower CP than CUBES (9.7% versus 10.5%, respectively). The CUBES diet had 14% lower NDF, and 17% lower ADF contents compared to CON, while DES had 16% higher ADF and 63% higher ADL content compared to CON (41 ± 2.8 g/kg DM). The CUBES diet had almost double EE content compared to CON and DES that had the same content. The DES diet had almost three times higher TEP, about six times higher TT and more than twenty times higher CT content (1.1%) compared to CON and CUBES (Table 3).

Feed intake, animal performance and milk composition

Dietary treatment influenced ($P < 0.05$) DM, OM, CP, NDF and ME intake but not ADF intake ($P = 0.64$, Table 4). The daily DMI and DM, OM and ME intake when expressed per 100 kg LW differed ($P < 0.05$) among the three treatments with DES having the lowest and CUBES having the highest intakes. Animals fed on CON had 13% lower CP intake compared to DES and 82% compared to animals fed on CUBES. The animals fed on DES had 19% and 29% lower total NDF intake compared to cows fed on CON and CUBES, respectively. The Treatment × Period interaction was significant ($P = 0.013$) for the ADF intake, with lower values reported in Period 1 for the DES treatment group as compared to the other two experimental periods.

Dietary treatment affected milk yield ($P < 0.001$), milk SNF ($P = 0.0069$) and lactose ($P = 0.02$) contents (Table 5). Animals fed on CUBES yielded 54% more milk per day while cows fed on DES produced 24% less milk per d, compared to the CON fed animals, even though milk production across all animal groups was low (<3 L per day). Animals fed on DES had about 6% lower milk lactose content compared to CON (4.34%) and CUBES (4.48%) that did not differ from each other. Feeding CUBES resulted in higher ($P < 0.05$) milk SNF compared to DES but feeding CON resulted in similar SNF content to the other two diets. Animals fed on CUBES had more than double ADG compared to animals fed on CON and DES that did not differ ($P > 0.05$) from each other (Table 5).

Nutrients apparent total tract digestibility and nitrogen balance

Animals fed on DES had lower ($P < 0.05$) apparent total tract digestibility of all measured nutrients (DM, OM, NDF and ADF) compared to animals fed on CON and CUBES that did not differ from each other (Table 6). The CP apparent digestibility differed ($P < 0.001$) among the three diets with DES resulting in the lowest (54.6%) and CUBES in the highest (70.3%) apparent digestibility.

TABLE 3 Chemical composition (g/kg DM, mean ± SE) of the feed ingredients and the formulated experimental diets consisting mainly of *Urochloa brizantha* var. *Xaraes* hay as the basal diet (CON) and supplemented either with *Desmodium intortum* hay (DES) or commercial dairy supplement (CUBES).

Component	DM	OM	CP	NDF	ADF	ADL	EE	TEP	TT	CT
Diet ingredients g/kg DM (*n*=6)										
Brachiaria hay	875 ± 2.8	905 ± 2.3	54 ± 0.5	626 ± 2.2	329 ± 0.8	39 ± 1.8	08.0 ± 0.14	15.3 ± 0.40	04.9 ± 0.23	00.2 ± 0.03
Desmodium hay	873 ± 3.4	936 ± 2.2	107 ± 3.0	566 ± 9.3	422 ± 6.3	96 ± 1.8	08.1 ± 0.23	44.8 ± 0.63	35.2 ± 0.61	30.2 ± 0.96
Dairy meal	907 ± 2.5	926 ± 3.0	175 ± 1.8	278 ± 6.0	116 ± 3.2	36 ± 0.7	32.3 ± 0.27	05.1 ± 0.05	01.3 ± 0.13	00.2 ± 0.04
Molasses	745 ± 1.4	975 ± 1.5	45 ± 0.5	_	_	_	02.6 ± 0.11	15.5 ± 1.06	05.1 ± 0.21	01.3 ± 0.18
Urea[1]	–	–	–	–	–	–	–	–	–	–
Experimental diets (g/kg DM) (*n*=12)										
CON	804 ± 4.9	915 ± 2.0	74 ± 1.1	590 ± 5.6	317 ± 4.0	41 ± 2.8	07.6 ± 0.05	11.4 ± 0.38	03.0 ± 0.21	00.3 ± 0.02
DES	809 ± 4.9	926 ± 2.5	97 ± 1.9	568 ± 3.8	369 ± 2.2	68 ± 2.4	07.6 ± 0.06	27.6 ± 0.52	18.2 ± 0.61	11.1 ± 0.77
CUBES	831 ± 3.4	916 ± 1.7	105 ± 1.0	510 ± 3.5	263 ± 2.1	37 ± 0.4	13.8 ± 0.03	11.1 ± 0.56	02.7 ± 0.10	00.4 ± 0.02

DM, dry matter; OM, organic matter; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; ADL, Acid detergent lignin EE, ether extract; TEP, total extractable phenols (expressed as tannic acid equivalents); TT, total tannins (calculated as TEP – non-tannin Phenols, Makkar et al. (1995)); CT, condensed tannins (expressed as leucocyanidin equivalents);
[1]Fertilizer grade urea with 46% nitrogen was used.

Like DMI, digestible DM and OM intake differed ($P < 0.001$) among the dietary treatments (Table 6) with animals fed on DES having 20% lower OM intake compared to CON and 56% lower than CUBES. Animals fed on CUBES had almost double digestible CP intake compared to the other two diets that did not differ from each other. Feeding DES resulted in a lower ($P < 0.05$) digestible NDF and ADF intake compared to feeding CON and CUBES that did not differ from each other.

Total N intake differed ($P < 0.001$) among the three dietary treatments (Table 7) with animals fed on CON having the lowest intake while feeding CUBES resulted in the highest N intake. Animals fed on CON excreted 23% lower N in feces compared to DES and CUBES that did not differ ($P > 0.05$) from each other. However, when fecal N was expressed as a proportion of N intake, CUBES resulted in the lowest fecal N excretion while DES having the highest ($P < 0.05$). Net urinary and milk N output for animals fed on CUBES were almost twice that excreted by cows fed on CON and DES. When expressed as a percentage of N intake, however, feeding DES resulted in a lower ($P < 0.05$) urinary N excretion compared to the other two diets that did not differ ($P > 0.05$) from each other (Table 7). Milk N output as a proportion of N intake was similar across the three dietary treatments ($P = 0.10$). Total N retained by animals fed on CUBES was almost double that reported with CON and DES that did not differ from each other ($P > 0.05$). When N retained was expressed as a percentage of N intake, however, the three diets differed from each other ($P < 0.001$) with CUBES still having the highest N retention and CON having the lowest. Animals fed on CUBES had about eight units higher N use efficiency (retained N + milk N/N intake; %) compared to animals fed on CON and DES that did not differ from each other ($P > 0.05$). Period had a significant effect ($P = 0.02$) on N retained as a proportion of N intake with the figures reported in Period 1 being lower compared to the other two feeding periods. Period and Treatment × Period effects were significant ($P < 0.05$) for net N intake and fecal N excretion with lower values reported

TABLE 4 Mean daily nutrient (kg/100 kg LW) and metabolizable energy intake (MJ/d) of crossbred cows (n=12) fed either on a control diet (CON) consisting mainly of *Urochloa brizantha* var. *Xaraes* hay alone or the control diet supplemented with either *Desmodium intortum* hay (DES) or a commercial dairy supplement (CUBES) over three periods with each period lasting for 28 d.

	Treatment				P value		
Parameter	CON	DES	CUBES	PSEM	*Treatment (T)*	*Period (P)*	*T × P*
DMI (kg/d)	8.91^{b}	7.74^{c}	10.85^{a}	0.37	<0.001	0.05	0.007
DMI (kg/100 kg LW[1])	2.55^{b}	2.20^{c}	3.22^{a}	0.086	<0.001	0.37	0.08
OMI	2.33^{b}	2.04^{c}	2.94^{a}	0.080	<0.001	0.32	0.07
CPI	0.20^{c}	0.22^{b}	0.36^{a}	0.008	<0.001	0.10	0.14
NDFI	1.48^{a}	1.24^{b}	1.60^{a}	0.048	<0.001	0.62	0.06
ADFI	0.79	0.79	0.81	0.029	0.64	0.08	0.013
MEI[2] (MJ/d)	83.7^{b}	66.2^{c}	116.1^{a}	4.17	<0.001	0.73	0.48

DMI, dry matter intake; OMI, organic matter intake; CPI, crude protein intake; NDFI, neutral detergent fiber intake; ADFI, acid detergent fiber intake; LW, live weight; MEI, metabolizable energy intake; PSEM, pooled standard error of mean; [a–c] Within a row, least square means without a common superscript differ (P < 0.05); [1]LW used was the average of the initial and final liveweight in each period; [2]ME was calculated using equation by AFIA (2014): ME = 0.203 * %OMD - 3.001, where OMD is the measured OM apparent total tract digestibility.

TABLE 5 Daily milk production, milk composition and average daily gain (ADG) of crossbred cows (n=12) fed either on a control diet (CON) consisting mainly of *Urochloa brizantha* var. *Xaraes* hay alone or the control diet supplemented with either *Desmodium intortum* hay (DES) or a commercial dairy supplement (CUBES) over 3 feeding periods with the measurements lasting for 28 d.

	Treatment				P value		
Parameter	CON	DES	CUBES	PSEM	Treatment *(T)*	*Period (P)*	*T × P*
Milk production parameters (kg/d)							
Milk yield	001.84^{b}	001.39^{c}	002.84^{a}	00.15	<0.001	00.29	0.29
FPCM	002.21^{b}	001.55^{c}	003.17^{a}	00.17	<0.001	00.50	0.64
Milk composition (%)							
Fat	005.04	005.11	005.06	00.304	00.99	<0.001	0.39
SNF	007.86^{ab}	007.53^{b}	008.16^{a}	00.196	00.007	00.67	0.55
Lactose	004.34^{a}	004.10^{b}	004.48^{a}	00.105	00.020	00.71	0.94
Protein	003.89	002.77	003.07	00.606	00.42	00.42	0.42
Total solids	012.7	012.5	013.1	00.46	00.25	00.003	0.54
ADG (g/d)	326^{b}	193^{b}	671^{a}	58.3	<0.001	00.012	0.53

[a–c]Within a row, least square means without a common superscript differ (P < 0.05); ADG, average daily gain; PSEM, pooled standard error of mean.

TABLE 6 Mean apparent total tract digestibility of nutrients and total digestible nutrient intake of crossbred cows (*n*=12) fed either on a control diet (CON) consisting mainly of *Urochloa brizantha* var. *Xaraes* hay alone or supplemented with either *Desmodium intortum* hay (DES) or a commercial dairy supplement (CUBES) with each measurement period lasting for 7 d.

	Treatment				P value		
Parameter1	CON	DES	CUBES	PSEM	*Treatment (T)*	*Period (P)*	*T×P*
Apparent digestibility (%)							
DM	63.7^{a}	59.3^{b}	66.2^{a}	0.86	<0.001	0.76	0.86
OM	66.7^{a}	61.3^{b}	69.4^{a}	0.81	<0.001	0.18	0.96
CP	60.0^{b}	54.6^{c}	70.3^{a}	1.06	<0.001	0.07	0.51
NDF	64.9^{a}	56.2^{b}	61.8^{a}	1.11	<0.001	0.07	0.92
ADF	63.5^{a}	50.6^{b}	60.3^{a}	0.98	<0.001	0.05	0.91
Digestible nutrient intake (kg/d)							
DDMI	05.67^{b}	04.78^{c}	07.30^{a}	0.307	<0.001	0.57	0.07
DOMI	05.53^{b}	04.60^{c}	07.16^{a}	0.297	<0.001	0.11	0.06
DCPI	00.42^{b}	00.44^{b}	00.86^{a}	0.033	<0.001	0.12	0.52
DNDFI	03.43^{a}	02.59^{b}	03.45^{a}	0.159	<0.001	0.003	0.09
DADFI	01.77^{a}	01.49^{b}	01.74^{a}	0.083	<0.001	0.001	0.03

DM, dry matter; OM, organic matter; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent; DDMI, digestible dry matter intake; DOMI, digestible organic matter intake; DCPI, digestible crude protein intake; DNDFI, digestible neutral detergent fiber intake; DADFI, digestible acid detergent fiber intake; PSEM, pooled standard error of mean. ^{a,b,c} Within a row, least square means without a common superscript differ (P < 0.05).

in Period 1 for the treatment groups CON and DES compared to the other two periods. There was also a period effect (P < 0.001) on net milk N output as well as when expressed as a proportion of N intake with values reported in Period 1 being lower than Period 3.

Enteric methane emissions

The daily enteric CH_4 emission (g CH_4/d) of the animals fed on the three experimental diets differed (P < 0.001) from each other (Table 8) with feeding CUBES resulting in the highest and DES the lowest daily CH_4 emissions. When CH_4 was expressed either per DMI (MY), OM intake (OMI), digestible OMI (DOMI) or as CH_4 conversion factor (Y_m: CH_4 MJ/GE intake (MJ)), animals fed on CON had 7 - 9% higher CH_4 emissions compared with DES and CUBES that did not differ from each other (P > 0.05). The CUBES diet had lower (P < 0.001) EI (g CH_4/kg CP out) compared to the other two diets that did not differ from each other but only showed a trend (P = 0.08) when emissions were expressed per unit FPCM (Table 8).

TABLE 7 Mean daily nitrogen (N) intake, N excretion and N retention of crossbred cows (*n*=12) fed on a control diet (CON) consisting mainly of *Urochloa brizantha* hay alone or supplemented with either *Desmodium intortum* hay (DES) or a commercial dairy supplement (CUBES) over three periods with the measurements lasting for 7 d.

	Treatment				P value		
Parameter	CON	DES	CUBES	PSEM	*Treatment (T)*	*Period (P)*	*T × P*
N intake (g/d)	110.8^{c}	127.6^{b}	195.1^{a}	6.52	<0.001	00.037	0.019
N excreted (g/d)							
Fecal N	044.1^{b}	057.7^{a}	057.6^{a}	2.78	<0.001	00.012	0.038
Urinary N	036.6^{b}	032.2^{b}	068.9^{a}	2.49	<0.001	00.99	0.91
Milk N	008.7^{b}	007.2^{b}	014.9^{a}	1.85	<0.001	00.021	0.24
N excreted as a proportion of N intake (%)							
Fecal N	040.0^{b}	045.4^{a}	029.7^{c}	1.12	<0.001	00.39	0.30
Urine N	033.4^{a}	026.3^{b}	035.5^{a}	1.45	<0.001	00.20	0.09
Milk N	007.9	005.6	007.6	1.21	00.10	<0.001	0.70
Retained N (g/d)	021.4^{b}	030.5^{b}	053.7^{a}	5.84	<0.001	00.12	0.26
Retained N/N intake (%)	019.3^{c}	023.9^{b}	027.5^{a}	1.40	<0.001	00.02	0.29
(Retained N + Milk N)/N intake (%)	26.9^{b}	028.4^{ab}	034.9^{a}	2.13	00.034	00.12	0.07

N, nitrogen; PSEM, pooled standard error of mean; $^{a-c}$ Within a row, least square means without a common superscript differ (P < 0.05).

TABLE 8 Mean daily methane production (DMP), methane yield (MY), methane produced per organic matter intake (OMI) and digested OMI (DOMI), methane conversion factor (Y_m) and methane emission intensity (EI; g CH_4/kg of fat and protein corrected milk; FPCM) of crossbred cows (n=12) fed on either a control diet (CON) consisting mainly of *Urochloa brizantha* var Xaraes hay alone or supplemented with either *Desmodium intortum* hay (DES) or a commercial dairy supplement (CUBES).

	Treatment				*P* value		
Parameter	CON	DES	CUBES	PSEM	*Treatment (T)*	*Period (P)*	*T × P*
g CH_4/d	245[b]	182[c]	272[a]	08.72	<0.001	0.09	0.11
g CH_4/kg DMI	027.6[a]	023.6[b]	025.2[b]	00.58	<0.001	0.07	0.09
g CH_4/kg OMI	030.2[a]	025.5[b]	027.6[b]	00.63	<0.001	0.09	0.08
g CH_4/kg DOMI	041.7[a]	038.1[b]	036.4[b]	00.85	0.001	0.10	0.034
Y_m(CH_4 MJ/GEI (MJ))	000.085[a]	000.074[b]	000.079[b]	00.002	<0.001	0.039	0.041
EI, (g CH_4/kg FPCM)[1]	253	297	175	62.4	00.08	0.012	0.98
EI, (kg CH_4/kg CP output)[2]	2.96[a]	2.83[a]	1.78[b]	0.28	<0.001	<0.001	0.81

CH_4, methane; DMI, dry matter intake, Y_m, methane conversion factor; [a-c] Within a row, least square means without a common superscript differ (P < 0.05); PSEM, pooled standard error of mean; [1]Calculated with the milk yield measured during the enteric CH_4 measurement week using the equation by Gerber et al. (2011):FPCM (kg)=raw milk (kg) x (0.337 + 0.116 x fat content (%)) + 0.06 x protein content (%)); [2]CP output is the sum of total CP in milk and CP deposited as live weight gain assuming dressing percentage of 52% (Muchenje et al., 2008), meat percentage of 85% (Mummed and Webb, 2019) and meat CP content of 21% on wet basis (Muchenje et al., 2008),

Discussion

Chemical composition and apparent total tract digestibility of the experimental diets

The low-quality CON diet (CP < 7.5% and ME = 8 MJ/kg) used in the present study was formulated to represent an average cattle diet in smallholder dairy systems in Sub-Saharan Africa, where high roughage diets form the main ration offered to ruminants. The two supplemented diets (DES and CUBES) were intended to contain a similar but higher CP content (10.5% CP) than CON and therefore support higher level of animal production (4 L vs 3 L of milk yield per d). While DES had marginally lower CP content (97 g CP/kg DM) than CUBES (105 g CP/kg DM), this was probably biologically too small to trigger any differences in animal's response. The lower CP content (10.7% DM) than expected (13.0-18.3% DM) (Osuji and Odenyo, 1997) in the Desmodium hay used necessitated a higher inclusion rate of about 58%. The low CP in the Desmodium hay was likely the case because the crop was harvested at six months of regrowth instead of the recommended three months (Heuzé et al., 2017) to allow for the primary crop in the push-pull system to mature. The possible loss of some leaf material that is generally higher in CP and tannins content than the stems during hay making and transportation could have also contributed to the rather low CP content (Kumar and Vaithiyanathan, 1990). The other chemical composition parameters such as NDF and ADF of the *Desmodium intortum* hay, though in line with what is reported elsewhere in literature (Perez-Maldonado and Norton, 1996; Kariuki et al., 1999; Kariuki et al., 2001; Nurfeta, 2010), were on the higher end. The PSC content of the leguminous hay (3.5% DM TT and 3.0% DM CT) agreed with findings by Perez-Maldonado and Norton (1996) who reported 3.2% DM CT and Tolera and Sundstol (2000) who reported 3.5% DM TT for *Desmodium intortum* hay. Getachew et al. (2000), however, found higher PSC contents in fresh *Desmodium intortum* forage (TEP: 8.5% and TT: 7.6% DM).

Despite the relatively low tannin content in DES (TT: 1.8% DM), where deleterious effects on feed intake would not be expected to manifest, DMI by animals fed the diet was depressed. The reduction in feed intake was accompanied by a reduction in CP apparent digestibility suggesting a possible suboptimal microbial activity in the rumen resulting in slow digestion playing a part in this. Several previous studies supplementing low quality basal diets offered to ruminants with *Desmodium intortum* reported no effect of feeding the legume on intake (Kariuki et al., 1999; Nurfeta, 2010), even at higher inclusion level (66%) than used in the present study (Tolera and Sundstol, 2000). Our finding, however, was in agreement with other studies where ruminants fed on diets rich in tannins also decreased feed intake (Grainger et al., 2009: *Acacia mearnsii* supplementation with dietary CT levels of 0.9 and 1.5% DM; Dschaak et al., 2011: supplementation with Quebracho tannin extract resulting in 3% DM dietary CT). The lower intake with Desmodium hay supplementation could be explained by the depressed DMD compared to the other two diets that could have slowed down rumen passage rate (Jayanegara et al., 2012). When tannins are present in ruminants' diet, these have been shown to bind to dietary and salivary protein during chewing in the mouth forming Protein-Tannin complexes that are quite stable at normal rumen pH (5.5-7.0) (Makkar et al., 1995). This binding, depending on activity and tannin concentration in the diet can significantly reduce proteolysis in the rumen and therefore influencing microbial growth and activity and hence fibre digestion. It is also thought that presence of tannins in feed reduces its palatability because of the astringent taste it exerts (Mueller-Harvey, 2006). We cannot substantiate this theory in the present study because the tannin content of the total ration offered to the animals was considered low and there was no evidence of animals selecting against the Desmodium hay.

The lower nutrient apparent digestibility observed in animals fed on the tannin-rich DES compared to the animals fed on CON was also reported in other studies where tannin - rich diets were fed to cattle. Pineiro-Vazquez et al. (2018) adding Quebracho tannin extract (91% CT) at an increasing rate of between 1-4% of the total ration to a basal diet consisting of *Pennisetum purpureum* observed a 2% decrease in DMD for every 1% increase of the extract. Ahnert et al. (2015) also while adding Quebracho tannin extract to the diet of crossbred heifers observed a reduced CP digestibility with dietary TT contents above 1.4% and a reduction in DM, OM and NDF digestibility above 2.7% dietary TT on DM basis. The suppressed nutrient apparent total tract digestibility observed with DES, despite the relatively low concentration of 1.8% dietary TT, could be due to the low CP content in the total diet. Anti-nutritional effects of tannins have been shown to be more pronounced in low protein diets (Hill et al., 1986; Butler, 1989). It is therefore possible that the reduced DMD observed in the present study at rather low TT content, was because of the tannin binding and reducing degradability of already marginal CP content in the diet, compromising on microbial nourishment and hence lower activity.

The increased DMI reported with CUBES agreed with findings elsewhere in the literature where animals fed on low quality basal diets were supplemented with highly digestible protein concentrates (Koster et al., 1996; Mathis et al., 2000). Protein supplementation has been shown to improve overall diet digestibility which in turn increases rumen passage rate and DMI (Koster et al., 1996; Mathis et al., 2000). The dairy supplement used being low in NDF content, could have also reduced overall diet bulkiness and therefore reducing the filling effect in the rumen and hence allowing for higher intake (Ketelaars and Tolkamp, 1992).

The OM digestibility of the control diet in the present study (67%) was higher compared to similar grass-based diets fed to cattle in experiments conducted in the tropics (48-63%, (Korir et al., 2016; Pineiro-Vazquez et al., 2018; Goopy et al., 2020) and this was likely a result of the inclusion of urea and molasses that could have provided readily available carbohydrate and N in the rumen and therefore supporting higher microbial growth and fibrolytic activity (Jetana et al., 2000).

Feeding CUBES resulted in higher animal performance (higher milk and ADG) and this was likely because of the higher digestible nutrient intake compared to animals fed on the other two diets. The lower milk yield by animals fed on DES on the other hand could be explained by the lower digestible nutrient intake, limiting production. With DES having the lowest milk yield yet not the lowest digestible CP intake, this is suggestive that available energy intake was the first limiting nutrient to animal production, and this could be related to the low digestible OM intake in the diet. Supplementation with better managed *Desmodium intortum* forage therefore need to be investigated to establish optimal inclusion levels that will improve nutrient intake and hence animal production in smallholder tropical context (Kariuki et al., 2001). Overall, the lower milk production (<3 L per day) by all animals used in the present study than what was predicted based on their nutrient intake was probably a result of poor nutrition during their early lactation that could have suppressed their peak and subsequent lactation (Duffield et al., 2009). When ME spent on milk production and weight gain were predicted using a feed formulation model (Moran, 2005), the animals seemed to partition more energy (4 – 17%) towards weight gain than lactation irrespective of dietary treatment. This observation however did not agree with the findings by Jenet et al. (2006) who observed a higher energy partitioning towards milk in cows of similar genotype that were fed on *Cynadon dactaylon* (CP: 46 g/kg DM; 7.5 MJ) and supplemented with wheat bran (CP: 161 g/kg DM; 10.1 MJ) at 35% of the ration. It should however be noted that the animals in the latter study were at an early stage of lactation (week 0 - 12 post calving) suggesting a more push by the animals towards nourishing their calves at this stage (Jenet et al., 2006) and were consistently in a better plane of nutrition; suggesting a modest loss of body reserves than what possibly occurred in the animals used in the present study. Especially under suboptimal feeding conditions, cows in their later stages of lactation have been shown to preferentially partition energy to replenishing body reserves at the expense of milk production in preparation for the next production cycle (Xue et al., 2011), and this could explain the trend observed in the present study

Effects of supplementation on nitrogen balance

Feeding either CON or CUBES did not affect the proportion of dietary N excreted in urine despite the animals fed on the latter diet having higher N intake. This was likely the case because animals fed on this diet had higher ADG suggesting a higher proportion of dietary N being captured and deposited in body tissues. In the present study, N fraction retained increased with both supplementation options (by 24% and 42% for DES and CUBES, respectively) in comparison to CON and was likely driven by different mechanisms related to the diets' chemical composition. The increased CP digestibility and higher ME content in the CUBES diet could have improved the N:energy ratio in the rumen, allowing for more dietary N to be captured for microbial protein synthesis and subsequently used by animals for their protein synthesis (Hoover and Stokes, 1991; Colmenero and Broderick, 2006). On the other hand, the presence of tannins in DES, could have improved N retention by reducing CP degradation in the rumen and therefore avoiding high ammonia tension in the rumen that encourages high N losses through the rumen wall into the portal systems and end up being excreted in urine (Stewart et al., 2019). In agreement with other studies involving diets rich in tannins, a shift in N excretion patterns from urine to feces was observed in the animals supplemented with Desmodium hay (Stewart et al., 2019; Herremans et al., 2020). It is not clear whether some tannin- protein complexes formed in the rumen fail to dissociate in the lower digestive tract or they dissociate, and new complexes formed, but what is generally observed is increased faecal N (Frutos et al., 2004). This shift in N excretion towards the fecal route could reduce environmental pollution because N in feces is less volatile compared to that excreted in urine and therefore reduces the proportion of excreted N being converted to ammonia or nitrous oxide (Rotz, 2004).

As the dietary protein was marginal for production in the three diets tested in the present study, the amount of N of dietary origin excreted in faeces may have been overestimated because the proportion of metabolic N in the faecal excretion is known to be higher in low quality diets (Ferrell et al., 2005). Caution therefore should be taken when interpreting CP apparent digestibility and N balance because metabolic N in feces were not measured and thus not accounted for in the calculations.

Effects of supplementation on enteric methane production

Animals fed on DES produced the lowest daily enteric CH_4 emission compared to the other two diets tested and this could be explained by the lower DMI and MY, which were likely effects of tannins as described earlier. On the other hand, the higher DMI associated with commercial dairy concentrate supplementation resulted in higher absolute CH_4 emissions despite having a lower MY than CON. Both supplementation options (CUBES and DES) resulted in lower MY and Y_m compared to the CON diet, which could be attributed to the presence of tannins in DES and possibly a combination of both presence of lipids and higher OM digestibility of the CUBES diet. Tannins and lipids have both been shown to have inhibitory effects on enteric CH_4 production when present in ruminants' diets (Beauchemin et al., 2008). Tannins have been shown to bind to CP and fiber in the diet reducing their digestibility and hence substrate availability for conversion to CH_4 by methanogens (Grainger et al., 2009; Williams et al., 2020). The simultaneous reduction in OMD and MY observed for the DES diet supports this explanation. Plant secondary metabolites also have the potential to suppress methanogenic activity by either intoxicating the protozoa or binding to microbial enzymes (McSweeney et al., 2001). The effect of the diets on rumen microbes was, however, not characterized in the present study.

The 9% reduction in MY for CUBES compared to CON was higher than the predicted 2.2 – 3.5% when using equations available in literature (Beauchemin et al., 2008; Moate et al., 2011) based on the EE difference of 6.2 g/kg DM between the two diets. Moate et al. (2011), from a meta-analysis using data from experiments with dairy and beef cattle supplemented with lipid-rich concentrates, observed a reduction of 3.5% in MY for every 10g/kg DM increase in dietary fat, while Beauchemin et al. (2008) in a similar review with cattle and sheep, reported a 5.6% reduction with the same magnitude of increase in dietary fat content. The higher EE content in CUBES than in the other two diets could have triggered several mechanisms suppressing CH_4 production per unit feed intake, including reduction in fiber digestion and protozoal numbers in the rumen (McGinn et al., 2004; Beauchemin et al., 2008). A trend towards lower NDF digestibility when feeding CUBES compared to CON supports this hypothesis. The higher response in MY (-9%) than expected when feeding CUBES in addition to the presence of fat, could be explained by higher DMI (than CON) that is also associated with shorter rumen retention time and hence lower MY (Goopy et al., 2014). Though passage rate was not measured in the present study, shorter rumen retention time has been observed to lead to a more propionate fermentation pathway and a reduction in the contact time between the methanogens and the substrate; both leading to a lower MY (Janssen, 2010).

The EI (g CH_4/kg FPCM), although not statistically different among the three diets because of huge variability within diets, was 31% lower for animals fed on CUBES compared to CON. This observation was consistent with other findings in the literature where increasing milk production in low yielding animals (< 6 L per d) is associated with a reduction in EI (Gerber et al., 2011). When emissions were, however, expressed per CP output, CUBES had lower EI compared to CON and DES and this is probably because the animals fed on CUBES had the highest ADG as well and therefore depositing more CP compared to the other two diets.

Conclusions and recommendations

The present study shows that supplementing lactating crossbred cows fed on a low-quality basal diet with either a commercial dairy concentrate or a tannin rich *Desmodium intortum* hay improved N use efficiency and reduced enteric MY and Y_m. While dairy concentrate supplementation improved feed intake and animal production, i.e., milk yield and weight gain, supplementation with almost 60% *Desmodium intortum* hay suppressed total nutrient intake and digestibility, and negatively affected animal production. The low CP and high lignin contents as well as the presence of tannins in the Desmodium hay used might explain these undesired effects on intake and animal performance. Desmodium hay supplementation, however, showed some positive environmental outcomes including reduced enteric CH_4 production and a shift in N excretion to the less volatile fecal route. Further animal experiments are needed to evaluate the potential of Desmodium hay as a supplement by using better quality *Desmodium intortum* hay to determine optimal supplementation levels for realization of the environmental gains reported without compromising animal production. The use of commercial concentrates could be a viable option to improve animal production and reduce enteric CH_4 emission intensity but its economic feasibility in smallholder context needs to be investigated.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation, upon request to the corresponding author..

Ethics statement

The animal study was reviewed and approved by the International Livestock Research Institute's Institutional Animal Care and Use Committee (IACUC, Reference number: IACUC 2019-09).

Author contributions

DK designed, planned and conducted the animal trial, processed, analysed the data and took the lead role in the writing the manuscript. RE contributed to designing and planning of the experiment, and revision of the manuscript to writing the manuscript. JG contributed to the designing of the experiment and revision of the manuscript. CA contributed to data analysis and revision of the manuscript. LM contributed to searching for funds and revision of the manuscript. SM contributed to the designing planning, data processing, writing and revision of the manuscript. All authors have approved the manuscript for publication.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Performance of food–feed maize and cowpea cultivars under monoculture and intercropping systems: Grain yield, fodder biomass, and nutritive value

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Livestock feeding in Burkina Faso is characterized by a recurrent deficit in both the quality and the quantity of fodder during the dry season, which affects animal performance. To overcome this, quality fodder/forage production is an alternative. Therefore, this study evaluated food- and feed-improved cultivars of maize and cowpea in intercropping trials using the "mother and baby trials" approach with crop–livestock farmers. The mother trial comprised a randomized block design with eight treatments and four replicates: two cowpea (KVx745-11P and Tiligré) and two maize cultivars (Barka and Espoir), and grown under two cropping systems (monoculture and intercropping). Baby trials were established on-farm and involved 30 farmers during two seasons (2019 and 2020) in four villages in the South Sudan zone of Burkina Faso. Data were collected on (1) weed density and biomass, (2) grain yield and fodder biomass, (3) intercropping efficiency, and (4) fodder nutritive value. Data were analyzed using ANOVA and the least significant difference (LSD) means separation at a 5% threshold. The results revealed that maize and cowpea intercropping significantly reduced weed biomass ($p \leq 0.05$). In monoculture, the maize cultivar Barka produced a greater grain yield (4980 kg/ha) and fodder biomass [6259 kg dry matter (DM)/ha] than the cultivar Espoir, which produced a grain yield of 2581 kg/ha and fodder biomass of 4952 kg DM/ha. The cowpea cultivars, KVx745-11P and Tiligré, were similar ($p \geq 0.05$) in terms of fodder biomass (2435–2820 kg DM/ha) and grain yield (1152–1163 kg/ha). For the intercropping system, land equivalent ratios for fodder biomass (1.18:1.41) and grain yield (1.02:1.44) were greater than 1; intercropping also had better productivity system indexes than the monoculture cropping system. The crude protein concentration of fodder was greater for Barka maize (9.5%–

9.8%) than for Espoir maize (8.5%–8.7%). The crude protein concentration was greater for cowpea KVx745-11P (19%–21.8%) than for cowpea Tiligré (15%–17%). Intercropping both Barka maize and cowpea KVx745-11P was the most productive cropping system for maximizing grain and fodder production for crop–livestock farmers in the South Sudan zone of Burkina Faso.

KEYWORDS

crop–livestock system, food–feed crops, intercropping, improved crop cultivars, Burkina Faso

1 Introduction

Livestock feeding in Burkina Faso uses mostly natural pastures and crop residues and is characterized by a recurrent deficit in fodder during the dry season that leads to a decrease in livestock productivity (Tamini et al., 2014). The three main livestock production systems are extensive, semi-intensive, and intensive systems (Kristjanson et al., 2012). The semi-intensive system involves integrating mixed-crop and livestock systems with two groups of actors, namely agropastoralists and sedentary crop–livestock farmers, who together manage about 70% of the national animal numbers (MRA, 2015). This system is a low-cost investment, with 10%–50% of household gross income coming from livestock activities (Sangaré et al., 2005). Livestock do not move far from the production site and their manure is used for soil amendment. Animals graze on natural pastures and are supplemented during the dry season with cereal stover and legume haulms (Mulumba et al., 2008; Kiéma et al., 2014). The dominant livestock species are local cattle (*Bos taurus* Linnaeus), sheep (*Ovis aries* Linnaeus), and goats (*Capra hircus* Linnaeus). The integrated crop and livestock system serves multiple roles, and benefits and strengthens farmers' resilience to risks related to the use of natural resources (Sanfo et al., 2015).

The major constraint of this production system is the scarcity of feed resources, especially in the dry season, when grazing distances become longer owing to the decline in pasture productivity and the growth of the livestock herd (Boote et al., 2021). This leads to the increased systematic use of crop residues as feed. These residues are cereal straws [sorghum, *Sorghum bicolor* (Linnaeus) Moench], millet [*Pennisetum glaucum* (Linnaeus) R Brown], and maize [*Zea mays* (Linnaeus)], as well as legume haulms [cowpea, *Vigna unguiculata* (Linnaeus) Walpers], groundnut [*Arachis hypogaea* (Linnaeus)], and Bambara beans [*Vigna subterranea* (Linnaeus), Verdc.]. They are used directly for livestock on a farm, collected and stored, or sold for livestock supplementation in the dry season (Sanou et al., 2011; Kiéma et al., 2014). The main cereal residues are maize, sorghum, and millet; these have a low crude protein concentration (2%–9%) compared with legumes (groundnut, cowpea, and Bambara bean) (9%–22%) (Nantoumé et al., 2000; Savadogo, 2000; Zampaligré et al., 2021). The incorporation of cereal and legume residues at 40% and 60%, respectively, in the diet of local sheep has been reported to give an average daily weight gain of 92–206 g/day (Kiéma et al., 2008).

The use of strictly forage-type crops remains limited despite extension and research efforts to improve adoption by farmers into their cropping systems (Cesar & Guiro, 2004). The rate of adoption has remained very low, reflecting the farmers' lack of interest in these forage-type crops due to land tenure, cropping calendar, and seed multiplication issues (Coulibaly et al., 2012). The low availability of cropland favors cereal planting to the detriment of forage plots. In an uncertain environment, the risk management strategy requires that small farmers give priority to dual-purpose crops over strict forage species so that they benefit from crop residues (feed) and grain (food) by cultivating the same unit of area. These residues, although systematically used, are generally from local cultivars and are often poorly preserved and lose their nutritive value over time. Akakpo et al. (2020) reported that crop residue preservation methods by farmers in the Sudan zone led to a loss of 14%–35% of dry matter and 15%–50% of crude protein (CP) because of a decrease in the leaf-to-stem ratio, which was linked to strong winds and intense sunshine. Improved feed–food cultivars of maize (i.e., Barka, Espoir, SR21, and Wari) and cowpea (i.e., KVx745-11P and Tiligré) have the advantage of having greater yield and quality fodder (Palé, 2017; Zampaligré et al., 2021). A better choice of cropping system could solve the problem of both arable land scarcity and soil fertility with a gain in energy and protein sources. Therefore, intercropping based on improved cultivars of maize and cowpea is proposed.

Intercropping is a combination of several crops on the same land area at the same time (Reddy et al., 1980). A complementarity of species is sought to make the system more resilient to soil physicochemical conditions (Matusso et al., 2014). Cereal and legume intercropping is suitable for small crop–livestock farmers for food and feed needs, with better land management (Nasir, 2019). An intercropping system based on improved cultivars compatible with the agricultural calendars of farmers would be a good alternative (Sangaré et al., 2005) to improve grain yield and fodder biomass (Baudron et al., 2009; Mbaye et al., 2014), as well as fodder protein (Louarn et al., 2016). The overall production (fodder plus grain) of maize–cowpea and sorghum–cowpea intercropping has been reported to increase grain yield and fodder biomass by 30%–60% compared with a monoculture of each crop (Obulbiga et al., 2015; Coulibaly et al., 2017). This cropping system improves soil fertility (Coulibaly et al., 2017) with better weed control (Matusso et al., 2014). To gain the multiple advantages of improved cultivars and intercropping, it is necessary to identify the best feed–food cultivars and suitable intercropping systems and adapt these to crop–livestock farmers' needs. It is in this context that the current study is conducted, with the objective of optimizing grain and fodder production under monoculture and intercropping among small-holder farmers using improved feed–food crop cultivars of maize and cowpea in Burkina Faso.

2 Materials and methods

2.1 Study site description: location, rainfall, soils, vegetation, animal husbandry, and farming characteristics

The study was conducted in southern Burkina Faso. The on-station trial was conducted at the Institut de l'Environnement et de Recherches Agricoles (INERA) in Farakoba, Bobo Dioulasso (11°07′00.0′′N 4°25′00.0"W), and the on-farm trials were conducted in Koumbia, Kongolikan, Sébédougou, and Makognadougou villages, with 90 km as the maximum radius from Farakoba (Figure 1). The INERA research station is located on the national road (NR1) Bobo-Dioulasso–Banfora, and 15 km from Bobo-Dioulasso.

The annual rainfall of the study site ranged from 900 to 1200 mm/year, with a 5- to 6-month dry season (from November to April). The annual rainfall recorded in 2019 (1308 mm) and 2020 (1210 mm) at the research station was greater than the long-term average of 1990–2020 (Figure 2), and the long-term mean temperature is 26°C–27°C. The rainy season typically spans May to October, with 67–69 rainy days (Figure 2).

Soils are mostly tropical ferruginous with low nitrogen, phosphorus, and organic matter content, and require fertilizer application for better crop production (Zampaligré et al., 2021). Soil analyses for the 0- to 30-cm horizon show that the study soils are acid and low in nitrogen, available phosphorus, and available potassium (Table 1).

The vegetation in this region consists of trees and shrubs, savanna, wooded savanna, and gallery forests. The climate zone is part of zone A of the Köppen climate classification (Beck et al., 2018). The dominant woody species are *Parkia biglobosa* Jacq., *Detarium microcarpum* Guill. et Perr, *Vittelaria paradoxa* CF Gaertn, *Gmelina arborea* Roxb, *Mangifera indica* Linnaeus, *Khaya senegalensis* A Juss., and *Tamarindus indica* Linnaeus, while the grass species are *Andropogon* spp, *Indigofera* spp, *Loudetia togoensis* Pilg., *Eragrostis tremula* Hochst., and *Urochloa* spp (Zampaligré et al., 2021).

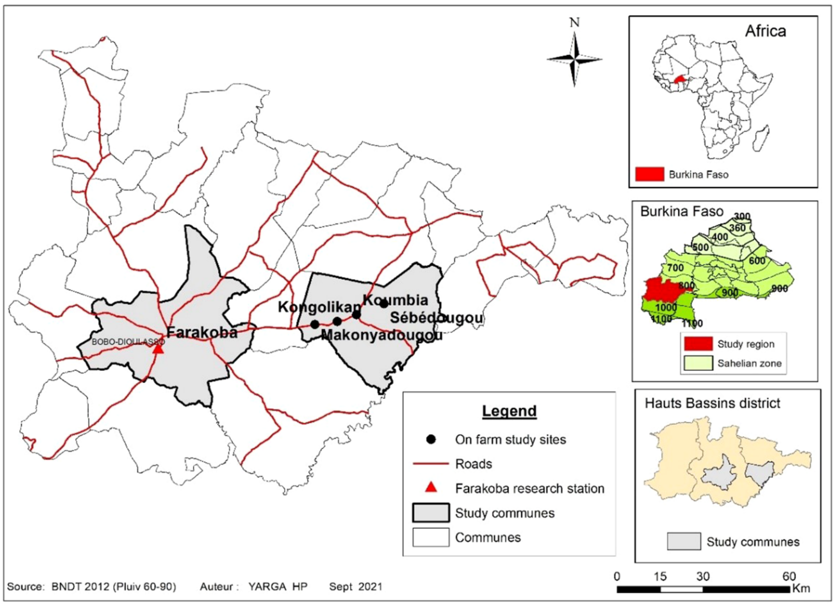

FIGURE 1
Map of the study location in Burkina Faso.

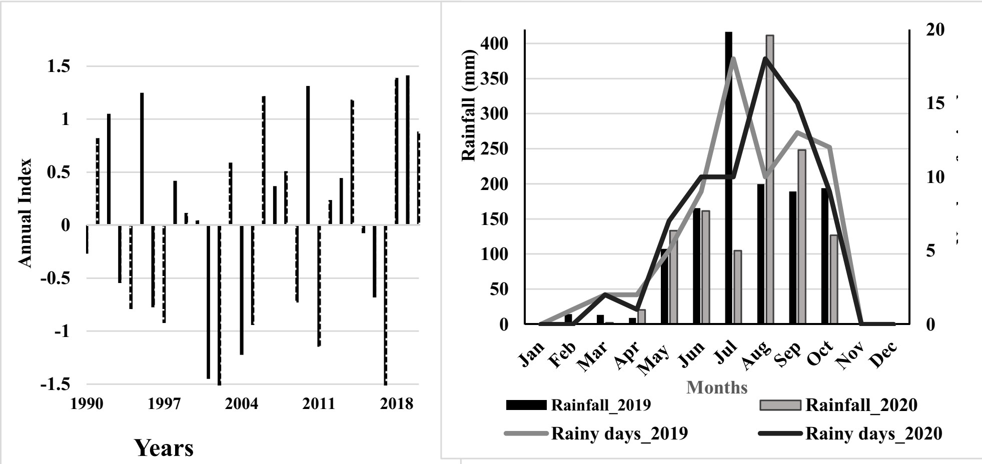

FIGURE 2
Annual rainfall indexes from 1990 to 2020 (left), and rainfall variation in 2019 and 2020 (right).

The two main livestock systems are the integrated mixed crop and livestock system and the agropastoral system; however, transhumant herders are welcomed every year during the dry season (Mulumba et al., 2008). The main animal species kept are local cattle, sheep, and goats. The national number of livestock in 2015 was estimated to be 33,455,000, with 69% of this total being small ruminants; this zone had 20% of this total number of livestock, with 13% being small ruminants (MRA, 2015). The main cereal and legume crops are maize and cowpea, respectively, and are grown by 70% of farmers in an extensive system (SP-CPSA, 2008). Plots sown for maize and cowpea represent 49.7% and 26% of the national area, respectively. Maize and cowpea are dominant staple food crops in the study area, representing about 58% and 19% of the national production, respectively, in 2015 (MA, 2015).

Agricultural systems are dominated by the small-holder farming and commercial crops, such as cotton, cashew, and mangoes.

2.2 Methodology

2.2.1 Conceptual framework

This research followed the "mother and baby trials" approach (Figure 3), and is a study of an on-farm participatory approach to introduce and test a range of technology suitable for heterogeneous communities, with two main steps (Snapp, 2002; Gonsalves et al., 2005):

The mother trial is a central test located in a village or at a nearby research station and is replicated at the site (i.e., three or four replications). The trial is designed, set up, and supervised by a research team to find the best-adapted technologies for farmers' conditions.

Baby trials are conducted on-farm and each farmer represents a replicate, comparing a subset of technologies (i.e., treatments) according to their choice from the mother trial, without replication on their farm, and are directly managed by them.

Farmers in Burkina Faso are a diverse group of individuals with distinct challenges and priorities, which has repercussions on their adaptation and adoption of new practices and technologies. In most cases, classical research approaches do not take into account this diversity. Therefore, technologies generated are not often applicable on-farm (Snapp, 2002). The "mother and baby trials" approach is a demonstration of alternative technologies and is the starting point to facilitate dialogue and collaboration among farmers, policymakers, extension, and researchers (Kerr et al., 2007). In our study, the mother trial was conducted at the Farakoba research station and the baby trials were conducted on farmers' plots in four villages

TABLE 1 Average soil chemical characteristics for two sites in Burkina Faso.

Site	pH (H_2O)	pH (KCl)	N[1] (%)	C/N[2]	Total P (mg/kg)	P-av. (mg/kg[3])	Total K (mg/kg)	K-av. (mg/kg)[4]
Farakoba research station	4.60	4.01	0.02	10.80	52.39	1.85	1263	68
On-farm	5.04	4.52	0.04	11.65	96.77	6.75	563	49

N, nitrogen; C, carbon; P,phosphoru; K, potassium; KCl, potassium chloride; av., available.

within a maximum radius of 90 km from the research station center (Figure 1).

2.2.2 Sampling methods and plant materials

A baseline survey was conducted in 2018–19 on forage production in crop–livestock systems involving 250 farmers in four (04) villages using a reasoned and stratified sampling approach with the following criteria: (1) farmers' willingness to engage in dual-purpose crop trials, with a plot size of at least 0.1 ha; (2) physical accessibility to farmers' plots; and (3) availability of at least three adult sheep for feeding trials, and a minimum of 30% of farmers being women. Following this survey, 30 farmers (20 men and 10 women) were selected to participate in a second study on the evaluation of their crops, cultivars, cropping systems, and fodder preservation method preferences (Sanfo et al., 2020). The same 30 farmers were then targeted to conduct this participatory research on fodder production using improved cultivars of dual-purpose maize and cowpea intercropping. The choice of plant material was based on farmers' preferences (Sanfo et al., 2020). Plant material was improved cultivars released by INERA that are currently being promoted in Burkina Faso. These cultivars are described in Table 2 (MRSI, 2014; Sanou, 2017a; Palé, 2017; Zampaligré et al., 2021).

2.2.3 Experimental design

The on-site mother trial followed a completely randomized block design with eight treatments in four replications. The size of each plot was 35 m² (7 × 5 m) and the treatments were:

- Treatment 1: Barka maize only.
- Treatment 2: Espoir maize only.
- Treatment 3: Tiligré cowpea only.
- Treatment 4: KVx745-11P cowpea only.
- Treatment 5: Barka maize intercropped with cowpea Tiligré.
- Treatment 6: Barka maize intercropped with cowpea KVx745-11P.
- Treatment 7: Espoir maize intercropped with cowpea Tiligré.
- Treatment 8: Espoir maize intercropped with cowpea KVx745-11P

The on-farm trials involved 30 farmers with individual plots of 1000 m². They were all trained in the agricultural practices and management of the farm on 12 June 2019, at Koumbia. Seeds, fertilizers, and pesticides were provided to the farmers by the project team. Field implementation was facilitated by extension workers (livestock and crop agents) under the supervision of the research team. Three treatments were selected from the central trial and were tested with 10 farmers per treatment: (1) Barka maize only, (2) KVx745-11P cowpea only, and (3) Barka maize intercropped with cowpea KVx745-11P. The study was conducted over two consecutive years (i.e., 2019 and 2020) during the rainy season both on-station and on-farm.

2.2.4 Trial establishment and agronomic management

Before trial implementation, information about cropping history was collected for each farmer's field. The intercropping system was the system most preferred by farmers: two rows of maize for one row of cowpea (Sanfo et al., 2020). The spacing for all the crops was 80 × 40 cm. Soils were plowed after rainfall by animal traction followed by sowing: 6–15 July for maize and 13–23 July for cowpea during the 2 years. Cowpea planting was shifted (7–10 days) later to optimize yield and fodder biomass (Mbaye et al., 2014). Seedlings were thinned at 15–20 days after sowing (DAS) to obtain the desired densities: 6.25 plants/m² in monoculture of maize and cowpea, and 4 plants/m² and 2.25 plants/m² for maize and cowpea intercropping, respectively. All the plots received compost made of cattle manure and crop residues (5 t/ha) in the first year, followed annually by mineral fertilization with nitrogen, phosphorous, and potassium (NPK; 14:23:14) at a rate of 100 kg/ha for cowpea and 200 kg/ha for maize between 15 and 20 DAS. In addition, maize plots received annually 150 kg/ha of urea (100 kg/ha between 25 and 35 DAS and 50 kg/ha between 40 and 45 DAS) in accordance with INERA recommendations (Sanou, 2017a). Weeding was carried out manually twice (15–20 and 25–35 DAS) followed by hoeing (40–45 DAS). Cowpea plots were treated twice (16 g/l of acetamiprid plus 30 g/l indoxacarb: 1 L per hectare) against parasites at flowering and pod formation. Specific treatment (15 g/l of lambda-cyhalothrin plus 20 g/l of acetamiprid) was carried out for other plots against armyworms (*Spodoptera frugiperda).* During the heading–flowering period (25–30 September), guided tours were organized for scouting and checking for insects.

2.3 Data collection

2.3.1 Grain yield (GY) and fodder biomass (FB) assessment

Grain yield was assessed at the grain maturity stage using three squares of 1 m² along the diagonal of each plot. Maize ears and cowpea pods were harvested separately and sun-dried for 10 days before the grain was shelled/threshed and winnowed. The

1 Kjeldahl procedure.

2 Walkley–Black procedure for carbon extraction.

3 Bray –^{I} method.

4 Acetate ammonium solution.

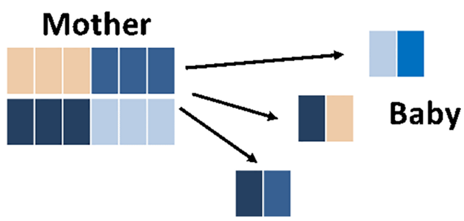

FIGURE 3
Illustration of the mother and baby approach.

resulting grains were further sun-dried to a constant weight and then weighed using a small scale of 2 kg ± 5 g to obtain a grain yield for each crop. Fodder biomass was also assessed after grain harvest on the same day and in the same area using the same three yield squares. Fresh biomass weight was measured using a 10 kg ± 10 g sensitive scale. A sample of 500 g of each square was then taken and oven-dried at 105°C for 48 hours to determine the dry matter concentration before fodder dry biomass (kg DM/ha) was computed.

2.3.2 Intercropping efficiency evaluation

Three parameters were used to evaluate the intercropping efficiency. These were weed control (WC), land equivalent ratio (LER), and system productivity index (SPI). WC describes weed density (WD) and weed biomass (WB):

WD was recorded between 75 and 80 DAS using yield square at three replicates in each plot along the diagonal.

WB was assessed using the same three yield squares. All the weeds in the squares were harvested and weighed; samples were oven-dried at 105°C for 48 hours to determine weed biomass (kg DM/ha).

LER is defined as the area of land under monoculture required to produce yields per ha achieved in intercropping (Wiley, 1979) and determined by Equation 1:

$$LER = \sum_{n=1}^{N} YN/SN \quad (1)$$

YA +............+ YN = yield of each component in the intercropping
SA + ... + SN = yield of each component in monoc ulture

SPI is an index used to evaluate the performance of the two crops in the intercropping treatment. It gives an overview of whole-system performance as both crop yields are standardized to allow comparison. Initially developed by Odo in 1991 (Khan et al., 2020), SPI has been successfully used in cereal and legume intercrop studies (Khan et al., 2020). The formula used for SPI calculation is as follows:

$$\mathbf{SPI} = (\mathbf{My} / \mathbf{Cy\ x\ Yc}) + \mathbf{Ym}$$

where My and Cy are the mean yields of maize and cowpea in monoculture, respectively, and Ym and Yc are the mean yields of maize and cowpea in intercropping, respectively.

These indexes were used to evaluate the relative advantages of intercropping compared with monocultures. The value in their appropriateness of use in our context comes from the fact that we used two crop types (i.e., cereal and legume) in a spatial arrangement. Table 3 summarizes the indices used for intercropping efficiency evaluation.

2.3.3 Fodder nutritive value assessment

For the fodder nutritive value assessment, two composite samples of the whole plant (stems plus leaves) from the three yield squares were taken in each plot, after biomass evaluation. The samples were first air-dried, then shade-dried, and finally ground at 1 mm. The near-infrared reflectance spectrometry (NIRS) method was used. The spectra of the samples were taken using the NIRS FOSS DS2500 F feed analyzer at the International Livestock Research Institute (ILRI). Global mixed-model calibrations were used. The following parameters were determined: dry matter (DM), crude protein (CP), ash, neutral detergent fiber (NDF), acid detergent fiber (ADF), acid

TABLE 2 Characteristics of crop cultivars used.

Cultivar	Cycle (day)	Seed color	Grain yield (t/ha)	Fodder biomass (t DM/ha)	CP (%)	IVOMD (%)
Maize Barka	88	White	2.7–5.5	3–7.3	8.7–9.4	52
Maize Espoir	97	Yellow	2.1–6.5	2.2–6.8	7.8–8.6	51
Cowpea Tiligré	70	White	1.5–2	2.5–3	13.–15.4	64
Cowpea KVx745-11P	75	White	0.8–1.6	3–4.5	16–21.6	65

CP, crude protein; DM, dry matter; IVOMD, in vitro organic matter digestibility.

detergent lignin (ADL), metabolizable energy (ME), and *in vitro* organic matter digestibility (IVOMD).

2.4 Statistical analysis

Microsoft Office Excel™ 2013 (Microsoft Corporation, Redmond, WA, USA) was used for data entry and for the preparation of tables and graphs. Statistical analysis was performed using IBM SPSS Statistics version 20.0 software (IBM Corp., Armonk, NY, USA).

Analysis of variance (ANOVA) (three-way ANOVA for on-station and two-way ANOVA for on-farm) was performed for a randomized complete block design (RCBD), followed by means comparisons for significant effects using the least significant difference (LSD) test. Significance was declared at a p-value of ≤ 0.05.

3 Results

3.1 Weather conditions during the two years

The 2-year period of the study received better rainfall than the mean of the last 20 years. The quantity of rain received was 1308 and 1210 mm in 2019 and 2020, respectively. These conditions were appropriate for maize production and allowed the cultivars to express their performance in the trials both on-farm and on-station. Details about the weather conditions are shown in Figure 2.

3.2 Statistical analyses of grain yield, fodder biomass, weed density, and weed biomass

For on-station trials, the year and cropping systems have significant effects on grain yield, fodder biomass, weed density, and biomass of maize (Table 4). The cultivar effect was significant only for the grain yield of maize. For cowpea, the year had a significant effect on grain yield, fodder biomass, weed density, and fodder biomass. The cultivar was significant only for fodder biomass, weed density, and weed biomass. The cropping system was also significant for grain yield and fodder biomass of cowpea. For on-farm trials, the year had a significant effect on maize grain yield only. The cropping system had a significant effect on the grain yield and fodder biomass of maize. For cowpea, the effect was significant for fodder biomass only.

3.3 Intercropping efficiency for weed control

Cropping systems did not affect weed density but did affect weed biomass (Table 5). Weed biomass in cowpea monoculture (124–328 kg DM/ha) was lower than that in maize (675–725 kg DM/ha), regardless of cultivar. In addition, cowpea and maize intercropping reduced weed biomass (261–343 kg DM/ha) compared with maize monoculture (675–725 kg DM/ha). The highest weed biomass was obtained with Barka maize monoculture (725 kg DM/ha), whereas the lowest value was observed with cowpea KVx745-11P monoculture (124 kg DM/ha) (Table 5).

3.4 Grain yield and fodder biomass

During the evaluation of the two cropping seasons, Barka grain yield (4980 kg/ha) and fodder biomass (6259 kg DM/ha) were greater than those of Espoir in monocultures. In intercropping, the best maize grain yields were obtained by Barka and KVx745-11P. The best maize fodder biomass in intercropping were Barka and KVx745-11P. Grain yield (1153–1162 kg/ha) and fodder biomass (2435–2821 kg DM/ha) were similar in monocultures of KVx745-11P and Tiligré cowpea. However, Tiligré had the least fodder biomass in intercropping. As expected, grain yield and fodder biomass from monocultures were greater than those from intercropping both on-station and

TABLE 3 Characteristics of the parameters for evaluating the efficiency of the crop association.

Parameter	Definition	Interpretation	Reference
Weed control	Weed density and biomass per unit of area	Intercropping and monoculture values comparison	Ekeleme et al., 2019
LER	Relative land area required by sole crops to produce the yields achieved in intercropping	LER = 1: equal advantages for intercropping and sole cropping TSE > 1: more advantage for intercropping than for sole cropping TSE < 1: less advantage for intercropping than for sole cropping	N'Goran et al., 2011
SPI	Standardization of the yield of the secondary crop "b" into the primary crop "a" Standardization of cowpea yield on maize yield for comparison purposes	Comparison between yields of the main crop in monoculture and the secondary crop in intercropping after standardization as indicated in the formula: a = b: yield of the primary and secondary crops are equal a > b: yield of the primary crop is greater than that of the secondary crop a < b: yield of the primary crop is less than that of the secondary crop	Khan et al., 2020

LER, land equivalent ratio; SPI, system productivity index; TSE, taux de surface equivalente.

on-farm. Barka maize fodder biomass was the best on-station for monoculture. However, cowpea KVx745-11P fodder biomass and grain yield were greater on-farm than on-station, regardless of the cropping system (Table 6).

3.5 Intercropping efficiency for land use, grain yield, and fodder biomass

Land equivalent ratio (LER) and SPI were used to evaluate intercropping efficiency in terms of land use, grain yield, and fodder biomass (Table 7). Maize and cowpea intercropping all had an LER for biomass (1.18:1.41) and yield (1.02:1.44) greater than 1 (the value of monocultures). Intercropping gave a greater SPI than monocultures for each of the maize cultivars evaluated (Table 7). The greatest SPI value for biomass was observed in Barka maize intercropped with KVx745-11P (7906). In terms of grain yield, Barka maize monoculture and Barka maize intercropped with KVx745-11P had the best SPI (4854–8787).

3.6 Fodder nutritive value

The effect of cultivar and cropping system on maize and cowpea fodder nutritive value is presented in Table 8. For the on-station trial, the main effect of the cultivar was significant only for maize CP. The main effect of cultivar was also significant for ash concentration and CP of cowpea, while only ash for their cropping system.

The nutritive value of cowpea fodder is presented in Table 8. Cowpea KVx745-11P fodder had higher CP levels than that of Tiligré (21.5% and 16.3%, respectively), with similar *in vitro* organic matter digestibility (56.8%–57.5%). Cowpea KVx745-11P fodder in intercropping had the greatest ash concentration, ranging from 14.7% to 15%. On-farm cowpea KVx745-11P fodder had a lower ash concentration than that of on-station cowpea KVx745-11P fodder (Table 9).

Barka maize fodder had higher CP levels (9.5%–9.8%) than that of Espoir (7.5%–8.5%). On-farm Barka maize fodder had a lower NDF concentration than on-station Barka maize fodder, regardless of the cropping system (Table 10).

4 Discussion

4.1 Effect of intercropping on weed control

The effect of cropping systems on weed control showed that cowpea monocultures and their intercropping with maize, regardless of cultivar used, reduced weed biomass in all the plots. Cover crops, such as cowpea, tend to occupy all the available space, resulting in a reduction in light, water, and mineral elements for weed development. Cowpea KVx745-11P is a creeping cultivar and would be the most effective in controlling these weeds in both monoculture and intercropping. The smothering of weeds inducing the reduction of their biomass would be linked to competition for light and the allelopathy phenomenon (Cordeau and Moreau, 2017). Competition for water, mineral elements, and light associated with allelopathy would affect the photosynthetic process of weeds, leading to a decrease in their development and growth (Cordeau et al., 2015). Ekeleme et al. (2019) showed that legume monocultures and their intercropping with cereals

TABLE 4 Statistical analyses of grain yield, fodder biomass, weed density, and weed biomass for cowpea and maize.

Trial	Source of variation	Cowpea				Maize			
		Grain yield (kg/ha)	Fodder biomass (kg DM/ha)	Weed density (plant/m^2)	Weed biomass (kg/ha)	Grain yield (kg/ha)	Fodder biomass (kg DM/ha)	Weed density (plant/m^2)	Weed biomass (kg/ha)
On-station	Year	***	***	***	***	*	***	***	***
	Cultivar	NS	*	*	**	***	NS	NS	NS
	Cropping system	***	***	NS	NS	***	***	***	***
	Year*cultivar	NS	**	*	*	NS	NS	NS	NS
	Year*cropping system	***	NS	NS	*	NS	**	NS	***
	Cultivar*cropping system	NS	NS	NS	NS	**	*	NS	NS
	Year*cultivar*cropping system	NS	NS	NS	NS	*	NS	NS	NS
On-farm	Year	NS	NS	–	–	*	NS	–	–
	Cropping system	NS	**	–	–	***	*	–	–
	Year*system	NS	NS			*	NS		

*p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001; NS, not significant at p ≤ 0.05.
DM, dry matter.

TABLE 5 Weed density and weed biomass (mean and standard deviation) from 2019 and 2020 under maize and cowpea monocultures and intercrops (on-station trial).

Cropping system		Weed density (plant/m^2)	Weed biomass (kg DM/ha)
Monoculture	Maize Barka	1084 ± 430	725^{a} ± 682
	Maize Espoir	943 ± 367	675^{ab} ± 585
	Cowpea Tiligré	1074 ± 129	328^{bc} ± 282
	Cowpea KVx745-11P	434 ± 237	124^{c} ± 90
Intercropping	Maize Barka and cowpea Tiligré	740 ± 390	343^{bc} ± 302
	Maize Barka and cowpea KVx745-11P	587 ± 326	260^{bc} ± 268
	Maize Espoir and cowpea Tiligré	561 ± 476	327^{bc} ± 336
	Maize Espoir and cowpea KVx745-11P	577 ± 341	261^{bc} ± 220
Statistic	*F*-value	1.54	2.30
	p-value	0.17	0.04

DM, dry, matter; LSD, least significant difference.
Values with the same letters in the same column are equal (LSD; p = 0.05).

reduced weediness and weed biomass compared with cereal monocultures and hoe-weeded plots. Chikoye et al. (2001) also found that cereal and legume intercropping reduced weed density by creating unfavorable conditions for their germination. The non-significant effect on weed density could be partly related to some short-cycle weeds having completed their life cycle, becoming dry and disappearing from the plots (Muhammad et al., 2013). In addition, in 2020, the sowing date of cowpea coincided with a dry spell, which did not allow their rapid growth (and, therefore, soil cover), which would, in turn, have had an impact on the germination of weed seeds (Ekeleme et al., 2019).

In cereal- and legume-based cropping systems for crop–livestock farmers, the use of legumes, such as cowpea, in either monoculture or intercropping presents advantages in terms of weed control that could increase yield and fodder biomass, and be time-saving for crop establishment (Muhammad et al., 2013). Odhinambo and Ariga (2001) showed that maize and cowpea intercropping for *Striga* control increased grain yield from 51% to 61%. In this study, the KVx745-11P cowpea cultivar would be the most suitable for weed control for crop–livestock farmers; therefore, this can be recommended to the farmers for better-integrated weed management in their production system instead of using chemical products.

4.2 Grain yield and fodder biomass improvement

Barka maize had the greatest fodder biomass and grain yield in monoculture, with greater fodder biomass in on-station trials than in on-farm trials. The cowpea cultivars KVx745-11P and

TABLE 6 Grain yield and fodder biomass of maize and cowpea in two cropping systems in on-station and on-farm experiments (mean from 2019 and 2020 cropping seasons).

	Cropping system	Grain yield (kg/ha)		Fodder biomass (kg DM/ha)	
Mother trial: research station		Maize	Cowpea	Maize	Cowpea
	Maize Barka monoculture	4980^{a} ± 1118	–	6259^{a} ± 1985	–
	Maize Espoir monoculture	2582^{b} ± 1466		4953^{ab} ± 2223	–
	Cowpea KVx745-11P monoculture	–	1162^{a} ± 445	–	2821^{b} ± 1281
	Cowpea Tiligré monoculture	–	1153^{a} ± 428	–	2435^{b} ± 587
	Barka and cowpea KVx745-11P intercropping	2842$^{bc=}$ ± 1360	744^{b} ± 234	3173^{bc} ± 1338	2147^{bc} ± 1055
	Barka and cowpea Tiligré intercropping	2095^{c} ± 1216	696^{b} ± 270	2736^{c} ± 1400	1804^{bcd} ± 723
	Espoir and cowpea KVx745-11P intercropping	2079^{c} ± 1261	753^{b} ± 253	2855^{c} ± 1081	2303^{bc} ± 1002
	Espoir and cowpea Tiligré intercropping	2261^{c} ± 1195	564^{b} ± 143	3471^{bc} ± 1335	1726^{bcd} ± 402
Baby trials: on farm	Maize Barka monoculture	5646^{a} ± 895	–	4840^{ab} ± 1153	–
	Cowpea KVx745-11P monoculture	–	1152^{a} ± 627	–	5352^{a} ± 1807
	Barka and cowpea KVx745-11P intercropping	3659^{bc} ± 1362	913^{ab} ± 391	3324^{bc} ± 1663	3149^{ab} ± 1048
Statistic	*F*-value	11.65	3.08	5.23	11.41
	p-value	0.0001	0.008	0.0001	0.0001

DM, dry matter; LSD, least significant difference.
Values with the same letters in the same column are equal (LSD; p = 0.05).

TABLE 7 Land equivalent ratio (LER) and system productivity index (SPI): monoculture versus intercropping.

Trial	Treatment	LER for forage biomass	LER for grain yield	SPI for forage biomass	SPI for grain yield
On-station	Cowpea Tiligré monoculture	1.00	1.00	2435	1153
	Cowpea KVx745-11P monoculture	1.00	1.00	2821	1162
	Maize Barka monoculture	1.00	1.00	6259	4980
	Maize Espoir monoculture	1.00	1.00	4952	2581
	Maize Barka and cowpea Tiligré intercropping	1.18	1.02	6974	4052
	Maize Barka and cowpea KVx745-11P intercropping	1.27	1.21	7907	4854
	Maize Espoir and cowpea Tiligré intercropping	1.41	1.36	6227	3450
	Maize Espoir and cowpea KVx745-11P intercropping	1.39	1.45	7046	4288
On-farm	Cowpea KVx745-11P monoculture	1.00	1.00	5352	1152
	Maize Barka monoculture	1.00	1.00	4839	5646
	Maize Barka and cowpea KVx745-11P intercropping	1.27	1.44	7021	8787

Tiligré also had similar grain yield and fodder biomass in monocultures. These results could be explained by genetic, agroclimatic, and cropping system factors (Coulibaly et al., 2012; Alidu et al., 2013; Coulibaly et al., 2020).

Barka and Espoir maize were identified as the best cultivars, performing well in the South Sudan zone of Burkina Faso for grain yield and fodder biomass, despite those performances being relatively low (Zampaligré et al., 2021). Cowpea KXv745-11P and Tiligré have been also identified as the best cultivar for grain yield and fodder biomass, respectively (Palé, 2017; Lalsaga and Drabo, 2017; Ramdé, 2019). These cultivars could have performed differently for grain yield and fodder biomass depending on the agro-ecological conditions of the site of production (Obulbiga et al., 2015; Ouattara, 2016; Sanou, 2017b; Traoré et al., 2020). Water and soil fertility influencing genetic potential are the most limiting factors for crop production, while the two cropping years of evaluation were wet seasons (Alidu et al., 2013; Kihindo et al., 2015). Zampaligré et al. (2021) reported less grain yield and fodder biomass for Barka and Espoir maize cultivars at Farakoba (INERA) research station in a 2-year experiment. This could be due to the level of fertilization. They used 50 kg/ha urea in addition to NPK, whereas we used 150 kg/ha urea. In addition, maize Espoir may be more sensitive to acidity, low levels of soil organic matter, and low concentrations of mineral elements, which are major causes of low maize productivity (Temegne et al., 2015; Coulibaly et al., 2017). According to the national statistics for staple crop production metrics, total maize production in the South Sudan zone of Burkina Faso is estimated at 1,124,824 t DM (MA, 2015) using approximately 367,504 ha, which represents approximately half (49.7%) of the national cropping areas dedicated to maize production (MA, 2015). In fact, this production is mainly carried out using traditional cultivars with lower grain yields and fodder biomass. The results of this study indicated that the use of this improved Barka maize cultivar in an appropriate crop management system would lead to an increase in maize biomass production up to 2,205,024–2,572,528 t DM. This represents an increase of 51% of the current maize biomass production in the country.

4.3 Intercropping efficiency evaluation

Intercropping had an LER greater than 1, which means the production capacity of a maize stand grown with cowpea is higher than that of a maize or cowpea stand grown in monoculture. Maize and cowpea intercropping, therefore, reflects a saving of 2%–45% of land area. The same grain yield and fodder biomass obtained with cowpea or maize monocultures can be produced by their intercropping while reducing the area sown by 2% to 45%. For fodder production, the highest area saving was obtained with Espoir and Tiligré intercropping (41%), while for grain production this was on-farm Barka with KVx745-11P (44%) and Espoir with KVx745-11P (45%). That means the need for an area of 1.17–1.41 ha for biomass and 1.02–1.45 ha for yield in monocultures to obtain the same production with 1 ha of intercropping. SPI values corroborate these results with higher values for intercropping than for monocultures of each entity. The highest fodder biomass (7906) and yield (8787) indexes were obtained with Barka and KVx745-11P intercropping. That means if Barka maize monoculture fodder biomass production is 6259 kg DM/ha and grain yielded 4980 kg/ha, then its intercropping with KVx745-11P would produce 7906 kg DM/ha as fodder biomass and grain yield between 4854 kg/ha and 8787 kg/ha. These gains could be explained by the beneficial complementary relationships between the two associated species for resource use (Justes et al., 2014; Louarn et al., 2016). Shifting the

TABLE 8 Statistical analyses of variation for cowpea and maize fodder nutritive value.

Trial	Source of variation	Cowpea								Maize							
		DM (%)	Ash (%)	CP (%)	NDF (%)	ADF (%)	ADL (%)	ME (MJ/kg)	IVOMD (%)	DM (%)	Ash (%)	CP (%)	NDF (%)	ADF (%)	ADL (%)	ME (MJ/kg)	IVOMD (%)
On-station	Cultivar	NS	**	***	NS	NS	NS	NS	NS	NS	NS	***	NS	NS	NS	NS	NS
	Cropping system	NS	*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	Cultivar*cropping system	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
On-farm	Cropping system	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

*=p ≤ 0.05, **=p ≤ 0.01, ***=p ≤ 0.001, NS, not significant at p ≤ 0.05.
ADF, acid detergent fiber; ADL acid detergent lignin; CP, crude protein; DM, dry matter; IVOMD, in vitro organic matter digestibility; ME, metabolizable energy; NDF, neutral detergent fiber.

sowing date between the two species reduces interspecific competition (Mbaye et al., 2014). Indeed, cowpea because it is a legume, has the capacity to fix nitrogen from the air, avoiding competition with maize for this resource. These results corroborate those obtained by N'Goran et al. (2011) and Diatta et al. (2019), who found an area saving of 30%–84% with cereal and legume intercropping. Considering the efficiency of intercropping in weed control, LER, and SPI, Barka maize and cowpea KVx745-11P or Tiligré intercropping would be the two most efficient systems.

4.4 Fodder nutritive value

Fodder nutritive values were affected by crop cultivar and cropping system. In fact, cowpea KVx745-11P had greater concentrations of ash and CP than Tiligré. Cowpea KVx745-11P fodder in intercropping had the highest ash concentration. Barka maize CP concentration was greater than that of Espoir, regardless of cropping systems. Cowpea KVx745-11P fodder content in ash was lower on-farm than on-station. However, Barka maize fodder was lower in NDF on-farm than on-station.

In total, 80%–90% of the stems and leaves of Cowpea KVx745-11P were green at the pod maturity stage (stay green), compared with 40–60% for Tiligré, which meant that better-quality fodder was obtained at harvest, including a higher CP concentration than many cultivars that do not have the same genetic characteristics (Obulbiga et al., 2015; Simian, 2017). However, the CP concentration of cowpea fodder for the two cultivars studied was higher than that obtained (11.9%) by Nantoumé et al. (2000), and was comparable to values recorded (13%–21%) by Gérad et al. (2001) using several cultivars. The concentration of CP in Barka maize fodder was similar to that of the cultivar studied by Zampaligré et al. (2021); however, in this study, Barka maize fodder had a greater concentrations of CP than Espoir. Nevertheless, both cultivars have greater concentrations of CP than those obtained by Nantoumé et al. (2000) and Savadogo (2000) with various maize cultivars fodders (4%–7%). In fact, once the maize grain is mature, there is rapid yellowing of its leaves. This could affect the nutritive value of its fodder if it is not harvested in time. For that reason, we recommend the timely harvest of maize and cowpea for fodder to maintain nutritive value.

Some authors have shown that agroecological conditions and the elapsed time between fodder sample collection at farms and their pre-drying or drying influence ash and CP content (Mehdadi et al., 2013; Schlegel and Wyss, 2013). It has been also shown that cereal–legume intercropping improves fodder nutritive value in terms of protein concentrations and levels of energy (Louarn et al., 2016). All cowpea and maize cultivar fodders at maturity have a CP level above the critical threshold of crude protein utilization, estimated at 7% (Van Soest, 1982), below which domestic ruminants' rumen microflora activity decreases. This would make it possible to avoid or reduce supplementation with an economic gain (Nantoumé et al., 2000). Thus, regarding agronomic and fodder performances of the cropping systems, Barka maize, and cowpea KVx745-11P intercropping would

TABLE 9 Nutritive value of cowpea haulms according to cropping systems: on-station and on-farm.

Cropping system		DM (%)	Ash (%)	CP (%)	NDF (%)	ADF (%)	ADL (%)	ME (MJ/kg)	IVOMD (%)
Monoculture	Cowpea Tiligré	91.3 ± 0.5	12.5^{b} ± 0.6	16.3^{b} ± 1.5	42.5 ± 1.9	36.0 ± 1.6	7.2 ± 0.5	8.0 ± 0.0	56.8^{b} ± 1.4
	Cowpea KVx745-11P	91.5 ± 0.6	13.5^{ab} ± 1.3	21.5^{a} ± 2.1	40.8 ± 3.3	37.7 ± 1.7	7.0 ± 0.0	8.3 ± 0.5	57.5^{b} ± 1.0
	Cowpea KVx745-11P*	91.0 ± 0.0	12.0^{b} ± 1.5	19.67^{a} ± 2.7	42.0 ± 3.9	32.6 ± 4.1	6.2 ± 0.9	8.5 ± 0.5	61.2^{a} ± 3.1
Intercropping	Cowpea Tiligré and maize Barka	91.3 ± 0.5	13.5^{ab} ± 1.3	15.5^{b} ± 2.5	42.7 ± 4.6	39.0 ± 4.7	7.7 ± 1.5	8.0 ± 0.0	56.0^{b} ± 2.7
	Cowpea KVx745-11P and maize Barka	91.5 ± 0.6	14.7^{a} ± 0.5	21.5^{a} ± 1.0	39.7 ± 2.1	36.2 ± 2.8	6.7 ± 0.5	8.3 ± 0.5	58.5^{b} ± 1.9
	Cowpea Tiligré and maize Espoir	91.5 ± 0.6	13.2^{ab} ± 1.7	17.3^{b} ± 1.0	41.3 ± 1.7	36.2 ± 1.3	7.0 ± 0.0	8.2 ± 0.5	57.8^{b} ± 0.9
	Cowpea KVx74511P and maize Espoir	91.7 ± 0.5	15.0^{a} ± 1.4	21.5^{a} ± 2.6	40.0 ± 2.5	38.5 ± 4.5	6.7 ± 0.9	8.3 ± 0.5	57.8^{b} ± 2.6
	Cowpea KVx745-11P and maize Barka*	91.2 ± 0.4	13.8^{ab} ± 1.9	21.8^{a} ± 1.6	40.6 ± 1.7	33.6 ± 2.5	6.4 ± 0.9	8.4 ± 0.5	60.2a ± 1.9
Statistic	*F*-value	1.17	2.42	6.94	0.61	2.33	1.61	0.67	3.07
	p-value	0.35	0.04	0.001	0.74	0.06	0.17	0.69	0.02

*on-farm trials.
ADF, acid detergent fiber; ADL acid detergent lignin; CP, crude protein; DM, dry matter; IVOMD, in vitro organic matter digestibility; ME, metabolizable energy; NDF, neutral detergent fiber.
Values with the same letters in the same column are identical ($p \leq 0.05$).

be the most suitable for crop–livestock farmers for better food–feed supply.

5 Conclusion

This study showed that intercropping with improved cultivars of maize and cowpea optimized grain and fodder biomass production, with high-quality fodder and better weed control. Although intercropping systems are better than monocultures of each crop, Barka maize intercropped with cowpea KVx745-11P was even more efficient. This will improve the availability of quality fodder and grain for crop–livestock farmers. Intercropping and use of the improved cultivars over local cultivars is an alternative that could contribute to ensuring sustainable food–feed security for humans and livestock in an integrated crop–livestock system. Thus, for the promotion of fodder production in quantity and quality based on dual-purpose cereal and legume crops that can meet human and livestock food–feed needs in the South Sudan zone, we recommend:

- ✓emphasizing the promotion of Barka maize and cowpea KVx74511P intercropping
- ✓considering Barka and KVx745-11P for monocultures if some farmers prefer this system
- ✓exploring with farmers, timely harvest, and best preservation methods needed to maintain produced fodder quality.

TABLE 10 Nutritive value of maize stover as affected by cropping systems in on-station and on-farm trials.

Crop system		DM (%)	Ash (%)	CP (%)	NDF (%)	ADF (%)	ADL (%)	ME (MJ/kg)	IVOMD (%)
Monoculture	Maize Barka	93.2^{a} ± 0.5	8.8 ± 1.7	9.8^{a} ± 1.0	67.3^{a} ± 1.5	40.0 ± 2.0	5.3 ± 0.5	7.3 ± 0.5	50.3 ± 1.5
	Maize Espoir	93.3^{a} ± 0.5	8.3 ± 1.0	8.5^{bc} ± 0.6	69.3^{a} ± 1.0	40.5 ± 2.0	5.3 ± 0.5	7.0 ± 0.0	50.0 ± 0.8
	Maize Barka*	92.0^{b} ± 0.0	11.7 ± 2.2	8.8^{bc} ± 0.8	63.2^{b} ± 3.0	40.3 ± 2.3	4.8 ± 0.8	7.3 ± 0.5	51.3 ± 3.1
Intercropping	Maize Barka and cowpea Tiligré	93.5^{a} ± 0.6	7.5 ± 0.6	9.5^{a} ± 1.0	72.3^{a} ± 3.6	40.8 ± 1.9	5.5 ± 0.6	7.0 ± 0.0	48.0 ± 2.7
	Maize Barka and cowpea KVx745-11P	93.3^{a} ± 0.5	10.5 ± 3.8	9.8^{a} ± 1.0	69.3^{a} ± 3.3	42.3 ± 3.8	5.3 ± 0.5	7.0 ± 0.1	48.3 ± 2.4
	Maize Espoir and cowpea Tiligré	93.3^{a} ± 0.5	9.5 ± 4.4	7.5^{c} ± 0.6	70.5^{a} ± 2.9	43.3 ± 3.4	5.5 ± 0.6	7.3 ± 0.5	48.0 ± 2.6
	Maize Espoir and cowpea KVx74511P	93.0^{a} ± 0.0	7.7 ± 1.0	7.8^{c} ± 1.0	70.0^{a} ± 2.6	41.3 ± 2.5	5.0 ± 0.0	7.0 ± 0.0	49.3 ± 1.3
	Maize Barka and cowpea KVx745-11P*	91.8^{b} ± 0.5	11.0 ± 2.4	8.4^{bc} ± 1.6	65.8^{b} ± 1.8	41.6 ± 2.1	4.8 ± 0.5	7.4 ± 0.6	50.4 ± 2.0
Statistic	*F*-value	11.38	1.9	3.03	6.13	0.78	1.17	0.90	1.48
	p-value	0.00	0.10	0.01	0.00	0.61	0.35	0.52	0.22

*, on-farm trials.
ADF, acid detergent fiber; ADL acid detergent lignin; CP, crude protein; DM, dry matter; IVOMD, in vitro organic matter digestibility; ME, metabolizable energy; NDF, neutral detergent fiber.
Values with the same letters in the same column are identical ($p \leq 0.05$).

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

Author contributions

AS: study concept and methodological design, data analysis, field work for data collection, and first draft of the manuscript, and its revision and editing. NZ: contributed to the study's concept and methodological design, data analysis, and first draft of the manuscript, and its revision and editing; was a PhD candidate adviser; and took part in country project co-ordination. AK: contributed to the study's concept and methodological design, manuscript revision and editing, and is a PhD research supervisor. SS and KT: contributed to data collection and curation. ER, JD, and KB: contributed to the study's concept and methodological design, manuscript revision, and manuscript editing. AA: contributed to manuscript revision and editing, project co-ordination and fundraising. All authors contributed to the article and approved the submitted version.

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Enhancing dairy productivity through best bet feeding interventions under smallholders in the central highlands of Ethiopia

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Dairy cattle production is an integral part of smallholder farming systems in the central highlands of Ethiopia, but it is characterized by low milk productivity mainly due to nutritional constraints. This study assessed the comparative advantage of using improved forage-based feeding system over the traditional feeding practices on feed intake, milk yield and quality, and cost benefit analysis of crossbred dairy cows under smallholder farmers. The study was conducted at Welmera and Ejere districts on 12 farmers and two cows per farmer, and two dietary treatments were assigned to each cow randomly, and the study lasted 8 weeks. The treatments were: 1) intervention diet composed of oats-vetch mixture hay with concentrate supplementation at the rate of 0.5 kg/l of milk and 2) basal diet following farmers' current feeding practices: crop residues and native hay, commonly soaked with local brewery residue ("Atela") and water. The results showed that there was no difference in total dry matter intake between the two diets. However, the basal feed intake was higher in the traditional feeding practice by 11% whereas the concentrate intake was higher in the intervention diet by 25%. Crude protein intake was significantly higher ($P<0.05$) in the intervention group by 23%. As a result, milk yield was increased by 36% under oat-vetch mixed fodder-based feeding system as compared to the traditional feeding practice. Consequently, the amount of feed required per unit of milk was lower in the intervention group, indicating higher feed conversion efficiency as compared to the traditional feeding practice. On the other hand, there was no significant variation in milk composition between the two groups. Partial budget analysis indicated that the benefit from the intervention diet (95 birr/cow/day) was greater by 266% than that obtained from the traditional practice (45 birr/cow/day). Utilization of improved forage varieties was proven to play significant role in supplying good quality and abundant feed resource that increased milk yield and also fetch additional economic return for smallholder farmers compared to the existing traditional practice. In general, this study demonstrates that adoption of improved forage production and feeding practices along with sufficient training on balanced ration formulation has been observed to increase dairy productivity and food security through provision of sustainable livelihood opportunity for smallholder farmers.

KEYWORDS

oats-vetch mixture, feed intake, milk yield, traditional practice, cost benefit

Introduction

Cattle production is an integral part of mixed crop livestock farming system in the central highlands of Ethiopia. The country has the largest livestock population in Africa (CSA, 2020)) and dairy has been identified as one of the priority commodities given attention by the government, with the aim to increase milk production. Given the potential, dairying is expected to significantly contribute to the national economy as well as to family food security, income and livelihoods. Among the various interventions required for improving smallholder dairy productivity, improving supply of adequate quantity of quality feed is placed at the top as feed cost accounts for more than 70% of all dairy production costs (Manaye et al., 2009; Yami et al., 2013; Seyoum et al., 2018). In view of this, utilization of technologies that can increase milk yield and lower production cost would be preferable. Evidence shows that it is possible to lower the cost of milk production by 4.4% without decreasing the output (Odero-Waitituh, 2017).

In the Ethiopian highlands, the traditional feeding system for dairy cattle is mainly based on the use of crop residues and natural pasture hay/grazing supplemented with a little or no concentrate. Feed resources are not only of low quality, but also do not last for the whole year (Mengistu et al., 2017). Consequently, smallholder dairy cattle milk yield is low, particularly during the dry season when feed scarcity hits the highest level. On average, an Ethiopian dairy cow produces only 15% of the milk produced by the average dairy cow in the world (FAOSTAT, 2012). Crop residues estimated at 57-million-ton DM per year, account for 59% of the national feed resource in Ethiopia (EIAR, 2017). However, crop residues from cereals are generally characterized by low crude protein content and low digestibility due to high cell wall constituents (Seyoum, 2007). According to FAO (2018), available feed resources in Ethiopia are still deficient by 21, 52 and 48% to meet the requirement of ruminants for dry matter, metabolizable energy and protein, respectively.

Significant successes have been registered in identifying various adaptable and high yielding fodder species belonging to grasses, herbaceous legumes and browse trees for the various agro-ecological zones of Ethiopia. Research results indicated that improved fodder species generally have higher herbage yield potential than natural pasture. The overall average productivity of the improved fodder crops per unit area has been found to exceed the productivities of seasonally rested and continuously grazed natural pastures by about 3 and 10-fold, respectively (Feyissa et al., 2015). In addition to their productivity, most of the improved forage crops are also nutritionally superior to that of natural pasture and crop residues. In a feeding trial that involved various levels of vetch (*Vicia dasycarpa*) hay supplementation in a Napier grass (*Penissetium purpureum*) based diet in lactating cows, provision of vetch hay at the rate of 20% resulted in higher daily dry matter intake and milk yield (Aemiro et al., 2010). Improved forage crops also have a long growing season and help to extend the green feed period so as to provide useful nutrients mainly in rural areas where availability and accessibility of agro-industrial by products are limited (Getnet et al., 2016).

Thus, the integration of improved forages into the existing dairy farming systems is a viable option to remedy the challenge in basal feed resource availability and unpredictable and rising prices often observed under various dairy production systems in Ethiopia. Forage species such as vetch (*Vacia sativa*) and oats (*Avena sativa*) are high potential feed resources to fill the gap of feed shortage (Negash et al., 2017). Promising improved forage oats varieties have been released from Holetta research center suitable and recommended for the central highlands of Ethiopia (Getnet et al., 2016). Thus, this study was conducted to evaluate the comparative advantage of feeding improved forage-based feeding system on milk productivity of crossbred dairy cows compared to the traditional feeding practices under smallholder management.

Materials and methods

Description of the study areas

The on-farm feeding experiment was conducted between April and June, 2020 for 60 days to demonstrate and evaluate oats-vetch mixed fodder-based feeding system on feed intake, milk yield, milk quality and cost benefit analysis using crossbred dairy cows under farmers' management condition. The study was conducted in two selected milk shed districts (Ejere and Wolmera), West Shewa Zone of the Oromia Regional State in the central highlands of Ethiopia (Figure 1). The two districts were typical representatives of market-oriented smallholder crop livestock production systems.

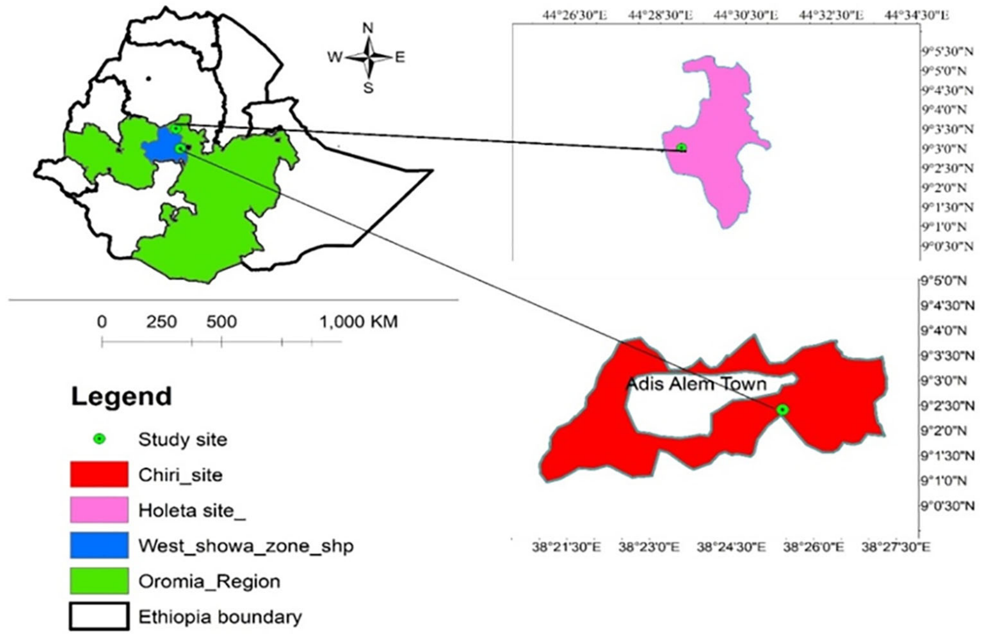

FIGURE 1
Map of the study areas.

Ejere district is located 40 km west of Addis Ababa between 8° 51'16"N to 9°14'53"N and 38°15'2"E to 38°28'45"E. The altitude of the area ranges from 2060 to 3185 masl with an average rainfall of 1100 mm. The minimum and maximum temperature of the district is 9 and 28°C, respectively (Ejere Agricultural Development Office, 2020). The district is characterized by a crop-livestock mixed farming system where cereal crops such as Teff, Wheat, Barley and Maize are commonly grown. Cattle, sheep and goat are the dominant ruminant species that support the livelihood of the farming community in the district. This district is one of the milks shed areas and well linked to fluid milk market. Moreover, from the various crops grown in the area, different crop residues are produced and conserved for use as feed during periods of feed shortage. The area is closer to Holetta Agricultural Research Center and easily accessible for monitoring and sampling of crop residues for laboratory assessment.

Welmera district is located 30 km West of Addis Ababa along the main road to Ambo. Geographically, the area is situated at 8°50' - 9° 15'N and 38°25' - 38°45'E with an altitude ranging from 2060 to 3380 masl. The district has a bimodal rainfall patterns. The minimum and maximum annual temperature is 0.1 and 27°C, respectively. Crop-livestock mixed farming is a typical agricultural practice in the area. Livestock production consists of cattle, sheep, goats, equines and poultry. Dairy production is a common practice in the district and because of its proximity to Addis Ababa with better market access, fresh milk sale is the major source of income for producers.

Oats-vetch mixed forage production

As part of the adoption and demonstration of forage technologies under the fodder component of the EQUIP project, improved forage materials were distributed and evaluated for their agronomic performance, yield and quality parameters under the farms of more than 140 dairy farmers in different locations around the Addis Ababa milk shed. Among the different improved forage materials demonstrated, further study was conducted to evaluate the feeding value of oats and vetch mixture at a ratio of 3:1 by seed weight. Twelve volunteer dairy farmers who were willing to allocate an average of 0.25 ha land for oats-vetch production, who own two crossbred dairy (Boran-Frisian cross) cows and who were willing to keep record and provide data were selected among the participants. The oats-vetch mixture was planted during the main rainy season (last week of June 2019) and fertilizer (diammonium phosphate) was applied at the rate of 100 kg/ha at the time of planting. The oats-vetch mixture was harvested 3 months after planting when the oat was at soft dough stage and when the vetch attained about 50% flowering stage. Before the total harvest, representative samples were collected using a 1m-by-1m quadrat, from three random areas per plot for yield and quality estimation. Oats-vetch mixed forage was then harvested, properly dried as hay and stored under shade until the feeding trial commenced.

Dietary treatments and experimental designs

Twelve farmers who own at least two lactating cows and who own sufficient amount of feed resources were considered for this study. From each of the 12 farmers, two lactating cows were selected, one of which was subjected to the traditional feeding practice and the other one to the oats-vetch based intervention diet. The intervention diet, T1, based on oats-vetch mixture as a basal diet supplemented with a concentrate mixture. The control group, T2, (farmer's practice which consisted of native pasture hay, crop residue and locally available supplemental feed resources). The cows were fed and watered individually. For the intervention diet, the basal feed was offered *ad libitum* and the concentrate mixture was offered at the rate of 0.5 kg per liter of milk produced. A concentrate mixture was prepared from wheat bran, noug *(Gizotia abyssinica)* seed cake and salt at a ratio of 60:39:1, respectively. The cows were exposed to the diet for an adaptation period of 14 days, followed by 60 days of actual data collection. Samples of offered feed ingredients were collected to determine chemical composition and nutritive value. Milking was conducted by hand twice per day at 6:00 am in the morning and at 6:00 pm in the evening. The data on feed intake, milk yield, milk quality, variable cost and milk sale price were collected and subjected to analysis using the independent t-test.

Stakeholder training and feed preparation

Both theoretical and practical training was provided to participant farmers (two members per household), development agents (three per district) and district experts (one per district) on improved forage production, management, ration formulation and utilization at Holetta Research Center. Concentrate mixture was provided to participants in the intervention group that is sufficient to supplement one lactating cow for the experimental period. Before feeding, the oats-vetch mixture was properly chopped, weighed and stored in clean sacks.

Laboratory analysis

The chemical composition and digestibility of the feed samples were analyzed at the national animal nutrition laboratory of Holetta Agricultural Research Center and International Livestock Research Institute. Dried samples were ground to pass through a 1mm sieve. Near Infrared Spectroscopy (NIRS) was used to analyze the samples. The NIRS machine used equations calibrated and validated by the conventional wet chemistry analysis (AOAC, 1990). The NIRS instrument used was a FOSS Forage Analyzer 5000 with software package WinISI II. Predicted nutritional variables were nitrogen (N) (Crude protein = N*6.25), neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL), dry matter (DM), total ash and *in vitro* organic matter digestibility (IVOMD). Metabolizable energy (ME) was estimated from digestible energy (DE) which in turn was estimated from the IVOMD (NRC, 2001): DE = (0.01*(OM/100) *(IVOMD+12.9) *4.4) - 0.3; ME (MCal/kg) = 0.82*DE.

Milk samples were collected in a clean plastic cup at both morning and evening milking times during the last seven days of the experimental period and immediately placed inside an icebox and taken to laboratory for milk composition analysis. The milk samples were analyzed for fat, protein, total solids, and lactose contents. NIR-Infrared milk product analyzer (user manual ver. 1.1, 2000) was used for analyzing milk composition.

Partial budget analysis

The partial budget analysis was performed to evaluate the economic advantage of the intervention diet over the traditional feeding practices. The analysis involved the calculation of the variable costs of experimental feeds and other inputs and milk sale. The feed cost for both treatments were computed by multiplying the average daily feed intake per cow with the current feed price at the local market. The total variable cost of improved forage, native pasture, crop residue and concentrate feeds was estimated using the local market price. Other input costs were calculated as 30% of the feed cost because 70% of the total cost of production for dairy production is assumed to be feed cost. The average daily revenue was calculated based on the amount of average milk yield/cow/day multiplied by farm gate milk selling price. Based on the price given by each dealer, the average selling price for each treatment was calculated. In the analysis, the average daily return (TR) was determined by calculating the difference between the average daily milk sale per cow per day and the average cost of variables per cow per day. The partial budget analysis measures profit or losses which were the net benefits or differences between gains and losses for the proposed change and includes calculating net return (NR), i.e., the amount of money left when total variable costs (TVC) are subtracted from the total returns (TR).

$$NR = TR - TVC$$

Total variable costs include the costs of all inputs that change due to the change in production technology. The change in net return (ΔNR) was calculated as the difference between the change in total return (ΔTR) and the change in total variable cost (ΔTVC), and this is to be used as a reference criterion for decisions on the adoption of new technology.

$$\Delta NR = \Delta TR - \Delta TVC$$

The marginal rate of return (MRR) measures the increase in net income (ΔNI) associated with each additional unit of expenditure (ΔTVC). This is expressed in percentages as:

$$MRR(\%) = (\Delta NR)/(\Delta TVC) * 100$$

Statistical analysis

The data was subjected to independent T-test analysis using the Stata procedure (StataCorp, 2017) to compare feed and nutrient intake, milk yield, milk quality and cost benefit analysis between intervention and control groups. The following statistical model was used for the analysis. $Y_{ij} = \mu + X_i + E_{ij}$. Where, Y_{ij} = is the response variable, μ = over all mean, X_i = the treatment effect and E_{ij} = random error.

Result

Feed types and proportion of the diet

The feed type and proportion of ingredients in the intervention and traditional diet are indicated in Table 1. For the intervention group, the feed type grown were oats-vetch mixed forage in the proportion of three to one respectively. In this group, the basal diet and the concentrate mixture consisted of 56 and 44% of the diet respectively. While in the traditional feeding system, the ration was formulated from basal diet and concentrate mixture at the rate of 62% and 38% respectively. Within the traditional system, the basal diet used were crop residue (55%) and natural pasture hay (45%). When the two groups are compared, the proportion of the basal diet was higher in the traditional group while the proportion of concentrate mixture was higher in the intervention group (Table 1).

Chemical composition, dry matter and nutrient intake of feed ingredients and dietary treatments

The chemical composition and *in vitro* dry matter digestibility of feed ingredients and dietary treatments are indicated in Table 2. Based on the laboratory evaluation, the CP content and IVDMD were observed to be higher for the intervention diet by 30% and 16% respectively. On the other hand, the NDF and ADF content was lower for the intervention diet by 13% and 14% respectively. Dry matter and nutrient intake of the intervention and traditional group are indicated in Table 3. There was no variation in total dry matter intake between the two feeding systems. However significant difference ($P<0.05$) was observed in basal diet and concentrate intake between the two groups.

Milk yield, milk quality and feed conversion ratio

Milk yield, milk quality and feed conversion ratio data of the intervention and traditional groups are indicated in Figure 2. The use of oats-vetch mixed fodder *adlibtum* and supplementation with 0.5 kg of concentrate mixture per liter of milk, increased daily milk yield by 4 liters per cow per day compared to the traditional practice.

The average milk yield in the intervention group increased from 8.5 to 11.6 liters per cow per day for the first three weeks and then remained stable afterwards while there was no change observed (7.5 liter per cow per day) in the traditional feeding practice (Figure 3). Similarly, the dry matter intake increased with increasing level of milk and remained constant after the third week, which indicates that the milk yield potential of the cow does not go beyond that level and feeding higher than that is not economical (Figure 4). In the intervention group, feed conversion efficiency decreased for the first three weeks and become stable for the following 5 weeks (Figure 5). While in the traditional diet it remained constant but higher than the conventional diet. This implies that the amount of feed required per liter of milk was higher for the traditional feeding practice.

Economic feasibility of the intervention diet over the traditional feeding practices

Total variable cost, gross return, net benefit and marginal rate of return are indicated in Table 4. Based on the partial budget analysis,

TABLE 1 Feed types and proportion of the diet used in the intervention and traditional feeding practices.

Feed type	Price/kg (Ethiopian birr)	Intervention diet (kg)	Traditional practice (kg)
Native pasture hay	2.7	–	3.52
Tef straw	3.0	–	0.38
Oats-vetch mixed hay	2.7	6.50	–
Oats hay	2.7	0.50	–
Pulse haulms	5	–	0.58
Noug seed cake	12	1.95	0.85
Wheat bran	8	3.76	1.57
Brewery by product	2	–	0.85
Molasses	3.5	–	0.10
Maize grain	10	–	0.182
Local brewery residue (“Atela”)	1	–	0.16
Total roughage intake		7.0	7.82
Total concentrate intake		5.71	4.29
Total feed intake on DM basis		12.71	12.11
Milk yield per cow per day		11.67	7.5
Milk price		16.5	16.5
Number of cows (sample size)		12	12

-, Home made brewery residue, non-conventional feed resource.

TABLE 2 Chemical composition and *in-vitro* dry matter digestibility of feed ingredients and dietary treatments used in the feeding trial.

Feed type	DM (%)	%DM					
		Ash	CP	NDF	ADF	ADL	IVDMD
Native pasture hay	89.60	13.21	5.50	67.47	40.31	5.62	49.48
Oats vetch mixture hay	91.66	10.76	8.13	61.67	40.78	5.67	57.79
Teff straw	92.25	8.39	5.02	72.66	45.20	5.57	51.09
Oat hay	92.0	10.23	6.13	62.51	41.22	5.21	54.97
Oats straw	92.48	10.98	2.38	74.15	49.65	5.86	34.19
Wheat straw	93.61	10.18	2.75	77.52	51.61	6.02	47.79
Wheat bran	89.83	5.7	16.02	48.03	15.24	4.51	71.73
Noug seed cake	92.01	9.08	26.5	41.5	32.3	8.1	65.5
Maize grain	89.90	5.7	8.8	19.4	3.8	1.0	95.3
Brewery grain	51	4.2	24.1	46	22	-	65.2
Molasses	57.7	14.25	1.63	0.0	0.0	0.0	99.2
Local brewery residue (Atela)	52.55		21.4	56.75	25.1	11	65.1
Traditional diet			9.18	62.41	37.06	5.68	53.41
Intervention diet			13.20	54.55	31.93	5.58	63.63
% Difference b/n traditional and intervention diets			30.48	12.6	13.86	1.71	16.06

TABLE 3 Dry matter and nutrient intake for the intervention and traditional feeding practice.

Parameters	Intervention diet		Traditional practice	
	Intake/cow/day (Kg)	% Intake	Intake/cow/day (Kg)	% Intake
Basal diet	7.00	57.30	7.82	63.11
Concentrate mixture	5.71	42.70	4.29	36.89
Total DM	12.71	100	12.11	100
CP	1.57	13.12	1.21	10.28
NDF	6.96	58.03	7.46	63.57
ADF	3.92	32.66	3.92	33.41

the highest net benefit was obtained from the use of intervention diet (ETB 95/cow/day) compared to the traditional group (ETB 45/cow/day). It was observed that the intervention diet had the highest variable cost, however the net benefit obtained was much higher than the variable cost. As indicated in Figure 6, income increased in the intervention group for the first three weeks and remained stable for the following weeks whereas in the traditional practice there was no change observed throughout the study period. This shows that the income in the intervention group remained higher compared to the traditional group. This confirms that the adoption and further scaling of such promising improved forage technologies bring change in milk productivity and economic benefit to small scale farmers.

Discussion

Traditional feed management and feeding practice

Soaking of crop residue with local brewery and water is commonly practiced in the traditional feeding system and such practices observed to improve intake. In the traditional feeding practice, feed provision was not based on the performance of the dairy cow and most farmers provided *adlibtum* amount of crop residue supplemented mostly with energy sources (molasses, wheat bran, pulse haulms or maize grain). On the other hand, some

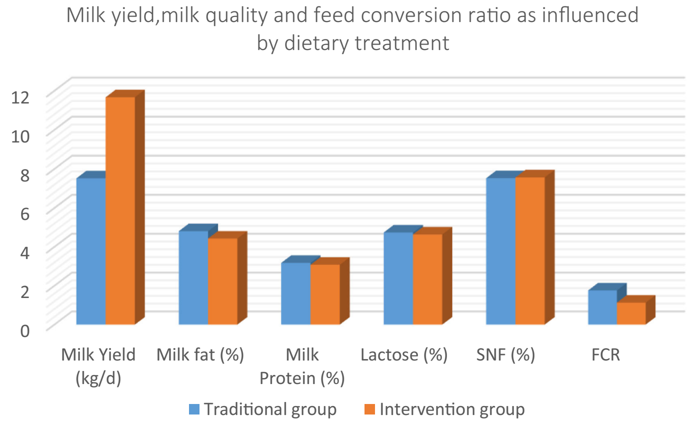

FIGURE 2
Milk yield, quality and feed conversion ratio as influenced by dietary treatments.

farmers used native pasture hay or crop residue as a basal diet and brewery by product as a supplement. This shows that the ration was not properly formulated in terms of protein and energy balance because of poor awareness on balanced ration formulation and higher cost of commercial supplements. It was also observed that ration formulated varied among smallholder farmers because of differences in available feed resources in each household and locality. Farmers usually purchase concentrate feeds that are cheaper and available in their localities without due consideration on quality parameters.

Oats-vetch fodder production

The dry matter and crude protein yield of oats-vetch mixed fodder was in the range of 6.2 to 6.7 t/ha and 0.5 to 0.54 t/ha respectively. This was in line with the report by (Shimelis et al., 2021) who indicated that dry matter (DM) yields of the oats-vetch mixed fodder ranged from 6.4 to 7.1 t/ha. Mixtures containing 25-50% legume produce more quality and quantity forage per unit area than those of pure sowings (Alemu et al., 2007). This report was in agreement with our study where we considered 25% of the basal diet from vetch. The current study revealed that the quality of oats-vetch mixed fodder is higher than pure oats hay, native pasture hay or crop residues. This is in agreement with previous report by (Negash et al., 2017) who stated that mixed cropping of cereals with forage legumes improved both quality and quantity of fodder over a pure cereal crop.

Smallholder farmers who participated in oats-vetch mixed fodder production and utilization indicated that they are well aware of the quality and higher dry matter yield compared to the existing traditional forage production practices. In support of our findings, previous study demonstrated that the use of grass legume mixture provides attractive opportunities for sustainable animal production as they contribute to increases in forage yield, substitute inorganic nitrogen inputs for the symbiotic fixation and also increase the nutritive value of the forage (Luscher et al., 2014).

Dry matter and nutrient intake

The basal feed intake in the intervention and traditional group was 57 and 63% of the total dry matter intake respectively. High values of fibrous materials in the traditional feeding practice are likely to depress digestibility of crop residue and native pasture that constituted large segment of the diet. On the other hand, the concentrate intake was 48 and 37% of the total dry matter intake in the intervention and traditional group respectively. This shows

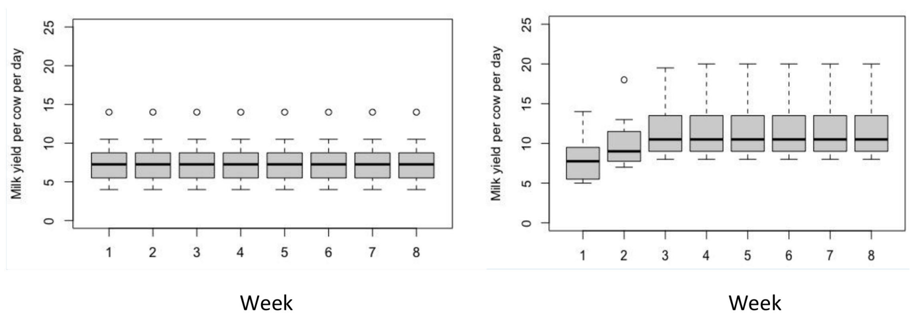

FIGURE 3
Comparison of effects of traditional feeding practice (left) and intervention diets (right) on milk yield (liter/cow/day).

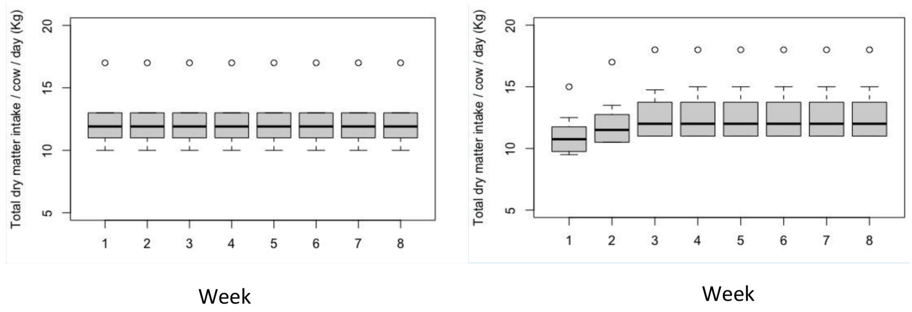

FIGURE 4
Comparison of effects of traditional feeding practice (left) and intervention diets (right) on dry matter intake (kg/cow/day).

that the basal diet intake was significantly higher in the traditional group whereas the concentrate intake was higher in the intervention group.

Crude protein intake for the intervention group was 1.57 kg per cow per day that was 13% of the total dry matter intake. On the other hand, the CP intake for the traditional feeding practice was 1.21 kg per cow per day that accounts for 10% of their total dry matter intake. Fiber (NDF) intake was higher for the traditional practice (64% of the total DM intake) than the intervention group (58% of the diet). In this study even though there was similar total dry matter intake between the two feeding systems, there was variation in nutrient intake. For instance, crude protein intake was observed to be higher in the intervention group by 23% compared to the traditional practice. Higher CP intake associated with higher digestibility has contributed to higher milk yield in the intervention group (11.67 kg per cow per day) compared to the traditional feeding practice (7.52 kg per cow per day). This indicates that promotion of improved forage development and supplementation with balanced concentrate mixture has enhanced supply of good quality feed resource.

The basal diets in the traditional feeding practices (wheat, barley and oat straw) were identified to be poor in quality that has low CP (< 2.8%) and digestibility (<48%). Such kind of traditional feeding practice based on poor quality fodder has limited the production potential of the crossbred dairy cows. In agreement with our findings, previous report indicated that traditional feeding of crossbred dairy animals suffer from under feeding in terms of nutrient requirement that caused the animals to produce below their potential (Mohamed et al., 2004).

Milk yield, milk quality and feed conversion ratio

In the current study, milk yield was significantly higher by 36% in the intervention group compared to the traditional practice. This difference in milk yield between the two groups is associated to the quality of the feed and proper formulation of balanced ration. In agreement to our findings, a study conducted in Kenya indicated that feeding oats and vetch fodder increased milk yield by up to 20% over the traditional practice and to realize adoption of such promising forage technologies, awareness creation and functioning forage seed systems need to happen concurrently (Mwendia et al., 2018). Similarly, a report by (Hintsa, 2016) indicated that the use of the oats-vetch mixed fodder as a supplement to traditional feeding resulted in daily additional milk yield of 2.33 liters per cow per day for crossbreed cattle, and 1.0 liter for local cows. Another study by Marishet et al., 2019 indicated that crossbred cows fed a basal diet of oats-vetch mixture supplemented with cottonseed cake-based concentrate mixture provided 7.97 kg of milk per cow per day

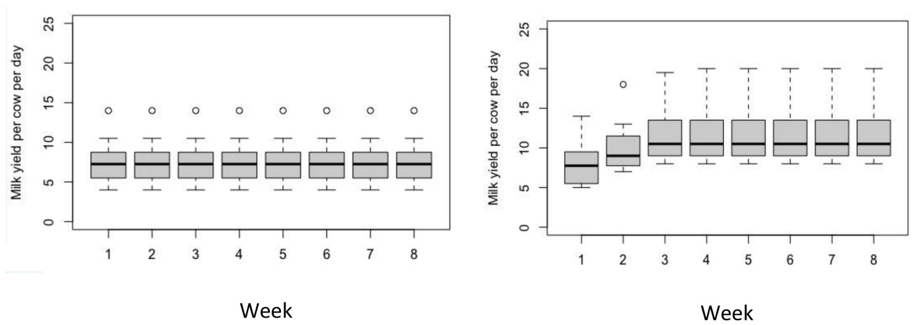

FIGURE 5
Comparison of effects of traditional feeding practice (left) and intervention diets (right) on milk income (Birr/day).

TABLE 4 Economic comparison of Intervention diet and farmers practice.

Parameter	Treatments			
	Farmers practice Mean ± SE	Intervention diet Mean ± SE	Overall mean ± SE	SL
DMI	12.11 ± 0.54	12.71 ± 0.62	12.41 ± 0.41	0.473
Cost of roughage/cow/day, Eth. Birr (A)	21.03 ± 0.83	18.9 ± 0.33	19.96 ± 0.49	0.027
Cost of concentrate mix/cow/day, Eth. Birr (B)	39.52 ± 4.44	56.01 ± 4.94	47.76 ± 3.68	0.021
Total feed cost/cow/day, Eth. birr, (D)= (A+B)	60.54 ± 4.66	74.91 ± 5.35	67.73 ± 3.71	0.05
Other costs/cow/day, Eth. Birr (C)	18.16 ± 1.40	22.47 ± 1.54	20.32 ± 2.08	0.05
Total variable costs/cow/day, Eth. birr, (D)= (A+B+C)	78.71 ± 6.06	97.38 ± 6.68	88.04 ± 4.82	0.05
Average daily milk yield (E)	7.52 ± 1.03	11.67 ± 0.81	9.60 ± 0.77	0.005
Farm gets milk selling price (F)	16.5 ± 0.00	16.5 ± 0.00	16.5 ± 0.00	
Gross return or Price of milk/cow/day, Eth. birr (G)= (E*F)	124.13 ± 13.34	192.5 ± 17.03	158.31 ± 12.8	0.005
Net profit/cow/day, Eth. Birr, H= (G-D)	45.42 ± 10.34	95.18 ± 12.78	70.27 ± 9.57	0.006
Change in total variable cost (TVC)		18.67		
Change in net profit (NP)		49.7		
Marginal rate of return (%) = (ΔNP/ΔTVC) *100		266.2		

G, represents gross return (milk price per cow per day; E, represents average daily milk yield; F, represents, farm get mil price.

which was lower than the present findings. These differences in milk yield might be associated to the variation in the level, type of ingredients, feeding management of animals among smallholder farmers and also the difference in the performance of dairy cows.

On the other hand, there was no difference observed in milk fat, milk protein, solid not fat as well as lactose content between the intervention and control group. The milk fat and protein content in the intervention diet were 4.43 and 3.15% respectively, which was in agreement with the previous report by Marishet et al., 2019, who indicated that the milk fat and protein content of dairy cows fed oats-vetch fodder were 4.44% and 2.9% respectively. The variation in milk protein in the present study can be attributed to the usual inverse relationship between milk yield and the content of milk solid components. In general, the feed conversion ratio was significantly ($P<0.05$) lower for the intervention group implying that less dry matter intake was required to produce a unit of milk compared to the traditional group.

Economic feasibility of the intervention diet over the traditional feeding practices

Economic visibility study is the main driving factor for adoption of feed technologies by smallholder farmers (Shimelis et al., 2021). Under the current price scenario for feed and milk, the higher benefit was obtained from the intervention diet. The marginal rate of return obtained from the intervention diet implies that for 1.0 birr invested in milk production, the producers obtained 2.66 Birr, an achievement that is above the minimum acceptable rate of return (CIMMYT, 1988). This study demonstrates that the adoption of improved feeding practices with sufficient training on formulation

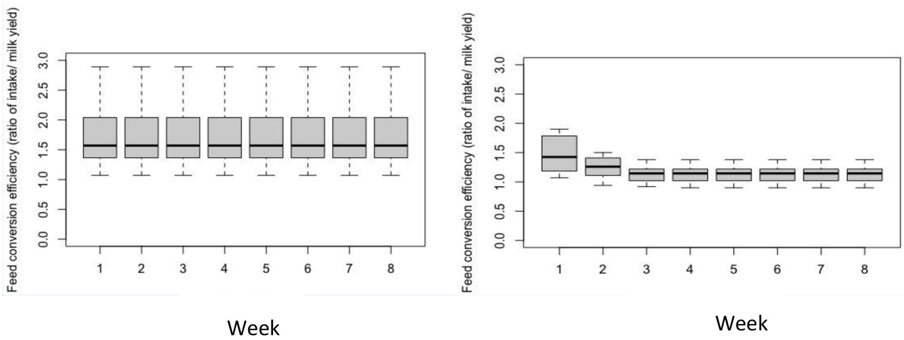

FIGURE 6
Comparison of effects of traditional feeding practice (left) and intervention diets (right) on feed conversion efficiency (ratio of intake/milk yield).

of balanced ration can result in higher economic benefit for smallholder dairy farmers. The cost benefit analysis of using oats-vetch mixed fodder for milk production resulted in a positive response. The variation in net return between treatments occurred due to the difference between the quality of feed and total milk yield per treatment.

Most of the participant smallholder farmers described that farm gate milk price is lower and it is decided by milk collectors despite the continuous increase in feed cost. This has been discouraging farmers from sustainable involvement in dairy production. Unless and otherwise feeding management is improved, the efficiency of livestock in terms of production and productivity as well as economic benefits are critically limited. This study demonstrated that changes in dairy feeding based on improved forage have shown significant improvement on farmer's livelihood through increased dairy productivity and economic return. In agreement with our study, Shimelis et al. (2021) reported that the use of good quality home grown forage can result in higher economic benefit for smallholder farmers.

At the end of this study, participant smallholder farmers visualized that, production of improved forage at their own farm land enabled them to obtain good quality fodder with higher dry matter yield at a cheaper price and that significantly increased milk yield performance and economic return. Thus, it was taken as a lesson learnt for further adoption and wider application of the technology.

Conclusion

Development and utilization of improved forage species such as oats and vetch mixture were proven to play significant role in alleviating feed scarcity in quantity and quality. The introduction of improved forage-based feeding practices has resulted in higher milk production with additional economic return to smallholder farmers compared to the traditional feeding practices. In order to address the present demand for milk, the engagement of a large number of smallholder dairy farmers on improved forage production and feeding is highly essential. In general, the study demonstrates that the adoption of improved feeding practices with sufficient training on balanced ration formulation has been observed to increase dairy productivity and food security through provision of sustainable livelihood opportunity for smallholder farmers. Development of suitable extension materials should be considered to ensure popularization of the results generated from the study to wider users.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

Ethical review and approval was not required for the animal study because we used best bet feeding system on dairy cows based on their requirement for milk yield to compare with farmers traditional practice.

Author contributions

AK did the experimental design work, conducted the experiment, analyzed the data, wrote and revised the manuscript. FF wrote and revised the manuscript. MB, ER, and AA revised and re analyzed the data. MD, GKi, DG, MM, GKe, GM, MFD, and KM revised the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Synergies of feed, management trainings, and genetics on milk production of dairy cows in the tropics: The case of Ethiopian smallholder farmers

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Efforts made so far to increase milk production of indigenous cattle by upgrading with exotic genotypes through crossbreeding under smallholder farmer conditions in Ethiopia have resulted in limited improvement. This study was conducted to determine if combining improved feed and management-related trainings with Holstein genetics will synergistically increase the milk production of dairy cows, and to examine the degree to which the increase or improvement is related to the level of exotic genetics involved. A total of 96 smallholder dairy farmers were purposively selected and allocated to one of three treatments reflecting differing degrees of capacity building support: (1) no training and no material support (Control); (2) training provided by another project (PAID) but without material support; (3) enhanced training plus material support (Feed-Mgt). Sixteen extension development agents and eight livestock experts were selected to provide training and weekly on-farm data collection, monitoring, technical support, and messaging reinforcement visits. Enhanced training addressed a broad range of topics related to feed and feeding, improved forage production, milk production and handling, construction and use of improved dairy housing, disease control, and dairy farm record keeping. Material support was in the form of formulated dairy concentrate feed and improved forage planting materials (seeds/seedlings). Data collection included variables describing animal performance, management practice adoption, and milk and butter quality. Tail hair samples for genetic profiling of breed makeup were also collected from all cows in the study. Results showed that there was a very significant effect of treatment ($P < 0.001$) on milk production, as well as treatment by week interaction ($P = 0.034$), with the effect of treatment markedly increasing over time ($P = 0.032$). That is, cows in Feed-Mgt group had up to 26.6% greater milk production compared to those in the Control group. Region and region by treatment interaction have no effects. Cows used in the experiment had varying proportions of Holstein-Friesian genome that ranged between 0 and 100%, suggesting prevalence of indiscriminate crossbreeding. Cows with a high proportion of Holstein genetics in treatment 3 produced more milk as compared to those in control group. Besides, supplemental feed improved body condition, reduced number of services per conception and resulted in higher lactation length of cows on treatment 3 compared with those on treatments 1 and 2 ($P < 0.001$). Overall, our findings suggest that an integrated approach of improved feed, feeding practices, and management training for smallholder dairy that goes beyond the improvement of genetics will increase milk production, improve milk quality and body condition, and resulted in higher length of lactation.

KEYWORDS

feed, genetics, training, smallholder, dairy cows, ethiopia

Implications

- To benefit from the genetic interventions implemented on smallholder farms, there is a need to optimize feed and management training programs
- Findings from this study could help government to enhance trainings so that smallholder dairy farmers can feed and manage productive cows in a sustainable and profitable way

1 Introduction

Dairy production in Ethiopia is predominantly undertaken by subsistence farmers, with a relatively small number of small and medium commercial dairy farms, and is characterized by low production and productivity. However, the increase of human population, urbanization and rising living standards of people are increasing demand for milk and milk products (Alexandratos and Bruinsma, 2012). This increase in demand for dairy products is becoming the main driver for changes in the dairy sector and may offer opportunities for smallholder dairy farmers to increase milk production and improve their income, livelihoods and create employment. Consequently, increasing the milk production of smallholder dairy farms is a high priority of the Ethiopian government as indicated in Growth and Transformation Plan II (National Planning Commission (NPC), 2016) and, more specifically, the Livestock Master Plan (Shapiro et al., 2015). Genetic improvement of indigenous cattle through cross breeding has been proposed as the most efficient and quickest ways of improving the productivity of local dairy herds. Various studies have shown that crossbred cows have greater milk yield and reproductive performance compared with local breeds in Ethiopia (Getahun et al., 2019; Getahun, 2022). As such, different interventions have been made by government, development partners, research institutions, and non-governmental organizations on the introduction of improved genetics into the nation's dairy herd through crossbreeding over the past five decades (Brannang et al., 1980; Schaar et al., 1981).

Milk production of these crossbred cows is yet to completely realize its full potential and produce enough milk and dairy products to meet the domestic demand. To fully achieve the milk productivity potential of the crossbred cows, commensurate improvements in management and feeding are needed (Tekeba et al., 2014). However, as illustrated by Haile et al. (2009), this is typically not the case with smallholder dairy farmers in Ethiopia. These authors reported that despite the potential for greater milk production with higher intervention levels, typical smallholder management and feeding practices are generally considered inadequate to sustainably support the greater productivity potential of crossbred cows. Similarly, Tekeba et al. (2014), based on a review of studies to assess the interaction of genetic potential and plane of nutrition, found that across studies and management systems, supplementation of conventional diets fed to cows with energy and protein-rich concentrates resulted in increased daily milk production. They also found that crossbred cows benefited substantially more than indigenous cows from better nutrition not only in terms of milk yield but also in maintenance of body condition, an important variable related to reproductive performance.

Apart from feed, it has been reported that training on dairy husbandry practices increased milk production, volume of milk processed, and milk income by about 21.7%, 56.5%, and 22.5%, respectively, relative to the untrained group (Seble et al., 2020). Other studies have also shown that training on dairy farming has a positive and significant relationship with the adoption of improved dairy husbandry practices in Ethiopia (Dehinenet et al., 2014; Samuel et al., 2016). In alignment with this, a study undertaken in another tropical country, Malawi, revealed that training improved the milk yield of dairy cows and the technical competence of dairy farmers (Kazanga, 2012). The Malawi study involved small scale dairy farmer members of six milk bulking groups (MBG) untrained and trained (three MBG replicates per treatment) in the proper management of water, feed, milk handling, animal welfare, farm hygiene and disease control. All farmers were milking cows with at least some degree of Holstein-Friesian genetics. Training was found to have a positive impact on farmer behavior with respect to improving water and feed availability, cleaning of milking equipment and utensils as well as barn facilities. These changes were, in turn, associated with increased milk yield and reduced rejections of milk by buyers. In contrast, the untrained dairy farmers continued to register low milk yield and to practice unhygienic milk handling with consequent high milk rejection rates.

Though crossbreeding of indigenous cattle with exotic breeds is established as an option commonly used by dairy cattle producers in milk-producing areas of the tropics, the national recommendation for optimum exotic genetics for high milk potential under favorable environmental and management conditions in Ethiopia is 50% (Haile et al., 2009; Hailu, 2013). However, previous research has suggested that the current genetic makeup of the Ethiopian dairy herd is highly variable, ranging from the incorporation of very low to very high levels of exotic genetics (Melku et al., 2017), implying that management skills and supportive resources required for cows to express their genetic potential also vary widely. This study will first test the hypothesis that increased knowledge and consequent changes in practices because of training in feeds and management will synergize with ongoing interventions in genetic improvement of dairy animals to further increase the quantity and improve quality of milk in Ethiopia. Secondly, we also hypothesize that level of genetic improvement will impact the response to level of nutrition. Hence, this study was conducted to investigate if the combination of improved feed and management-related trainings with exotic (Holstein) genetics would synergistically increase milk production and improve the health of dairy cows, and to explore the degree to which the increase or improvement is related to the level of exotic genetics involved.

2 Materials and methods

2.1 Study location

This study was conducted at the farm level in the primary milk shed areas in four regions of Ethiopia, namely Oromia, Amhara, Tigray, and Southern Nations Nationalities and Peoples' (Figure 1). The study specifically focused on smallholder farms that had been targeted and were close to those targeted by the Feed Enhancement for Ethiopian Development (FEED[1]) I and II projects implemented by ACDI/VOCA and were a subset of those involved in the African Dairy Genetic Gains (ADGG) and Public Private Partnership for Artificial Insemination Delivery (PAID) projects. The ADGG and PAID participants were targeted because of the opportunity to leverage the training, registration and recording of pedigree and performance data introduced through these projects. Proximity to the FEED I and II woredas (second smallest administrative unit in Ethiopia, equivalent to a district) allowed leveraging of the FEED project farmers' cooperative unions and extensive resource provision and capacity building in these woredas. In addition, other selection criteria such as possessing at least two cross-bred dairy cows (milking and/or pregnant cows), male and female headed household, proximity to the farmers' cooperative unions (FCUs), farmers who were, or intend to be, market oriented and are early adopters to serve as disseminators, farmers trained earlier by different projects (like FEED II, PAID and ADGG), untrained previously, and location (proximity for data collection, follow up and assessment) were also used.

Two woredas in each of the four regions, two kebeles (smallest administrative unit in Ethiopia, equivalent to a ward) in each woreda, and six farms in each kebele were purposely selected, resulting in a total of 96 smallholder dairy farms. The study protocol was reviewed and approved by Institutional Research Ethics Committee (IREC) of International Livestock Research Institute (ILRI). Written informed consent was obtained from the owners for the participation of their animals in this study.

2.1.1 Baseline study

After the research farms were selected a baseline study was conducted to assess feed resources and availability, feeding practices, and various aspects of dairy farm milk management on the farms. The survey and key participant interviews targeted small-scale dairy farmers and extension workers to inventory and catalogue their feeds and feeding practices, and milking management and milk handling practices.

2.1.2 Extension staff recruitment

Livestock Experts (LEs) for each woreda and Development Agents (DAs) for each kebele were recruited to support project activities in the study areas. Specifically, the DAs were selected for a Training of Trainers (TOT), assisted in the support and monitoring, participated in data collection, and conducted outcome assessments. Support and monitoring visits were scheduled so that each farmer was visited by the assigned DA once per week. This weekly visit was intended to reinforce the management practice lessons covered during the training. Monitoring included a survey of farmers to assess adoption of practices, quantity and quality of milk produced, practices used at the milk collection centers, and soliciting feedback on potential improvements. Specific variables of interests included milk production, milk quality assessment from the cooperative (including rejection rates), animal health, feed intake, and adoption rates of feed and management techniques.

2.2 Research design

The research design consisted of three treatments reflecting differing degrees of capacity building support: 1) no training and no

1 FEED was implemented with support from the USDA Food for Progress program, and PAID and ADGG projects were funded by Bill & Melinda Gates Foundation.

external support (Control), 2) training delivered by another project (PAID) but without material support, and 3) received enhanced training and material support (Feed-Mgt). In addition to their previous training by different projects (like FEED II, ADGG, etc.) or government extension services, farmers on treatment 3 were provided with enhanced trainings on feed production and feeding, dairy management, hygienic milk production, milk handling and processing, animal health, and record keeping plus support on formulated concentrate feed (Table 1) and supplied with improved forage seeds/seedlings to produce forage during year 1 followed by continuous advice, follow up and monitoring until the end of year 3. Control group farms received no training and no other support. Farmers on treatment 2 received training from the PAID project in year 1 or had previously as a standard part of the program. Persistence in animal performance improvement and farmer behavior change were monitored and data were collected for all treatments through to the end of year 3. To avoid heterogeneity of supplied formulated concentrate feed, all experimental feed required for the research was manufactured at the same feed mill and distributed to the 32 farmers (treatment 3) across the four regions. Supplied seeds/cuttings were oats (*Avena sativa*), vetch (*Vicia sativa*) and elephant grass (*Cenchrus purpureus*) that were acquired through FEED II affiliated nurseries. However, these planting materials were not supplied to all farmers in treatment 3 since some did not have a plot of land or were hesitant to allocate a plot of land to grow these forages.

A farm was considered the experimental unit. Due to the variability inherent in comparing different levels of crossbred cows, at least 32 farms per treatment were required to have an 80% chance of detecting a 20% difference in daily milk production (2 liters/day; $P < 0.05$). Based on previous training history, selected farmers per kebele were allocated to the three treatment groups. Farms were selected from different villages to avoid possible spillover effects likely to occur between farms within the same village.

TABLE 1 Ingredient and nutritional composition of concentrate feed.

Ingredient composition (%, unless otherwise stated)	
Ingredient	**Composition**
Wheat bran	19.5
Noug seed cake	48.0
Corn/maize grain	27.0
Molasses	2.0
Limestone	2.0
Salt	0.5
Premix Intraco Ruminant 1%*	1.0
Nutritional composition (%, unless otherwise stated)	
DM	90.6
Ash	8.3
CP	21.5
NDF	32.6
ADL	7.4
ADF	22.0
IVOMD	57.9
ME (KJ/kg)	8.1

*Contained per kilogram of premix: 2,000,000 IU of vitamin A; 400,000 IU of vitamin D3; 3,775 IU of vitamin E; 3,758 mg of Fe; 3,932.3 mg of Cu; 14,939.8 mg of Zn; 10,259.2 mg of Mn; 200. 9 mg of Co; 385. 0 mg of I; 52. 3 mg of Se.

2.2.1 Feed, management-related trainings, and genetics interaction

After the farm selection and baseline study were completed, and DAs were recruited training modules on feeds, feeding, and different dairy husbandry practices were developed or adapted from previously developed materials to reflect the local conditions

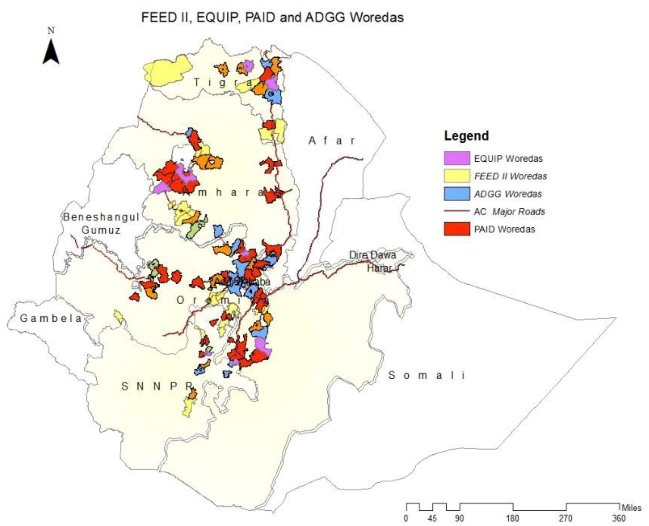

FIGURE 1
Study areas (the current study is represented by EQUIP).

and address the specific educational needs of the farmers. The training was divided into two levels. The first level of training was a Training of Trainers (ToT) targeting DAs and woreda livestock experts who were directly linked to farmers and supervise the DAs, respectively. This training lasted for 4 days, and an interactive training approach was used. In this approach, trainers were first exposed to basic information about the different training modules (Table 2) in a formal setting followed by guided assessment of actual dairies and development of a consulting report for producers to apply the knowledge gained in the formal setting. This method is believed to enhance the learning by trainers, but also to be a model for them to use in training producers at the farm level.

The second phase of training was given to farmers and lasted for 6 days. The curriculum for smallholder farmers was the same as that which was presented to DAs during the ToT. However, farmers were not asked to develop consulting reports; rather they were guided through specific improvements on their farms by the trainers. These training courses aimed at equipping farmers with knowledge, skills and practices in basic feeding and dairy management, health, and data recording, so that they could achieve higher milk yields with higher quality. This phase of the training was supported by modules, posters, brochures, and short videos. All the training materials were prepared in three local languages, namely Amharic, Afan Oromo and Tigrigna. Farmers were first exposed to the theoretical understanding of the module materials, posters and brochures followed by discussion and a short video presentation and finally practical show. At the end of the training farmers took the flyers and posters to their home to be used as a reference. The venue for this second phase of the training was at the respective farmer's home thus creating an opportunity for all family members of the household to be trained and to avoid the need for one or two members of the household to travel away from their home for such training. The ratio of trainer to trainees was one DA per household family member. The training in this research was more comprehensive with more detailed feed and management components included and more frequent (weekly) farm support visits and this is deemed as an enhanced training approach.

2.3 Data collection

During the study, DAs made visits to each farm per week. The visits were intended to provide advice and reinforce the improved practices and guide farmers in implementation of better feeding, data collection and other management practices covered during training to fully realize the genetic gain of animals. The monitoring also included a survey of farmers to assess the adoption of practices, quantity and quality of milk produced, and for soliciting feedback on potential improvements. Data collection on-farm included variables describing milk production, milk quality, and cow health. Each farmer was supplied with locally available and socially acceptable equipment to measure daily milk production of each cow. Besides, all farmers were also provided with paper forms at the beginning of each month to record daily milk production and quantity of supplemental concentrate fed. Then, training on data recording was offered to all farmers. Records were performed by farmers and checked by DAs during their weekly visit. Supplemental concentrate feed was either bought by some farmers in Control and PAID groups (treatment 1 and 2) and in this study

TABLE 2 Training modules used for the training of trainers (ToT) and smallholder dairy farmers.

Module 1. Feeds and feeding including feed troughs	Module 2. Keeping cows breeding and healthy	Module 3. Milking and milk post-harvest	Module 4. Dairy farm record keeping and housing
1. Fodder production & utilization • Fodder production strategies • Planting and managing forages • Selected improved forages and their recommended best practice • Division of family labor and role of gender in feeds and feeding	1. Natural mating versus artificial insemination • Selection of cows for breeding • Nutrition • Body condition score	1. Clean milk production and handling techniques • How to produce high quality milk? • Milk contaminants	1. Essential information for farm record keeping
2. Feed conservation • Hay making • Silage making	2. Detecting heat signs	2. Handling the milk • Milk equipment cleaning • How to keep milk cool	2. Calving and calf management • Calving • Calf management • Heifer management
3. Crop residue treatment 4. Concentrate feed mixing • Milking cows concentrate feed mixing options • Heifers and dry cows ration formulation • Calves and growers ration formulation 5. Feeding dairy cows • Feeding roughage to cows • How to feed concentrate feeds to dairy cattle • Timely feeding of cows	3. Keeping cows healthy • Mastitis • Milk fever • Vaccinations • Diarrhea and pneumonia in calves • Worm prevention • Tick control • Hoof problems	3. Small scale milk processing • How to make butter • How to take cream from milk • Preparation of cottage cheese • Roles of gender	3. Housing dairy cows • How to construct dairy barns

supplied for Feed-Mgt group (treatment 3). Though milk composition analysis was not done due to lack of laboratory facilities at or close to the study sites, quality was assessed based on organoleptic tests (visual inspection/appearance, and smell), hygienic characteristics (cleanliness and quality). It is established that good-quality raw milk must be free of debris and sediment, free of off-flavors and abnormal color and odor. Furthermore, butter quality was assessed based on indicators such as color, smell and consistency though there are no formally established standards and grades to determine the quality of butter.

2.3.1 Estimation of breed composition

Tail hair samples for genetic profiling were collected from all experimental cows and reference breeds, including samples from Arsi, Fogera, and Ethiopian Boran, all considered local indigenous breeds. Hair samples are easily collected and are amenable to long term storage at ambient temperature, making them the ideal sample for our purposes. All samples were sent to Neogen GeneSeek Operations (Lincoln, Nebraska, USA) for genotyping using the GeneSeek® Genomic Profiler™ Bovine 100K array that includes roughly 100,000 genetic markers.

Genotype data for 95,236 single nucleotide polymorphism (SNP) markers across the entire genome were generated for all the experimental cows and the reference breeds, i.e., both pure indigenous cows and pure Holstein cows. SNP markers with a minor allele frequency of less than 1% or with a calling rate of less than 95% were removed from the dataset. After quality control, a total of 86,957 SNP markers were retained for subsequent genomic analyses. The Genetic Relationship Matrix (GRM) was computed using the R package “gaston” by the formula XX’/q, with X the standardized genotype matrix and q the total number of SNPs. Note that the GRM is a symmetric square matrix of dimension equal to the number of individuals and each entry can be interpreted as an estimated kinship coefficient between individuals. The breed composition of the experimental cows was determined using Principal Component Analysis (PCA) executed in the R package “FactoMineR”. The PCA plot shows that the experimental cows cluster between the two reference breeds (indigenous breeds and US Holstein), and scores from the first principal component were used to calculate the exact Holstein composition of each experimental cow (between 0% and 100%).

3 Statistical analysis

Average milk production per week was analyzed using a linear mixed model implemented with the R package “nlme”. Note that in this first analysis farm was considered as the experimental unit. The statistical model included region, lactation, treatment, the interaction of region by treatment, week, the interaction of treatment by week, and farm as explanatory variables. An autocorrelation structure of the order 1 was used to model the residual variance-covariance matrix. Moreover, to evaluate the synergistic effects of breed composition and treatment on performance, average milk production per cow per week was also analyzed using a linear mixed model implemented with the R package “nlme”. Note that in this second analysis cow was considered as the experimental unit. The model included region, lactation, treatment, breed composition, the interaction of treatment by breed composition, week, the interaction of treatment by week, and cow as explanatory variables. Again, an autocorrelation structure of the order 1 was used to model the residual variance-covariance matrix.

4 Results and discussion

Demographic and some baseline farm characteristics of smallholder dairy farmers are presented in Table 3. The data

TABLE 3 Demographic and some baseline farm characteristics of the smallholder dairy farmers.

Parameter	Treatment		
	Control	PAID	Feed-Mgt
Age (year): Mean ± SD	47.7 ± 12.96	47.3 ± 13.20	47.5 ± 11.09
Gender: Number (%)			
Male	15 (22.4)	16 (23.9)	19 (28.4)
Female	8 (11.9)	3 (4.5)	6 (9.0)
Household family size (mean)	6.4	5.5	6.0
Education: Number (%)			
Primary school	11 (16.4)	7 (10.4)	13 (19.4)
Secondary school	5 (7.5)	9 (13.4)	8 (11.9)
College/university	2 (3.0)	0 (0.0)	0 (0.0)
Read and write	4 (6.0)	2 (3.0)	2 (3.0)
Illiterate	1 (1.5)	1 (1.5)	2 (3.0)
Family income from dairy farming: Number (%)			
Most important	9 (13.4)	9 (13.4)	13 (19.4)
Very Important	10 (14.9)	4 (6.0)	5 (7.5)
Important	4 (6.0)	4 (6.0)	5 (7.5)
Fair	0 (0.0)	0 (0.0)	2 (3.0)
Least important	0 (0.0)	2 (3.0)	0 (0.0)
Family income from other livestock: Number (%)			
Most important	1 (1.5)	0 (0.0)	3 (4.5)
Very important	6 (9.0)	6 (9.0	3 (4.5)
Important	12 (17.9)	9 (13.4)	15 (22.4)
Fair	3 (4.5)	3 (4.5)	2 (3.0)
Least important	1 (1.5)	1 (1.5)	2 (3.0)
Pastureland ownership: Number (%)			
Yes	16 (23.9)	14 (20.9)	20 (29.9)
No	7 (10.4)	5 (7.5)	5 (7.5)

given in this table show the percentages of the respondents interviewed, unless otherwise stated. There was no significant difference in the age of respondents. Most of the respondents (38%) were found to be in the productive working-age category of 18 - 55 years. This agrees with a previous study (Yohanis and Tilahun, 2021) that reported that a large proportion of dairy producers are up to age of 50. This implies the sector can provide job opportunities and serves as an income generation for a wide range of age groups. Both male and female-led dairy farms were purposively included in this study.

On-farm feed resources, consisting primarily of crop residues (straw and stover) and native grasses were the primary feed resources used as basal diet. Grass hay of medium to poor quality, mostly overgrazed communal pasture, agro- and milling by-products, and ‘atella’ (spent grains from home brewed beer) are some of the other feed resources identified in the study area. This agrees with other studies such as Tolera et al. (2012) and Gizaw et al. (2017), that reported crop residues as the major contributor to total feed supply followed by grazing, and the remaining was supplied by other agricultural and agro-industrial by-products, non-conventional feeds such as ‘atella’, and green feeds (such as weeds, sugarcane leaves, false banana leaves, etc.). These often do not meet maintenance, let alone production requirements. Formulated concentrates have been shown to improve dairy cattle performance (Assaminew and Ashenafi, 2015). However, most farmers in the study rarely supplement their cows with concentrates, mainly because of its high price and lack of local availability. They often provide saltwater solution-soaked wheat bran and oilseed cakes mixtures at milking time, presumably to encourage intake.

However, farmers assigned to treatment 3 were provided with inputs, specifically formulated compound feed (Table 1). Also, based on willingness of the farmers to allocate a small plot of land for forage production some farmers in treatment 3 were supplied with improved forage planting materials (seed/seedlings) necessary to compliment on-farm feed resources and optimize nutrition required for greater productivity, health, and reproductive performance. Though planted improved forages contributed to the total feed basket to a certain extent for those famers in treatment 3, it was difficult to quantify area of land planted, quantity fed, and proportion included in the daily basal diet. Because the type of improved forages adopted, and adoption rate varied from region to region. For instance, farmers in SNNP preferred perennial forages whereas farmers in central highlands (such as Oromia) favored annual forage crops mainly owing to the nature of the open livestock production system. During the dry season, other groups of animals are completely dependent on grazing on arable and pasture lands. In addition, farmers were also hesitant to grow the improved forages for all seasons. This is mainly because cultivable land is prioritized for food crops over forages, as forages are assumed to be less profitable than food crops.

The formulated compound/concentrate feed replaced grain and oilseed byproducts like wheat bran and noug (*Guizotia abyssinica*) seed cake typically purchased individually and mixed at home in varying amounts and fed (regardless of the production level of the animal) after being soaked in a saltwater solution. It should be noted that after our intervention, farmers adopted practices, such as feeding of cows according to production level and use of feed troughs. The mean milk production for each group weeks after intervention is shown in Table 4 and Figure 2. There was an effect of treatment ($P < 0.001$), treatment by week interaction ($P = 0.034$) on milk production and the effect of treatment markedly increases with time ($P = 0.032$; Table 4). It is evident that supplementation with improved feed substantially improved milk yield. The mean daily milk yield for treatment 3 in the current study was 11.1 L/day. That is, a net benefit in milk production for farms on treatment 3 of as much as 26.6% relative to controls was observed, and region and region by treatment interaction had no significant effect. This agrees well with the production level reported by Lobago et al. (2007) who evaluated lactation performance of smallholder crossbred dairy cattle in Oromia region. In contrast, 9.6 L/day in the Tigray region (Girmay and Gebrekidan, 2014), 5.1 L/day in North Shewa zone (Mulugeta and Belayeneh, 2013), 5.0 L/day in the central highlands (Zelalem, 1999), and 7.7 L/day in the Southwestern region (Belay et al., 2012) were reported. The relatively high maximum daily milk yield recorded from crossbred cows managed under the smallholder production level in the current

TABLE 4 Effect of feed, management-related trainings, and genetics on milk production (L/d) of dairy cows under smallholder farmers.

Treatment	Week	LSM	SE	Lower CL	Upper CL
Control	1	9.6	0.37	8.8	10.3
PAID	1	9.2	0.36	8.5	9.9
Feed-Mgt	1	10.5	0.32	9.9	11.2
Control	2	9.8	0.37	9.1	10.6
PAID	2	9.0	0.36	8.3	9.7
Feed-Mgt	2	10.9	0.32	10.2	11.5
Control	3	9.7	0.37	9.0	10.4
PAID	3	9.1	0.36	8.4	9.8
Feed-Mgt	3	11.2	0.32	10.6	11.9
Control	4	9.8	0.37	9.0	10.5
PAID	4	9.1	0.36	8.4	9.9
Feed-Mgt	4	11.7	0.32	11.1	12.4
Source		NumDF	DenDF	F- value	P-value
Baseline		1	72	889.0	< 0.0001
Region		3	72	0.8	0.5030
Treatment		2	72	11.7	< 0.0001
Week		3	241	3.0	0.0320
Region × Treatment		6	72	0.9	0.4890
Treatment × Week		6	241	2.3	0.0340

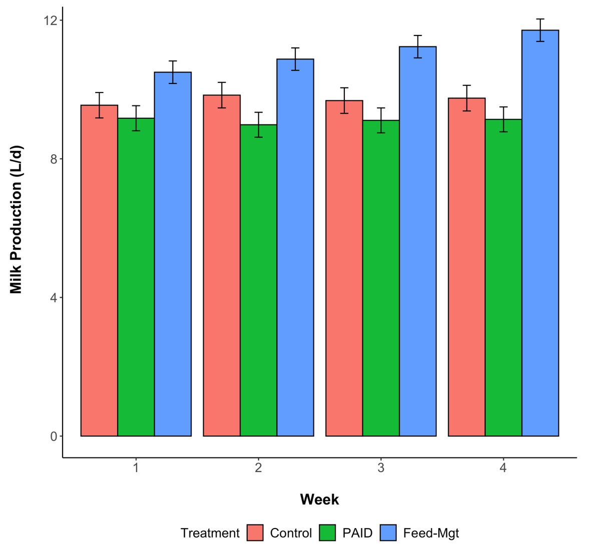

FIGURE 2
Milk production (L/d) per treatment per region weeks after intervention.

study clearly demonstrates that improved feeding and management (housing, use of feed trough and improved husbandry practices) allowed the crossbred cows to express their genetic potential to a greater extent. The results found in the current study were also higher than those reported for other tropical countries with mean daily milk yields of 6.6 L/day in Kenya (Muraguri et al., 2004) and 9.5 L/day in Tanzania (Msangi et al., 2005). The type and amount of improved feed supplemented, level of exotic genes and other management factors might have contributed partly to the difference among the studies.

Feeding 0.5 kg concentrate feed per liter of milk produced per day was cost effective if there was an increase in milk yield by at least 1.5 liter per day. On average supplementation increased milk yield by about 1.5 L/cow/d. In terms of cost, this was equal to 32 to 44.8 Ethiopian birr (ETB) at a price of 20 to 28 ETB for a liter of milk. The cost of feeding 0.5 kg concentrate feed per day was ETB 15.0 leaving a profit margin of 5.0 to 13.0 ETB which is cost effective under Ethiopian smallholder conditions. Other advantages gained by supplementation and reported by farmers were improved body condition, reduced number of services per conception in the treatment group (1 to 2 for treatment 1 versus 3 to 5 services per conception for treatment 2 and 3) and resulted in higher length of lactation as compared to other groups. Famers also indicated that the quality of butter has improved due to intervention (treatment 3). The butter becomes yellow-red which is preferred by buyers as compared to white butter in most of the study areas. This agrees well with the finding by Gebremedhin et al. (2014) who assessed butter value chain in Ethiopia. Regarding smell, farmers indicated that butter made from milk produced by cows in treatment 3 (Feed-Mgt) has got a pleasant smell.

The number of records (number of experimental cows) in each region per treatment and per breed composition is presented in Figure 3 Plot A and Plot B, respectively. Although the national recommendation for optimum exotic inheritance for high milk potential under favorable environmental and management conditions is 50% (Haile, 2006) and Beyene (1992) reported that Friesian crosses with exotic inheritance of 50 to 62.5% were

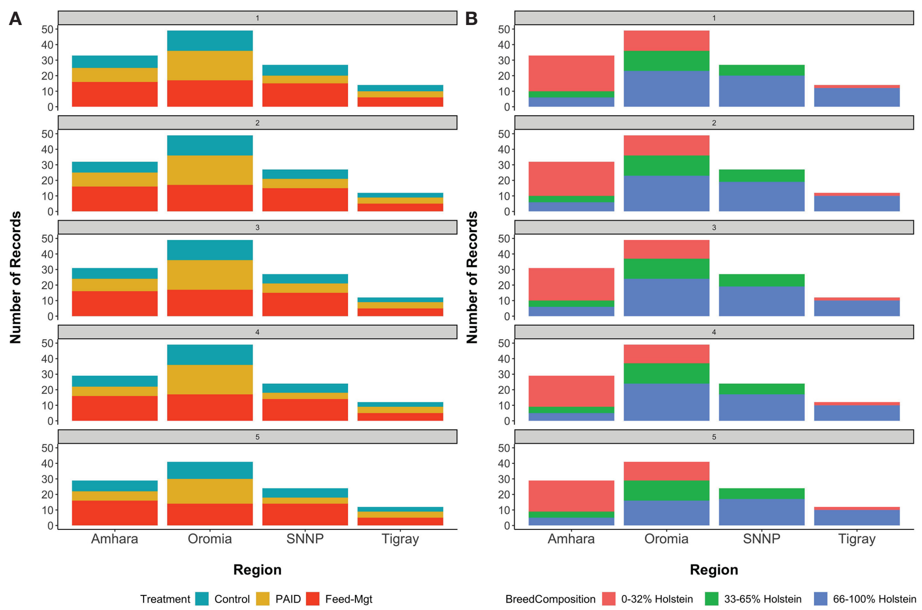

FIGURE 3
Number of records in each region per treatment (Plot A) and per breed composition (Holstein-Friesians; Plot B).

appropriate for smallholder dairy production in Ethiopia, our results revealed a large variation in breed composition, ranging from almost 0% to almost 100% Holstein (Figure 4). Of the cows involved in this study, about 33.9%, 18.6%, and 47.5% contained less than 32%, 33 - 65%, and greater than 66%, respectively, of Holstein genetics. This huge variation might be due to indiscriminate crossbreeding (Mengistu, 2019) and indiscriminate mating of indigenous cattle with exotic breeds (Alemayehu and Kebede, 2015) owing to either lack of appropriate breeding policy or binding breeding strategies (Desta, 2002; Alilo, 2019; Mengistu, 2019). Most of the cows in SNNP and Oromia regions, unlike Tigray and Amhara regions, had a large proportion (66 - 100%) of Holstein genetics (Figure 3 Plot B). This might suggest that feeding and genetic improvement for increased milk output interventions targeting smallholder dairy farmers should be implemented with breeding strategies adapted to the environment and production systems. However, it should be noted that this study did not characterize for genetics composition of cows for other exotic breeds like Simmental and Jersey.

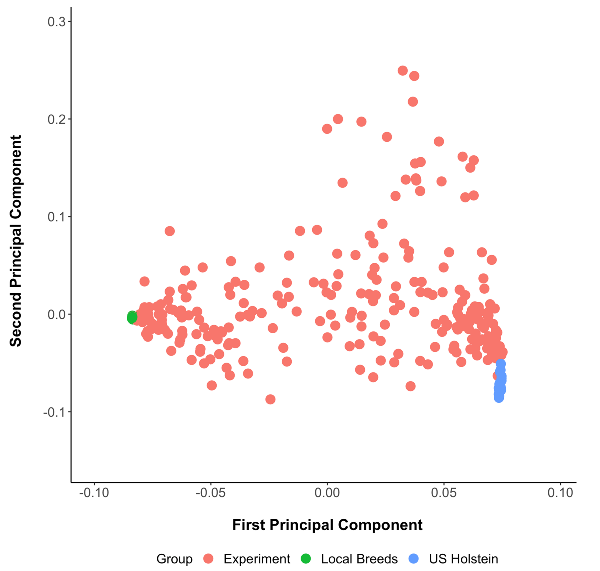

FIGURE 4
Principal component analysis looking at the breed composition (Holstein-Friesians) of cows in the study.

Daily milk production weeks after intervention per region per treatment is shown in Figure 5 and milk yield per region per breed composition is presented in Figure 6. Specially, milk production for treatment 3 in Oromia region increased 3 to 4 weeks after the beginning of the experiment as compared to the control group (Figure 7). As shown in Figure 6, at least in two regions, SNNP and Oromia, there is a clear relationship between milk production and breed composition, cows with a high proportion of Holstein genetics tend to produce more milk. This agrees with earlier studies (Tadesse and Dessie, 2003; Aynalem et al., 2009; Getahun et al., 2020) who reported that daily milk yield increases with an increasing proportion of exotic genetics. It clearly shows that there is an interaction between breed composition and training (treatment 2), and training, improved feeding, and breed

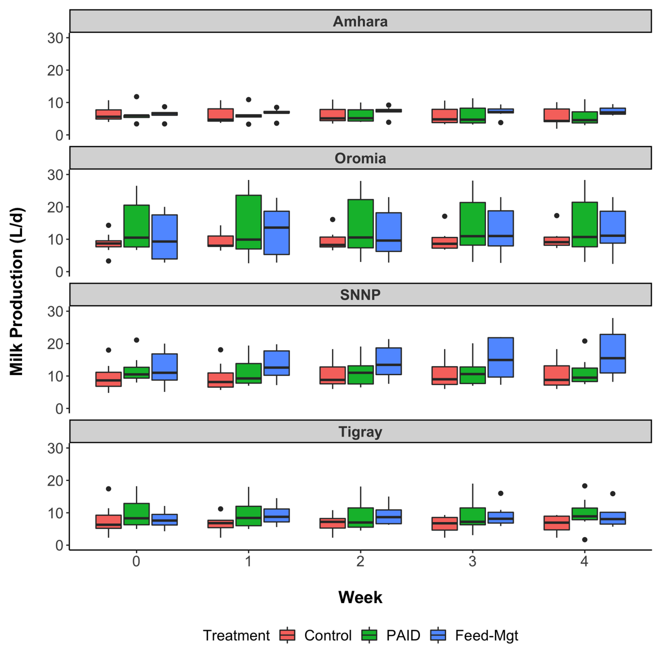

FIGURE 5
Milk production (L/d) per treatment per region weeks after intervention.

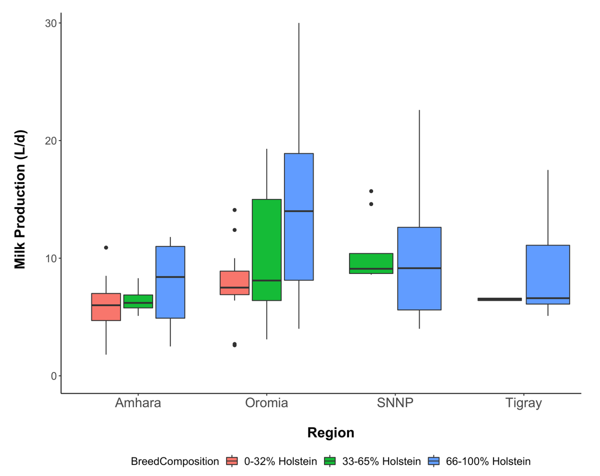

FIGURE 6
Milk production (L/d) per region per breed composition (Holstein-Friesians).

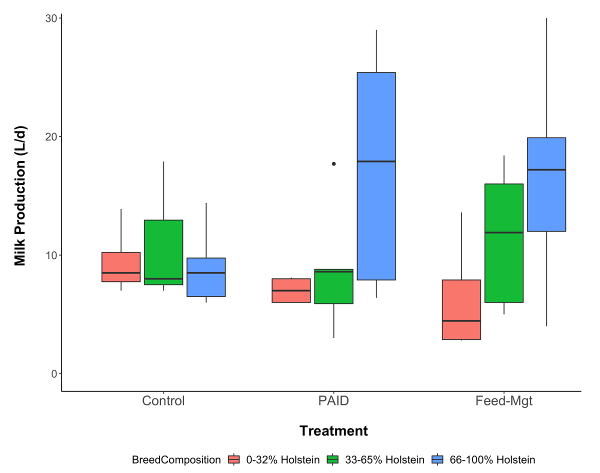

FIGURE 7
Milk production (L/d) in Oromia region 3 - 4 weeks after the beginning of the experiment.

composition (treatment 3) on the milk production. However, the reason for lack of the relationship in the other regions (Tigray and Amhara) is unclear.

Though it was observed that training and improved feeding increased milk yield and improved the adoption of practices, farmers in some study sites stated shortage of dry season forage, high cost of concentrate feed, unreliable insemination services, and low price of milk are the main limitations to improving milk production. But training of farmers in efficient feed utilization using improved feed troughs helped the farmers to reduce feed waste, feeding time and labor required to feed. Cows tended to eat more with the improved troughs because of less feed waste. However, the main challenge with the construction of improved feed troughs is lower perception of farmers about the benefits of the improved feed trough. Notably, after looking at farmers in treatment 3, other famers have approached the project team to make requests for the improved feed trough.

5 Conclusion

This study showed that improved feeding and management increased milk production of cross-bred cows. It indicates that interventions in Ethiopian smallholder dairy farms need to optimize feed and management training programs to match crossbreeding efforts to fully exploit the genetic potential of crossbred dairy cows. Findings from the current study will help extension agents as well as development project implementers to improve smallholder dairy training programs so that dairy farmers can manage highly productive cows in a sustainable and profitable way and supply both the household as well as communities with nutritious dairy foods.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

Ethics statement

The animal study was reviewed and approved by Institutional Research Ethics Committee (IREC) of International Livestock Research Institute (ILRI). Written informed consent was obtained from the owners for the participation of their animals in this study.

Author contributions

BH: Site selection, conceptualization, set up of experimental design, delivery of trainings, follow up of the experiment, data collection, and writing of original draft preparation. FP: Contributed to designing of experiment, data analysis, and revision of manuscript. MG: Project administration and revision of manuscript. CJ: Supervision, visualization, and revision of manuscript. GD: Contributed to designing of the experiment, and revision of manuscript. AA: Contributed to designing of the experiment, supervision, revision of manuscript, and funding acquisition. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Effect of the *DGAT1 K232A* mutation and breed on milk traits in cattle populations of Ethiopia

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Non-conservative *K232A* substitution in the *diacylglycerol acyl-CoA acyltransferase 1 (DGAT1)* gene has been reported to explain variation in milk traits in cattle. The objective of this study was to estimate allele and genotype frequencies and to assess associations between *K232A* variants and milk yield and composition in cattle populations of Ethiopia. Blood samples for genomic DNA extraction and milk samples for analysis of milk components were collected from 92 randomly selected cattle of five Ethiopian breeds. Gene-specific primers were used to amplify 278 bp of the exon 8 region of *DGAT1*. Allele and genotype frequencies were calculated using Power Marker, and the GLM function of SAS software was used to assess the associations of detected genetic variation with milk traits. Boran * Holstein Friesian (HF) crosses produced a higher daily milk yield than the other breeds ($p< 0.05$), whereas the Boran and Begait breeds produced milk with higher fat and protein contents ($p< 0.05$), and Horro produced milk with a higher content of lactose, than the other breeds studied ($p< 0.05$). Alleles K and A and genotypes AA, KA, and KK were detected. The frequency of *K232* ranged from 0.50 in Boran * HF crosses to 0.97 in the Horro population. The frequency of the KK and KA genotypes in the zebu population ranged from 0.50 to 0.94 and from 0.03 to 0.50, respectively. The AA genotype was associated with higher milk yield in Boran * HF crosses, whereas the KA genotype was associated with higher milk yield in the zebu populations ($p< 0.05$). The fat and lactose contents of milk produced with the KA genotype were lower than those of milk produced with the KK genotype in all genetic groups ($p< 0.05$). Substitution of one copy of the K allele led to a significant ($p< 0.05$) increase in fat content, of up to 0.81%, a decrease in daily milk yield of up to 3 L, and a decrease in lactose content of 0.58% in the sampled populations. The association study confirmed that the *DGAT1 K232A* marker had significant effects on daily milk yield, milk fat and lactose contents in the investigated cattle. These results suggested that the *DGAT1 K232A* marker may be utilized to accelerate future molecular breeding of dairy cattle, tropical zebu, their crossbreeds, after validation in a larger population.

KEYWORDS
DGAT1 K232A, Ethiopian cattle, frequency distribution, milk yield, milk quality

Introduction

The cattle agriculture sector in the tropics faces the challenges of growing productivity and profitability, in order to meet increased demand from a growing human population. Ethiopia is home to a large variety of cattle populations that experience an assortment of harsh ecological conditions, but interventions aimed at animal genetic improvement are scarce.

Increasing milk volume increment is the principal goal in the breeding of dairy cattle worldwide (Meredith et al., 2012). However, newer breeding objectives, notably to improve milk composition traits, are a response to the demand for a healthier human diet, and the basis of the milk-pricing system has shifted from a standard amount to milk composition, with a direct effect on farms' financial performance (Krovvidi et al., 2013).

Local breeds' performance levels can be increased through genetic improvement in milk performance traits, but efficient genetic improvement requires information on genetic variation and its effects on milk yield. It is known that genetic selection increases the frequency of alleles with a positive effect on a given attribute (Dekkers, 2004).

Improving productivity through genetic selection is a common goal of many animal breeding programs around the world (Meredith et al., 2012; Narayana et al., 2017; Heimes et al., 2019). Several genes affecting economically important traits in livestock species have been localized within quantitative trait loci (QTL) dispersed throughout the genome.

In cattle breeds, the *diacylglycerol O-acyl transferase 1* (*DGAT1*) gene encodes acyl coenzyme A:diacylglycerol acyltransferase, a protein involved in fat metabolism (Grisart et al., 2004; Yu and Ginsberg, 2004; Coleman and Mashek, 2011; Yen et al., 2015). The important role of this gene in the metabolism of milk fat makes it an interesting candidate for the genetic manipulation of milk characteristics. The mutation, which results in a lysine to alanine substitution at position 232 (*K232A*), has been linked to differences in the kinetics of the enzymes encoded by the two allelic variants (Grisart et al., 2004). Grisart et al. (2004) demonstrated that the lysine variant, which represents the "wild type" and is designated the K allele, is distinguished by a higher maximum rate of reaction of the enzyme involved in triglycerides synthesis than the alanine variant, thereby increasing the fat percentage in the animal's milk. The lysine variant has been linked to higher fat yield and fat and protein percentages, whereas the alanine variant has been linked to higher milk and protein yields (Grisart et al., 2002; Krovvidi et al., 2021; Elzaki et al., 2022; Gothwal et al., 2022).

The distribution of the allele frequencies of the *DGAT1 K232A* mutation has been studied in various Holstein populations and other exotic breeds, and the effects of *DAGT1* polymorphisms on milk yield traits have been researched (Bovenhuis et al., 2016; Krovvidi et al., 2021; Li et al., 2021; Gothwal et al., 2022). Information on the effect of *DGAT1 K232A* on the daily milk yield and milk composition of different cattle breeds is scarce in tropical zebu and their crossbred population. The *K232A* genotypes of *DGAT1* have been shown to have significant effects on milk composition traits in some tropical zebu populations, including Sudanese Kenana, Butana, Butana–Holstein, and Benin cattle breeds (Rahamattalla et al., 2015; Houaga et al., 2017; Houaga et al., 2018; Elzaki et al., 2022). However, whether or not the known *DGAT1 K232A* mutations segregate and are associated with milk yield and milk quality traits in zebu and their crossbred cattle populations in Ethiopia has not been well studied. Before the aforementioned marker is used for the genetic improvement of Ethiopian cattle productivity, its effect should be clearly examined.

Ethiopia is endowed with indigenous cattle, such as the Boran, Begait, Horro, and Fogera breeds, that have promising yield potential (Rege and Tawah, 1999; Zerabruk et al., 2007). A recent study revealed the polymorphic nature of the *DGAT1* gene in cattle populations in Ethiopia (Samuel et al., 2022), but further study on the influence of *DGAT1 K232A* on milk traits is required. The detection of mutations affecting dairy traits will facilitate the application of marker-assisted selection and provides insights into the genetics of milk traits in tropically adapted cattle breeds. Therefore, the present study aimed to estimate the milk composition variation as well as allele and genotypic frequencies of the *DGAT1 K232A* mutation and to assess the effects of the two allelic variants on daily milk yield and milk composition in cattle populations in Ethiopia.

Materials and methods

Sample collection and DNA extraction

The animal study was reviewed and approved by the Ethical Review Board of Adama Science and Technology University (certificate reference number RECSoANS/BIO/07/2021). Written informed consent was obtained from the owners for the participation of their animals in this study.

A total of 92 animals from five cattle populations (17 Boran, 16 Begait, 18 Fogera, 17 Horro, and 24 Boran * HF cross (75% F1 HF** (HF * Boran) were sampled from state-owned farms in Ethiopia.

Boran cattle are predominantly distributed in the semi-arid and arid areas of southern Ethiopia, northern Kenya, and south-western Somalia, and are large East African zebu cattle known for their fast growth, high milk yield, and heavy muscling. They are known to need a calm environment (Rege and Tawah, 1999). The Horro cattle breed is widely distributed in south-western and west Ethiopia. It is one of the sanga * zebu intermediate types and is used for milk and meat yield, and to provide draft power (Rege and Tawah, 1999; Edea et al., 2012; Mwai et al., 2015). The Fogera cattle breed is found in the north-west highlands of Ethiopia and is reared mainly for dairy yield and for its draft power. It belongs to the zenga group, which is a cross between sanga and zebu (Rege and Tawah, 1999; Zerabruk et al., 2007). The Begait breed is grouped under large East African zebu (Rege and Tawah, 1999) and is used primarily for milk and meat yield (Zerabruk et al., 2007; Assefa and Hailu, 2018).

Blood and milk samples were obtained only from randomly selected and sampled lactating cows. The studied animals were raised on natural grazing without concentrate supplementation and were sampled at the same time, eliminating the effect of season. Blood samples (4 mL)were collected from the tail head of each animal under aseptic conditions and gently mixed with ethylene diamine tetraacetic acid (EDTA) anticoagulant placed into an ice box containing ice. Extraction of genomic DNA was carried out using the salting-out extraction procedure (Nasiri et al., 2005). The quality of the DNA and its concentration were assessed using a NanoDrop1000 spectrophotometer and electrophoresis in 1.5% agarose gels. Those DNA samples with good quality and quantity were used for amplification and sequencing.

PCR amplification and sequencing of the *DGAT1* gene region

Based on the reference sequence (AJ318490) the primers were designed to amplify a 278-bp fragment enclosing the p.Lys232Ala mutation (forward: 5′-AAGGCCAAGGCTGGTGAG-3′; reverse: 5′-GGCGAAGAGGAAGTAGTAG-3′) using Primer3Plus software (Rozen and Skaletsky, 2000). PCR was carried out in a total volume of 25 µL containing, 5* PCR buffer (5 µL), 1.5 mM $MgCl_2$ (3 µL), 10 mM dNTP mix (1 µL), forward primer 10 pmol/µL (0.5 µL), reverse primer 10 pmol/µL (0.5 µL), genomic DNA 25 ng/µL (2 µL), Taq DNA polymerase 5 U/µL (0.3 µL) and DNAase-free water (12.7 µL). The optimized thermal profile comprised an initial denaturation at 94°C for 3 min, 30 cycles of denaturation at 94°C for 1 min, annealing at 57°C for 45 s, elongation at 72°C for 1 min, and a final extension at 72°C for 7 min. Finally, the PCR products were visualized post electrophoresis on 1.7% agarose gel in TAE buffer (Tris base, acetic acid, and EDTA) followed by GelRed staining. The PCR products were sequenced on an ABI 3730 sequencer (Applied Biosystems, Foster City, CA, USA) using the Sanger dideoxy chain termination method at Konkuk University, Seoul, South Korea. The sequences were analyzed and deposited in GenBank with the accession numbers ON262825–ON262849.

In the early morning and late afternoon, milk samples (50 mL) were collected from each animal in clean, dry, grease-free, and labeled milk collection vials. The milk samples were collected in the mid-milking stage to avoid sampling fluctuations in the milk composition data. No milk was collected from the animals that were in the terminal or early stage of lactation. Milk samples were stored at 4°C and then sent to the Holeta dairy research laboratory for milk composition analyses. The percentages of milk fat, protein, lactose, and non-fat solids were determined using a Lactoscan ultrasonic milk analyzer (Milkotronic Ltd, Nova Zagora, Bulgaria).

Data management and statistical analysis

Prior to analysis, all chromatograms were visualized and sequence fragments were edited using Bio-edit version 7.0.5.3 and aligned using the ClustalX2 software package (Larkin et al., 2007). Genotype and allele frequencies were calculated using Power Marker (version 3.25) (Liu and Muse, 2005). The standard error (SE) of allelic frequency was calculated as $[p(1-p)/2n]^{1/2}$, where n is the sample size and p is the frequency of allele (Spiess, 1977).

To investigate the effect of breed type and *DGAT1* genotypes on milk components, we used a generalized linear model (GLM), and the difference between means was calculated using the Tukey honestly significant different (HSD) assess with the statistical software package SAS version 9.3 (SAS, 2011). Breed type and genotype were fitted as fixed independent variables, while the observed traits (daily milk yield, fat percentage, protein percentage, non-fat solids percentage, and lactose percentage) were fitted as dependent variables. The effects of fixed factors were fitted in the statistical model below:

$$Y_{ij} = \mu + B_i + G_j + (B_i \times G_j)_{ij} + \epsilon_{ijk}$$

where Yij is the observed trait (daily milk yield, fat percentage, protein percentage, non-fat solids percentage, or lactose percentage), µ is the population mean, B_i is the fixed effect of ith breed type ($i = 5$), G_j is the fixed effect of *DGAT1* genotype (KK, KA, or AA), $(B \times G)_{ij}$ is the fixed interaction effect between breed and *DGAT1* genotype, and ϵ_{ijk} is the random residual associated with each record.

The quantitative effects of one copy of the K allele of *DGAT1 K232A* were also calculated. The linear model was used, with the replacement of genotype effect by the linear regression on the number of desired alleles: $Y_{ijk} = \mu + b^{*}x_i + e_{ij}$
where Y_{ij} is a phenotypic observation (milk traits) of the animal, µ is the overall mean, x_i is the number of desired alleles (0, 1, or 2), b is the regression coefficient representing the allele substitution effect, and e_{ij} is a random residual effect. The results of breed and *DGAT1* genotype effects are presented as least-squares means ± SE. A probability of less than 0.05 was considered to indicate a significant difference.

Results

Effect of breed on milk yield and composition

Statistically significant differences in milk components were observed among the studied breeds (Table 1). The Boran lowland breed produced milk with a significantly higher fat content than the other breeds ($p< 0.05$), while the other lowland breed, Begait, produced milk with a significantly higher protein content ($p< 0.05$). On the other hand, Horro produced milk with higher lactose content than the other breeds ($p< 0.05$). The Boran, Begait, and Horro breeds all produced milk with a higher content of non-fat solids than other breeds.

Least-squares means of milk components across breeds are reported in Table 1. Boran * HF crosses produced a higher daily milk yield than the other breeds ($p< 0.05$).

TABLE 1 Effect of breed on DMY and milk composition traits in Ethiopian cattle.

Variable	Breed				
	Boran (17)	Begait (16)	Horro (18)	Fogera (17)	Boran * HF cross (24)
DMY (L/day)	2.12 ± 0.12^{ab}	2.82 ± 0.12^{bc}	2.42 ± 0.26^{b}	2.68 ± 0.12^{bc}	7.21 ± 0.10^{d}
Fat (%)	6.21 ± 0.04^{d}	4.68 ± 0.042^{c}	3.47 ± 0.09^{b}	4.63 ± 0.041^{c}	3.18 ± 0.03^{a}
Protein (%)	3.54 ± 0.03^{bc}	3.63 ± 0.03^{c}	3.39 ± 0.08^{b}	3.04 ± 0.03^{a}	3.14 ± 0.03^{a}
Lactose (%)	4.72 ± 0.05^{b}	4.43 ± 0.05^{a}	5.31 ± 0.11^{c}	4.29 ± 0.05^{a}	4.70 ± 0.04^{b}
Non-fat solids (%)	8.99 ± 0.09^{c}	8.86 ± 0.09^{c}	9.35 ± 0.18^{c}	7.90 ± 0.08^{a}	8.57 ± 0.08^{b}
Ash (%)	0.71 ± 0.008^{a}	0.76 ± 0.008^{b}	0.74 ± 0.01^{b}	0.69 ± 0.008^{a}	0.70 ± 0.007^{a}

DMY, daily milk yield. [a,b,c,d]Values within a row with different superscripts differ significantly at p< 0.05.
p-value indicates that daily milk yield and milk composition varied significantly in different genetic groups within a row.

Allele and genotype frequencies of the *K232A* protein variant

The genotypic and allelic frequencies of the *DGAT1* gene in Boran, Begait, Horro, Fogera, and Boran * HF cross cattle are presented in Table 2.

The *DGAT1* K allele, corresponding to lysine substitution, was the predominant allele in all zebu populations. All three possible genotypes of *DGAT1 K232A* were observed in Boran * HF crosses, although the KA genotype predominated. The KK genotype predominated in the Boran and Fogera populations, but was even more prevalent in Horro. In Begait, the KK and KA genotypes have identical frequency distributions (Figure 1; Table 2).

Effect of *DGAT1 K232A* variants on daily milk yield and composition

Variability at the *DGAT1* K232A polymorphic site indicated that this locus can be considered for association studies only in Boran, Begait, Fogera, and Boran * HF crosses. In Horro populations, the minor allele frequency is less than 0.1 and, hence, does not qualify for association studies (Table 3).

The AA genotype was associated with greater milk yield in Boran * HF crosses, whereas the KA genotype was associated with greater milk yield in the zebu populations ($p< 0.05$). The fat and lactose contents of milk produced with the KA genotype were lower than those of milk produced with the KK genotype in all genetic groups ($p< 0.05$). The percentages of milk protein and non-fat solids were lower in milk produced with the KA genotype than in milk produced with the KK genotype in all the genetic groups ($p > 0.05$).

Substitution of the K allele showed that even one copy of this allele leads to a significant ($p< 0.05$) increase in fat content in Boran * HF crosses (of up to 0.81%) and a significant increase of protein content (of up to 0.32%) in Boran breeds. In contrast, substitution of the K allele leads to a decrease in daily milk yield of up to 3 L in Boran * HF crosses and a decrease in milk lactose content of 0.58% in Horro cattle.

Discussion

Effect of breed on milk yield and composition

The milk fat content of the studied breeds was within the range of the standard milk composition requirement for cows (3.18%–6.21%). Zebu cows can give milk containing up to 7% fat (O'Mahony, 1988). Significant differences among the studied breeds were observed for all milk components. Similar studies have reported the effect of breed on milk composition in South African (Myburgh et al., 2012) and Benin cattle breeds (Houaga et al., 2017). Boran cows in Ethiopia presented a higher fat content

TABLE 2 Genotypic and allelic frequencies in exon 8 of the *DGAT1* gene.

Breed	*N*	Genotypic frequencies			Allele frequencies		SE
		KK	KA	AA	K	A	
Boran	17	0.59	0.41	–	0.80	0.20	0.07
Begait	16	0.50	0.50	–	0.75	0.25	0.08
Horro	17	0.94	0.06	–	0.97	0.03	0.03
Fogera	18	0.56	0.44	–	0.78	0.22	0.07
Boran * HF cross	24	0.25	0.50	0.25	0.50	0.50	0.07
Overall	92	0.54	0.39	0.07	0.74	0.26	0.03

N, number of animals; SE, standard error of the allele frequencies.

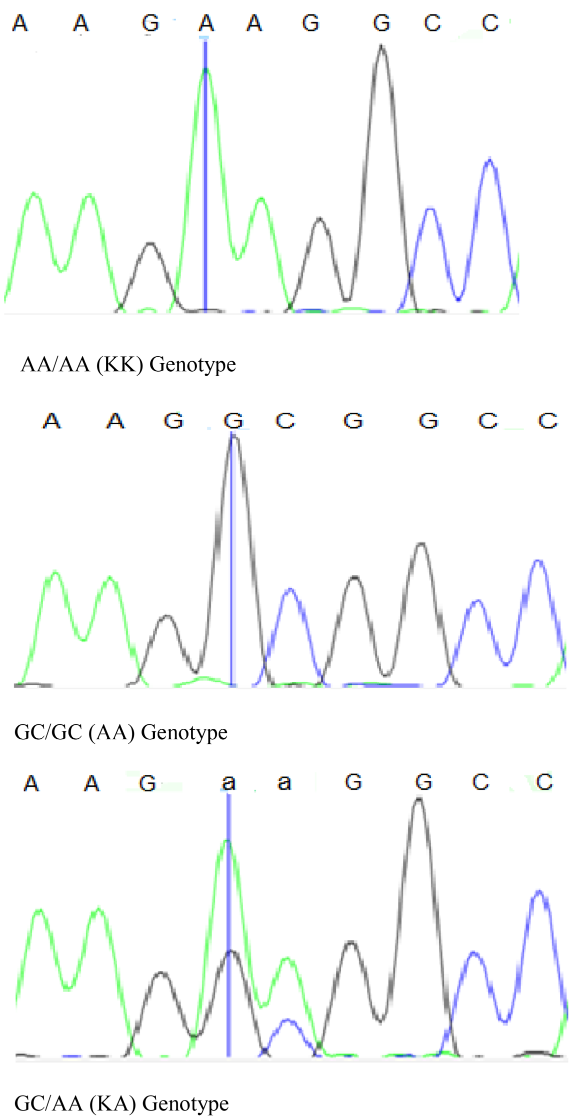

FIGURE 1
Sequence trace views of sequencing traces for positions 10,433 and 10,434. The vertical line indicates nucleotide position 10,433. Positions 10,433 and 10,434 are responsible for the *K232A* substitution. The positions of individual mutations were named according to the sequence available in GenBank (AJ318490).

(6.21%) than Boran cows in South Africa (2.68%) and Borgou and White Fulani cows in Benin (4.78% and 4.85%, respectively) (Myburgh et al., 2012; Houaga et al., 2017). Thus, according to the present study, Boran * HF cross cows are to be favored for daily milk yield, whereas Boran cows are best in terms of milk fat percentage. The observed differences between breeds are therefore likely to be due to differences in genetic background. However, another the reason for the variation in milk components among cattle breeds could be differences in the forage nutrient composition of natural grazing. The investigated cattle breeds are reared in different agro-ecological zones with different floristic compositions. Cow's milk composition could be affected by forage species and variety, climate, and growth stage of forage plants.

Allele and genotype frequencies of the *K232A* protein variant

The variants of the analyzed gene were found in all the five breeds, and therefore it is the allelic distribution that characterizes the differences between breeds. The present study revealed that the lysine variant of the *DGAT1* gene, i.e., the *K232* allele, was common in Horro, Boran, and Fogera cattle. This is in agreement with studies in other *Bos indicus* breeds, in which the frequency of the lysine variant *K232* ranged from 0.80 to 1 in four types African of zebu (Banyo Gudali, Kenana, Butana, and White Fulani) (Abu et al., 2015; Rahamattalla et al., 2015; Elzaki et al., 2022) and three types of Indian cattle (Sahiwal, Gir, and Kankrej) (Ganguly et al., 2013; Patel and Chauhan, 2017; Gothwal et al., 2022), and was comparable to the frequencies of *K232* in Borgou (0.77) and White Fulani (0.92) cattle of Benin (Houaga et al., 2018). The higher frequency of the K allele seen in those three Ethiopian breeds might reflect the exceptional adaptive value of these breeds. The overall immunity of zebu cattle to diseases, and their ability to adapt to and survive on a coarse feed diet, could be due to increased acyltransferase activity of the *DGAT1* gene, as DGAT catalyzes the synthesis of retinol esters and thus regulates the synthesis of vitamin A. Manga and Řiha (2011) reported a favorable association of the *DGAT1* K allele with low somatic cell count in lactating cows that partly explains the exceptional genetic resistance of native cattle to mastitis (Liu et al., 2007).

In the present study, the observed frequencies of the K allele (0.50–0.97) are higher than those observed (0.210–0.380) in the native Turkish and European *Bos taurus* breeds (Kaupe et al., 2004), with the exception of the Modicana and Jersey breeds (Valenti et al., 2019). The finding in the present study indicated the tendency of fixation of *DGAT1 K* allele in Horro cattle did not support its use as a reliable universal marker for milk yield and composition traits for the population. Fixation of the *DGAT1* K allele in ongole and buffalo breeds in India was noted by Krovvidi et al. (2021), whereas Kaupe et al. (2004) found fixation of the *DGAT1* A allele in five *Bos taurus* breeds (Belgian Blue beef, Gelbvieh, Hereford, Pinzgaurer, and Slavonian Syrmian). The high proportion of the lysine variant *K232* observed in the current study might contribute to the higher fat content in native cattle in Ethiopia and other *Bos indicus* breeds than in HF cross cattle. The frequency of the lysine variant *K232* in the investigated Boran * HF cross cattle is similar to that found in studies of other HF populations or their crosses, which have reported a range from 0.21 to 0.63 (Ahani et al., 2015; Akyüz et al., 2015; Komisarek and Kolenda, 2016; Bhat et al., 2017; Li et al., 2020; Krovvidi et al., 2021). The differences in the frequency of the lysine variant *K232* among breeds might result from different breeding goals and the considerable Holstein contribution to the blood level of Boran * HF cross cattle.

Allele A is a rare allele detected in Boran, Begait, Fogera and Boran * HF crosses. A locus is considered polymorphic if the most common allele has a frequency of ≤ 0.95 and therefore the less common allele (at a locus with two alleles) has a frequency of ≥ 0.05 (Hartl and Clark, 1997). Accordingly, in the present study, the reason why the *DGAT1* locus was not found to be polymorphic in

TABLE 3 Effect of *DGAT1 K232A* genotypes on DMY and composition traits (mean ± SE).

Breed	*N*	Genotype			Genotype		
		DMY			Fat (%)		
		AA	KA	KK	AA	KA	KK
Boran	17	–	2.40 ± 0.19[b]	1.84 ± 0.16[a]	–	6.08 ± 0.06[a]	6.35 ± 0.05[b]
Begait	16	–	2.91 ± 0.18[b]	2.74 ± 0.18[a]	–	4.46 ± 0.06[a]	4.9 ± 0.06[b]
Fogera	18	–	2.89 ± 0.18[b]	2.47 ± 0.16[a]	–	4.44 ± 0.06[a]	4.83 ± 0.05[b]
Boran * HF cross	24	10 ± 0.21[c]	7.62 ± 0.15[b]	4 ± 0.21[a]	2.41 ± 0.07[a]	3.12 ± 0.05[b]	4.03 ± 0.07[c]
		Lactose (%)			Protein (%)		
Breeds	N	AA	KA	KK	AA	KA	KK
Boran	17	–	5.00 ± 0.08[b]	4.45 ± 0.07[a]	–	3.38 ± 0.06	3.69± 0.05
Begait	16	–	4.49 ± 0.08[b]	4.36 ± 0.08[a]	–	3.60 ± 0.06	3.66 ± 0.06
Fogera	18	–	4.34 ± 0.08[b]	4.23 ± 0.07[a]	–	2.99 ± 0.06	3.11 ± 0.05
Boran * HF cross	24	5.11 ± 0.09[c]	4.59 ± 0.06[b]	4.40 ± 0.09[a]	3.12 ± 0.06	3.03 ± 0.05	3.28 ± 0.06

N, number of cows, DMY, daily milk yield, Predicted means and the SE of those means were derived from the GLM.
[a,b,c]Values within a row with different superscripts indicate significant differences (i.e., $p < 0.05$), as is the case for the traits DMY, fat content (%), and lactose content (%). In the case of protein content (%), all p-values were > 0.05.

Horro cattle populations may be that one animal among the sampled population had the A allele. The A allele frequency observed in the zebu populations was relatively similar to that found in the indigenous cattle of Sudan (0.037–0.15) (Abu et al., 2015). The A allele frequency observed in Boran * HF cross cattle was higher than reported by Li et al. (2021) in Kiwi cross cattle in New Zealand and lower than in Butana–Holstein crossbred cattle in Sudan (Elzaki et al., 2022). To reiterate, the low A allele frequency observed in native breeds of Ethiopia might be attributed to *Bos taurus* cattle introgression.

The KK and KA genotypic frequencies observed in in the zebu populations in the present study ranged from 0.50 to 0.94 and from 0.03 to 0.50, respectively, and are comparable to the frequencies observed in most *Bos indicus* cattle (Abu et al., 2015; Rahamattalla et al., 2015; Houaga et al., 2017; Elzaki et al., 2022). An excess of heterozygotes over homozygotes in Boran * HF cross breeds indicates that heterozygotes are preferred, which could be the effect of natural selection over the years of evolution and would be effective in selecting for or against polymorphism.

Effect of *DGAT1 K232A* on daily milk yield and composition

The association study confirmed that the *DGAT1 K232A* marker had significant effects on daily milk yield and milk fat and lactose contents in the investigated cattle. Similar effects of the KK genotype on milk composition content traits, and an even greater effect of the AA and KA genotypes on average milk yield, have been observed in Holstein, Holstein crosses, Jersey and its local Kashmiri crosses, Holstein *Jersey crosses, Gir, and Kankrej cattle populations in different parts of the world (Molee et al., 2012; Akyüz et al., 2015; Bhat et al., 2017; Patel and Chauhan, 2017; Li et al., 2021).

In contrast, Manga and Řiha (2011) have reported that the *DGAT1*K allele (lysine variant) is associated with higher milk yield in Holstein cows.

Substitution of the K allele showed that even one copy of this allele led to a significant ($p< 0.01$) increase in fat and protein content and a decrease in milk yield and lactose and non-fat solids content in all breeds considered. Similar effects of allele substitution were reported by Grisart et al. (2002). They observed that substitution of one copy of A allele by K allele reduced average milk yield by 130 L, increased average milk fat content by 5.76 kg, and reduced milk protein by 2.45 kg in HF cattle, and reduced milk yield by 110 L, increased milk fat by 3.30 kg, and reduced milk protein by 2.48 kg in a Jersey cattle population. Moreover, an increase in milk yield of 548 kg and in protein yield of 12.6 kg, accompanied by a decrease in milk fat of 15.4 kg, has been reported in HF animals homozygous for the A allele of the *DGAT1* gene (Hradecká et al., 2008).

Conclusion

Our results show that Boran * HF cross cows are the best breed in terms of daily milk yield, whereas Boran cows are best in terms of milk fat percentage. Allele K and genotype KK were prevalent in the zebu cattle populations. The overall diversity indices showed that the *DGAT1* locus was polymorphic in the Boran, Begait, Fogera, and Boran * HF cross populations. The association study confirmed that the *DGAT1 K232A* marker has significant effects on daily milk yield, milk fat and lactose content in Boran, Begait, Fogera and Boran * HF cross populations. The *DGAT1 K232A* marker could be utilized for breeding and improvement of milk yield and composition traits in local cattle populations in Ethiopia after validation in a larger population.

Data availability statement

The datasets presented in this study can be found online at https://www.ncbi.nlm.nih.gov/genbank/, under the accession numbers ON262825–ON262849

Ethics statement

The animal study was reviewed and approved by the Ethical Review Board of Adama Science and Technology University (certificate reference number RECSoANS/BIO/07/2021). Written informed consent was obtained from the owners for the participation of their animals in this study. Animal samples were obtained in compliance with local/national laws in force at the time of sampling. Data exchange was in accordance with national and international regulations, and approved by the owners. The procedure involving sample collection followed the recommendation of directive 2010/63/EU. All methods were carried out in accordance with relevant guidelines and regulations. All authors gave their informed consent prior to their inclusion in the study.

Author contributions

Conceptualization: BS, HDi, and HDa. Data curation: BS. Formal analysis: BS. Funding acquisition: BS. Investigation: BS. Methodology: BS, HDi, and HDa. Project administration: BS. Resources: BS, HDi, and HDa. Supervision: HDi and HDa; Visualization: BS. Writing – original draft: BS. Writing – review and editing: BS, HDi, and HDa. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Livestock feed markets across agro-ecological zones of Burkina Faso: feed provenance, price and quality

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Livestock form a major means of livelihood in Burkina Faso where almost 80% of the rural population keep sheep and goats. However, the major constraint to livestock productivity is seasonal feed scarcity. To bridge the increasing feed gap, particularly in the dry season, livestock keepers are increasingly turning to purchase of feeds from markets, which has led to emergence of feed markets in peri-urban towns and cities in the Sahel. To characterize the dynamics of these livestock feed markets, to inform and guide the development of efficient feeding systems in peri-urban livestock production, we conducted surveys in feed markets in four major towns and cities of Burkina Faso across all the agro-ecological zones. Results from this study showed that the feed types on sale included browse, bush hay, bran of maize, millet, sorghum, rice and wheat, legume residues, cereal straw and agro-industrial by-products such as cottonseed cake and concentrate feed for ruminants. The average number of feeds sold per vendor ranged from 1.5 ± 0.1 in early dry season to 3.5 ± 0.4 in late dry season. More than 80% of the feed sellers in each location reported that generation of additional income for the household was the principal reason for feed sale. The price of livestock feeds sold at different locations in Burkina Faso varied significantly with season, market location and feed type. Cottonseed cake had the highest crude protein content in all locations and across seasons ranging from an average of 16.3 to 31.9% while cereal straw (millet, maize and sorghum straw) had the lowest crude protein content of 3.5%. The results also showed a lack of a clear trend in terms of relationship between price and nutritional quality which confirms the absence of quality standardization for feeds on sale in our study sites. In developing feed industry in the region, the issue of quality standardization needs to be addressed to facilitate establishment of nutritive value-based pricing and to ensure that consumers pay a premium for quality products.

KEYWORDS

livestock, feed resources, feed markets, nutritive value, Sahel

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1 Introduction

Livestock form a major means of livelihood in Burkina Faso where almost 80% of the rural population keep sheep and goats, as they are key household assets for multiple functions such as food supply, income generation and as a store of wealth and insurance (Ayantunde et al., 2020). The major constraint to livestock productivity in Burkina Faso and other West African Sahelian countries is seasonal scarcity of feed, both in terms of quantity and quality (Ouedraogo et al., 2021; Amole et al., 2022) to varying extents in different agro-ecological zones. Generally, feed biomass including natural pastures and crop residues, is abundant in the South-Sudan agro-ecological zone of Burkina Faso for almost 10 months of the year (Savadogo et al., 1999), which makes the zone a popular destination for transhumant pastoralists from the Sahelian zone (Zampaligré, 2012). The Sahelian zone of Burkina Faso and other neighbouring countries (Mali and Niger) is characterized by feed deficit for livestock for almost for 4 to 7 months of the year (Ayantunde et al., 2011; Amole et al., 2022) as natural pastures are not sufficient to satisfy animal requirements in the dry season when the quantity decreases by 25 to 50% of peak biomass (Savadogo et al., 1999).

The increasing feed gap for livestock in the West African Sahel could be partly attributed to increase in livestock population (Ayantunde et al., 2022). To mitigate the problem of feed scarcity, particularly in the dry season, livestock keepers are increasingly turning to purchase of feeds from markets (Ayantunde et al., 2014). The increasing demand for feeds has contributed to the emergence of livestock feed markets in many peri-urban towns and cities in West African Sahel (Ayantunde et al., 2022). These feed markets are characterized by sale of crop residues, cereal bran, browses, bush hay and agro-industrial by-products. The feed markets are often located close to livestock markets and sometimes along the roads, while agro-industrial by-products are sold at agro-dealer shops (Ayantunde et al., 2022).

In a well-functioning livestock system, feed price varies based on classic supply-demand characteristics. Shortage of overall feed, as may occur during the dry season, leads to higher prices for feed (Ayantunde et al., 2014). Similarly, feeds of higher quality (both within and between feed types) attract higher prices since they support higher levels of livestock production. Previous studies have been able to show that higher quality feeds attract higher price. For example, Blummel and Rao (2006) reported that a difference of 5% units increase (range 47 to 52%) in *in vitro* Organic Matter Digestibility (IVOMD) of sorghum straw was associated with a higher price of about 25%. In the United States of America, alfalfa hay buyers and sellers often use Relative Feed Value (RFV) index for benchmarking marketing grades of alfalfa hays, which consequently informed the price (Rocateli and Zhang, 2017). Hay with higher value of RFV indicates better nutritional quality and vice versa. Lack of close relationships between price and quality, particularly for crop residues as reported in previous studies on livestock feed markets in West Africa (Wane et al., 2010; Ayantunde et al., 2014; Jarial et al., 2016; Ayantunde et al., 2022) suggest market failures, due to lack of information. In the case of feed, the key information relates to reasonable estimates of feed quality and in the absence of feed standards and their enforcement, buyers may have inadequate information on which to base price decision.

This study aimed at characterizing the livestock feed markets in different agro-ecological zones of Burkina Faso and to collect information on the price and quality of livestock feeds at different markets across seasons. The underlying hypothesis for this study is that feed price varied with agro-ecological zone (market location), season, feed type and provenance. Unlike previous studies on livestock feed market in the Sahel (Wane et al., 2010; Ayantunde et al., 2014; Jarial et al., 2016; Ayantunde et al., 2022) that were mainly conducted in peri-urban areas in one or two agro-ecological zone(s), this study covered all the agro-ecological zones in Burkina Faso and will therefore provide a national outlook of livestock feed price and quality across seasons and can inform policy on feed marketing in the country. In addition, the results of this study have implications beyond Burkina Faso as the agro-ecological gradients in Burkina Faso are typical of other West African Sahelian countries.

2 Materials and methods

2.1 Study sites, feed market surveys and laboratory analysis

Surveys were conducted in livestock feed markets in four peri-urban areas across the four agro-ecological zones (South-Sudan, Sudan, Sahel-Sudan and Sahel) of Burkina Faso (Figure 1) in the late dry season (April – May 2019), wet season (July – August 2019) and early dry season (November-December 2019). The surveys were conducted in Bobo-Dioulasso (commonly referred to as Bobo) in South-Sudan zone, Ouagadougou in Sudan zone, Ouahigouya in Sahel-Sudan zone and Dori in the Sahel zone of Burkina Faso. Five feed markets each were surveyed in Bobo and Ouagadougou, while three markets each were surveyed in Ouahigouya and Dori. In each location, independent feed sellers and buyers were randomly

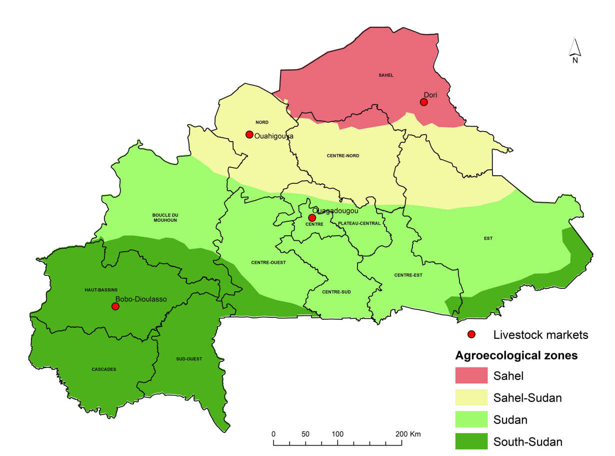

FIGURE 1
Map of study sites in different agro-ecological zones of Burkina Faso.

selected and interviewed in their local language by an enumerator. The feed sellers were asked questions about the types of feed being sold, the price in FCFA (Franc Communauté Financiére Africaine; currency for French speaking West and Central African countries; 1 USD=550 FCFA as at the time of this study), the reasons for selling feeds and provenance of the feeds. The feed buyers were asked about the reasons for buying feed and frequency of purchase of feed. At least five women feed sellers were interviewed in each market except for Ouahigouya where no female feed seller was found in wet and early dry seasons. In each season, about 30, 25, 27 and 14 feed vendors were interviewed in Ouagadougou, Bobo, Dori and Ouahigouya, respectively, which in total were 90, 75, 87 and 42 interviewees across seasons. The total number of buyers interviewed across the three seasons were 75 in Ouagadougou, 84 in Bobo, 59 in Dori and 89 in Ouahigouya. The total number of feed sellers and feed buyers interviewed varied with location depending on the available number of feed sellers and buyers. In all the markets, the vendors and buyers were dominated by male adults across seasons. The age of the feed sellers ranged from 10 to 80 years in Ouagadougou, 20 to 77 years in Bobo, 17 to 72 years in Dori and 15 to 77 years in Ouahigouya. The average year of experience in selling feed ranged from 4.3 ± 0.4 years in the early dry season in Bobo to 15.7 ± 3.2 years in the late dry season in Ouahigouya.

Samples of major feeds on sale at the markets were collected at different seasons for laboratory analysis and were analysed using Near Infrared Reflectance Spectroscopy (NIRS) technique after grinding (2 mm sieve) for dry matter (DM), ash, total nitrogen (N), fiber components (neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL)), metabolizable energy (ME) and *in vitro* organic matter digestibility (IVOMD). Crude protein was estimated from nitrogen content (nitrogen x 6.25). The NIRS model used is FOSS DS 2500F with spectral range from 800 to 2498 nm at International Livestock Research Institute (ILRI), Ouagadougou, Burkina Faso.

2.2 Statistical analysis

Data analysis was performed with SAS (Statistical Analysis System (SAS) institute, 1987) using Means and Frequency Procedures for descriptive statistics. The general linear model (GLM) procedures for variance and regression analyses were used to assess the relationship between market location, season, feed provenance, vendor-related variables and price. For this GLM model, the response variable was price while market location, season, feed type, age of vendor, sex of vendor, years of experience of the vendor and provenance of feed were the independent variables. In the analysis, market location, season, feed type, sex, and feed provenance were considered as a binary categorical variable with a value of either 0 or 1. The GLM model was also used to establish whether there was a relationship between the feed price and nutritional quality. For this GLM model, price was the response variable while crude protein content and IVOMD were the independent variables. Correlation procedure was also used to establish the relationship between price and nutritional quality. Duncan Multiple Range Test was used for comparing feed price and nutritional quality means for different market locations and seasons. Unless otherwise specified, the level of significance was set at $P < 0.05$.

3 Results

3.1 Inventory of feed sold at different locations

The feed types sold in the study sites at different seasons are presented in Table 1. The feed types included browse (leaves of *Fardherbia albida*, *Pterocarpus lucens*, *Piliostigma reticulatum* and *Parkia biglobosa* fruit); bush hay (dry herbaceous species collected from grazing area or roadside vegetation); bran of maize, millet, sorghum, rice and wheat; legume residues (cowpea hay, cowpea pod and groundnut haulm); cereal straw (mainly sorghum straw, millet straw and rice straw); and agro-industrial by-products such as cottonseed cake and concentrate feed for ruminants. Green pasture (freshly harvested herbaceous species from roadside or grazing area) was sold in the wet and early dry seasons in nearly all the locations. Irrigated fodder was reported in Ouagadougou by a feed vendor in the late dry season. The irrigated fodder sold was *Brachiaria ruziziensis*. In Bobo, one of the main cotton growing areas of Burkina Faso, there were residues from cotton which are commonly sold as animal feed. These included cotton straw and cotton grain (largely spoilt). Legume residues, cereal bran, cottonseed cake, bush hay and straw were the most commonly sold feed types in all the locations across seasons (Table 1).

TABLE 1 Inventory of feeds sold at different markets in Burkina Faso according to the respondents across seasons (number of response).

Feed type[a]	Ouagadougou	Bobo	Dori	Ouahigouya
Wet season				
Browse	3	4	1	0
Cereal bran	9	16	13	4
Concentrate	0	1	0	2
Cereal grain	0	2	7	0
Cottonseed cake	2	14	8	12
Cotton grain	0	8	0	0
Green pasture	15	10	0	4
Irrigated fodder	0	0	0	0
Legume residues	27	8	16	3
Bush hay	8	0	0	1
Sweet potato vines	0	2	0	0
Straw	1	17	3	2

(Continued)

TABLE 1 Continued

Feed type[a]	Ouagadougou	Bobo	Dori	Ouahigouya
Early dry season				
Browse	0	4	1	2
Cereal bran	12	10	12	3
Concentrate	1	1	0	0
Cereal grain	0	0	9	0
Cottonseed cake	1	7	6	6
Cotton grain	0	3	0	0
Green pasture	9	12	3	1
Irrigated fodder	0	0	0	0
Legume residues	25	13	13	10
Bush hay	1	5	1	1
Sweet potato vines	0	6	0	0
Straw	3	10	9	12
Late dry season				
Browse	2	7	2	0
Cereal bran	11	20	7	7
Concentrate	1	3	0	1
Cereal grain	0	4	1	0
Cottonseed cake	4	8	9	10
Cotton grain	0	0	0	0
Green pasture	0	0	0	0
Irrigated fodder	1	0	0	0
Legume residues	32	21	11	11
Bush hay	17	7	5	2
Sweet potato vines	0	2	0	0
Straw	5	17	5	6

[a]Feed type: Browse (leaves of Fardherbia albida, Pterocarpus lucens, Piliostigma reticulatum and Parkia biglobosa); Bush hay (dry herbaceous specie collected from grazing area or roadside vegetation; Cereal bran (Wheat bran, sorghum bran, millet bran, maize bran and rice bran); Cereal grain (mainly spoilt maize and sorghum grain); Straw (mainly cereal straw such as sorghum straw, millet straw and rice straw but in Bobo it was mainly cotton straw); Green pasture (freshly harvested herbaceous species from roadside or grazing area); Irrigated fodder was Brachiaria ruziziensis; Legume residues (cowpea hay, cowpea husk and groundnut haulm).

The average count of feed types sold per vendor ranged from 1.5 ± 0.1 in early dry season in Dori to 3.5 ± 0.4 in late dry season in Bobo (Table 2). Generally, more diverse feeds were sold in Bobo than in other locations. Dori had a significantly lower average number of feeds sold than the other locations (Table 2). In Ouagadougou, significantly more feeds were sold per vendor in the late dry season than in the early dry season. There was no significant seasonal effect in the average number of feeds sold per vendor for all the other locations. The trend in average number of feeds sold tended to follow agro-ecological zone, that is the highest number was observed in the wettest South-Sudan zone while the lowest number was observed in the Sahel zone with the least rainfall of all the zones.

TABLE 2 Number (count) of feed types sold per vendor at different markets in Burkina Faso (mean ± standard error).

Location	Wet season	Early dry season	Late dry season
Ouagadougou	2.1 ± 0.2^{b}	1.9 ± 0.2^{b}	2.5 ± 0.2^{ab}
Bobo	3.3 ± 0.4^{a}	2.8 ± 0.4^{a}	3.5 ± 0.4^{a}
Dori	1.6 ± 0.2^{b}	1.5 ± 0.1^{b}	1.9 ± 0.3^{b}
Ouahigouya	1.9 ± 0.4^{b}	2.6 ± 0.7^{a}	3.0 ± 0.4^{a}

Values followed by different superscripts (a,b) on the same column (season) are statistically significant ($P < 0.05$).

3.2 Provenance of feed sold

Feeds sold at different markets in our study came from diverse sources. The most common sources were crop residues from other's farms, another feed vendor, grazing area, agro-industrial by-product shop and household food processing (Figures 2A–C). Legume residues came mainly from other's farms or from another feed vendor. Cereal bran came mainly from household food processing, another feed vendor or an agro-industrial by-product shop. The sources of bush hay were grazing area and roadside vegetation. Cottonseed cake and concentrate for ruminants came mainly from agro-industrial by-product shops or another feed vendor. Sourcing feeds from another vendor suggests the existence of some large-scale feed vendors from where feed retailers can buy and then sell to the final consumers. The provenance of crop residues from the respondent own's farm accounted for 25% and 14% of responses by the respondents in Dori and Ouahigouya, respectively in the early dry season, which corresponds to the period of crop harvest. The provenance of different feeds tended to vary with location and season (Figures 2A–C).

3.3 Reasons for sale and purchase feed

More than 80% of the feed sellers in each location reported that generation of additional income for the household was the principal reason for feed sale (Figure 3). Other reasons given for the sale of feed included urgent household need for money, difficulty in conserving crop residues and excess household feed biomass. There was no major variation in the response of the feed sellers at different seasons so we combined the data for all seasons. According to the feed buyers, the principal reasons for purchase of feed were insufficient household feed biomass, inaccessibility to grazing areas, need for supplementary feed to meet requirement for animal performance and lack of household labour to herd the animals for grazing (Figure 4). The reasons for purchase of feed varied with location. For example in Ouagadougou, the major reasons given by the respondents were inaccessibility to grazing areas, need for supplementary feed to meet animal performance, insufficient household feed biomass and lack of household labour to herd the animals for grazing in descending order (Figure 4) whereas in Dori where there was availability of herding labour in view of their pastoral tradition, the two major reasons for purchase of feed were insufficient household feed biomass and need for supplementary feed to meet animal performance. The primary

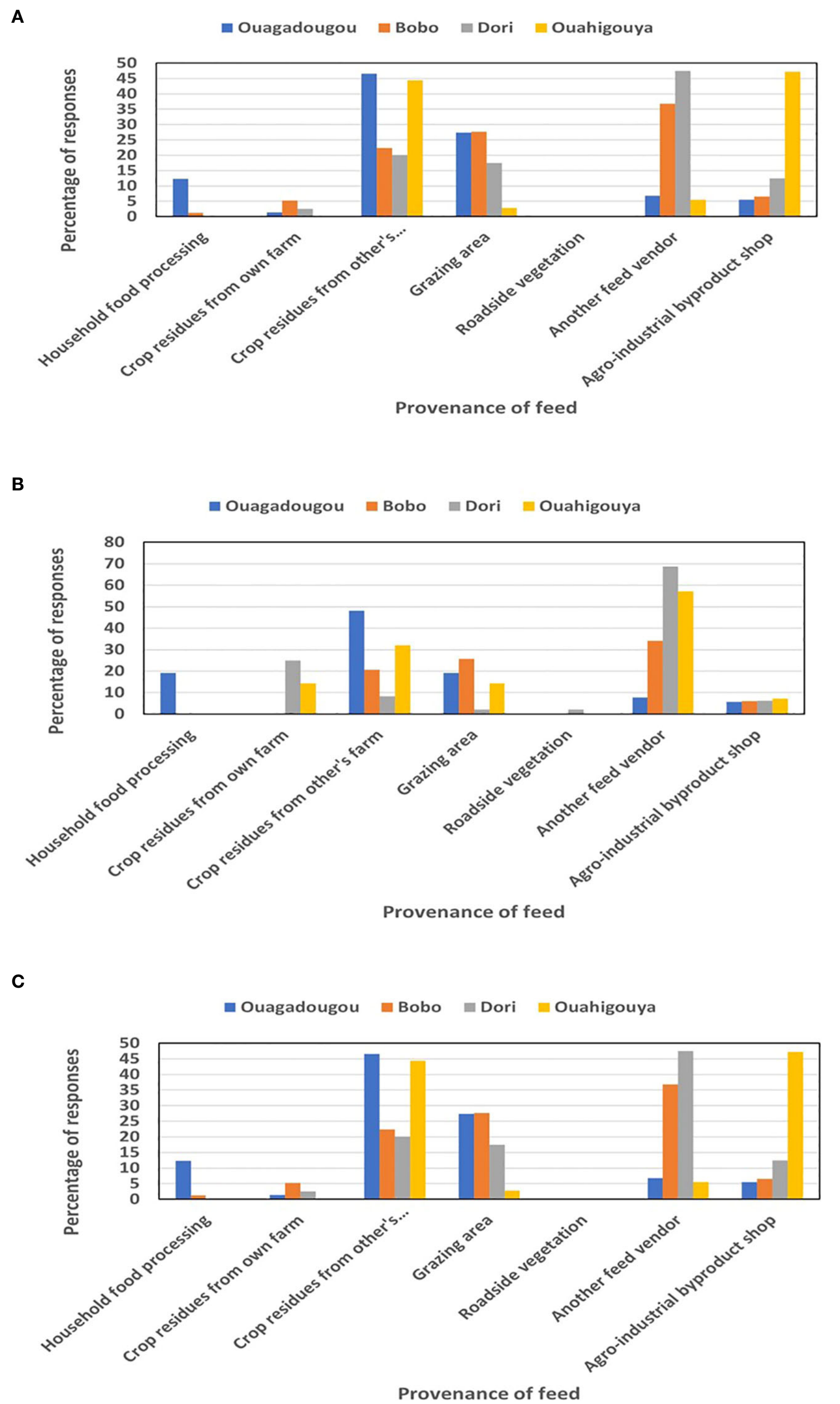

FIGURE 2
(A) Provenance of feeds sold at different markets in Burkina Faso in the wet season (number of responses are 75, 82, 48, and 28 for Ouagadougou, Bobo, Dori and Ouahigouya, respectively). **(B)** Provenance of feeds sold at different markets in Burkina Faso in the early dry season (number of responses were 52, 71, 54 and 35 for Ouagadougou, Bobo, Dori and Ouahigouya, respectively). **(C)** Provenance of feeds sold at different markets in Burkina Faso in the late dry season (number of responses were 73, 76, 40 and 36 for Ouagadougou, Bobo, Dori and Ouahigouya, respectively).

occupations of feed buyers in our study sites were diverse including crop and livestock farming, livestock farming, livestock trade, salaried work, and small-scale commerce. The feed buyers in Ouagadougou and Bobo were dominated by the livestock traders (Figure 5). In Dori where the pastoralist ethnic group is dominant, almost one-third of the feed buyers were livestock farmers.

Buying feed every week in both dry and wet seasons was most frequent according to the respondents (Table 3), which suggests high reliance on feed purchase in the study locations irrespective of season. Regular purchase of feed across seasons is a common feature of keeping livestock in peri-urban areas of West African Sahel. In Ouahigouya, about 28% of the respondents reported buying feed a

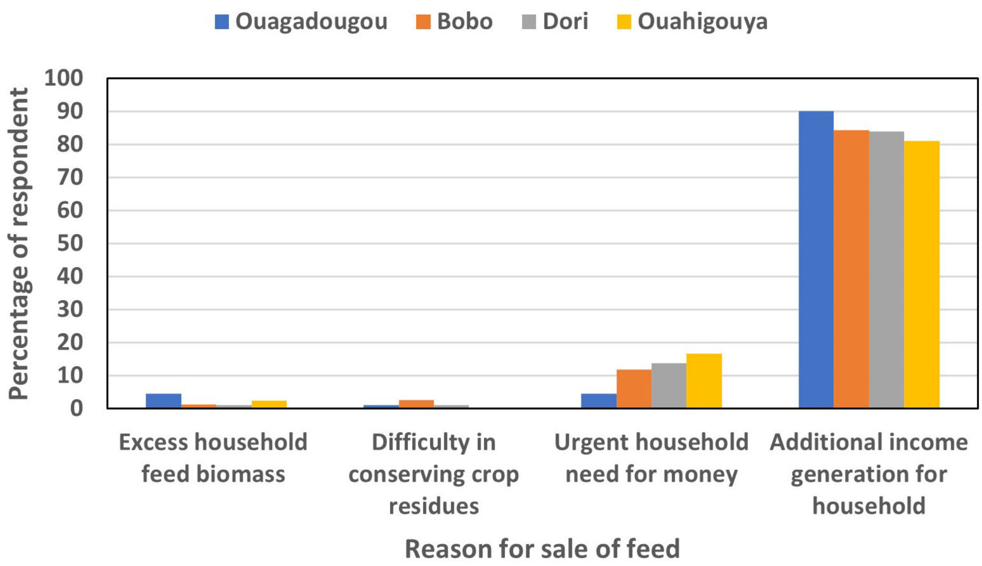

FIGURE 3
Reasons given by the respondents for sale of feed at different markets in Burkina Faso for all seasons (Ouagadougou n=90; Bobo n=76; Dori n=87; Ouahigouya n=42).

few times in the dry and wet seasons (Table 3), that is less frequent than in the other three locations.

3.4 Price of livestock feeds at different locations

The price of livestock feeds sold at different locations in Burkina Faso varied significantly with season, market location and feed type (Table 4). Legume residues (cowpea hay, cowpea husk and groundnut haulm) consistently had higher price than all other feed types in all locations irrespective of season. The prices of legume residues were similar in wet and late dry seasons when they are not available but significantly lower in early dry season which corresponds to crop harvest when residues are generally in abundance. Consequently, the prices of crop residues were lowest in early dry season. The price of bush hay was highest in wet season corresponding to the period of lowest availability as rain has washed away the herbaceous litters while some herbaceous litters have decomposed. The prices of cereal bran (millet, maize, sorghum, rice and wheat) were consistently lower

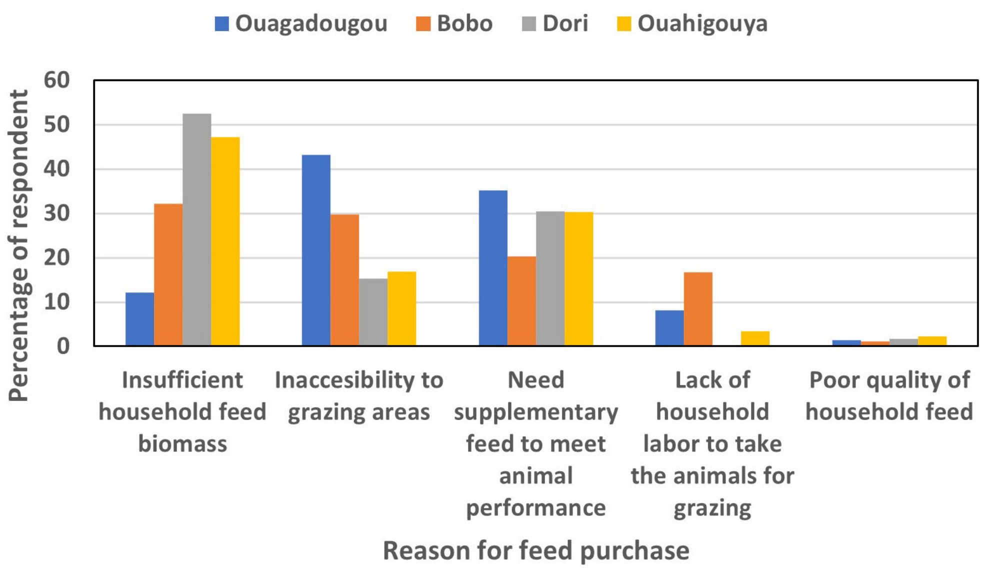

FIGURE 4
Reasons given by the respondents for purchase of feed at different markets in Burkina Faso for all seasons (Ouagadougou n=74; Bobo n=84; Dori n=59; Ouahigouya n=89).

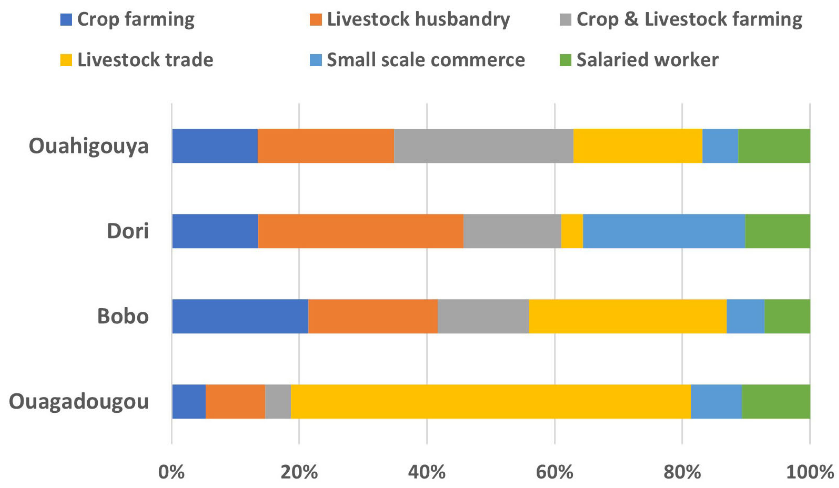

FIGURE 5
Primary activity of the feed buyers at different markets in Burkina Faso for all seasons (Ouagadougou n=75; Bobo n=84; Dori n=59; Ouahigouya n=89).

in Ouagadougou across seasons than in other three study sites, as they (particularly millet, maize and sorghum bran) were mainly sourced from household food processing. The price of cottonseed cake tended to be higher in Bobo than in other locations despite the fact that Bobo is one of the major cotton producing areas in Burkina Faso.

The results of feed price at different locations showed that legume residues were sold at lowest price of 186 ± 9 FCFA/kg DM in early dry season in Ouagadougou while it was sold for highest price of 367 ± 27 FCFA/kg DM in the late dry season in Dori, suggesting the significant effect (p<0.05) of season and location on price. The results further showed the significant effect of feed type on price. For example, the prices of legume residues were significantly (p<0.05) higher than that straw across seasons (Table 4). Provenance of the feed was significant at p<0.10 suggesting that provenance might also have effect on feed price. This is expected as feeds bought from other vendors for resale such as legume residues tended to have higher price than feeds collected from grazing areas and roadside vegetation such as bush hay and green pastures. The results also showed that sex of the vendor did not have significant effect on the feed price.

3.5 Livestock feed quality across seasons

The results of crude protein content (%) and *in vitro* organic matter digestibility (%, IVOMD) of different feed types at the study sites across seasons are presented in Table 5. As expected, crude protein content and IVOMD varied with feed type and season. Cottonseed cake had the highest crude protein content in all locations and across seasons ranging from an average of 16.3 in Ouagadougou in the wet season to 31.9 in Ouahigouya in the early dry season (Table 5). Cereal straw (millet, maize and sorghum straw) had the lowest crude protein content of 3.5 in Ouahigouya in the wet season. IVOMD of the feeds ranged from about 43 for cotton straw in Bobo in the early dry season to 74 for browse in Dori, also in the early dry season. There were no significantly differences in crude protein content and IVOMD of each feed type across locations for a given season except for crude protein content of cottonseed cake in the wet season for Ouagadougou. In addition, the crude protein content of bush hay in early and late dry seasons in Dori was significantly lower than in other locations. Based on correlation analysis, the results also showed lack of clear relationship between price and nutritional quality (crude protein content and IVOMD; p>0.05 for a given feed except for roughage (bush hay and straw; crude protein correlation coefficient (r) = 0.356; P = 0.028; IVOMD: r = 0.286; P = 0.042) as presented in Figures 6A, B. However, there tended to be relationship between price and nutritional quality when feed types are compared. For example, in Figures 6A, B, roughage (bush hay and cereal straw) which had lowest crude protein content and IVOMD also largely had lowest price compared to other feed types namely cereal bran, legume residues and cottonseed cake.

TABLE 3 Frequency of buying feed by the respondents at different markets in Burkina Faso (%, with number of respondents in bracket).

Frequency	Ouagadougou	Bobo	Dori	Ouahigouya
Every week in the dry season	16% (12)	17% (14)	9% (5)	27% (24)
A few times in the dry season	8% (6)	2% (2)	5% (3)	3% (3)
Every week in both dry and wet seasons	69% (52)	74% (62)	83% (49)	42% (37)
A few times in both dry and wet seasons	7% (5)	7% (6)	3% (2)	28% (25)

TABLE 4 Price (FCFA, mean ± standard error; 1 USD = 550 FCFA as at the time of this study) of common feed types at different markets in Burkina Faso across seasons.

Feed type	Ouagadougou	Bobo	Dori	Ouahigouya
Wet season				
Browse	105 ± 1^{cd}	119 ± 18^{d}	114 ± 2^{d}	–
Cereal bran	121 ± 5^{c}	145 ± 4^{cd}	233 ± 16^{b}	154 ± 5^{b}
Concentrate	–	161 ± 6^{bc}	–	170 ± 3^{b}
Cereal grain	–	188 ± 28^{b}	164 ± 15^{c}	–
Cottonseed cake	201 ± 39^{b}	202 ± 10^{b}	205 ± 17^{b}	179 ± 4^{b}
Cotton grain	–	182 ± 28^{b}	–	–
Green pasture	82 ± 11^{d}	41 ± 3^{e}	–	58 ± 4^{d}
Legume residues	321 ± 18^{a}	257 ± 21^{a}	291 ± 14^{a}	317 ± 7^{a}
Bush hay	165 ± 15^{c}	–	–	95 ± 1^{c}
Sweet potato vines	–	69 ± 2^{e}	–	–
Straw	123 ± 2^{c}	122 ± 3^{d}	120 ± 9^{d}	79 ± 3^{cd}
Early dry season				
Browse	–	120 ± 22^{b}	75 ± 1^{d}	89 ± 1^{c}
Cereal bran	114 ± 4^{c}	118 ± 6^{b}	134 ± 10^{c}	137 ± 4^{b}
Concentrate	180 ± 1^{ab}	160 ± 4^{a}	–	–
Cereal grain	–	–	188 ± 12^{b}	–
Cottonseed cake	160 ± 3^{b}	184 ± 9^{a}	150 ± 12^{c}	147 ± 4^{b}
Cotton grain	–	102 ± 22^{bc}	–	–
Green pasture	88 ± 9^{c}	68 ± 5^{d}	83 ± 6^{d}	101 ± 2^{c}
Legume residues	207 ± 11^{a}	186 ± 9^{a}	222 ± 11^{a}	224 ± 6^{a}
Bush hay	103 ± 3^{c}	82 ± 6^{c}	105 ± 1^{d}	93 ± 2^{c}
Sweet potato vines	–	54 ± 2^{d}	–	–
Straw	104 ± 3^{c}	85 ± 5^{c}	67 ± 12^{d}	58 ± 3^{d}
Late dry season				
Browse	148 ± 1^{c}	151 ± 19^{c}	153 ± 1^{c}	–
Cereal bran	137 ± 6^{c}	134 ± 9^{c}	143 ± 19^{c}	163 ± 4^{c}
Concentrate	198 ± 3^{b}	193 ± 18^{b}	–	220 ± 3^{b}
Cereal grain	–	206 ± 29^{b}	258 ± 4^{b}	–
Cottonseed cake	169 ± 8^{bc}	200 ± 13^{b}	167 ± 10^{c}	161 ± 5^{c}
Irrigated fodder	64 ± 5^{e}	–	–	–
Legume residues	327 ± 31^{a}	271 ± 12^{a}	367 ± 27^{a}	348 ± 5^{a}
Bush hay	105 ± 8^{d}	110 ± 16^{d}	119 ± 4^{d}	88 ± 3^{d}
Sweet potato vines	–	77 ± 15^{e}	–	–
Straw	153 ± 5^{c}	142 ± 15^{c}	122 ± 5^{d}	97 ± 4^{d}

Values followed by different superscripts (^{a,b}) for the location on the same column are statistically significant ($P < 0.05$).
Significant ($P < 0.05$) factors for feed price were market location, season, feed type, and years of experience of vendors. Provenance of feed was significant at $P < 0.10$.

TABLE 5 Crude protein (%) and *in vitro* organic matter digestibility (%, IVOMD) of different feed types at different markets in Burkina Faso, across seasons (mean ± standard error).

Wet season								
	Ouagadougou		Bobo		Dori		Ouahigouya	
Feed type	Crude protein	IVOMD	Crude protein	IVOMD	Crude protein	IVOMD	Crude protein	IVOMD
Browse	7.1 ± 2.1^{c}	63.5 ± 4.2^{b}	7.9 ± 0.6^{d}	69.2 ± 1.3^{a}	10.4 ± 1.2^{c}	63.5 ± 2.6^{b}	–	–
Cereal bran	13.4 ± 0.3^{ab}	68.4 ± 0.3^{a}	12.0 ± 1.5^{c}	65.8 ± 2.8^{ab}	14.6 ± 0.7^{b}	70.0 ± 0.5^{a}	14.2 ± 1.3^{b}	68.2 ± 1.2^{a}
Cereal grain	–	–	13.4 ± 2.1^{bc}	70.3 ± 2.5^{a}	10.5 ± 0.1^{c}	65.2 ± 0.5^{ab}	–	–
Cowpea hay	11.1 ± 1.4^{b}	61.2 ± 2.1^{bc}	8.7 ± 0.6^{d}	57.8 ± 0.4^{c}	–	–	–	–
Cowpea husk	6.6 ± 0.4^{c}	56.3 ± 0.4^{c}	–	–	6.7 ± 0.1^{d}	56.7 ± 0.6^{c}	–	–
Cottonseed cake	16.3 ± 1.3^{a}	67.1 ± 0.3^{ab}	24.8 ± 2.4^{a}	66.6 ± 0.9^{ab}	27.5 ± 2.5^{a}	67.4 ± 0.9^{ab}	24.7 ± 0.5^{a}	65.36 ± 0.74^{ab}
Cotton grain	–	–	21.8 ± 4.5^{a}	65.7 ± 2.6^{ab}	–	–	–	–
Green pasture	10.4 ± 0.8^{b}	55.6 ± 1.1^{c}	11.2 ± 0.2^{c}	56.0 ± 0.1^{c}	–	–	14.4 ± 1.4^{b}	60.6 ± 1.2^{bc}
Groundnut haulm	11.5 ± 1.0^{b}	60.3 ± 0.9^{bc}	12.8 ± 0.6^{bc}	60.8 ± 0.7^{bc}	–	–	–	–
Bush hay	2.3 ± 0.9^{d}	47.4 ± 0.6^{e}	–	–	5.9 ± 0.9^{d}	47.2 ± 1.2^{d}	2.7 ± 0.1^{c}	47.2 ± 1.2^{c}
Sweet potato vines	–	–	13.8 ± 1.8^{b}	61.8 ± 2.9^{b}	–		–	–
Cereal straw	4.2 ± 0.3^{d}	51.4 ± 0.8^{d}	–	–	5.4 ± 1.6^{d}	50.1 ± 0.5^{d}	3.5 ± 0.2^{c}	49.6 ± 0.6^{c}
Cotton straw	–	–	5.6 ± 2.8^{d}	54.8 ± 2.0^{c}	–	–	–	–
Early dry season								
	Ouagadougou		Bobo		Dori		Ouahigouya	
Feed type	Crude protein	IVOMD	Crude protein	IVOMD	Crude protein	IVOMD	Crude protein	IVOMD
Browse	–	–	4.1 ± 1.3^{d}	71.2 ± 2.1^{a}	7.78 ± 0.7^{d}	73.6 ± 0.9^{a}	–	–
Cereal bran	14.3 ± 0.2^{a}	68.1 ± 0.5^{a}	13.9 ± 1.0^{b}	64.5 ± 2.7^{ab}	15.6 ± 0.5^{b}	69.1 ± 0.6^{a}	14.5 ± 1.4^{b}	67.7 ± 1.4^{a}
Cereal grain	–				11.2 ± 0.3^{bc}	62.8 ± 0.8^{b}	–	–
Cowpea hay	10.4 ± 1.8^{b}	69.3 ± 1.8^{a}	12.9 ± 1.0^{b}	60.1 ± 1.5^{b}	9.8 ± 1.0^{c}	59.9 ± 2.0^{bc}	14.6 ± 3.9^{b}	62.4 ± 4.7^{ab}
Cowpea husk	6.5 ± 0.3^{c}	55.9 ± 1.0^{b}	10.2 ± 2.0^{c}	55.4 ± 0.7^{c}	5.6 ± 0.5^{e}	55.3 ± 1.4^{c}	–	–
Cottonseed cake	–		31.6 ± 0.9^{a}	68.5 ± 0.5^{a}	27.5 ± 2.5^{a}	64.7 ± 1.2^{ab}	31.9 ± 1.4^{a}	69.9 ± 1.3^{a}
Green pasture	–		15.0 ± 0.7^{b}	55.2 ± 0.3^{c}	–	–	–	–
Groundnut haulm	13.3 ± 1.7^{a}	53.9 ± 1.7^{bc}	13.9 ± 0.8^{b}	57.7 ± 2.1^{bc}	12.0 ± 0.4^{bc}	59.4 ± 1.2^{bc}	10.2 ± 1.0^{c}	58.9 ± 0.3^{b}
Bush hay	12.0 ± 2.1^{ab}	58.4 ± 1.6^{b}	–	–	5.9 ± 0.9^{e}	47.9 ± 1.3^{d}	8.1 ± 0.4^{d}	57.2 ± 3.4^{b}
Sweet potato vines	–	–	15.8 ± 0.4^{b}	62.0 ± 0.7^{b}	–	–	–	–
Cereal straw	5.15 ± 0.21^{c}	48.0 ± 2.5^{c}	–	–	5.4 ± 1.6^{e}	51.3 ± 2.7^{cd}	5.0 ± 0.4^{e}	48.9 ± 1.6^{c}
Cotton straw			4.1 ± 0.6^{d}	43.5 ± 0.9^{d}	–	–	–	–

(Continued)

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TABLE 5 Continued

	Wet season							
	Ouagadougou		Bobo		Dori		Ouahigouya	
Feed type	Crude protein	IVOMD	Crude protein	IVOMD	Crude protein	IVOMD	Crude protein	IVOMD
Late dry season								
	Ouagadougou		Bobo		Dori		Ouahigouya	
Feed type	Crude protein	IVOMD	Crude protein	IVOMD	Crude protein	IVOMD	Crude protein	IVOMD
Browse	8.1 ± 0.0^{d}	62.7 ± 1.8^{ab}	6.6 ± 1.1^{d}	68.6 ± 2.7^{a}	11.2 ± 0.6^{b}	61.0 ± 1.0^{ab}	–	–
Cereal bran	15.9 ± 0.5^{b}	65.1 ± 0.5^{a}	13.1 ± 1.8^{c}	60.9 ± 3.5^{b}	14.6 ± 0.9^{a}	65.3 ± 0.8^{a}	17.5 ± 1.9^{b}	69.7 ± 1.1^{a}
Concentrate	20.1 ± 1.6^{a}	64.6 ± 0.4^{a}	18.2 ± 5.2^{a}	64.7 ± 3.1^{a}	–	–	22.5 ± 0.3^{a}	63.5 ± 0.2^{ab}
Cereal grain	–	–	16.1 ± 2.5^{b}	59.8 ± 2.0^{b}	9.5 ± 1.0^{b}	59.1 ± 1.6^{b}	–	–
Cowpea hay	10.5 ± 5.0^{c}	56.7 ± 1.4^{bc}	10.2 ± 1.1^{d}	61.7 ± 5.9^{b}	9.5 ± 0.9^{b}	59.8 ± 1.3^{b}	11.9 ± 0.3^{c}	60.0 ± 0.4^{b}
Cowpea husk	6.5 ± 0.5^{e}	50.5 ± 0.5^{c}	7.1 ± 0.8^{d}	52.5 ± 0.4^{c}	5.7 ± 0.5^{c}	51.6 ± 0.2^{c}	–	–
Cottonseed cake	20.3 ± 1.6^{a}	63.2 ± 0.8^{ab}	21.3 ± 2.2^{a}	66.7 ± 1.8^{a}	17.0 ± 1.7^{a}	59.3 ± 2.2^{b}	23.1 ± 1.8^{a}	65.2 ± 0.7^{a}
Groundnut haulm	11.5 ± 0.6^{c}	60.0 ± 0.7^{b}	11.5 ± 2.1^{c}	61.6 ± 0.6^{b}	11.0 ± 0.5^{b}	60.3 ± 0.1^{ab}	10.0 ± 0.7^{c}	58.4 ± 0.7^{b}
Bush hay	8.1 ± 0.5^{d}	50.4 ± 1.4^{c}	4.5 ± 2.1^{e}	56.6 ± 0.9^{bc}	3.8 ± 0.4^{d}	45.9 ± 0.1^{d}	3.8 ± 1.0^{d}	49.3 ± 1.2^{cd}
Cereal straw	7.7 ± 1.1^{de}	50.4 ± 1.7^{c}	–	–	4.2 ± 0.5^{cd}	48.7 ± 0.1^{d}	5.2 ± 1.5^{d}	51.1 ± 1.3^{c}
Cotton straw	–	–	5.3 ± 2.7^{de}	53.3 ± 1.3^{c}	–	–	–	–

Values followed by different superscripts (^{a,b}) for the same variable on the same column (quality parameter) for each location and season are statistically significant ($P < 0.05$).

4 Discussion

The dominance of crop residues in the feed markets is consistent with previous studies (Ayantunde et al., 2014; Jarial et al., 2016) and this reaffirms the growing importance of crop residues as source of livestock feed in the Sahel (Amole et al., 2022). According to these authors, crop residues can account for up to 45% of ruminant livestock diet in arid and semi-arid zones of West Africa, which can be attributed to significant decline in grazing areas in the region due to encroachment from cropping. Legume residues, cereal bran, cottonseed cake, bush hay and straw were the most commonly sold feed types in all the locations across seasons (Amole et al., 2022). The near total absence of irrigated fodder at the feed markets in our study sites is an indication of a generally low cultivation of forage species, which may be attributed to their low adoption in West African Sahel as observed by Amole et al. (2022). The presence of cotton residues at the markets in Bobo is a reflection of the widespread cultivation of cotton in Bobo area. The trend of more diverse feeds sold in Bobo than in other locations suggests that agro-ecological zone is an important factor in feed availability as this affects floristic composition of vegetation, and the types of crops grown (Savadogo, 2000; Hiernaux et al., 2009). The declining number of feeds sold in our study sites from South-Sudan to Sahel zone is therefore expected as this reflects decline in the length of growing period.

Given that the major provenance of crop residues is crop field, removal of crop residues from the crop field may impact soil fertility as the soil is exposed to erosion (Valbuena et al., 2015). Besides, removal of the crop residues from the crop field leads to loss of organic matter that could be returned to the soil to enhance soil fertility. The increasing use of crop residues as animal feed raises the issue of trade-offs with using crop residues as soil amendment strategy. Removal of crop residues from the field for sale also undermines the previous practice of allowing the animals to graze the crop field after harvest to deposit manure on the field (Ayantunde et al., 2018), and if this persists it will result in nutrient mining from the crop field. To address the problem of nutrient mining from the crop field, Valbuena et al. (2015) suggest increasing the crop residue biomass by using improved dual purpose crop varieties, and this will allow having enough crop residues biomass for mulching after removal for animal feeding. The other possible solution is developing feed industry to boost production of concentrates and agro-industrial by-products to reduce reliance on crop residues as animal feed. To facilitate development of feed industry in Burkina Faso, the government has been supporting the establishment of small-scale feed mills around peri-urban areas in terms of provision of grants to prospective entrepreneurs, particularly young

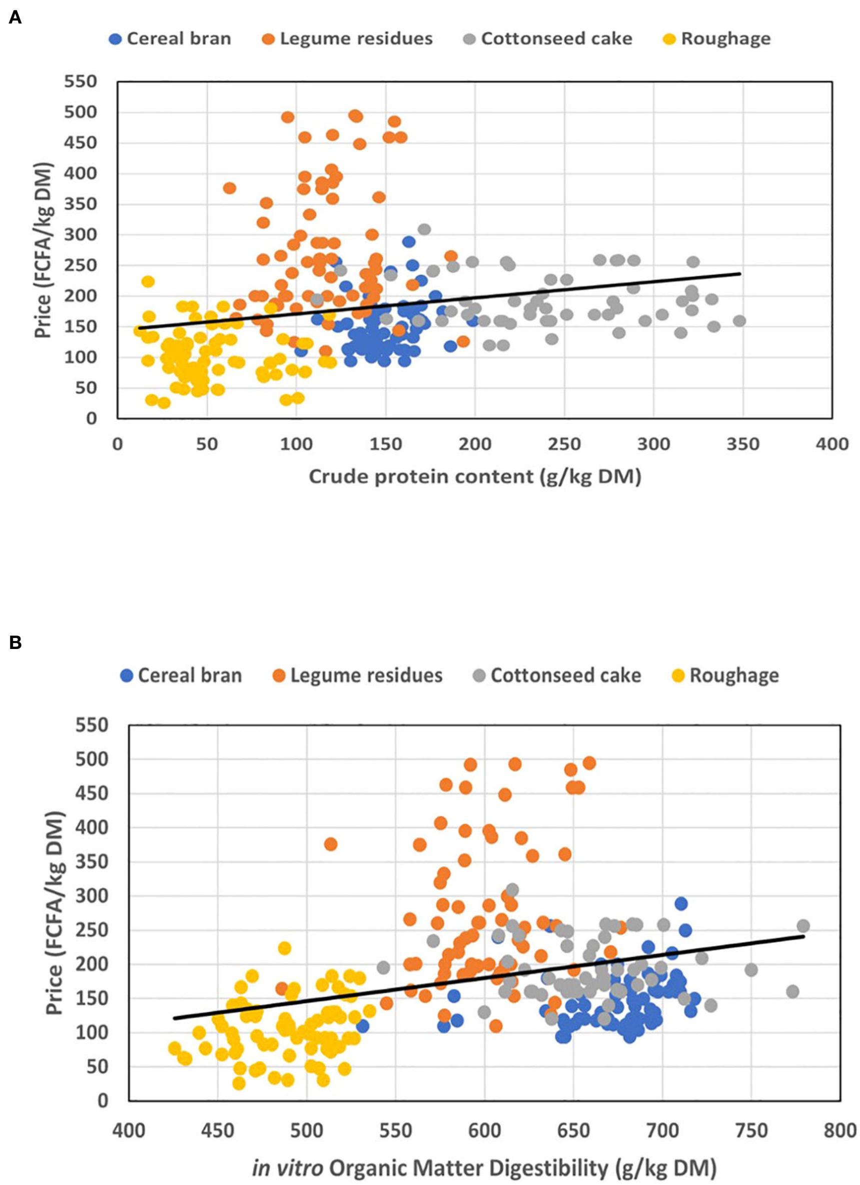

FIGURE 6
(A) Relationship between price and crude protein content of major feed types in the study sites for all seasons combined. **(B)** Relationship between price and in vitro organic matter digestibility of major feed types in the study sites for all seasons combined.

agricultural graduates. However, funding for the grants depends mainly on external donors for livestock projects, which raises the issue of sustainability. The means of transport of crop residues and bush hay from farms to the feed markets is mainly by donkey in all the study locations and the distance is normally not more than 50 km. Thus, there is no fuel cost incurred but for labor in collecting and transporting the crop residues. However, transport of agro-industrial by-products such as cottonseed cake, concentrate feeds and cereal bran is by vehicle and this implies additional transport cost. The additional transport costs partly explains the high cost of cottonseed cake, and cereal bran in Dori markets in view of long distance (about 400 km) from the sources (Bobo and Ouagadougou).

The necessity for experience in sale of feed is supported by our results showing the average year of experience of the feed sellers of about 4 to 15 years. Generally, experience is required particularly in conservation and packaging of different feed types, and in negotiation of the price with the buyers in view of the absence of quality standardization for feeds on sale. Our results on the primary activity of the feed buyers confirm the involvement of those with non-farming profession in livestock production in peri-urban areas of West African Sahel. The involvement of people with non-farming background in livestock production necessitates the purchase of feeds for the animals and this is driving the demand for livestock feeds, particularly in the peri-urban areas of the region. The domination of feed buyers in Ouagadougou and Bobo by livestock traders suggests an increasing commercialization of livestock production as observed in the peri-urban towns and cities in the Sahel by Valerio et al. (2020). As Burkina Faso is a net exporter of live animals to the coastal countries in West Africa, increasing numbers of people are engaging in the livestock trade. Livestock trade in West African Sahel is largely informal and entails buying animals (cattle, sheep and goats) from smallholder livestock farmers in rural areas and fattening of the animals in peri-urban areas before being sold to final buyers who move them to the coastal countries for sale to consumers (Valerio et al., 2020). This trend of domination of feed buyers by livestock traders is expected to continue in the peri-urban towns and cities of West African Sahel. Therefore, the development of the feed industry is necessary to meet the growing feed demand for feed in peri-urban areas and to assure stability in feed supply.

Selling feeds as a means of generation of additional income for the household, as reported by feed vendors in our study, confirms that sale of feeds can be a livelihood strategy, particularly in peri-urban areas. The main challenge to this is seasonal availability of some of the feeds, especially crop residues, which can affect income generation for the vendors. However, with necessary support to the feed vendors in accessing credit to set up shop for sale of feed concentrates and agro-industrial by-products, the feed business can be a viable venture. The results on major reasons given for the purchase of feed by the buyers in our study sites are consistent with findings from similar study in Niger by Ayantunde et al. (2022), as purchase of feed is necessary to meet household feed deficit in peri-urban livestock production. The increasing population of livestock in the peri-urban areas of the Sahel in the past three decades has exacerbated demand for feed and there is also the need for purchase of supplementary feeds as smallholder dairy production and sheep fattening are common, particularly in Ouagadougou and Bobo, in view of the increasing commercial orientation of peri-urban livestock production. The increasing dependence on feed purchase by many peri-urban livestock keepers in West African Sahel raises the question of profitability of peri-urban livestock production in the region (Ayantunde et al., 2014; Roessler et al., 2016). Two of the regions (Haut Bassins and Sahel, see Figure 1) where this study was conducted had the highest population of livestock in the country and were reported to have significant feed deficit in 2019 (Amole et al., 2022). With reported trend of climate change in West African Sahel of increased likelihood of rise in temperature and unpredictable rainfall pattern (Zougmore et al., 2016), feed availability in the study sites and the whole country will be precarious in the coming decades. This will necessitate either moving the animals to where the surplus feed is available through the continued practice of transhumance, or moving the surplus feed to where the animals are, which implies transportation of feed to feed-deficit areas and the associated increase in feed costs at the destination markets.

Our findings that legume residues (cowpea hay, cowpea husk and groundnut haulm) consistently had higher price than all other feed types in all locations irrespective of season is consistent with published results from other studies on livestock feed markets in Mali (Ayantunde et al., 2014), Niger (Jarial et al., 2016; Ayantunde et al., 2022) and in Senegal (Wane et al., 2010). The higher price of legume residues is not necessarily because of their better quality compared to other feeds but largely can be attributed to perception of the buyers, and tradition of using legume residues for small ruminant fattening in the Sahel. For example, concentrate feed, cereal bran and cottonseed cake have better nutritional quality in terms of crude protein content and *in vitro* organic matter digestibility but their prices are consistently lower than those of legume residues. However, it should be emphasized that legume residues do provide needed bulk without potential digestive upset that concentrates may have and have value beyond crude protein alone. The generally lower prices of crop residues in the early dry season compared to other seasons is a reflection of their abundance at this period which corresponds to the harvest period.

The variability of the nutritional quality of feeds, particularly crop residues, in terms crude protein content and *in vitro* organic matter digestibility with season and feed type is expected as chemical composition varies with feed materials (Amole et al., 2022). Lack of a clear trend in terms of relationship between price and nutritional quality for nearly all feeds confirms absence of quality standardization for feeds on sale at the markets in our study sites. Similar observation of lack of clear relationship between price and quality of feed has been reported for feeds sold in peri-urban areas of Bamako, Mali (Ayantunde et al., 2014) and for peri-urban areas of Maradi and Niamey in Niger (Jarial et al., 2016). This suggests that other factors besides laboratory fodder quality traits such as season, market location or agro-ecological zone, feed provenance, perceptions of farmers, traders and livestock keepers are determinants of feed price in West African Sahel. In developing feed industry in the region, the issue of quality standardization and labelling needs to be addressed by the policy makers to facilitate establishment of nutritive value-based pricing and to ensure that consumers pay for quality products. In addition, systematic support in terms of access to credit and incentives such as subsidy for the establishment of small and medium scale feed mills can facilitate the development of feed industry in the country. The government policies can also provide support for transport of feed from the feed-surplus areas of the country to the feed-deficit areas through improvement of road infrastructures and fuel subsidy. Provision of incentives for investment by big feed companies in the country can also enhance growth of feed industry in the country but this has to be managed well to avoid stifling the growth of small and medium scale feed mills.

5 Conclusion

In conclusion, to bridge the increasing feed gap for livestock, particularly in the dry season in West African Sahel, livestock keepers are increasingly turning to purchase of feeds from markets, which has contributed to the emergence of livestock feed markets in many peri-urban towns and cities in the region. Results from our surveys on livestock feed markets in Burkina Faso showed that adult male dominated feed markets in all locations and the youths (≤ 25 years) were mainly involved in sale of feed during the wet season in all the four locations. The results further showed that the determinants of feed price were season, market location, feed type, year of experience of feed vendor and to some extent the provenance of feed. As a result, there is lack of clear relationship between price and nutritional quality (crude protein content and *in vitro* organic matter digestibility) of feed. In developing feed industry in the region, the issue of quality standardization and labelling needs to be addressed to facilitate establishment of nutritive value-based pricing and to ensure that consumers pay for quality products.

Data availability statement

The original contributions presented in the study are included in the article. Further inquiries can be directed to the corresponding author.

Author contributions

AA designed the study, supervised data collection and cleaning, analysed the data, wrote the original draft and contributed to review of the draft. TA and AD reviewed and edited the draft manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Exploration of gender dynamics in the production and marketing of forage technologies in Kenya

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Inadequate and poor-quality feed resources are a key constraining factor in livestock production that compromises the livelihoods of many women and men dependent on livestock, who can be found in most low- and middle-income countries (LMICs). Several forage varieties have been developed, which are targeted to smallholder farmers in developing countries, yet the level of adoption remains low, particularly among women farmers. Adoption of varieties could enhance livelihoods by increasing livestock productivity and also by providing an income from the sale of surplus forage. However, evidence on gendered barriers to and incentives for the adoption of forage varieties is scarce. This study explores the gender dynamics influencing the uptake and commercialization of a forage variety, *Brachiaria* (syn. *Urochloa)*, among women and men farmers in four subcounties in Kenya. Through a mixed-methods approach, 260 individuals (59 women and 201 men) belonging to a household where at least one individual is a member of a dairy cooperative were interviewed. Complementary data from qualitative interviews engaging 16 single-sex focus group discussions (FGDs) and 8 key informant interviews (KIIs) were collected. Results reveal that *Brachiaria* is accessible to both women and men. Dairy cooperatives are important means for women and men to access extension services, useful information, and planting materials, but only a few women are cooperative members. Men face constraints owing to small land areas, rather than the ownership and access problems that constrain women. These challenges privilege men as *de facto* owners of resources while subordinating women and disenfranchising them in terms of adopting this technology. Complex yet important pathways for women's and men's empowerment exist through the sale of planting materials, hay bales, and an increased volume of milk obtained when cows are fed with *Brachiaria*. We conclude that cooperative membership for men should be supported, while at the same time gender norms that reduce women's engagement with cooperatives, and their access to and control over land, should be challenged.

KEYWORDS

forage improvement, gender, Kenya, *Brachiaria*, marketing

1 Introduction

Livestock is an important asset for the livelihood of farmers in low- and middle-income countries (LMICs) and has the potential to empower women through the multiple roles involved in livestock production (Galiè et al., 2015). This is in line with Sustainable Development Goal (SDG) 5 on “Women’s Equality and Empowerment” (UN Women, 2019). In the last decade, the discourse on women’s empowerment (WE) in livestock production has gained interest among researchers and development partners, and research to establish the link between the two has intensified (Price et al., 2018; Galiè et al., 2019; Baltenweck et al., 2021; Baltenweck et al., 2022). Some of the documented pathways to realize WE through livestock production include women’s livestock ownership and management; women’s participation in the livestock market and the commercialization of live animals, animal products, and animal inputs; and the opportunity for women to control and make decisions over these products and over the income generated from their sale (Njuki & Sanginga, 2013). For example, women often sell surplus eggs and milk and control the generated revenues, which they can use to meet their own or their household’s needs (Njuki and Sanginga, 2013; Tavenner et al., 2021).

This potential is, however, threatened by insufficient and poor-quality feed in LMICs, which limits livestock productivity and the ability of livestock keepers to sell surplus products and earn a living. Quality forages are needed to provide the nutrients and fiber essential for increased livestock production, enhanced body-weight gain, efficient reproduction, and possibly increased income from the sale of livestock and livestock products (Balehegn et al., 2020). Uptake of cultivated forages by farmers is being increasingly promoted by governments and development organizations in collaboration with research institutions (Nguku et al., 2016; Njarui et al., 2016; Maina et al., 2020) as one way of addressing the problem of livestock feed scarcity. *Brachiaria* is a valuable alternative to Napier grass, the dominant forage grass in Kenya (Maass et al., 2015). *Brachiaria* is reputed for its high regeneration potential of up to 7–10 times annually, high biomass, palatability, and drought tolerance. It can enhance the income of smallholder farmers through the sale of both produced milk and excess forage (Maina et al., 2020).

Little is known about women and men farmers’ access to and adoption of *Brachiaria*. Research has shown that agricultural technologies, such as plant varieties, are often developed with a focus on enhancing productivity and may fail to cater to the different and gendered needs of various types of farmers (Teklewold et al., 2020; Neway & Zegeye, 2022). Gender-blind plant breeding is unlikely to result in varieties that fully reflect the needs of women farmers or that benefit poor women and men equally (Ragasa, 2012). This is one of the reasons why women farmers are generally less likely to adopt plant varieties than men in their communities (Radović-Marković et al., 2020). Similarly, research shows that forage variety improvement pays little attention to gendered differences in trait needs and priorities, gendered constraints to adoption, the gender dynamics that affect such adoption, and the ability of women to benefit from growing the varieties (Balehegn et al., 2020; Harris-Coble et al., 2022; Njuguna-Mungai et al., 2022).

This study explores the gender dynamics influencing *Brachiaria* uptake and commercialization among women and men farmers and members of dairy cooperatives in four subcounties in Kenya using the framework of empowerment. Specifically, the study (i) assesses the rate of adoption and commercialization of *Brachiaria* among women and men farmers, (ii) analyses the determinants of adopting *Brachiaria* among women and men farmers, (iii) examines the power relations among women and men in the context of feed technology, and (iv) explores how the adoption of feed technologies contributes to women’s economic empowerment.

Gender is understood as the characteristics of women, men, boys, and girls that are socially constructed by society (Johnson, 2020)—affected by other individual characteristics such as marital status and ethnic group—and shape an individual’s behavior, their access to resources, and opportunities. Gender relations are the social relations and power dynamics between women and men in both private and public spheres (Kabeer, 2009). Gender relations are affected by and, in turn, affect gender norms. Gender norms are the social rules and expectations that govern the behavior of individuals and restrict their gender identities to what is considered to be appropriate (Agarwal, 1997). We define WE as a process of change in terms of the ability of women to make meaningful and strategic life choices and act to reach preferred results (Kabeer, 2009).

The following sections cover the study’s method, followed by the study findings, discussion, conclusion, and recommendations. We organize our results starting with the socioeconomic profile of the study participants, then focus on *Brachiaria* establishment and commercialization. This is followed by power relations among women and men farmers in the context of forage technologies, and then by the factors influencing farmers’ decision to adopt *Brachiaria*. Finally, we focus on opportunities for and constraints to benefiting from forage technologies. In the discussion section, we expound on how the gender dynamics that affect *Brachiaria* adoption and commercialization relate to empowerment.

2 Materials and methods

2.1 Study area

This study was carried out in four subcounties in Kenya (Figure 1) that were selected owing to their high potential for commercialized dairy production, which is associated with the likelihood of households cultivating forage. Such potential stems from favorable climatic conditions that support crop and livestock production as the primary source of livelihood (County Integrated Development Plan, 2017; National Drought Management Authority, 2020). Each subcounty had some active dairy cooperatives through which a development project, the Accelerated Value Chain Development, implemented by ILRI (Auma et al., 2018), promoted the adoption of *Brachiaria* technology by smallholder farmers. The project supplied the planting materials and piloted a private agricultural extension

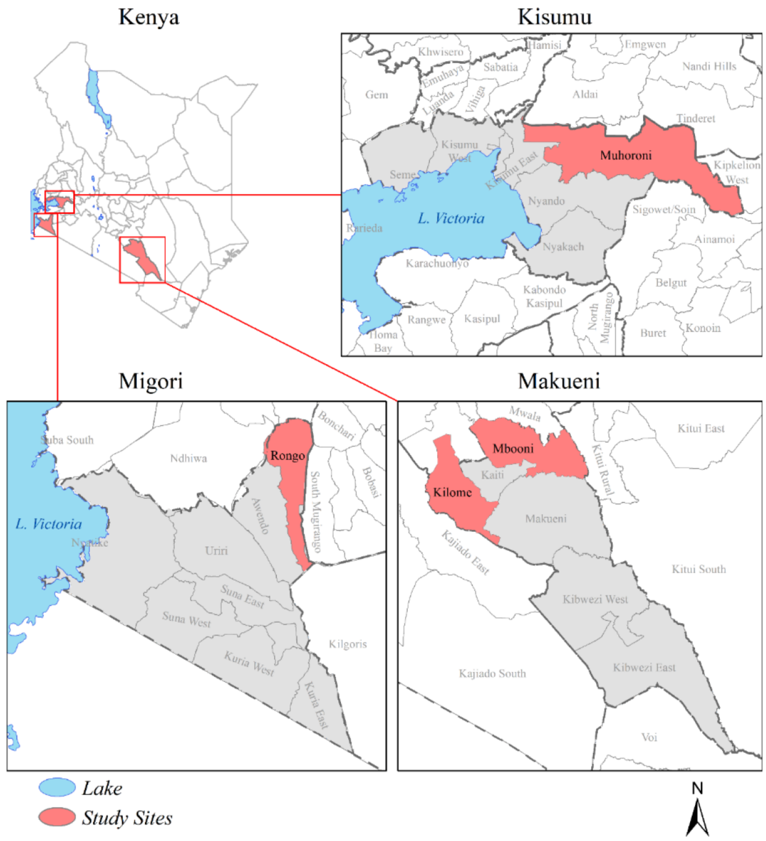

FIGURE 1
Map showing the study site locations in Kenya.

model that uses dairy farmer assistants (DFAs) attached to each dairy cooperative. Under each DFA, community mobilizers were issued with *Brachiaria* seeds for propagation, followed by dissemination to farmers in their respective villages. Anecdotal evidence from the project implementers revealed a disparity in *Brachiaria* adoption levels across the study subcounties (Muhoroni, Rongo, Mbooni, and Kilome), as well as in feed commercialization in Mbooni and Kilome, which have a relatively dry climate.

2.2 Study design and sampling

Gender-sensitive mixed-methods research, with both quantitative and qualitative research designs, was applied to gain a more complete and contextually significant picture of the topic under investigation (Bonis-Profumo et al., 2022). Both study components were conducted concurrently between November/December 2019 and February/March 2020.

2.2.1 Quantitative research method

The quantitative method used in this study is grounded in a positivist paradigm (Alharahsheh and Pius, 2020). We implemented a quantitative survey using a predesigned questionnaire to assess the rate of *Brachiaria* technology adoption and commercialization and to further identify determinants of *Brachiaria* adoption by households in which at least one person (either the household head or the spouse) was a cooperative member. The study employed a multistage sampling technique to select a random sample, guided by the sampling formula provided by Cochran for proportions of large samples with unknown variability (Cochran, 1963). A total of 260 individuals (59 women and 201 men) were randomly selected from a list of all dairy cooperative members, whether men or women, provided by the DFA (Table 1). A questionnaire was administered to the household head; in their absence, an adult woman or man (above 18 years of age) was interviewed.

2.2.2 Qualitative research method

The study adopted an exploratory research design underpinned by a qualitative approach and situated in the interpretivism paradigm (Alharahsheh and Pius, 2020) to obtain in-depth details and new insights on the study topic. Focus group discussions (FGDs) were the primary method of data collection, guided by semistructured interview questionnaires. With the support of DFAs, we purposively selected research respondents following the study selection criteria: (i) women and men from households with membership of a local dairy cooperative and (ii) farmers of *Brachiaria* varieties (whom we call “adopters”) or farmers who did not grow any *Brachiaria* variety (whom we call “non-adopters”). By including both *Brachiaria* adopters and non-

TABLE 1 Research questions and tools used.

Research question	Tool used	Respondents			
		Location	Adopters (*n*)	Nonadopters (*n*)	Total (*n*)
Rate of adoption and commercialization of *Brachiaria*	Household survey	Muhoroni	FHH = 8 MHH = 26	FHH = 1 MHH = 22	260
		Rongo	FHH = 9 MHH = 20	FHH = 15 MHH = 22	
		Kilome	FHH = 2 MHH = 18	FHH = 6 MHH = 45	
		Mbooni	FHH = 5 MHH = 21	FHH = 13 MHH = 27	
Analysis of determinants of adopting *Brachiaria*	Household survey	Total of 260 respondents for the four study subcounties as above			
Examination of power relations among women and men	FGDs and KIIs	69 women (34 adopters, 35 non-adopters) and 59 men (23 adopters, 36 non-adopters) for a total of 128 participants			
How the adoption of feed technologies contributes to women's economic empowerment	FGDs and KIIs	69 women (34 adopters, 35 non-adopters) and 59 men (23 adopters, 36 non-adopters) for a total of 128 participants			

adopters, we aimed to compare what influenced and facilitated the adopters to take up *Brachiaria* technology with what barriers non-adopters faced that prevented adoption. We conducted 16 single-sex FGDs: one each with adopting and non-adopting women and one each with adopting and non-adopting men in each study site. Each FGD had between 5 and 15 participants, totaling 69 women (34 adopters, 35 non-adopters) and 59 men (23 adopters, 36 non-adopters) for an overall total of 128 participants. The gender issues explored included activity profiles; perceptions of the attributes of *Brachiaria*; access to and control over resources; and benefits, constraints, and opportunities, and their influence on the decision of whether or not to adopt *Brachiaria*. Our study explored the inter-household gender dynamics and perceptions of female-headed households (FHHs) and male-headed households (MHHs) to understand the gender dynamics surrounding the adoption of *Brachiaria*.

In addition, key informant interviews (KIIs) were conducted with the subcounty livestock production officers, DFAs, and leading farmers in *Brachiaria* production and commercialization to corroborate information gathered from the FGDs and to provide additional nuanced information on social relations and their effects on technology adoption. We conducted a total of eight KIIs with two livestock production officers (one woman and one man), two DFAs (one woman and one man), and four leading *Brachiaria* adopters (four men: there were no leading women *Brachiaria* farmers in any of the study sites). The lead author conducted all the FGDs and KIIs in person, assisted by one temporarily hired and trained research fellow. The number of KIIs was guided by the principle of data saturation (Saunders et al., 2018), and interviewing was stopped when saturation, with no emergent new information, was achieved.

During the study, the confidentiality and anonymity of all study participants was assured. This study received ethical approval from ILRI's Institutional Ethics Committee (Ref. ILRI-IREC 2019-47).

2.3 Data analysis

2.3.1 Quantitative data analysis

To evaluate the determinants of the adoption decision, a logistic regression model was used to assess the association between the binary adoption outcome and factors with the potential to impact it, including demographic, geographic, socioeconomic, institutional, and technology-related attributes. In technology adoption, a farmer's decision to adopt can be described as a binary outcome —to adopt or not adopt. Therefore, the dependent variable, adoption, is a discrete variable that informed the choice of a discrete model. Therefore, a binary logistic model was used in this study, which is popular among other adoption studies (Conteh et al., 2015). A household will adopt *Brachiaria* if the utility derived is higher than that of non-adoption. As we can only observe a household's actual choice and not the derived utility, we therefore specified the logistic model in its linear form as:

$$y(0,1) = \beta_0 + \beta_i x_i + E_i \quad (1)$$

where y is a binary dependent variable taking the value of 1 when a household adopts *Brachiaria* and 0 otherwise, β_0 is the intercept, x_i is a vector of the household's socioeconomic and environmental factors, β_i is a vector of the respective coefficient parameters, and E_i is the error term. The study hypothesized that factors likely to influence adoption decisions include land ownership and access (+); extension support (+); the number of dairy animals owned (+); and respondents' demographic characteristics, such as education (+/–), age (+), gender (+/–), occupation (+/–), sources of income (+/–), and ability to take a decision (+/–) (Melesse, 2018). In parenthesis, we indicate the expected direction of influence, positive (+) or negative (–), based on the literature.

The study further reports marginal effects on the estimated logistic model to better interpret the model—i.e., using the probability scale and not the odds scale. In the probability scale, all the effects are non-linear because, conditional on covariate values, the probability must be bound between 0 and 1. The logistic model used in the study is the log-odds, but we interpreted the model in the probability scale. The log-odds are expressed as:

$$log\left(\frac{p}{1-p}\right) = \beta_0 + \beta_1 x \tag{2}$$

Solving for p to go from log-odds to probability, we get:

$$p = \frac{e^{\beta_0 + \beta_1 x}}{1 + e^{\beta_0 + \beta_1 x}} = \frac{1}{1 + e^{-}(\beta_0 + \beta_1 x)} \tag{3}$$

The logistic regression model was estimated using the Stata® (StataCorp LP, College Station, TX, USA) computer package.

2.3.2 Qualitative data analysis and interpretation

Qualitative data collected were transcribed and translated into English. Coding, based on predetermined and emerging themes, was carried out using QSR International's (Warrington, UK) NVivo 11 software. The findings were then organized and analyzed inductively based on emerging themes. For example, gender norms emerged during the FGDs without the authors having explored them intentionally.

3 Results

The findings from the explanatory study are presented first, then corroborated and integrated with those from the exploratory study. We begin with a characterization of study participants; followed by a characterization of forage production, with a focus on *Brachiaria* adoption in terms of preferences, constraints, and benefits; and lastly, we present the discussion, conclusion, and recommendations.

3.1 Characterization of study participants

Most surveyed respondents came from MHHs (77%), with 23% from FHHs. Sampled households had at least one member registered in the dairy cooperative. Overall, 260 households were surveyed; of them, 109 were adopters of *Brachiaria* (24 FHHs and 85 MHHs) and the rest were non-adopters (35 FHHs and 116 MHHs). Rongo had the highest number (9.2%) of FHHs, whereas only 3.1% of respondents in Kilome were from FHHs (Table 2). The majority (61.2%) of the study respondents were household heads (20% female heads and 41.2% male heads). The other respondents were either spouses (28.1% with 27.7% men and 0.4% women), adult children (7.7% young man/young woman above 18 years of age, the age where adulthood begins in Kenya), or other household members (3.1% either a close relative or a non-relative).

Overall, more MHHs (22% of adopters and 25% of non-adopters) than FHHs (4% of adopters and 6% of non-adopters) engage in non-agricultural activities. Women mostly work on family farms and engage in mixed farming (Figure 2). Food crops, such as maize, beans, green gram, and mung bean, are grown by women, whereas cash crops, such as sugarcane and fruit trees, are grown by men. These results were corroborated by qualitative findings, which indicated that women often engage in on-farm activities for their livelihood. Commonly reared animals are cattle, sheep, goats, and chickens, with the majority (94%) of respondents rearing cows that have calved at least once (96.61% of FHHs and 92.04% of MHHs), with the next most popular livestock being heifers (51% of households; 52.54% of FHHs and 50.25% of MHHs), followed by female calves (40.68% of FHHs and 33.83% of MHHs). Bulls were slightly more popular among MHHs (29.85%), only being raised by 11.86% of FHHs. Forage and food crops were commonly termed as the "women's crops for subsistence", whereas cash crops were for men. Men in Muhoroni and Rongo consider sugarcane as their valued cash crop and more important than any other crop or livestock. Approximately three-quarters of the land

TABLE 2 FHH and MHH households surveyed.

Subcounties		Adopters		Non-adopters		Total number
		Number	Percentage	Number	Percentage	
Muhoroni	FHH	8	23.5	1	4.4	**9**
	MHH	26	76.5	22	95.7	**48**
Rongo	FHH	9	31	15	40.5	**24**
	MHH	20	69	22	59.5	**42**
Kilome	FHH	2	10	6	11.8	**8**
	MHH	18	90	45	88.2	**63**
Mbooni	FHH	5	19.2	13	32.5	**18**
	MHH	21	80.8	27	67.5	**48**
Total	**FHH**	**24**	**40.68**	**35**	**59.32**	**59**
MHH	**85**	**42.29**	**116**	**57.71**	**201**	**260**

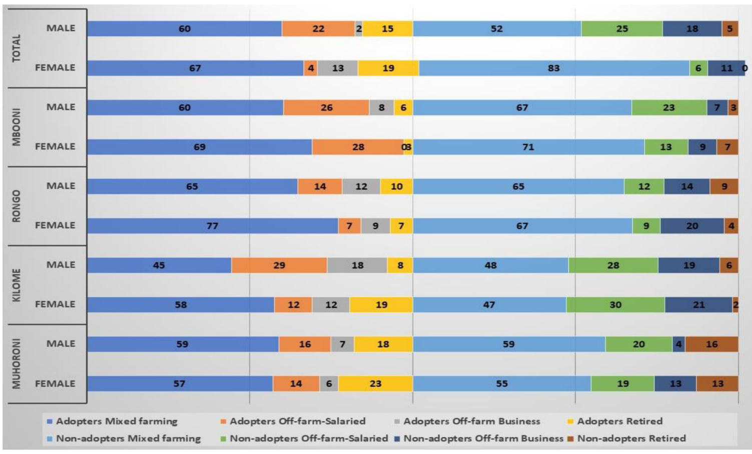

FIGURE 2
Occupation of the study participants.

grows sugarcane, whereas the rest is occupied by the homestead, with little or no space left to grow forage and food crops. In Mbooni and Kilome, mango and orange trees are "crops for men". Regarding marital status, the majority (> 90%) of male and female respondents, as self-identified, were married.

FHHs had less access to extension services than MHHs. More adopters (83.33% of FHHs and 84.71% of MHHs) than non-adopters (54.29% of FHHs and 62.07% of MHHs) had access to extension services (Figure 3), with a chi-squared test further confirming that the differences between the two sets of proportions were statistically significant (chi with one degree of freedom = 20.2836; p = 0.000). The qualitative results revealed that both women and men have access to extension services in principle, through which training and information are disseminated.

Cooperatives are important avenues for accessing inputs, credit, information, and a market for products. Overall, dairy cooperative membership comprises 75% men and 25% women, of which 5% are aged between 18 and 35 years. The men, as registered members, commonly attend important cooperative meetings, but for meetings that they deem less important or when they are engaged with other commitments, they ask their spouses, the women, to attend instead.

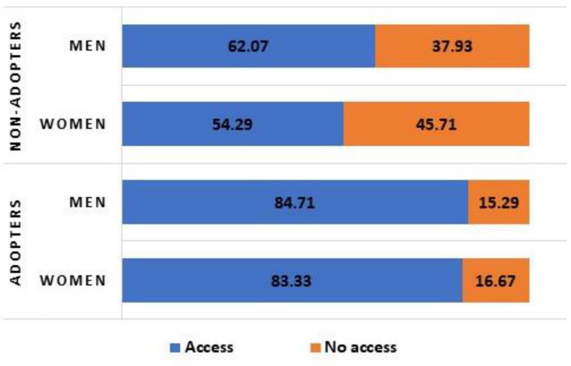

FIGURE 3
Access to extension services.

Women and men with higher education levels have a higher rate of *Brachiaria* adoption. Often men have higher education levels than women, which is reflected in men's slightly higher adoption rate. More adopters (82%) than non-adopters (54%) had attained at least a secondary school level of education. *Brachiaria* adoption rate is low (see below) in study areas where women have low education levels. Adopters in Muhoroni had relatively higher levels of formal education, with 60% of FHHs and 53% of MHHs having at least a college education, in contrast to their counterparts in the other three subcounties. Men in Rongo often had a lower level of education, with 47% of them attaining at most a primary school level of education, compared with 18% of men in Muhoroni. Similarly, most women from Rongo (43%) had at most a primary-level education, and 9% were illiterate (Figure 4). The gender of the respondents and their education level had a statistically significant relationship (chi with degrees of freedom = 26.2573; p = 0.000), with men having higher levels of education than women.

3.2 Characterization of forage production

3.2.1 Forage crops cultivated

The study assessed the crops and feed materials grown by households for livestock feeding. Napier grass was most commonly produced (56% of FHHs and 55% of MHHs), followed by *Brachiaria* (41% FHHs and 42% MHHs), which was a recent introduction. There was no significant difference between FHHs and MHHs. These two forage types are grown as "cut and carry" forage. Pasture–ley grasses, forage shrubs, and forage legumes [i.e., greenleaf desmodium (*Desmodium intortum*) and lucerne (*Medicago sativa*)] were produced in only 7% of FHHs and 9% of MHHs (Table 3).

3.2.2 Women and men's roles and responsibilities in forage production

Women in all the study sites are traditionally assigned the roles of forage harvesting, transporting crops home, and feeding the

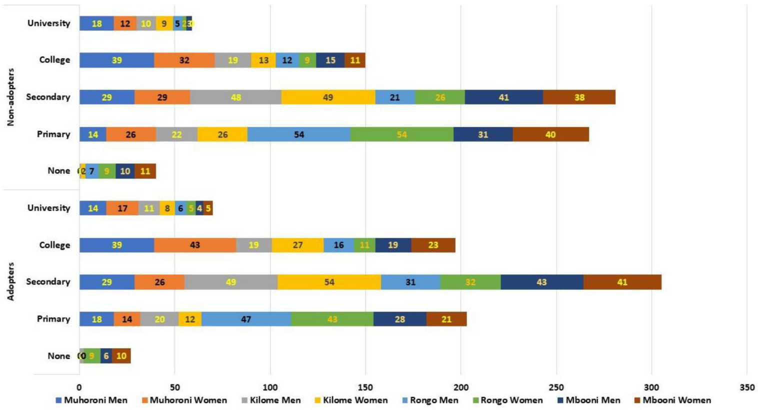

FIGURE 4
Levels of education.

animals. Less than one-third of men, both husbands and workers, in Muhoroni and Rongo harvest and carry forage. Men mostly prepare the land and handle the planting, whereas transplanting, weeding, and forage gathering and its utilization is often assigned to women (Table 4). Women also engage in milking, milk delivery to the dairy cooperative, chicken feeding, and egg collection. During weekends and school holidays, children help with some of the chores. Boys and, in a few instances, girls help the women harvest forage, collect eggs, feed the chickens, and deliver milk to the dairy cooperative. The FGDs revealed some gender stereotypes behind labor patterns. These include men considering women to have strong and flexible backbones, which enable them to bend, and harvest forage and transport it. Women mentioned some gender norms around such labor patterns: men generally find it shameful to transport forage because carrying forage would undermine the image of a man's power in the eyes of his community; also, men do not engage in low-profit activities, such as forage carrying, but prefer high-revenue off-farm activities.

Some of the gender norms discouraging men from carrying forage were expressed by two women in FGDs:

Men like to relax; it is challenging to find a man carrying forage. They view it as a shameful activity. Other men will perceive such a man as weak and one whom his wife oppresses. They think it is not befitting for them to carry forage. (FGD woman, Mbooni subcounty, March 2020)

Older women said that, although it is shameful for men to carry forage, they hire young men to harvest and carry forage because of their physical strength, as explained by one woman KII:

I can say it is culture [that dictates that women carry forage …] but then I can say cutting forage like this Napier grass is heavy. Young men are stronger than me. I must call them to come and help […]. My husband is not involved in this work. I have to make him breakfast because he is still with the government. So, when the young man I hire brings the forage [home], I start feeding the cows; there is no sitting down, and there is no time to go to other homesteads to gossip. You are busy until evening. That is why you need the youth to come and help you to harvest. (KII woman, Rongo subcounty, February 2020)

3.2.3 Focus on *Brachiaria* adoption: preferences, constraints, and benefits

3.2.3.1 Rate of adoption and preferences

The FHHs had a slightly lower rate of adoption (40.7%) than the MHHs (42.3%), with no significant difference in their means (p = 0.6463). The overall adoption rates among the subcounties were significantly different (chi with three degrees of

TABLE 3 Forage crops grown.

Forage crop		Percentage
Napier	FHH	55.9
	MHH	54.7
Brachiaria	FHH	40.8
	MHH	43.1
Pasture–ley grass	FHH	6.8
	MHH	8.5
Other forage	FHH	11.9
	MHH	15.4

TABLE 4 Participant's activity profile relating to dairy production, forage production, and utilization.

Activity		Who mostly implements the activity				
		Women	Men	Girls/boys (children)	Employee	
					Female	Male
Preparing land		Sometimes	x	Sometimes		x
Planting		x	x	Sometimes	x	x
Weeding		x		Sometimes	x	x
Harvesting forage		x	Sometimes	x	x	x
Carrying harvested forage		x				
Feeding and management of cattle		x	Sometimes	x		
Milking		x	Sometimes			x
Feeding and management of poultry		x	Sometimes	x		x
Selling the produce	Milk	x	x			x
Cattle		x				
Shoats	x	x				
Traditional chicken	x					
Commercial chicken	x	x				
Constructing animal houses			x			x
Collecting milk proceeds from the dairy cooperative		x	Sometimes			

Source: Focus group discussions with study participants.

freedom = 13.1561; p = 0.004); the highest was in Muhoroni (89% for FHHs and 54% for MHHs) and the lowest was in Kilome (25% for FHHs and 29% for MHHs). The adoption rate for FHHs in Rongo and Mbooni was 38% and 28%, respectively (Figure 5).

In general, the surveyed households have small areas of land, with an average of 0.72 acres. The FHHs have smaller land areas (0.46 acres) than MHHs (0.80 acres), which limits their adoption rate. Households in Kilome had the largest properties (1.46 acres), followed by Mbooni (0.65 acres), then Muhoroni (0.52 acres), and, lastly, Rongo (0.49 acres). In all study sites, land allocated to *Brachiaria* cultivation was smaller for FHHs (0.28 acres) than for MHHs (0.43 acres) (Table 5). Overall, few women and men grow forage on a dedicated plot. Commonly it is grown as a hedge or terrace in crop fields along contour lines to conserve soil and water. This limits the ability to increase the quantity of feed produced and compromises year-round feed availability:

I would like to produce [*Brachiaria*], but the limiting factor is land. (FGD woman, Muhoroni subcounty, November 2019).

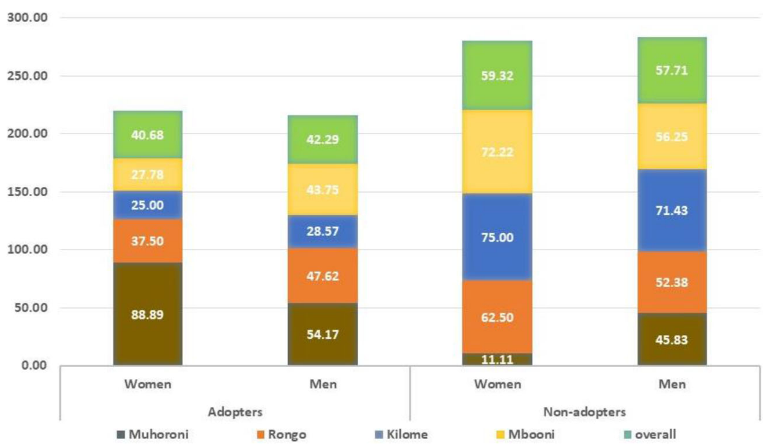

FIGURE 5
Rate of *Brachiaria* adoption.

TABLE 5 Average area of *Brachiaria* in the surveyed households.

Gender of household head	Mean area in subcounty (acres)				
	Muhoroni	Kilome	Rongo	Mbooni	Overall
FHH	0.52	0.15	0.18	0.12	0.28
MHH	0.48	0.58	0.29	0.38	0.43

We have planted *Brachiaria* on small portions of land since we have a challenge with land. (FGD man, Muhoroni subcounty, November 2019)

We enquired about gendered preferences for forage crops through the FGDs. *Brachiaria* is preferred by both women and men adopters, and it is much more frequently traded than other forage crops (67% FHHs and 56% MHHs) (Table 6). It was also evident among the KII traders that there is high demand for processed *Brachiaria* (in the form of hay) rather than silage because of its preservability and ease of transportation. Through the FGDs in Kilome and Mbooni, we also identified a preference for *Brachiaria* by both women and men adopters over food crops. Maize, beans, and vegetables were being replaced with *Brachiaria* because "milk has higher income", which can be used to purchase the grains and pulses. However, men prioritize cash crops over *Brachiaria*.

We further explored *Brachiaria* characteristics liked and disliked by women and men farmers. The women and men farmers mentioned their preferred characteristics according to their experience using the grass or what they had heard from neighbors, friends, and relatives. *Brachiaria* was assessed positively by both women and men in all the study subcounties because of (i) low labor intensity, (ii) high productivity, (iii) high palatability, (iv) resistance to drought and disease, and (v) ease of storage. However, respondents from Kilome, which is relatively drier, said that it is not drought tolerant. Low labor requirements were particularly important to women in all sites because *Brachiaria* suppresses weeds, so in turn women's traditional work of weeding was reduced. *Brachiaria* is preferred to other grasses because of its productivity: it is high yielding and increases milk production. *Brachiaria* is more palatable to cattle, which translates to higher productivity, as animals eat more. Farmers mentioned that it is resistant to pests and diseases and has high regeneration potential even with little rainfall. Finally, *Brachiaria* is easy to bale and store. In the words of two FGD respondents:

It [*Brachiaria*] kills the indigenous weeds, reducing our [women's] work. (FGD woman, Kilome subcounty, November 2019)

The roots are deep, so no other grass can grow … it is drought resistant, it regenerates quickly after harvesting, and it helps in soil conservation by preventing soil erosion. (FGD man, Mbooni subcounty, March 2020)

Additional attributes preferred by most women respondents include the lack of hairiness and non-stemminess, making it easier to harvest and transport. These characteristics were also mentioned by only a few men as being important. Some women FGD respondents argued that the *Brachiaria* variety they were growing had these characteristics, whereas less than one-quarter of the respondents said *Brachiaria* was hairy and stemmy. Women and men clearly expressed preferences for characteristics associated with the roles they played in managing the crop. Some had the right variety for their preferences, and some did not:

I know *Brachiaria* Basilisk, Mulatto, but I do not know where they came from. There is one that is smooth, and the other is hairy … I think [Basilisk] is the one that is hairy. I have planted both: one bench has Basilisk and the other bench has the other one. I only know this grass is *Brachiaria*. We have not been able to monitor … the first, *Brachiaria*, is a bit tough; one has to chop it for cows to be able to feed on it well. (FGD man, Mbooni subcounty, March 2020)

TABLE 6 Commercialization of *Brachiaria*.

		FHH percentage	MHH percentage	Overall percentage
Forage trading—main business		100	17	14
Source of traded forage	Self-produced	100	100	100
Forage type sold	Ley grass	33	17	10
	Napier	0	17	14
	Brachiaria	67	56	52
	Other	0	11	9
Forage business ownership	Sole ownership	100	78	
	Partnership	0	6	
	Group activity	0	11	
	Other	0	6	

In terms of the characteristics that respondents did not like about *Brachiaria*, although environmental resilience (*Brachiaria* does not dry completely and regenerates very fast even with little rainfall) was one of the positive attributes of *Brachiaria*, the poor growth rate was highlighted, particularly by respondents from Kilome and Mbooni, which are areas that experience dry and salty/saline environmental conditions. These conditions do not favor growth for most crops, including *Brachiaria*:

I planted *Brachiaria* splits and seedlings, but they dried off. The few that grew were retarded because of the salty water prevalent in our environment. (FGD woman, Kilome subcounty, December 2019)

The high demand for manure by the grass was also a concern for both women and men.

3.2.3.2 Factors that influence the adoption of *Brachiaria*

The study quantitatively evaluated the factors that influence women's and men's decisions to adopt the production of *Brachiaria* forage. Two models were estimated—one for FHHs and another for MHHs. Multicollinearity was tested before the estimation by evaluating the partial correlation coefficients between pairs of explanatory variables in the model. One of the variables was dropped when the partial correlation coefficients were too high (> 0.6 or< –0.6) between the two variables. Table 7 shows the results of the econometric model. The likelihood ratio (LR) chi-squared test (LR χ^2 = 46.70 for women and 90.19 for men, degrees of freedom = 12; p = 0.0000) for both models was statistically significant, indicating the suitability of the model for explaining adoption. Logistic results for FHHs and MHHs revealed a slight difference in some of the factors that influence the adoption decision.

For both women and men, four main factors were positively and significantly associated with the probability of adopting *Brachiaria* forage, but mostly at varying significance levels. These factors were ownership and access to resources (including land, lactating animals, and forage technology); access to services (including extension services); access to education; and low labor demand (e.g., method of land preparation). Other factors uniquely influenced either men's or women's adoption decisions. For example, how adopters got the planting materials and the sale of forage influenced MHHs only. Group membership positively and significantly influenced the FHHs' adoption decision. The significant variables (factors) are explained in detail below.

When analyzing ownership and access to resources, a woman's or man's land tenure status and ownership of lactating cows were considered independent variables for the logistic model. In general, married women lack access to and control over productive resources such as land, which constrains their rate of adoption. Land tenure (private, non-private leased, or communal) was positively associated with adoption (p< 0.05 for FHHs and p< 0.01 for MHHs). When a woman or a man privately owns the land, the probability of adopting *Brachiaria* increases by 36.10%. However, FGD respondents from all sites revealed gendered inequalities in land ownership, land management, and the right to sell land. Land ownership is considered to be for men in all the subcounties. Less than one-quarter of the women respondents owned land with the title deed in their names. FGD results revealed that land inheritance is a common means of owning land; traditionally, men and boys inherited property. Unmarried, separated, divorced, or widowed women (i.e., unmarried women) can acquire land with their income only, as explained by some men respondents:

TABLE 7 Factors influencing the decision to adopt *Brachiaria*.

Explanatory variables	Odds ratio		Standard error		*z*-value		Marginal effects
	MHH	FHH	MHH	FHH	MHH	FHH	
Main occupation (1 = mixed farming; 0 = otherwise)	1.34	0.13	0.59	0.18	0.67	–1.50	0.0693
Extension contact (access = 1; 0 = otherwise)	2.38	12.11	1.17	14.73	1.76*	2.05**	0.1932
Number of lactating cows	1.43	5.08	0.25	4.67	2.07**	1.77*	0.0839
Grow Napier grass	12.09	184.44	5.83	407.89	5.16***	2.36**	0.5245
Schooling years	1.17	1.57	0.07	0.30	2.59***	2.35**	0.0368
Household's land tenure (1 = private; 0 = otherwise)	11.13	44.84	11.27	93.61	2.38**	1.82*	0.3610
Method of land preparation (1 = manual; 0 = otherwise)	0.36	0.01	0.22	0.03	–1.66*	–1.88*	0.2486
Source of labor for land preparation (1 = family; 0 = otherwise)	0.83	0.21	0.36	0.22	0.42	-1.49	0.0431
Source of planting materials (1 = dairy cooperative; 0 = otherwise)	9.17	7.23	5.09	12.04	3.99***	1.19	0.5033
Group membership (1 = member; 0 = otherwise)	1.38	47.33	0.69	100.78	0.64	1.81*	0.0743
Engagement in sale of forage (1 = sell forage; 0 = otherwise)	6.90	108.87	7.01	2024.76	1.90**	0.25	0.4388
Constant	0.00	8.22	0.00	4.24	–4.47***	–2.72***	
Men: observations = 180, χ^2 = 0.0000; pseudo-R^2 = 0.3485. Women: observations = 58, χ^2 = 0.0000; pseudo-R^2 = 0.5837.							

*, **, and *** are significance levels: *p< 0.05, **p< 0.01, ***p< 0.001.

… men own ancestral land, but if a woman works hard and buys her own land, then she is the rightful owner of the land. (FGD man, Mbooni subcounty, March 2020)

Culturally land is owned by men. Upon marriage, women surrender land they might have owned before getting married to the man. There is no land owned by women, a woman might have bought land before you married her, but with time, she must transfer to your name … all the woman's property becomes mine once we get married. (FGD man, Kilome subcounty, December 2019)

The study further assessed the patterns of individual livestock ownership among FHHs and MHHs. This revealed the intra-household dynamics that have significant implications for the decision to adopt *Brachiaria*, a critical resource for livestock production. The rate of adoption for both FHHs and MHHs increases with an increase in the number of lactating animals, probably due to increased livestock resources and the subsequent high demand for feed. An increase in the number of lactating animals increases the probability of adopting *Brachiaria* for FHHs ($p< 0.05$) and for MHHs ($p< 0.01$) by 8.39%. The women FGD respondents added that an increase in lactating animals means more demand for feed/forage and, also, more work for the women who collect forages, as stated below:

The men have left everything to be done by women … it is like a donkey in the house. (FGD woman, Kilome subcounty, December 2019)

Although men are the owners of land and cattle, women own indigenous chickens, which are perceived to be of low economic value. The high cost of purchasing an animal (specifically a cow) was blamed for limiting women's ownership. Even with limited ownership rights, as it is a woman's responsibility to mostly fetch forage and feed the animals, women expressed the desire to grow forage grass. This would reduce the work of traveling far to fetch forage from community land or collect forage purchased from neighbors.

Access to services, such as extension services, social capital (such as group membership), and access to education and markets were assessed as valuable channels for accessing relevant information and influencing adoption decisions. Contact with extension services ($p< 0.01$) and group membership ($p<0.05$) positively and significantly influenced the decision to adopt *Brachiaria*. For men, only access to extension services was significant ($p< 0.05$). The probability of participating in *Brachiaria* production increases with access to extension services by 19.32% (for both women and men). In comparison, group membership increases the adoption decision for women by 7.43%.

When analyzing how labor affects adoption decisions, the model result shows that the manual land preparation method had adverse and significant effects on the adoption decision for women and men ($p< 0.05$). The probability of adoption increases by 24.86% with a shift from manual to mechanized or chemical land preparation methods. This is probably due to reduced workload, particularly for women who are overburdened with numerous other activities (both paid and unpaid, as discussed above). Most FHHs (85%) and MHHs (83%) prepare land manually; only a few have mechanized land preparation (15% of FHHs and 17% of MHHs), and only 0.38% of the respondents use herbicides as a land preparation method. FGD respondents indicated that dependence on family labor or both family labor and hired labor combined is common for land preparation. Most FHHs (51%) are dependent on family labor for land preparation, compared with 40% of MHHs, whereas 42% of FHHs and 50% of MHHs depend on hired labor (women and men employees). The others depend on a combination of hired labor and family labor.

We qualitatively explored the constraints the respondent farmers faced when wanting to adopt *Brachiaria*. Results from the FGDs revealed that both the women and the men are faced with land constraints: women lack land ownership, whereas men complained about the small area of land they owned. Persistent gender norms in the study communities restrict women from owning or renting land as men do. Men, on the other hand, complained that their ability to grow more *Brachiaria* and its commercialization are constrained by the small area of the land they own and the different uses of the land that compete for space. For example, most men in Muhoroni engage in sugarcane production, which occupies most of the family land. In three-quarters of the households, men have prioritized their crops, mainly the cash crops, and the rest of the land is occupied by the homestead, with little or no land left for women's crops, which are mostly the food crops. Men can afford to hire land, whereas women cannot, especially given that they do not seem to have cash crops as men do.

Overall, men make major decisions, and they must be consulted by women when decisions are made. The decision to adopt *Brachiaria* is subject to the existent decision-making patterns:

I went somewhere to a meeting and took *Brachiaria*, I did not consult my wife, I did not consult my mother, I just came and planted. But in case it is the woman who went to that meeting and heard the benefits of *Brachiaria*, she must convince me [her husband] that it is good before we collectively choose a place to plant it. (FGD man, Rongo subcounty, February 2020)

FGD respondents clarified that another challenge to the adoption of *Brachiaria* relates to access to the planting materials by women and men farmers. The commonly used planting materials were seeds, splits, and seedlings. Approximately one-quarter (25%) of *Brachiaria* adopters, mostly men, received *Brachiaria* planting materials directly through the dairy cooperatives, whereas women mostly sourced splits or seedlings from fellow farmers, owing to their group membership. However, it was highlighted that access to *Brachiaria* planting materials was a challenge, particularly among non-adopters who said, "for non-cooperative members, it is difficult to access *Brachiaria* seeds":

I started with *Brachiaria* in 2017 when ILRI and Heifer International brought seeds to a group where I was a member. We were 30 [members] but only four were given the seeds, I was not among them. I borrowed some seeds from the other members, and I was given *Brachiaria* 'Xaraes' [one of the varieties]. (KII woman, Rongo subcounty, February 2020)

3.2.3.3 Benefiting from *Brachiaria*

We sought to understand the potential benefits that can accrue from the uptake of *Brachiaria* technology for a woman and a man, because this might influence the adoption decision. An increase in milk production was a benefit reported by women and men FGD respondents when an animal was fed with this grass. Farmers mixed *Brachiaria* with other feed (either Napier grass, wild grasses, or concentrates), making it difficult to determine the effects of *Brachiaria* on milk production. However, more than three-quarters of the FGD and all the KII respondents reported an increase in produced milk by approximately 2–8 L of milk (an approximately 40% to 80% increase) when *Brachiaria* was part of the dairy cattle diet:

Generally, this grass is good, you get a bumper harvest, and it increases milk production. It also makes you feel proud … when you have a lot of grass, everyone with dairy cows will look for you … it is resistant to termites, common during the dry spell … milk production has increased and the cost of dairy meal reduced by half … I have transformed my farm to a registered forage model farm where I charge access and training fee to individuals and groups who visit to learn about *Brachiaria* production. (KII male farmer, Makueni County, December 2019)

Another benefit highlighted by participants, which they noticed when animals were fed on *Brachiaria*, was the expediated expression of heat signs (estrus) and improved body condition (smooth skin).

Increased income and reduced cost of production (in terms of labor needed to weed the grass and the cost of buying concentrates) were highlighted by women and men as benefits reaped from the production of *Brachiaria*. The quantity of concentrates fed to an animal (lactating animal) reduces by half when an animal is fed *Brachiaria*, yet an increase in milk production is evident.

Respondents said that the production of *Brachiaria* could offer an opportunity for a woman or a man to become an entrepreneur. Approximately three-quarters of women include the sale of milk as a source of income from *Brachiaria*, whereas approximately one-quarter of men said they received income from selling *Brachiaria* seedlings and splits. The cost of splits was approximately US$10 for a 90-kg bag, whereas the retail price of a seedling is approximately US $0.05. Survey results revealed, however, a modest sale of the grass in the form of seedlings or splits. Overall, only 21 (8.08%) out of 260 participants sell forage of any type. Among *Brachiaria* adopters, only two (8.33%) FHHs and 15 (17.65%) MHHs sell forage (Figure 6). However, the difference in the proportion of forage traders between FHHs and MHHs is not statistically significant.

The potential earnings from the sale of *Brachiaria* seedlings and splits were explained by a man involved in a KII:

One day my son wanted school fees, so I decided to sell. I got 25 bales of *Brachiaria* 'Xaraes' and sold them for US$170. I realized *Brachiaria* had money, so I decided to expand … It has been giving me approximately US$250 per month … I grow seedlings and sell them. I have earned US$400 from that … this has made me do away with cereals. I sell forage and buy grains, and I can still pay school fees … Seeds are not locally available. (KII man, Rongo subcounty, February 2020)

All FHHs and 78% of MHHs that sell *Brachiaria* operate their forage business on their own or are solely assisted by their spouse, a family member, or a hired worker (who are often men).

Another business opportunity that *Brachiaria* can provide for farmers is, according to FGD respondents, the ability to train other farmers about the grass, as was the case with one man from Makueni county, who has commercialized *Brachiaria* production and engaged in all three activities i.e., production of brachiaria for own use, sale, and training of other farmers.

The study further explored the power dynamics surrounding produced milk and how this influenced the benefits of growing *Brachiaria*. The results show that a woman or a man can deliver milk to dairy cooperatives where at least one of them is a member. Although the men constitute the majority of registered members of the cooperatives, women mostly collected the milk proceeds from the dairy cooperatives. Often, the woman remits the money from milk and forage sales to her husband, for him to direct its use, either solely or jointly. In a few instances, the woman keeps the money, but often she is required to purchase food, pay children's school fees, or buy clothes (roles "traditionally" assigned to a man). Even though a man directs the use of income from the sale of milk/forage, women's access to it is a motivation for the woman to continue taking care of the cow:

… there are some [husbands] who just want a little money so I can just keep the income from milk if it is little. But when such

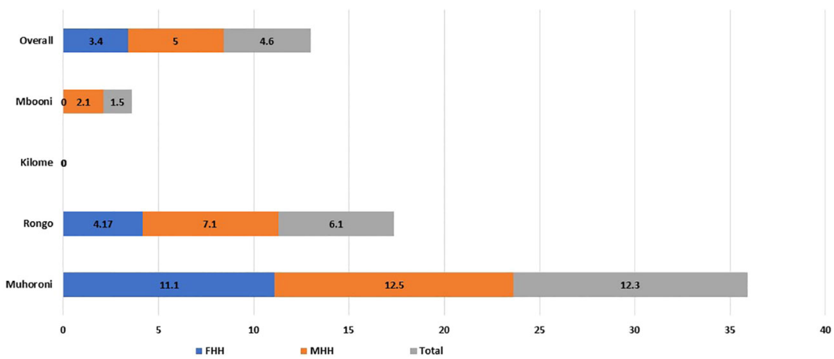

FIGURE 6
Commercialization of *Brachiaria* (percentage of adopters selling).

income is a lot, then he must know about it, and when I get the money, I must show him and tell him to take some while I remain with the rest. (KII Woman, Rongo subcounty, February 2020).

Remitting the milk money to my husband is a sign of respect for him. (FGD woman, Kilome subcounty, December 2019)

In our culture, we leave some activities to women, like selling milk. A man does not sell milk, so they [the women] know that if they get milk and sell it, then they will get the money. That is why women like milking because they will milk and sell. I will not ask my wife how many liters the cow produced because, normally, that is little money that women can use in the household. (FGD man, Kilome subcounty, November 2019)

4 Discussion

We explored the gender dynamics influencing *Brachiaria* technology uptake and commercialization among women and men farmers. These results can inform future breeding and development efforts to enhance the benefits that women and men can reap from such agricultural technologies. This section summarizes and interprets the key findings, guided by the research questions. The implications of the results and the study's limitations and recommendations are also discussed.

This study assessed several components of *Brachiaria* production among women and men farmers. We specifically focused on the rate of *Brachiaria* adoption, forage characteristic preferences, *Brachiaria* establishment and commercialization, and perceptions about *Brachiaria*. These components are important, and they potentially reveal the opportunities and limitations for women and men farmers that might either enhance or hinder forage production. *Brachiaria* has attributes liked by both women and men. In addition, women had preferences related to their role as forage providers. This shows that, although women may not be the official owners of the cattle, the intra-household gender division of labor may affect forage variety preferences. Breeding programs may therefore need to provide forage solutions by involving household members engaged in various forage-related tasks. This is in line with the literature on gender-responsive plant breeding (Ceccarelli et al., 2013).

It is important that women and men farmers receive information on what varieties are available and what varieties reflect their needs so that the adoption of new *Brachiaria* varieties is increased, in turn enhancing farmers' livelihoods. We also found that FHHs have adopted *Brachiaria* to a slightly lesser extent than MHHs in three out of four subcounties. The reason for the slightly higher adoption rate among MHHs is possibly related to the fact that dairy cooperatives were found to be an important means of accessing extension services, useful information about the production, and forage planting materials. However, mostly men and only a few women are cooperative members. Female heads of households from three sites said they accessed *Brachiaria* planting materials through women's groups. This arguably limits women's access to new technologies and advice, as also discussed by Farnworth and Colverson (2015) and Lamontagne-Godwin et al. (2019). This is in line with Wossen et al. (2017), who posit that access to extension services by a household exposes them to new technologies and trains them on best agricultural practices. Our findings support the evidence from the literature pointing to the importance of integrating gender-sensitive approaches into extension services in order to reach female and male farmers with agricultural innovations.

In the Muhoroni subcounty only, FHHs adopted *Brachiaria* to a greater extent than their male counterparts. This is possibly related to women's higher education levels in this subcounty: our quantitative analysis across all sites shows that higher education levels correspond to higher rates of *Brachiaria* adoption, regardless of the gender of the head of the household. This is in line with findings from Melesse (2018), who posits that an educated farmer can obtain, process, and efficiently use information that supports the adoption of new technologies. Although qualitative analysis could have shed light on the ways in which the respondents thought higher education related to variety adoption, our qualitative study was undertaken at the same time as the quantitative study. Consequently, we were not able to explore in depth our quantitative findings through qualitative interviews. We therefore agree with Galiè et al. (2019) that qualitative and quantitative analysis can be best used complementarily: qualitative analysis can be best undertaken before quantitative to contextualize the quantitative component; another round of qualitative work can then also be undertaken after the completion of the quantitative survey to both validate and explain the quantitative results.

Another methodological consideration relates to our aggregation of quantitative results according to household headship, that is, as either FHHs or MHHs. Such an approach does not allow for intra-household exploration of the topics the study focused on. An exploration of intra-household dynamics can present a more complete and unbiased assessment of who is likely to be constrained, the extent of constraint, and why they are constrained. For this to happen, we recommend that questions asked to individual household members do not focus on the household but highlight the respondent's own perception, in line with the recommendation by Doss and Kieran (2014).

One of the main constraints to growing *Brachiaria* (and, therefore, commercializing it) mentioned by both women and men was the availability of land. However, a gender dimension emerged in such land-related constraints. Men are limited by small land areas coupled with competing uses for land, where cash crops are prioritized over *Brachiaria*. Women, on the other hand, are limited by a lack of formal ownership of, access to, and control over land. As such, whereas men complained about small land areas, women complained about a lack of land altogether. Those interested in intensifying their *Brachiaria* production are left with the option to rent land. Even then, women are still limited by a lack of capital to pay land rent. These findings are consistent with results from the literature, where gender norms are shown to limit women's ability to accumulate and retain control over assets [see, e.g., Herrero et al. (2013) and Quisumbing et al. (2015)].

Among both adopter and non-adopter respondents, women seemed more frustrated about the inability to grow *Brachiaria* (or expand its cultivation) than men. This can be explained by the fact that, in all sites, women control income from milk, and women are therefore interested in growing *Brachiaria* to produce more milk for sale; however, they have no say on the use of land. Interestingly, men showed a keen interest in growing more *Brachiaria* in sites where they had adopted it because they had seen the contribution of *Brachiaria* to household expenses and food security: men traditionally are in charge of providing food and money to the household. On these bases, we recommend that in order to support adoption of forage grasses such as *Brachiaria*, women need to be supported in their control over land and men need to be sensitized to the benefits of forage grasses. Interventions that challenge the gender norms that limit women's access and control over land can enhance the adoption of forage technology by women. This may result in increased women's income from milk sales. Such interventions could also support the enforcement of the existing land ownership policies in Kenya, which recognize spouses as equal property owners and protect women's rights to land ownership during marriage, divorce, and separation. In addition, sensitization of male farmers to the importance of forages, including *Brachiaria*, and the possible benefits to household income and nutrition would contribute to nutrition security and also align men's and women's crop interests. Aligning men's and women's interest in growing forages could also satisfy women's preference for forage crops owing to their normative role as weeders and forage providers.

Overall, our findings indicate three main potential pathways offered by *Brachiaria*—and, possibly, other forage varieties—toward the economic empowerment of the respondents. These pathways concern (i) the sale of *Brachiaria* planting materials and hay bales, (ii) the sale of the increased volume of milk obtained when cows are fed with *Brachiaria* (which is particularly effective for women who control milk income), and (iii) the training of other farmers as another, more limited way of generating revenue from *Brachiaria*. For men to be able to enjoy such progress toward empowerment through *Brachiaria*, it is important that their cooperative membership continues to be supported, which enhances access to information and extension services. For women to also progress toward economic empowerment through *Brachiaria*, their control over milk revenues needs to continue to be supported together with their access to and control over land. Norms reducing women's engagement with cooperatives and meetings need to be addressed for women to access good forage-planting materials.

The fact that gender norms emerged from our discussion as strongly affecting the ability of women and men to learn about, adopt, grow, and benefit from *Brachiaria* is an important finding in itself. Our findings also show that these gender-based norms are flexible. Although older men cannot be seen carrying forage or risk losing power in the eyes of the community, young men can be hired as daily laborers to do such work. This speaks to how gender and age intersect to shape individuals' access to opportunities (such as working as a daily laborer to carry forage). Wealth may also be another intersectional component, as young men may be allowed to engage in such work when in need of money. Also, the findings seem to show how gender-based power dynamics are relational: married men, unlike young men, may be seen as losing power to their wives when carrying forage because a married man "should" have the power to get his wife to do the work.

5 Conclusions and recommendations

This study explored the gender dynamics influencing *Brachiaria* uptake and commercialization among dairy cooperative FHHs and MHHs. More MHHs than FHHs often engage in non-agricultural activities, whereas women mostly work on family farms and engage in mixed farming. Food crops are grown by women, whereas cash crops are grown by men, with little land left for forage production. Dairy cooperatives are important means to access information and forage-planting materials. There exists land-holding differences between women and men. Women are disenfranchised by an inability to own, access, and control land use, whereas men are limited by small land areas. Women's land ownership and access is subject to traditions that dictate who can inherit and own land. The same culture assigns the roles of forage harvesting, transportation, and feeding of animals to women. Education emerged from the quantitative analysis as important to the adoption process.

In this regard, our study recommends the need for holistic efforts to address the identified barriers often faced by women farmers. The barriers regarding land tenure, decision-making, and control rights function in favor of men. Hence, challenging the social structures that produce, and reproduce, such inequalities between women and men is necessary. Enforcement of existing land ownership law in Kenya that recognizes spouses as equal property owners is needed. Gender-sensitive approaches to extension services are essential, as extension services have not successfully reached women and men equally, meaning women are often not reached by technologies and related information received by their men counterparts. The systematic implementation of qualitative studies, followed by quantitative studies, then another qualitative exploration and validation of the results yielded in the first two phases, is recommended. Such holistic efforts can contribute to the ongoing momentum of women's mobility out of poverty and advancing equality in the study areas.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by ILRI's Institutional Ethics Committee (Ref. ILRI-IREC 2019-47). The patients/participants provided their written informed consent to participate in this study.

Author contributions

Conceptualization: NN, MT, BB, and JB. Methodology: NN, FW, MT, and AG. Software: NN. Validation: NN, FW, AG, IB, BB, and JB. Formal analysis: NN. Investigation: NN and MT. Resources: IB. Data curation: NN. Writing—original draft preparation: NN. Writing—review and editing: NN, FW, AG, IB, MT, BB, JB, and EN-M. Supervision: AG, IB, BB, JB, EN-M, and CJ. Project administration: IB. Funding acquisition: IB. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Morphological characteristics, dry matter yield, and nutritive value of maralfalfa grass (*Pennisetum* spp.) grown under different planting densities in the central highlands of Ethiopia

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Introduction: Maralfalfa grass (*Pennisetum* spp.) is known for its high dry matter productivity and nutritive value. However, information on agronomic management practices to improve yield and nutritive value in Ethiopia is sparse.

Materials and method: The experiment consisted of 7 plant densities (33,333 [75 cm × 40 cm]; 26,667[75 cm × 50 cm]; 25,000 [100 cm × 40 cm]; 22,222 [75 cm × 60 cm]; 20,000 [100 cm × 50 cm]; 16,667 [100 cm × 60 cm]; and 13,333 [125 cm × 60 cm] plants per hectare, and arranged in randomized complete block design with three replications.

Results: The results indicated that the year had a significant ($P<0.001$) effect on measured traits. Plant densities had no significant ($P>0.05$) effect on the number of nodes and leaves per plant, leaf length, and basal diameters. The number of tillers per plant varied significantly among plant densities only in the first year at the 1st harvest. Plant height in the first year was significantly ($P<0.01$) greater than in the second year.

Discussion: Annual dry matter production and annual crude protein production of Maralfalfa grass were not significantly affected by plant density. Nutritive value parameters (CP, Ash, NDF, ADF, ADL, and IVDMD) were not significantly ($P > 0.05$) influenced by plant density. Using a lower plant density could reduce the amount of planting material, transport, and labor costs.

Conclusion: However, further studies on Maralfalfa grass should be conducted in multi-locations of Ethiopia both under rain-fed and irrigated conditions with various agronomic practices.

KEYWORDS

establishment, plant spacing, management practices, maralfalfa grass, yield, quality

1 Introduction

Maralfalfa grass (*Pennisetum* spp.) is a perennial tropical grass that belongs to the Poaceae family. It is a hybrid between napier grass (*Pennisetum purpureum*) and pearl millet (*Pennisetum glaucum*) with 2n = 3x = 21 chromosomes (Hanna et al., 1984; Clavero and Razz, 2009). In different studies, maralfalfa is referred to as *Pennisetum* spp. (Ramirez et al., 2006), with the genotype of *Pennisetum purpureum* (Marquez et al., 2007) and the giant king grass (Geren and Kavut, 2015). Maralfalfa grass can be grown under a wide range of ecological conditions up to 3,000 m above sea level. Maralfalfa can be grown on a wide range of soil types, but it grows best in deep, well-drained, friable loams with a pH of 4.5 8.2 (Nyambati et al., 2010). It is propagated using stem cuttings, root splits, or rhizomes (Singh et al., 2013). The species is widely cultivated in numerous countries in Latin America (Correa, 2006), sub-tropical regions of Asia (Karforma, 2018), Southern Europe (Clavero and Razz, 2009), and Africa (Obok et al., 2012).

Maralfalfa has a great potential as a forage for ruminant animals, as it has a high level of fodder production and provides 40–60 tons of dry matter ha^{-1} year^{-1} (Bolonio et al., 2015). Moreover, due to its fast growth rate, intake potential, and nutritive value, it is the best option to increase feed availability for livestock production (Ramirez et al., 2006; Bolonio et al., 2015). Maralfalfa can reach up to 3–4 m in height and has an acceptable nutritional content for animal feed. It is also tolerant to heat and flooding (Correa et al., 2004). Most importantly, maralfalfa has a 14.4% crude protein (CP) content, which is high for grasses but lower than those of forage legumes, such as alfalfa, which have a CP content ranging from 15% to 22% (Scholtz, 2008).

Studies by Clavero and Rass (2009) and Marquez et al. (2007) indicated that due to its high biomass and nutritive value, maralfalfa grass could provide a considerable amount of quality feed for ruminant livestock producers. Maralfalfa grass can be cut and fed in several forms, such as chopped fresh, without chopping, made into silage, and dried and baled into hay (Santos et al., 2013; Geren and Kavut, 2015). It is best used in a “cut-and-carry” system directly or mixed with other legume forages to achieve the necessary daily nutrient requirement of the animal (Criscioni et al., 2016). Dry matter yield varies depending on genotypes, edaphic and climatic factors, and management practices (Malley et al., 1996). The studies performed by Palacios-Diaz et al. (2013) and Clavero and Razz (2009) suggested that the nutritive value of maralfalfa grass is influenced by management practices.

In Ethiopia, this species was recently introduced from Spain and tested under different agro-ecologies in the country. Then, it was registered as a variety by the Ministry of Agriculture (MOA, 2018). However, despite its introduction and registration, the grass is not well used by livestock producers. Moreover, no detailed studies have been made on the agronomic requirements of maralfalfa grass in different agro-ecological regions of the country. Because of its potential contribution to livestock feed, an understanding of the effects of various management practices on the yield and nutritive value of maralfalfa grass is very important.

Using the right planting density is an important factor in maximizing the herbage yield of *Pennisetum* species (Danalatos et al., 2007; Tessema, 2008). Plant density is one of the important agronomic factors affecting the yield and quality of forage crops (Tessema, 2008; Ansah et al., 2010; Palacios-Diaz et al., 2013). Currently, the spacing often used for napier grass in Ethiopia is 100-cm inter-row and 50-cm intra-row spacing. Owing to their morphological and genetic differences, understanding the effect of plant density on growth, development, yield, and forage quality will allow for improving management and benefits from potential forage crops such as maralfalfa grass. Therefore, we conducted this study in the central highlands of Ethiopia with the objective of determining the optimal plant densities of maralfalfa grass for dry matter yield (DMY) and nutritive value.

2 Materials and methods

2.1 Description of the study site

The study was performed at Holetta Agricultural Research Center, located in the central highlands of Ethiopia. The center is located at an elevation of 2,400 m above sea level with the coordinates 9° 3′ 47.9′N and 38° 30′ 24.9′E. The long-term (30 years) mean annual rainfall of the area is 1,055 mm, with a bimodal rainfall distribution with the majority (70%) falling in the periods from June to September, whereas the remaining (30%) falls from February to April (EIAR, 2005). The area has an average relative humidity of 60% and mean maximum and minimum temperatures of 22.2°C and 6.1°C, respectively. The weather data during the experimental periods

(2019 and 2020) at the Holetta experimental station are indicated in Figure 1. Compared with the long-term records, both 2019 and 2020 had greater annual rainfall with means of 1,344 mm and 1,265 mm, respectively. Comparing the single months of the experimental years, the greatest rainfall was recorded in August with 356 mm and 334 mm in 2019 and 2020, respectively. The mean monthly maximum temperature in April was 17.8°C and 17.1°C for 2019 and 2020, respectively. The mean monthly minimum temperature in October and December was 13.2°C in 2019 and 2020. However, no major differences were observed between the experimental years in mean annual temperatures. The farming area is characterized by a mixed crop–livestock production system.

The soil of the area is mainly nitisol with an acidic pH of 4.9, an organic carbon content of 1.8%, total nitrogen of 0.18%, and available phosphorus of 5.6 parts per million (ppm), and with different proportions of clay, sand, and silt (Table 1).

2.2 Experimental treatments, design, and forage establishment

The treatments consisted of seven planting densities, as indicated in Table 2. The experiment was laid out as a randomized complete block design with three replicates, in a total of 21 3.0 m × 7.5 m experimental units spaced 1 m and 1.5 m apart between plots and blocks, respectively. Maralfalfa grass, which was registered as a variety by the Ministry of Agriculture, was planted under rain-fed conditions in mid-July (13 July 2018) in a well-prepared experimental field. We planted the grass in its vegetative form using root splits (Singh et al., 2013). We applied phosphorus fertilizer at the rate of 100 kg/ha to all plots at planting in the form of diammonium phosphate [DAP: 18% N, 20% P, and 1.5% S Urea (46% N)] fertilizer at the rate of 109 kg ha^{-1}. This was applied in split applications, with one-third applied during the short rainy season and the remaining two-thirds at the beginning of the main rainy season at every harvest. Uniform trial management to all treatments, such as hand weeding and hoeing between rows, was carried out during establishment and after every harvest of the grass to facilitate regrowth.

TABLE 1 Physical–chemical properties of the soil of the study area.

Parameters	Values	Methods of analysis
pH (1 : 2.5 soil: H_2O)	4.94	Potentiometric method
Organic carbon (OC %)	1.90	Dichromate oxidation method (Walkley and Black 1934)
Total nitrogen (TN %)	0.18	Kjeldhal method (Jackson, 1958)
P (mg kg^{-1})	5.60	Bray II (Bray & Kurtz, 1945)
K (mg kg^{-1})	5.03	NH_4OAc method (Okalebo et al., 1993)
Ca (mg kg^{-1})	29.50	NH_4OAc method (Okalebo et al., 1993)
Mg (mg kg^{-1})	13.70	NH_4OAc method (Okalebo et al., 1993)
Na (mg kg^{-1})	0.16	NH_4OAc method (Okalebo et al., 1993)
Soil texture (%)		
Clay	67	Bouyoucos hydrometric method
Sand	18	Bouyoucos hydrometric method
Silt	15	Bouyoucos hydrometric method

2.3 Data collection, sampling, and measurements

Experimental data were taken for each treatment on morphological, agronomic, and nutritional quality traits. The survival rate and percentage of plot cover were observed at 8 weeks to assess the establishment performance. The number of nodes per plant (NNPP), number of leaves per plant (NLPP), leaf length (LL), number of tillers per plant (NTPP), and basal diameter (BD) were recorded on 10 randomly taken samples plants and their means were recorded for all treatments. The leaf-to-stem ratio (LSR) was measured on five sample plants and partitioned into leaf and stem fractions to determine the ratio of the dry weight of the leaves to the weight of the stems. The plant height (PH) was measured from 10 randomly selected plants in each plot from the ground to the tip of the plant, whereas leaf length (LL) records of flag leaf length were taken from the bottom, middle, and top of the sample plants and

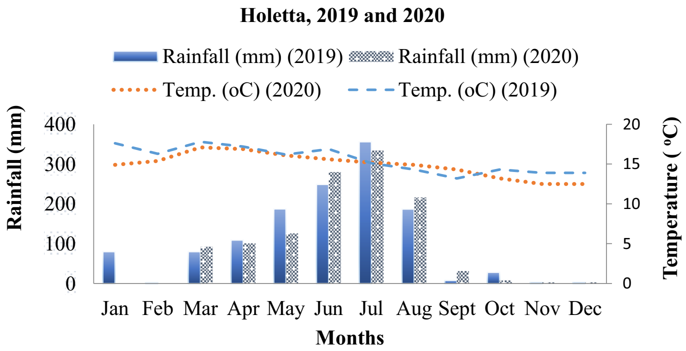

FIGURE 1
Mean monthly rainfall and temperature during 2019 and 2020 in Holetta. *Source: Holetta Agricultural Research Center Metrological data records.*

TABLE 2 Experimental treatments.

Treatments	Spacing (cm)		Area/plant (m^2)	Number of plants ha^{-1}
	Inter row	Intra row		
T1	75	40	0.75 × 0.4 = 0.30	33,333
T2	75	50	0.75 × 0.5 = 0.38	26,667
T3	75	60	0.75 × 0.6 = 0.45	22,222
T4	100	40	1.00 × 0.4 = 0.40	25,000
T5	100	50	1.00 × 0.5 = 0.50	20,000
T6	100	60	1.00 × 0.6 = 0.60	16,667
T7	125	60	1.25 × 0.6 = 0.75	13,333

their means were used for the analysis (Aklilu and Alemayehu, 2007). For DMY determination, whole plants from each plot size of 22.5 m^2 were cut at about 10 cm above ground manually using a sickle, and the fresh yield was measured using a spring balance. A 500 g fresh weight from each plot was taken and chopped into small sizes to facilitate drying. The samples were dried using an oven at 65° C for 72 h (Getnet and Ledin, 2001). The DMY was computed from the samples in each plot. CP yield (CPY) was computed using the formula: CPY (t ha^{-1}) = CP% × DMY divided by 100.

2.3.1 Chemical analysis

Chemical analyses were carried out at the International Livestock Research Institute (ILRI) feed nutrition laboratory. The dried samples were ground in a mill to pass through a 1-mm sieve size used for laboratory analyses. The ground samples were dried overnight at 65°C in an oven to constant moisture. The DM, ash, and organic matter contents of the feed samples were determined following the procedure of AOAC (1990). The total ash content was determined by combusting the samples in a muffle furnace at 550°C for 6 h. The N content of the samples was determined according to the micro-Kjeldahl method AOAC (1990) and the percentage of N was multiplied by 6.25 to get the total CP content. Neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) were determined according to the procedure of Van Soest and Robertson (1985). *In vitro* dry matter digestibility (IVDMD) was made according to Tilley and Terry (1963).

2.4 Statistical analysis

The data were subjected to analysis of variance using SAS software (SAS, 2011). Prior to the analysis we tested the homogeneity of error variances using Bartlett (1947) test. Least square means (ls-means) were calculated, and the significance level was determined as a p-value < 0.05. The following statistical model (Equation 1) was used:

$$Y_{ijk} = \mu + T_i + B_k + Y_j + (TY)_{ij} + e_{ijk}, \quad (1)$$

where Y_{ijk} is the response variables, μ is the overall mean, T_i is the effect due to plant density ith, B_k is the effect due to block kth, Y_j is the effect due to the year (j is 2019 and 2020), $(TY)_{ij}$ is the effect due to the interaction effect between ith treatment and jth year, and e_{ijk} is the random error.

3 Results

3.1 Survival rate and plot cover

The survival rate and plot cover of the maralfalfa grass planted in different plant densities are shown in Figure 2. There was no statistical difference (p > 0.05) among the plant densities for survival rate or plot cover of the grass. The survival rate ranged from 90% to 100%, and plot cover ranged from 46% to 68%.

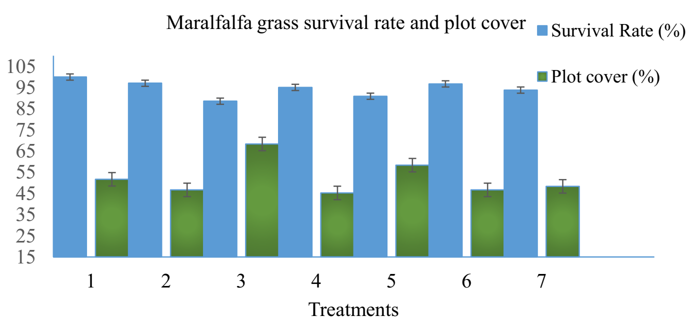

FIGURE 2
Survival rate and plot cover percentages of maralfalfa grass planted under different plant densities observed 8 weeks after planting.

3.2 Effects of planting density, year, and their interactions

The mean square for planting density over the years and their interactions with the measured morphological traits are indicated in Table 3. The measured agronomic characteristics were not significantly influenced by planting densities. Similarly, there was no significant ($p > 0.05$) interaction effect between planting density and year. On the contrary, PH, NTPP, total DMY, and crude protein yield (CPY) were significantly ($p < 0.001$) affected by the year.

3.3 Year effects on yield and yield components

The overall means of the NTPP, PH, annual DMY, and annual CPY of maralfalfa grass planted at different plant densities during 2019 and 2020 are shown in Table 4. The NTPP, PH, DMY, and CPY were highly different ($p < 0.01$) across the years. The means of NTPP recorded in year 2 increased by 89.74% compared with those of year 1. The DMY and CPY differed significantly ($p < 0.001$), and the DMY and CPY in the second year (2020) increased by 65% and 66% compared with those of the first year (2019), respectively.

3.4 Planting density effects on the morphological characteristics of maralfafa grass

The morphological characteristics of maralfalfa grass under the different planting densities are shown in Table 5. The planting densities had no significant ($p > 0.05$) effect on the NNPP, the NLPP, LL, BD, and LSR of maralfalfa grass.

3.5 Plant height at harvest and the number of tillers per plant

The mean PH and mean NTPP of maralfalfa grass under seven planting densities in 2019 and 2020 are indicated in Table 6. There was no significant difference ($p > 0.05$) among planting densities for plant height in the establishment year, while PH was significantly ($p < 0.05$) affected by planting densities in the following year. Accordingly, the tallest mean PH was recorded in T7 (125 cm × 60 cm), followed by T6 (100 cm × 60 cm) and T5 (100 cm × 50 cm). The lowest plant height was recorded in T4, but was statistically similar among T1 (75 cm × 40 cm), T2 (75 × 50 cm), and T2 (75 × 60 cm). However, for the 2019 and 2020 cropping seasons, the overall mean PH was not affected ($p > 0.05$) by planting density. The NTPP increased with the decreasing plant density, and this was pronounced only in the establishment year.

3.6 Dry matter and crude protein yields

The DMYs and CPYs showed insignificant ($p > 0.05$) differences among planting densities in both years (Table 7). The mean annual DMY in the establishment year under different planting densities was 13.8 t ha^{-1} ± 0.8 t ha^{-1}, whereas in the annual cumulative yield in the second-year harvest achieved 22.0 t ha^{-1} ± 0.7 t ha^{-1}. The overall mean of the DMY obtained in 2019 and 2020 under different planting densities was 17.9 t ha^{-1} ± 0.7 t ha^{-1}. The greater competition associated with greater planting densities did not

TABLE 3 Statistical analysis of the planting density and year effects and their interactions.

Morphological measures	Mean squares			Mean	CV (%)
	Treatment	Year	Treatment * year		
Plant height (cm)	34.10^{ns}	8,401.8**	13.63 ^{ns}	128.58	3.5
Number of tillers per plant	64.97^{ns}	84,641.0**	62.83 ^{ns}	80.3	14.9
Dry matter yield (t/ha)	5.20^{ns}	786.6**	8.30^{ns}	17.68	22.7
Crude protein yield (t/ha)	0.21^{ns}	19.0**	0.15 ^{ns}	2.69	24.4

Ns, non-significant (p > 0.05); **, significant (p < 0.001); CV (%), coefficient of variation in percentage.

TABLE 4 Number of tillers per plant, plant height, annual dry matter yield, and annual crude protein yield of maralfalfa grass in 2019 and 2020.

Years	NTPP	PH (cm)	DMY (t ha^{-1})	CPY (t ha^{-1})
2019	35.7^{b} ± 1.4	142.1^{a} ± 0.5	13.8^{b} ± 0.8	2.1^{b} ± 0.1
2020	125.2^{a} ± 2.2	114.4^{b} ± 1.3	22.0^{a} ± 0.7	3.4^{a} ± 0.1
Mean	80.5 ± 1.3	128.3 ± 0.7	17.9 ± 0.7	2.7 ± 0.1
CV (%)	14.9	3.5	22.7	24.4
p-value	0.001	0.001	0.001	0.001

NTPP, number of tillers per plant; PH, plant height; DMY (t ha^{-1}), dry matter yield tons per hectare; CPY (t ha^{-1}), crude protein yield tons per hectare; CV, coefficient of variation. The means within a column followed by different supper script letters are significant different (p < 0.001).

TABLE 5 Morphological characteristics of maralfalfa grass under different planting densities.

Treatments	Mean ± SE				
	NNPP	NLPP	LL (cm)	BSD (mm)	LSR
T1 (75 cm × 40 cm)	3.9 ± 0.3	7.6 ± 0.3	74.6 ± 1.8	21.2 ± 1.2	2.7 ± 0.2
T2 (75 cm × 50 cm)	3.6 ± 0.2	7.6± 0.2	82.0 ± 6.3	22.3 ± 0.5	1.65 ± 0.5
T3 (75 cm × 60 cm)	4.1 ± 0.3	7.8 ± 0.5	73.3 ± 3.1	22.6 ± 0.9	2.7 ± 0.5
T4 (100 cm × 40 cm)	3.6 ± 0.2	7.4 ± 0.2	75.7 ± 4.2	20.9 ± 0.4	3.3 ± 0.4
T5 (100 cm × 50 cm)	3.5 ± 0.1	7.4 ± 0.2	76.6 ± 4.2	24.2 ± 1.8	2.2 ± 0.3
T6 (100 cm × 60 cm)	3.8 ± 0.3	7.8± 0.2	79.3 ± 2.2	21.5 ± 0.6	2.5 ± 0.2
T7 (125 cm × 60 cm)	4.4 ± 0.1	7.4 ± 0.2	74.1 ± 2.8	20.9 ± 1.3	2.4 ± 0.7
Mean	35.7 ± 1.4	7.6 ± 0.2	13.8 ± 0.8	2.1 ± 0.1	142.1 ± 0.5
CV (%)	10.2	6.1	7.8	8.6	29.2
p-value	0.12	0.88	0.07	0.36	0.27

LSR, leaf-to-stem ratio; NNPP, number of nodes per plant; NLPP, number of leaves per plant; LL, leaf length (cm); BD, stem basal diameter; SE, standard error; CV, coefficient of variation.

result in lower dry matter production than lower planting densities. The CPY did not vary significantly among the different planting densities. The mean CPY in the first year was 2.1 t ha^{-1} ± 0.1 t ha^{-1}, whereas in the following year it was 3.4 t ha^{-1} ± 0.1 t ha^{-1}. The overall annual mean of CPY attained over the 2019 and 2020 seasons under different planting densities was 2.7 t ha^{-1} ± 0.1 t ha^{-1}.

3.7 Trends of dry matter and crude protein yields at harvest

The trends of DM and CPYs of maralfalfa grass under different plant densities during 2019 and 2020 are shown in Figures 3, 4. Three harvests were made in the establishment year, whereas six harvests were achieved in the second year. However, plant density did not affect the DMYs or CPYs at each harvest. During the 2019 cropping season, the DMY at first harvest (DMY1) was slightly increased for T3 (75 cm × 60 cm) relative to T1 (75 cm × 40 cm), T2 (75 cm × 50 cm), and T4 (100 cm × 40 cm) to T7 (125 cm × 60 cm). For harvest 2 (DMY2), the DMY decreased for T3 (75 cm × 60 cm) but slightly increased from T4 to T7. The DMY3 showed curvilinear decreasing trends from T3 (75 cm × 60 cm) to T6 (100 cm × 60 cm). In the second year, DMY1 and DMY2 showed almost similar trends and were greater than DMY4 and DMY6, whereas DMY3 showed inconsistent trends among treatments. In the DMY5 harvest, T1 (75 cm × 40 cm), T4 (100 cm × 40 cm), and T7(125 cm × 60 cm) showed increasing trends as compared with the DMYs achieved in the rest of the treatments. The CPY3 in the first year showed a

TABLE 6 Plant height and number of tillers per plant (mean ± SE) of maralfalfa grass under seven planting densities during the 2019 and 2020 cropping years.

Treatments	Mean ± SE					
	2019		2020		Overall	
	PH (cm)	NTPP	PH (cm)	NTPP	PH (cm)	NTPP
T1 (75 cm × 40 cm)	142.7 ± 3.4	30.0 ± 0.6	111.8^{b} ± 1.1	129.7 ± 6.9	127.3 ± 7.1	79.9 ± 22.5
T2 (75 cm × 50 cm)	142.1 ± 4.9	34.8 ± 0.9	112.7^{b} ± 1.7	114.4 ± 12.2	127.4 ± 6.7	74.6 ± 18.6
T3 (75 cm × 60 cm)	143.8 ± 1.8	35.8 ± 4.2	113.0^{b} ± 3.1	121.0 ± 7.7	128.4 ± 7.1	78.4 ± 19.5
T4 (100 cm × 40 cm)	141.3 ± 4.4	35.9 ± 1.2	110.7^{b} ± 0.8	130.1 ± 10.2	126.0 ± 7.1	83.0 ± 21.6
T5 (100 cm × 50 cm)	143.3 ± 3.1	38.2 ± 2.6	115.7^{ab} ± 1.5	128.4 ± 13.5	129.5 ± 7.1	83.3 ± 21.1
T6 (100 cm × 60 cm)	139.7 ± 5.9	33.6 ± 2.4	116.5^{ab} ± 2.6	125.5 ± 3.9	128.1 ± 5.9	79.5 ± 20.7
T7 (125 cm × 60 cm)	146.1 ± 2.4	39.9 ± 1.6	120.7^{a} ± 2.1	127.5 ± 11.7	133.4 ± 5.9	83.8 ± 20.3
Mean	142.1 ± 0.5	35.7 ± 1.4	114.4 ± 1.3	125.2 ± 2.2	128.3 ± 0.7	80.5 ± 1.3
CV (%)	4.8	10.9	3.0	13.7	3.4	14.1
p-value	0.94	0.12	0.04	0.91	0.13	0.79

PH, plant height; NTPP, number of tillers per plant; DMY, dry matter yield; CPY, crude protein yield; SE, standard error; CV, coefficient of variation. The means within a column followed by different supper script letters are significantly different (P<0.05).

TABLE 7 Annual dry matter yield and annual crude protein yield (t ha^{-1}) of maralfalfa grass under seven planting densities during the 2019 and 2020 cropping years.

Treatments	Mean ± SE (t ha^{-1})					
	2019		2020		Overall	
	DMY	CPY	DMY	CPY	DMY	CPY
T1 (75 cm × 40 cm)	13.6 ± 0.8	2.1 ± 0.2	22.8 ± 1.2	3.5 ± 0.1	18.2 ± 2.8	2.8 ± 0.5
T2 (75 cm × 50 cm)	13.5 ± 1.2	1.9 ± 0.1	20.7 ± 2.1	2.9 ± 0.3	17.1 ± 1.9	2.5 ± 0.3
T3 (75 cm × 60 cm)	14.0 ± 2.6	2.1 ± 0.3	20.3 ± 2.1	3.1 ± 0.4	17.1 ± 2.1	2.3 ± 0.3
T4 (100 cm × 40 cm)	12.1 ± 1.6	2.0 ± 0.2	24.3 ± 3.5	3.9 ± 0.5	18.3 ± 3.2	3.0 ± 0.5
T5 (100 cm × 50 cm)	12.7 ± 2.1	1.9 ± 0.3	21.7 ± 1.7	3.3 ± 0.3	17.2 ± 2.3	2.6 ± 0.4
T6 (100 cm × 60 cm)	12.1 ± 2.3	1.9 ± 0.4	20.1 ± 1.1	3.2 ± 0.3	16.1 ± 2.1	2.6 ± 0.3
T7 (125 cm × 60 cm)	15.5 ± 1.8	2.3 ± 0.2	24.1 ± 3.9	3.5 ± 0.5	19.8 ± 2.7	2.9 ± 0.4
Mean	13.8 ± 0.8	2.1 ± 0.1	22.0 ± 0.7	3.4 ± 0.1	17.9 ± 0.7	2.7 ± 0.1
CV (%)	24.3	23.2	22.9	25.4	21.4	22.8
p-value	0.87	0.96	0.88	0.85	0.08	0.08

DMY, dry matter yield; CPY, crude protein yield; t ha^{-1}, ton per hectare; SE, standard error; CV, coefficient of variation.

curvilinear decreasing trend from T3 (75 cm × 60 cm) to T6 (100 cm × 60 cm). In the second year, CPY1 and CPY2 were equivalent but were greater than CPY4 and CPY6, whereas CPY3 showed inconsistent trends among treatment. The CPY consistently followed similar trends as those of DM production in both cropping seasons.

3.8 Chemical composition and *in vitro* dry matter digestibility

The nutritional contents and *in vitro* DM digestibility of maralfalfa grass under different planting densities are shown in Table 8. The contents of DM, ash, CP, neutral detergent fiber (NDF), acid detergent fiber (ADF), and *in vitro* dry matter digestibility (IVDMD) of maralfalfa grass were not influenced by planting density. The overall mean was 15.3 ± 0.2 for CP%, and 58.2 ± 0.4 and 60.4 ± 0.3 for NDF and IVDMD %, respectively.

4 Discussion

The survival rate of maralfalfa grass was not influenced by the plant densities. Similarly, the plot cover measured at 8 weeks after the establishment date was not affected by planting density. The insignificant contribution of different planting densities of the maralfalfa grass on the survival rate and plot canopy cover might be due to the good establishment and tillering performances of the grass in the study area. A study done by Kebede et al. (2016) suggested that the establishment performance of the same species or variety varied across different locations due to soil and climatic variations. The establishment and survival rate are strongly related to the rooting ability of seedlings. This indicated that maralfalfa grass is well established and highly adapted to nitisol soil types consisting of clay soil texture and tolerant to acidic soils. The results of this study agree with Geren and Kavut (2015), who noted that maralfalfa grass can adapt to a wide range of soil particles, from sandy to clay, and a pH range of very acidic to alkaline in Ethiopia.

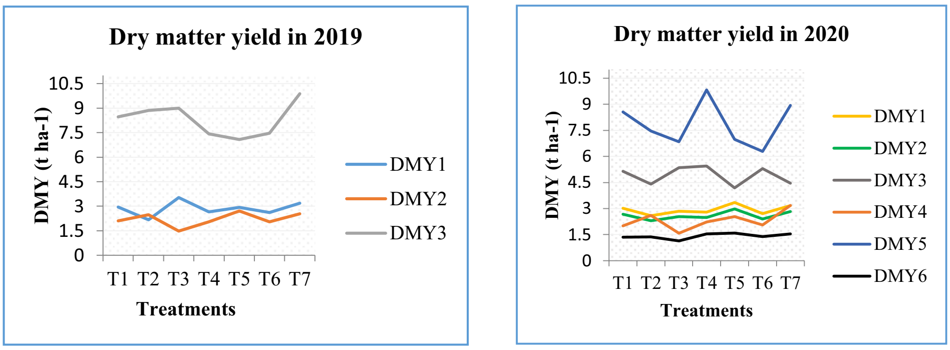

FIGURE 3
Dry matter yield (DMY) of maralfalfa grass at three or six harvest dates under different planting densities during 2019 and 2020, respectively.

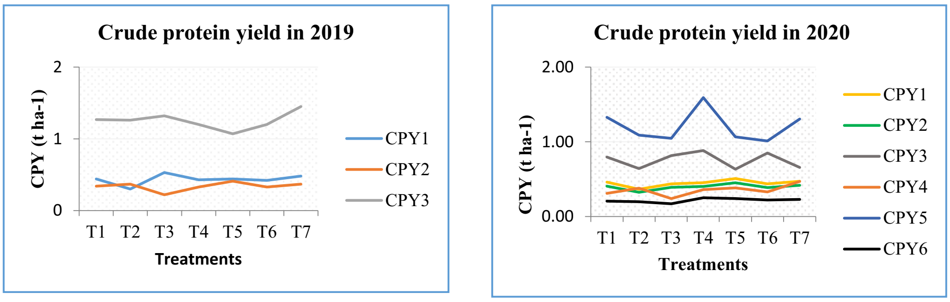

FIGURE 4
Crude protein yield (CPY) of maralfalfa grass at three or six harvest dates under different planting densities during 2019 and 2020, respectively.

The significant effect of years on PH, NTPP, DM, and CPY may be due to the variation in weather effects (Tessema, 2005); however, it is more likely related to cumulative time from establishment. Thus, significant differences were observed in yield-related traits across the years. Similar to the current study, Habte et al. (2020) indicated that year had a significant (p<0.01) effect on the NTPP, PH, and the LSR of napier grass. Our finding also agrees with Geren and Kavut (2015), who reported that the interactions of year and planting density of maralfalfa were not significant.

The year effect on the measured traits of maralfalfa grass could be due to new tillers emerging from the mother plants with more developed root systems in terms of depth and width in the soil profile and utilizing nutrient reserves available in the soil. Geren and Kavut (2015) indicated that the average number of tillers in the first year (44 tillers) of maralfalfa grass was significantly lower than the second year (62 tillers). Tessema (2008) also reported that the number of tillers of napier grass attained in the second year was approximately seven times higher than in the first year. The DM production also varied over the year, and this might have been due to the regeneration of stems and a greater number of tillers and developed roots to take up available water and nutrients from the soil (Tegami and Mello, 2007; Wijitphan et al., 2009). Tessema (2005) reported that due to its perennial nature, as the pasture period increases, napier grass produces more tillers and greater vegetative growth of napier grass accessions in the north-western parts of Ethiopia. The DMY obtained in the first year was almost similar (13.06 t ha^{-1}) to the first-year DMY of napier grass accessions planted at 100 cm × 50 cm spacing reported by Gezahagn et al. (2017).

In contrast to the present study, Karanja (1984) indicated that narrow spacing resulted in a greater number of, and longer, internodes than wider spacing in napier grass. The study done by Tessema (2008) indicated that plant densities affected leaf length, basal circumference, and nodes per plant in the second year. The presence of a greater proportion of leaves is a good nutritive value indicator, but the planting density in the current study did not significantly influence the LSR of maralfalfa grass. The LSR of plants mainly depends on the number of leaves per plant, leaf length, and thickness of the stem. The nutritive value of any forage crop is highly influenced by leafiness, leaf length, and the nature of the stem. In contrast to the present study, Tessema and Baars (2003) and Taye (2004) found that the LSR of napier grass planted at 100

TABLE 8 Nutritional contents and *in vitro* dry matter digestibility of maralfalfa grass under different planting densities.

Trt	Nutritional composition and *in vitro* dry matter digestibility (%)						
	DM	Ash	CP	NDF	ADF	ADL	IVDMD
T1	91.8 ± 0.1	12.7 ± 0.3	15.2 ± 1.1	59.7 ± 1.5	43.8 ± 1.3	6.1 ± 0.3	59.5 ± 1.1
T2	91.9 ± 0.3	12.8 ± 0.9	14.6 ± 1.4	58.8 ± 1.9	42.8 ± 1.3	6.1 ± 0.4	60.0 ± 1.3
T3	91.8 ± 0.1	12.6 ± 0.5	15.1 ± 0.9	58.8 ± 0.7	42.9 ± 0.6	6.1 ± 0.2	59.7 ± 0.1
T4	91.9 ± 0.1	14.0 ± 0.7	16.3 ± 0.2	56.0 ± 1.1	41.1 ± 0.5	5.8 ± 0.1	61.6 ± 0.5
T5	91.9 ± 0.1	12.3 ± 0.4	15.1 ± 0.3	57.6 ± 0.6	42.4 ± 0.6	6.2 ± 0.2	60.2 ± 0.2
T6	91.8 ± 0.1	12.8 ± 0.5	16.0 ± 0.5	58.2 ± 1.4	42.4 ± 1.5	5.9 ± 0.1	60.6 ± 0.9
T7	91.8 ± 0.1	13.5 ± 0.1	14.8 ± 0.5	58.1 ± 0.2	42.7 ± 0.3	5.9 ± 0.1	60.8 ± 0.2
Mean	91.8 ± 0.1	12.9 ± 0.2	15.3 ± 0.2	58.2 ± 0.4	42.6 ± 0.1	6.0 ± 0.1	60.4 ± 0.3
CV (%)	0.2	7.1	7.3	3.6	3.7	4.8	1.9
p-value	0.95	0.33	0.52	0.52	0.64	0.61	0.47

T1 = 75 cm × 40 cm, T2 = 75 cm × 50 cm; T3 = 75 cm × 60 cm; T4 = 100 cm × 40 cm; T5 = 100 cm × 50 cm; T6 = 100 cm × 60 cm; T7 = 125 cm × 60 cm.
DM, dry matter; NDF, neutral detergent fiber; ADF, acid detergent fiber percentage; ADL, acid detergent lignin; CP, crude protein; IVDMD, in vitro dry matter digestibility; SE, standard error; CV, coefficient of variation.

cm × 50 cm spacing was greater than that planted at 50 cm × 50 cm, with values of 1.91 and 1.49, respectively.

The NTPP was not influenced by plant densities in our study, although one may hypothesize that because plants with wider spacing in the establishment season may have reduced inter-tiller competition for space, moisture, and nutrients during early growth. Although different from the current study, Geren and Kavut (2015) reported that the planting density significantly affected the NTPP. Similarly, Tessema (2008) reported that planting density significantly influenced the NTPP from napier grass: he reported 62.3 tillers from wider rows (175 cm × 25 cm) versus 24.3 tillers from narrower (50 cm × 75 cm) rows.

In agreement with Tessema (2008), there was no significant effect on the PH of napier grass due to planting density in the first year but there was in the second year. The PH range obtained in the first and second years of the present study lies between the reported heights (100 cm to 150 cm) of napier grass (Muia et al., 1999; Fekede et al., 2005; Kariuki et al., 2016).

The DMY difference across the years might have been due to improving vegetative growth as the pasture period increased in perennial grass (Ndikumana, 1996). The greater DMYs obtained at the third harvest (DMY3) during the establishment year and DMY5 during the following year might have been due to the harvests in September and August during the main rainy seasons when rainfall was sufficient for growth. In agreement with the present finding, Berihun (2005) reported that an insignificant difference in DMY of napier grass was attained under different plant densities. In contrast to the present study, Wijitphan et al. (2009) reported that napier grass in different planting spaces (50 cm × 40 cm, 50 cm × 60 cm, 50 cm × 80 cm, and 50 cm × 100 cm) had a significant effect on DM yield, and the greatest total DMY (70.84 t ha^{-1}) was obtained from 3 years' cumulative harvest from a 50 cm × 40 cm row spacing in Thailand. A study done by Tessema (2008) on napier grass under different planting densities indicated that the DM yield increased as plant density increased. Generally, the variations of DMY at different harvest times may have been due to harvesting at different growth periods. The trends of CPY were consistent with similar trends for DMY as the CPY was computed from the DMY and because the CP content was relatively non-varying.

The insignificant differences in the chemical analysis and IVDMD of maralfalfa grass at different plant densities is likely because all the treatments were harvested at similar growth stages with similar LSRs. The nutritional quality of forages is influenced by the environment (climate), management factors, soil characteristics, and age at harvest (Getnet and Ledin, 2001; Tessema et al., 2011; Keba et al., 2013). Tessema (2008) and Wijitphan et al. (2009) reported that the DM, NDF, ADF, CP, and ADL contents of napier grass were not influenced by plant densities. Marafalfa grass is known to have a high CP content and the present study also indicated that the grass had an overall treatment mean value of 15.3 ± 0.2 CP% content, which is above the minimum CP level of 7% required for optimum rumen function for ruminant animals (Van Soest, 1984). The grass is categorized as a medium protein source of forage (Lonsdale, 1989). Except for the CP of T1 and T3 plant densities, the CP of the planting densities can satisfy the minimum CP content (150 g/kg DM) required for lactation and growth of cattle (Norton,1982). However, compared with high nutritional quality grasses, such as Kikuyu grass (*Pennisetum clandestinum*) with 21.9% CP and 27.4% ADF, maralfalfa grass had lower CP and higher fiber contents (Echavarria et al., 2020). The mean values of the NDF content of maralfalfa under different plant densities lie between 45% and 65%, a range that is recommended as medium-quality roughage (Singh and Oosting, 1992). The IVDMD value of maralfalfa in the current study was almost similar to the digestibility of tropical grasses, which lies between 50% and 60% (Owen and Jayasuria, 1989).

5 Conclusion

Plant densities had no significant effect on the measured morphological traits, DM production, and nutritional quality of maralfalfa grass. It is recommended that the farmers should consider the availability of planting materials, labor, and transportation cost for the planting materials without compromising the DM production. Thus, using 125 cm × 60 cm inter- and intra-row spacing with 13,333 plant population ha^{-1} can be an appropriate planting density for an optimum DM production in the study area and similar agro-ecologies of the country. However, further studies on maralfalfa grass should be conducted in multiple locations in Ethiopia under both rain-fed and irrigated conditions and with various agronomic practices.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

GM: Data curation, Writing – original draft, Formal analysis, Methodology. GK: Conceptualization, Writing – review & editing. MF: Methodology, Writing – review & editing. FF: Funding acquisition, Project administration, Writing – review & editing. KM: Data curation, Writing – review & editing. AK: Formal analysis, Supervision, Writing – review & editing. DG: Resources, Writing – review & editing. MM: Conceptualization, Data curation, Methodology, Writing – review & editing. MB: Funding acquisition, Project administration, Writing – review & editing. ER: Data curation, Visualization, Writing – review & editing. KB: Data curation, Writing – review & editing. JD: Methodology, Writing – review & editing. AA: Project administration, Resources, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Productivity and economic feasibility of cultivated forage and food crops in the central highlands of Ethiopia

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The study was conducted to evaluate the yield performance of improved forage and food crops and to estimate the profitability of fodder and food crops in Holetta and Ejere areas, central highlands of Ethiopia during 2019 and 2020 cropping seasons. The experiment was laid out in randomized complete block design with three replications and evaluated two annual forage crops, two perennial forage crops, and two cereal food crops. Data on herbage dry matter (DM) yield was collected for forage crops while straw and grain yields were measured for food crops. Partial budget analysis was made to evaluate the economic feasibility of forage and food crops production. The result revealed that the herbage DM yield varied ($P<0.05$) at each location during each production year and combined over years and between the two locations. The straw and grain yields of food crops also varied ($P<0.05$) between the two production years and locations. The nutritive values of herbage and straw yields of forage and food crops differed ($P<0.05$) for all measured parameters. The crude protein and in-vitro dry matter digestibility of perennial forage crops were relatively higher than annual forage crops and straw of food crops. The partial budget analysis result indicated that the gross revenue (GR) and net return (NR) obtained from food crops were the highest followed by annual forage crops while the least was recorded from perennial forage crops during the first year of production. However, perennial forage crops produced the higher GR and NR than food crops and annual forage crops in the second year of production. In the second year of production, among the forage crops, Desho grass generated 308, 293, 287, and 232% while Rhodes grass generated 99, 92, 90, and 62% more NR than wheat, sole oat, barley, and oat/vetch mixtures, respectively. The benefit-cost ratio (BCR) of perennial forage crops was the lowest (3.0 for Desho and 1.6

for Rhodes) in the first year of production but the ratio was the highest (17.2 for Desho and 8.6 for Rhodes) in the second year of production. This confirms the better economic feasibility of perennial forage crops as they can be maintained using minimal management cost once they have been established.

KEYWORDS

dry matter yield, grain yield, nutritive value, partial budget analysis, straw yield

1 Introduction

Agriculture is the dominant sector of the Ethiopian economy, contributing about 35% to GDP and 68% to employment, and 90% to the export value (FAO (Food and Agricultural Organization of the United Nations), 2019). Similarly, livestock are an integral part of agriculture, accounting for about 45% of the agricultural GDP, 31 to 48% of the total household income and 10% of the total export earnings (FAO (Food and Agricultural Organization of the United Nations), 2019). Livestock play a significant role as a source of food and income in addition to several other important economic and socio-cultural functions. The report of the Central Statistical Agency indicated that 98.24% of the total cattle in the country are local breeds with low productivity and production (CSA (Central Statistical Agency), 2018) due to poor quality and inadequate quantity of available feed (FAO (Food and Agricultural Organization of the United Nations), 2019). Ethiopia has a huge livestock population that requires considerable amounts of good quality feed and appropriate feeding systems for increased productivity, production value addition, and transformation of the livelihood of the farmers. Improving the supply and quality of the available feed resources will significantly contribute to enhance productivity and production of the livestock sector in the country (Seyoum et al., 2001; Getnet, 2007; Alemayehu et al., 2017).

The potential feed resources in Ethiopia include natural pastures, crop residues, and stubble grazing, cultivated forages, agro-industrial by-products, grain, and grain screenings, compound or formula feeds. Natural pasture grazing accounts for the major share of feed supply (54.5) followed by crop residues (31.1) and hay, which contribute 7.4% of the total feed (CSA (Central Statistical Agency), 2021). Agro-industrial by-products, improved forage crops, and non-conventional feed resources like animal by-products, vegetable and fruit wastes contribute the remaining 2.0, 0.6, and 4.4% of the total feed, respectively (CSA (Central Statistical Agency), 2021). Livestock feeding in Ethiopia mainly depends on natural pasture and crop residue, both of which have low nutritional value (Adugna et al., 2012b; Alemayehu et al., 2017). Moreover, feed production and processing industries are not well developed and good quality supplemental feeds are in short supply and very expensive in Ethiopia. Feed cost accounts for about 60 to 70% of the total cost of livestock production (Adugna et al., 2012a; Fekede, 2013; Seyoum et al., 2018) and it increases to 75-80% during the critical feed shortage period in the country (Demisse, 2017). Cultivated forage crops have a tremendous contribution to boosting and sustaining livestock productivity by filling the gap of good quality feed resources available in the country (Getnet et al., 2012).

Over the past five decades, the adaptability and yield performance of different improved forage crops have been tested in areas ranging in altitude from 600-3000 meters above sea level, and many promising species have been selected for high, medium, and low altitudes (Getnet and Gezahagn, 2012; Alemayehu et al., 2017). The selected forage crops are generally well adapted to the different agro-ecologies and are high-yielding and have better quality compared to natural pastures (Getnet et al., 2012; Fekede et al., 2015). So far, a total of 82 forage varieties have been officially registered in the crop variety register book in Ethiopia (EAA (Ethiopian Agricultural Authority), 2022). However, the adoption of released forage technologies in Ethiopia is extremely low. The overall adoption rate of forage crops in the Oromiya region during the 2014 production year was 10% (Agajie et al., 2016) and the adoption rate in the same region reached 12% in 2017 (Agajie et al., 2018). Generating tangible evidence to show that forage crops are competitive and better enterprise choices to ensure adoption by various target groups is very important. Hence, generating compelling empirical evidence on yield performance and economic feasibility of improved forage crops is crucial to inform policy makers and practitioners.

2 Materials and methods

2.1 Description of the study areas

The experiment was conducted under rainfed conditions during the main cropping season (June to January) of 2019 and 2020 at the experimental field of the Holetta Agricultural Research Center (HARC) and Ejere under on-farm conditions. HARC is located at 9°00'N latitude, 38°30'E longitude, and at an altitude of 2400 meters above sea level (masl). It is 34 km west of Addis Ababa and is characterized by a long-term (30 years) average annual rainfall of 1055 mm, and average minimum and maximum air temperatures of 6.1 and 22.2°C, respectively (EIAR (Ethiopian Institute of Agricultural Research), 2005). The rainfall is bimodal and about 85% of the precipitation falls from June to September, while the remaining 15% falls from March to May (Getachew, 2017). The soil type of the area is predominantly acidic Eutric Nitosol, which is characterized by an average organic matter content of 1.8%, total nitrogen of 0.17%, pH of 5.24, and available phosphorus of 4.55 ppm (Gemechu, 2007). The experiment was also conducted under on-farm conditions at Ejere. It is located at 9°03'N latitude, 38°26'E longitude, and at an altitude of 2400 masl. The site is located 40 km west of Addis Ababa and is characterized by a long-term average annual rainfall of 1044 mm, average minimum and maximum air temperatures of 6.5 and 22.4°C, respectively. The soil type of the area is Nitosol, which is characterized by an average organic matter content of 3.0%, total nitrogen of 0.13%, pH of 5.0, and available phosphorus of 5.46 ppm. The monthly precipitation and mean temperature of the study areas during the experimental years are indicated in Figure 1.

The farming system of the study areas is mixed crop-livestock production where tef [*Eragrostis tef* (Zucc.) Trotter] is the main staple crop complemented by other cereals such as barley (*Hordeum vulgare* L.), wheat (*Triticum aestivum* L.), and maize (*Zea mays* L.). Also, faba bean (*Vicia faba* L.), field pea (*Pisum sativum* L.), chickpea (*Cicer arietinum* L.), and horticultural crops such as potatoes (*Solanum tuberosum* L.) are the major crops grown in the study areas. The main feed resources in the areas are natural pasture, crop residues, and cultivated forage crops mainly oat/vetch mixture (*Avena sativa* L./*Vicia dasycarpa* L.), Napier grass (*Pennisetum purpureum*), Desho grass (*Pennisetum glaucifolium*), Rhodes grass (*Chloris gayana*), and tree lucerne (*Chamaecytisus palmensis*) which are grown by some farmers for their crossbred dairy cows (HARC (Holetta Agricultural Research Center), 2009). Map of the study areas is indicated in Figure 2.

2.2 Experimental treatments and design

The study was conducted using the best adapted four forage crops and two cereal food crops (Table 1). The experiment was laid out in a randomized completed block design (RCBD) with three replications. The treatments were assigned to plots at random within each block. Each treatment was sown to a plot area of 5 m x 4 m = 20 m^2. All treatments were sown with their recommended sow spacing. Except for desho grass, the treatments were sown with 20cm row spacing. Desho grass was planted using root splits in 50-cm inter and 25-cm intra row spacing. The spacing between plots and blocks was 1 m and 1.5 m, respectively. The oat-vetch mixture was sown with 75 and 25% of the recommended seeding rate for oat (75 kg/ha) and vetch (6.3 kg/ha), respectively (Astatke, 1979; Lulseged, 1981). The recommended fertilizer rate of 18 kg N/ha + 20 kg P/ha in the form of diammonium phosphate (DAP) was uniformly applied at sowing for all forage crops. In addition, the recommended fertilizer rate of 45 kg N/ha + 50 kg P/ha and 27 kg N/ha + 30 kg P/ha in the form of diammonium phosphate (DAP) was uniformly applied at sowing for wheat and barley, respectively. Moreover, 50 kg N/ha in the form of urea was applied to wheat and barley crops at tillering stage. For perennial forage crops (Desho grass and Rhodes grass), one-third of 50 kg N/ha in the form of urea was applied during the short rainy season and the remaining two-third were applied at the active vegetative growth stage. The sowing of the first-year experiment was done on July 10, 2019, and July 12, 2019, at Holetta and Ejere, respectively. Similarly, the second-year sowing was done on June 22, 2020, and June 26, 2020, at Holetta and Ejere, respectively. The first-hand weeding was done 30 days after full crop emergence and the second-hand weeding was performed 30 days after the first weeding.

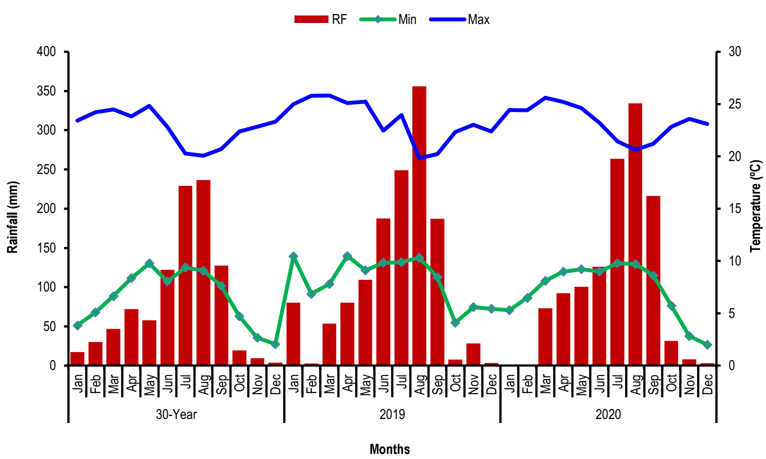

FIGURE 1
Monthly total precipitation and mean minimum and maximum temperatures during the cropping seasons and 30-year (1989-2018) at the study areas.

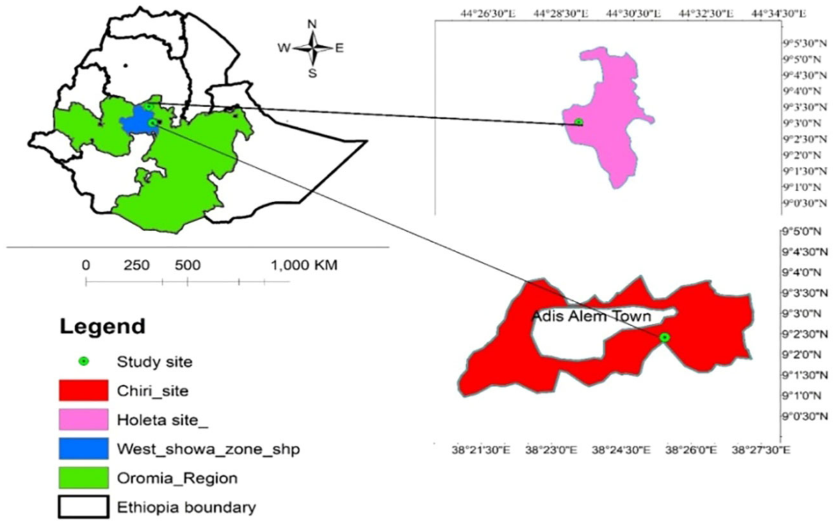

FIGURE 2
Map of the study areas.

2.3 Yield determination and nutritive value analysis

Yield data were collected on a plot basis (excluding the two border rows) at forage and grain harvesting stages for forage crops and food crops, respectively. The soft dough stage has been recommended to be the best stage for forage harvesting of oat and oat/vetch mixtures because optimum yield and quality are obtained at this stage for haymaking in Ethiopia (Astatke, 1976; Lulseged, 1981). For the determination of herbage dry matter accumulation, the forage crops were harvested manually using a sickle at the soft dough stage for sole oat and oat/vetch mixtures, 50% heading for Rhodes grass and at 1-m height for Desho grass. The weight of the total fresh biomass was recorded from each plot in the field. To isolate the panicle from the total biomass of the food crops, the panicle portion of the plant was harvested first manually using a sickle and separately collected. The remaining aftermath was harvested from the ground level and its fresh biomass was measured and recorded in the field. About a 500 g sample of the herbage and aftermath was taken and oven-dried at 105°C for 24 h to determine the herbage and aftermath dry matter accumulation. Grain samples were taken and oven-dried at 100°C for 48 h to adjust the moisture concentration to 12.5%, a recommended percentage level for cereals (Biru, 1979). Grain yield was then estimated at an adjusted moisture concentration of 12.5% for safe storage. The residue remaining after grain threshing (chaff) was oven-dried at 100°C overnight and added to the aftermath dry matter to estimate straw dry matter biomass.

The oven-dried samples of forage and straw at 65°C for 72 hours were ground to pass through a 1-mm sieve for laboratory analysis. Total ash and crude protein (CP) concentrations were determined using standard procedures of AOAC (1995). Accordingly, ash was determined upon complete burning of the feed samples in a muffle furnace at 550°C for six hours (AOAC, 1990). Nitrogen concentration was determined by the Kjeldahl method involving acid digestion and distillation (Nelson and Sommers, 1982). The CP concentration was calculated as 6.25 multiplied by N concentration assuming that protein contains 16% N. The structural plant constituents such as neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) were analyzed using the detergent extraction method

TABLE 1 List of experimental treatments used for the study.

SN	Crop	Latin name	Variety	Life cycle	Seeding rate (kg/ha)
1	Sole Oat	*Avena sativa*	SRCPX80Ab2806	Annual	100
2	Oat/vetch mix	–	–	–	–
	- Oat	*Avena sativa*	SRCP X 80Ab 2806	Annual	75
	- Vetch	*Vicia dasycarpa*	Lana	Annual	7.5
3	Desho grass	*Pennisetum glaucifolium*	Kulumsa (DZF-592)	Perennial	80,000 splits
4	Rhodes grass	*Chloris gayana*	Massaba	Perennial	15
5	Wheat	*Triticum aestivum*	Ali Doro	Annual	150
6	Barley	*Hordeum vulgare*	HB-37	Annual	200

described by Goering and Van Soest (1970). The *in-vitro* dry matter digestibility (IVDMD) was determined by the two-stage *in-vitro* fermentation techniques of Tilley and Terry (1963). The metabolizable energy (ME) of the feed was estimated according to the equation proposed by Wiseman (1987).

2.4 Partial budget analysis

An economic analysis was used to compare the economic benefits of food and forage crops using the partial budget analysis (CIMMYT (International Maize and Wheat Improvement Center), 1988). Partial budget analysis involves the calculation of the variable costs and benefits. The benefits were calculated based on the market value of natural pasture hay, grain, and straw of wheat and barley crops after harvesting time (January) of each growing season. The natural pasture hay price was used to estimate the price of Desho and Rhodes grass hay. Costs of pre-basic seed, inorganic fertilizers, and labor costs for land preparation, weeding, and threshing were considered for estimation of production cost. The cost structure is different when comparing annual and perennial forage crops. For annual forage crops, all the production costs are incurred during the production cycle for a single crop. But for perennial crops, costs can be separated into the start-up or establishment cost and the annual cost incurred thereafter. The total costs (i.e., seeds, fertilizers, and labor) were calculated based on local market prices. Labor cost for each activity was taken based on the local daily wage per person to perform the activity per hectare and multiplied by the total man-days required to complete the activity. The gross benefit was calculated by multiplying the forage, grain, and straw yields (kg/ha) by the respective average open market price (presented in ETB/kg). In the economic analysis, the average forage, grain, and straw yields were adjusted downwards by 10%, taking into consideration that farmers could obtain 10% less than the experimental yield (CIMMYT (International Maize and Wheat Improvement Center), 1988). This is because yields from experimental plots are often higher than the yields that farmers could expect using the same treatments. The forage, grain, and straw yields were the average of two growing locations, gross income was estimated based on local market prices, and net income was obtained by deducting total costs from the gross income. Moreover, the benefit-cost ratio (BCR) was calculated by dividing gross revenue (GR) by total variable cost (TVC) (Ozpinar, 2006). If the ratio is less than one, then the costs exceed the benefit (not cost-effective), and such an intervention should be rejected. However, if the ratio is more than one, then the benefit exceeds the costs and the investment is therefore worthwhile (Jehanzeb, 1999). A BCR value of greater than one suggests an intervention whose benefits exceed its costs. Conversely, a BCR of 1 meant that the intervention would not add or take away any value from the farmers and should not be promoted unless there would be wider social or economic benefits (other than financial).

2.5 Statistical analysis

Analysis of variance (ANOVA) procedures of the SAS general linear model (GLM) version 9.4 was used for analysis to compare treatment means (SAS (Statistical Analysis System), 2012). To determine the validity of each location and year analysis, Bartlett's test for homogeneity of variance was done by the SAS computer packages prior to computing ANOVA. Moreover, the homogeneity of error variance for combined ANOVA was tested using the F-max method of Hartley (Hartley, 1950), which is based on the ratio of the larger mean square of error (MSE) from the separate analysis of variance to the smaller MSE. If the larger MSE is not three-fold larger than the smaller MSE, the error variance is considered homogeneous (Gomez and Gomez, 1984). A separate analysis was made for forage and food crops. The statistical significance was determined using the F test. Only traits that show a significant difference in ANOVA were promoted to mean comparisons using the least significance difference (LSD) at a 5% probability level following Gomez and Gomez (1984). The statistical models for individual location, year, and combined over locations and years are presented below. The statistical model of the RCBD design for individual location and/or year is given by:

$$Y_{ij} = \mu + B_i + S_j + \varepsilon_{ij}$$

Where Y_{ij} = measured response of species j in block i; μ = grand mean; B_i = effect of block i; S_j = effect of species j; ε_{ij} = random error effect of species j in block i. The statistical model of the RCBD design for over years and locations is given by:

$$Y_{ijkl} = \mu + B_i + S_j + L_k + Y_l + (SL)_{jk} + (SY)_{jl} + (LY)_{kl} + (SLY)_{jkl} + \varepsilon_{ijkl}$$

Where Y_{ijkl} = measured response of species j in block i at location k and year l; μ = grand mean; B_i = the effect of block i; S_j = the effect of species j; L_k = the effect of location k; Y_l = the effect of year l; $(SL)_{jk}$ = the interaction effect between species j and location k; $(SY)_{jl}$ = the interaction effect between species j and year l; $(LY)_{kl}$ = the interaction effect between location k and year l; $(SLY)_{jkl}$ = the interaction effect among species j, location k and year l and ε_{ijkl} = the residual or effects of random error.

3 Results

3.1 Productivity of forage and food crops

The analysis of variance indicated that year, species, and the interaction effect of the year by species showed significant (P<0.05) differences for herbage dry matter accumulation of forage crops (Table 2). In addition, the year and location had significant effects for straw and grain yields of the food crops. Moreover, the interaction effects of the year by location, year by species, and location by species had a significant (P<0.05) effect on straw yield. The grain yield also varied (P<0.05) for species and the interaction effect of the year by location by species.

The mean herbage DM accumulation of forage crops at the recommended forage harvesting stage differed (P<0.05) at each location during each production year and combined over years and locations (Table 3). The combined analysis at Holetta showed that the mean herbage DM accumulation ranged from 7.3 to 15.8 t DM/

TABLE 2 Analysis of variance for year, location, species, and their interaction effects on yield and yield components of improved forage and food crops.

Factors	FDMY (t/ha)	SY (t/ha)	GY (t/ha)
Year (Y)	*	**	***
Location (L)	NS	**	*
Species (S)	***	NS	*
Y*L	NS	*	NS
Y*S	***	*	NS
L*S	NS	***	NS
Y*L*S	NS	NS	*
Mean	9.3	4.1	2.1
CV (%)	23.6	18.9	25.3
R^2	0.85	0.80	0.86

FDMY, forage dry matter yield; SY, straw yield; GY, grain yield; Y*L, year by location interaction; Y*S, year by species interaction; L*S, location by species interaction; Y*L*S, year by location by species interaction; CV, coefficient of variation; R^2, R-square; * ≤ 0.05; ** ≤ 0.01; *** ≤ 0.001; NS, non-significant.

ha with a mean of 9.9 t DM/ha. The highest herbage DM accumulation at Holetta was recorded from Desho grass followed by Rhodes grass and oat/vetch mixtures while the lowest was obtained from sole oat. In the combined analysis, Desho grass at Holetta had 80, 108, and 116% herbage DM accumulation advantages over Rhodes grass, oat/vetch mixtures, and sole oat, respectively. Similarly, Rhodes grass at Holetta showed yield advantages of 16 and 21% over oat/vetch mixtures and sole oat, respectively. Furthermore, the combined analysis done at Ejere indicated that Desho grass produced the highest herbage DM accumulation followed by oat/vetch mixtures, and sole oat while the least was recorded from Rhodes grass (Table 3). According to the result of combined analysis at Ejere, Desho grass had 71, 89, and 100% herbage DM accumulation advantages over oat/vetch mixtures, sole oat, and Rhodes grass, respectively. Likewise, oat/vetch mixtures at Ejere had DM yield advantages of 10 and 17% over sole oat and Rhodes grass, respectively.

The combined analysis of the two locations indicated that herbage DM accumulation in both production years and combined over years varied ($P<0.05$) among forage species (Table 3). The result combined over years showed that Desho

TABLE 3 Mean herbage DM yield (t/ha) of forage crops at each site, year, and combined over years and locations.

Location	Species	Year		
		2019	2020	Combined
Holetta	Sole oat	7.7^{b}	6.8^{b}	7.3^{b}
	Oat/vetch mixtures	8.5^{b}	6.7^{b}	7.6^{b}
	Desho grass	11.6^{a}	19.9^{a}	15.8^{a}
	Rhodes grass	7.4^{b}	10.2^{b}	8.8^{b}
	Mean	8.8	10.9	9.9
	CV (%)	16.2	25.3	30.8
	P-value	0.04	0.003	0.0004
Ejere	Sole oat	8.3^{a}	5.7^{c}	7.0^{b}
	Oat/vetch mixtures	9.2^{a}	6.2^{bc}	7.7^{b}
	Desho grass	9.0^{a}	17.3^{a}	13.2^{a}
	Rhodes grass	4.1^{b}	9.2^{b}	6.6^{b}
	Mean	7.6	9.6	8.6
	CV (%)	16.1	16.6	35.3
	P-value	0.003	<.0001	0.005
Combined	Sole oat	8.0^{ab}	6.2^{c}	7.1^{b}
	Oat/vetch mixtures	8.9^{a}	6.5^{c}	7.7^{b}
	Desho grass	10.3^{a}	18.6^{a}	14.5^{a}
	Rhodes grass	5.7^{b}	9.7^{b}	7.7^{b}
	Mean	8.2	10.3	9.3
	CV (%)	23.5	23.7	34.1
	P-value	0.006	<.0001	<.0001

Different superscript letter/s in the same column at each location represent significant differences at P<0.05.

grass had yield advantages of 88 and 104% over oat/vetch mixtures/ Rhodes grass and sole oat, respectively. The herbage DM accumulation produced at Holetta was relatively higher than Ejere during each production year and combined over years. The result showed that yield advantages of 16, 14, and 6% were recorded at Holetta in the first year, the second year, and combined over years, respectively. Moreover, the herbage DM accumulation of forage species varied (P<0.05) between the two production years. Accordingly, the forage species had a 26% yield advantage in the second year of production.

The mean straw yield of wheat and barley at each location during each production year and combined over years and locations is indicated in Table 4. The mean straw yield of wheat and barley at the grain harvesting stage varied (P<0.05) during the first production year and combined over years at Holetta. On the other hand, the straw yield in the second year of production and combined over years differed (P<0.05) between food cops at Ejere. A higher straw yield was recorded for wheat at Holetta while the barley crop gave a higher straw yield at Ejere during both production years and combined over years. At Holetta, the wheat crop had 31, 43, and 35% straw yield advantages in the first year of production, the second year of production, and combined over years, respectively. Similarly, straw yield advantages of 11, 100, and 46% were recorded at Ejere for barley in the first year of production, the second year of production, and combined over years, respectively. The straw yield was slightly higher at Ejere than Holetta in both production years and combined over years. The combined over years analysis indicated that the straw yield advantage of 28% was recorded at Ejere. The mean straw yield varied (P<0.05) between the two production years and accordingly, 28% yield advantage was recorded in the first year of production.

The mean grain yield of wheat and barley at each location during each production year and combined over years is indicated in Table 4. The result indicated that the grain yield varied (P<0.05) for the first year of production and combined over years at Holetta. In the first year of production and combined over years, the wheat crop gave a higher grain yield while the barley crop produced a higher grain yield in the second year of production at Holetta. The grain yield advantages of the wheat crop at Holetta were 63 and 36% over barley crop during the first year of production and combined over years, respectively. At Ejere, the barley crop produced a higher grain yield in the first year of production, but the wheat crop produced a higher grain yield in the second year of production and combined over years. Barley crop had a 5% grain yield advantage in the first year of production while 86 and 18% grain yield advantages were recorded for wheat crop in the second year of production and combined over years, respectively. The combined over locations at each year and combined over years analysis indicated that wheat crop produced a higher grain yield than barley crop. Accordingly, 29, 24, and 27% grain yield advantages for wheat were recorded in the first year of production, the second year of production, and combined over years, respectively. The grain yield was higher at Holetta than Ejere in both production years and combined over years. The grain yield advantages at Holetta were 33, 12, and 26% in the first year of production, the second year of production, and combined over years, respectively. The mean grain yield varied significantly between the two production years and a higher yield

TABLE 4 Mean straw yield (t/ha) and grain yield (t/ha) of food crops at each site, year and combined over years and locations.

Location	Species	Straw yield			Grain yield		
		2019	2020	Combined	2019	2020	Combined
Holetta	Wheat	5.1^{a}	3.3	4.2^{a}	4.2^{a}	1.3	2.7^{a}
	Barley	3.9^{b}	2.3	3.1^{b}	2.6^{b}	1.4	2.0^{b}
	Mean	4.5	2.8	3.6	3.4	1.3	2.4
	CV (%)	7.5	25.4	19.2	9.5	21.1	20.6
	P-value	0.05	0.22	0.03	0.03	0.51	0.04
Ejere	Wheat	4.4	3.0^{b}	3.7^{b}	2.5	1.6	2.0
	Barley	4.9	6.0^{a}	5.4^{a}	2.6	0.8	1.7
	Mean	4.7	4.5	4.6	2.5	1.2	1.9
	CV (%)	26.9	8.7	17.4	31.0	22.1	25.9
	P-value	0.67	0.01	0.01	0.88	0.08	0.32
Combined	Wheat	4.8	3.1^{b}	3.9	3.3	1.4	2.4^{a}
	Barley	4.4	4.1^{a}	4.3	2.6	1.1	1.9^{b}
	Mean	4.6	3.6	4.1	3.0	1.3	2.1
	CV (%)	20.4	13.9	18.9	23.3	22.8	25.3
	P-value	0.53	0.02	0.35	0.11	0.16	0.03

Different superscript letter/s in the same column at each location represent significant differences at P<0.05.

was obtained in the first year of production. Accordingly, a 133% grain yield advantage was observed in the first year of production.

3.2 Nutritive value of herbage and straw yields

The nutritive values of herbage for forage crops and straw for food crops were significantly different for all measured parameters at each location and combined over locations (Table 5). Among treatments, Desho grass had higher values of ash, CP, and IVDMD while ME was higher for sole oat at each location and combined over locations. On contrast, NDF and ADL concentrations were higher for wheat and barley straw, respectively. The location had a significant effect on NDF, IVDMD, and ME of herbage DM and straw yields. Furthermore, ash and IVDMD were significantly affected by the interaction effect of species by location. Among the tested crops, perennial forage grasses (Desho and Rhodes grasses) had better ash, CP, and IVDMD followed by annual forage crops (sole oat and oat/vetch mixtures) while the lowest values were produced from wheat

TABLE 5 Chemical composition (%), *in-vitro* dry matter digestibility (%), and metabolizable energy (MJ/kg) of forage crop herbage yield and straw yield of food crops at each site and combined over locations.

Location	Species	Ash	CP	NDF	ADF	ADL	IVDMD	ME
Holetta	Sole oat	10.8^{c}	7.7^{b}	52.2^{d}	40.6^{b}	5.4^{c}	57.2^{b}	8.1^{a}
	Oat/vetch	10.5^{c}	8.5^{b}	63.6^{d}	41.4^{b}	5.6^{c}	57.8^{b}	7.9^{a}
	Desho	17.8^{a}	14.5^{a}	67.6^{c}	34.6^{d}	4.4^{d}	60.8^{a}	7.9^{a}
	Rhodes	15.8^{b}	14.4^{a}	68.6^{c}	37.1^{c}	5.2^{c}	59.9^{a}	7.9^{a}
	Wheat	8.4^{d}	4.0^{c}	76.3^{a}	50.5^{a}	6.8^{b}	48.4^{d}	7.0^{b}
	Barley	8.9^{d}	4.3^{c}	74.5^{b}	51.0^{a}	7.7^{a}	49.7^{c}	7.1^{b}
	Mean	12.0	8.9	68.8	42.5	5.8	55.6	7.6
	CV (%)	6.8	10.2	1.3	1.9	4.7	1.2	1.9
	P-value	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0002
Ejere	Sole oat	11.2^{cd}	7.6^{c}	63.7^{d}	40.4^{b}	5.1^{c}	56.7^{a}	7.9^{a}
	Oat/vetch	12.0^{c}	9.3^{b}	62.7^{d}	41.6^{b}	5.6^{c}	56.8^{a}	7.7^{ab}
	Desho	16.9^{a}	13.7^{a}	69.0^{c}	35.3^{d}	4.4^{d}	57.8^{a}	7.5^{b}
	Rhodes	15.0^{b}	13.7^{a}	70.0^{c}	37.9^{c}	5.3^{c}	56.9^{a}	7.5^{b}
	Wheat	9.2^{e}	4.3^{d}	78.3^{a}	52.3^{a}	7.0^{b}	47.2^{b}	6.7^{c}
	Barley	10.4^{d}	4.3^{d}	74.2^{b}	51.4^{a}	7.7^{a}	46.8^{b}	6.9^{c}
	Mean	12.5	8.8	69.6	43.1	5.9	53.7	7.4
	CV (%)	4.7	6.4	1.5	2.6	5.5	1.3	2.7
	P-value	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.001
Combined	Sole oat	11.0^{c}	7.6^{c}	62.9^{d}	40.5^{b}	5.2^{d}	57.0^{c}	8.0^{a}
	Oat/vetch	11.2^{c}	8.9^{b}	63.2^{d}	41.5^{b}	5.6^{c}	57.3^{c}	7.8^{ab}
	Desho	17.4^{a}	14.1^{a}	68.3^{c}	35.0^{d}	4.4^{e}	59.3^{a}	7.7^{b}
	Rhodes	15.4^{b}	14.0^{a}	69.3^{c}	37.5^{c}	5.2^{d}	58.4^{b}	7.7^{b}
	Wheat	8.8^{e}	4.1^{d}	77.3^{a}	51.4^{a}	6.9^{b}	48.0^{d}	6.8^{c}
	Barley	9.7^{d}	4.3^{d}	74.3^{b}	51.2^{a}	7.7^{a}	48.2^{d}	7.0^{c}
	Mean	12.2	8.8	69.2	42.8	5.8	54.7	7.5
	CV (%)	5.6	8.4	1.2	2.4	5.1	1.3	2.5
	P-value	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
	Loc	NS	NS	*	NS	NS	***	***
	Sp * Loc	*	NS	NS	NS	NS	**	NS

CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; ADL, acid detergent lignin; IVDMD, in-vitro dry matter digestibility; ME, metabolizable energy; Loc, location; Sp* Loc, Species by location interaction; CV, coefficient of variation.
* ≤ 0.05; ** ≤ 0.01;*** ≤ 0.001; NS, non-significant.

and barley straws. The metabolizable energy value was higher for sole oat followed by oat/vetch mixtures while the lowest was recorded for wheat and barley straws.

3.3 Partial budget analysis for food and forage crops

The partial budget analysis for food and forage crops during the first year of production is presented in Table 6. The total input cost (TIC) required for annual forage crops was generally lower than for food and perennial forage crops. Accordingly, the TIC required for the production of Desho grass, wheat, barley, and Rhodes grass were 296, 288, 266, and 192% higher over oat/vetch mixtures, respectively. Similarly, the TIC for Desho grass, wheat, barely, and Rhodes grass were higher by 257, 250, 230, and 164% over sole oat, respectively. The total labor cost (TLC) was generally higher than the TIC required for food and forage crops production. The TLC required for the production of oat/vetch mixtures, sole oat, Rhodes grass, barley, and wheat were 211, 166, 74, 38, and 8% higher than the TIC for the respective crops. Likewise, the TLC for the production of Rhodes grass, barley, wheat, and Desho grass were increased by 72, 71, 42, and 32% over sole oat production, respectively. Furthermore, the TLC of 64, 62, 35, and 25% over oat/vetch mixtures were recorded for Rhodes grass, barley, wheat, and Desho grass, respectively. Thus, the TVC for the production of barley, wheat, Rhodes, and Desho were increased by 114/112, 99/97, 97/95, and 93/91% over sole oat and oat/vetch mixtures, respectively.

The income obtained from the selling of food and forage crops during the first year of production is indicated in Table 6. The result showed that relatively a higher mean gross revenue (GR) was generated from annual forage crops when compared to perennial forage crops during the first year of production. Among forage crops, the highest GR was recorded from Desho grass followed by oat/vetch mixtures and sole oat while the lowest was obtained from Rhodes grass. The GRs earned from Desho grass were 81, 29, and 8% more than Rhodes grass, sole oat, and oat/vetch mixtures, respectively. Similarly, oat/vetch mixtures generated 67 and 19% more GR than Rhodes grass and sole oat, respectively. Furthermore, sole oat generated 40% more GR than Rhodes grass. The wheat crop generated 16% more income from grain sold than barley while barley earned 13% more income from straw sold than wheat. The GR obtained from food crops was higher than forage crops during the first year of production. The result revealed that wheat generated 180, 99, 67, 55, and 10% more GR than Rhodes grass, sole oat, oat/vetch mixtures, Desho grass, and barley, respectively. Likewise, barley crop produced 155, 82, 52, and 41% more GR than Rhodes grass, sole oat, oat/vetch mixtures, and Desho grass, respectively. On the other hand, the net return (NR) obtained from wheat was the highest followed by barley, oat/vetch mixtures, Desho grass, sole oat while Rhodes grass generated the lowest. The wheat crop generated 463, 100, 81, and 60% while barley produced 386, 72, 56, and 38% more NR than Rhodes grass, sole oat, Desho grass, and oat/vetch mixtures, respectively. Similarly, oat/vetch mixtures produced 251, 24, and 13% more NR than Rhodes grass, sole oat, and Desho grass, respectively. Furthermore, Desho grass generated 212, and 10% more NR than Rhodes grass, and sole oat, respectively, while sole oat generated 182% more NR than Rhodes grass.

The benefit-cost ratio (BCR) of food and forage crops during the first year of production is shown in Table 6. The result indicated that the BCR for annual and perennial forage crops and food crops

TABLE 6 Partial budget analysis for food and forage crops in 2019 production year (Ethiopian birr).

Items	Sole oat	Oat/vetch	Desho	Rhodes	Wheat	Barley
Seed expense	1800	1508	8000	5200	6000	6600
Fertilizer expense	1200	1200	2710	2710	4500	3300
TIC (ETB) (A)	3000	2708	10710	7910	10500	9900
Land preparation expense	3190	3203	3783	3770	2787	3446
Expense for weeding	4800	5213	6762	10009	4419	4666
Expense for threshing	–	–	–	–	4167	5555
TLC (ETB) (B)	7990	8416	10545	13778	11372	13667
TVC (ETB) (C = A+B)	10990	11123	21255	21688	21872	23568
Income from forage	49680	59274	63963	35397	–	–
Income from seed	–	–	–	–	80960	69660
Income from straw	–	–	–	–	18144	20592
GR (ETB) (D)	49680	59274	63963	35397	99104	90252
NR (ETB) (D-C)	38690	48151	42708	13709	77232	66685
BCR (D/C)	4.5	5.3	3.0	1.6	4.5	3.8

TIC, Total input cost; TLC, Total labor cost; TVC, Total variable cost; GR, Gross revenue; NR, Net return; BCR, Benefit-cost ratio; ETB, Ethiopian birr; 1 kg DM Sole oat = 6.90 ETB; 1 kg DM Oat/vetch mixtures = 7.40 ETB; 1 kg DM Desho = 6.90 ETB; 1 kg DM Rhodes = 6.90 ETB; 1 kg wheat straw = 4.20 ETB; 1 kg barley straw = 5.20 ETB; 1 kg wheat grain = 27.0 ETB; 1 kg barley grain = 30.0 ETB; Labor cost for 8 hours = 50 ETB; January 31, 2020 exchange rate ($ 1 USD = Br. 32.0991).

were greater than one, but the highest ratio was recorded for annual forage crops followed by food crops while perennial forage crops gave the lowest ratio. The highest BCR was recorded for oat/vetch mixtures followed by wheat, sole oat, barley, Desho grass while the lowest was from Rhodes grass. The BCR for oat/vetch mixtures was 3.3 and 1.8; wheat/sole oat 2.8 and 1.5; and barley 2.3 and 1.3 times higher than Rhodes grass and Desho grass, respectively. For every one Ethiopian Birr (ETB) spent for the production of oat/vetch mixtures, wheat, sole oat, barley, Desho grass, and Rhodes grass, the farmers generated ETB of 5.33, 4.53, 4.52, 3.83, 3.01, and 1.63 returns or the profit of 4.33, 3.53, 3.52, 2.83, 2.01, and 0.63 ETB on every one ETB spent for the production of the respective crops.

The partial budget analysis for food and forage crops during the second year of production is indicated in Table 7. For wheat production 608, 278, and 242% more TIC were spent than Desho/Rhodes grass, oat/vetch mixtures, and sole oat, respectively. Likewise, for barley production 571, 258, and 224% more TIC were disbursed than Desho/Rhodes grass, oat/vetch mixtures, and sole oat, respectively. Furthermore, sole oat production required 107% more TIC while oat/vetch mixtures production required 88% more TIC than Desho/Rhodes grass production. The sole oat production required 48 and 40% while oat/vetch mixtures production required 44 and 36% more labor cost than Desho grass and Rhodes grass, respectively. The TVCs for the barley were 242, 227, 123, and 112% while for wheat were 214, 200, 104, and 94% more than Desho grass, Rhodes grass, oat/vetch mixture, and sole oat, respectively. The sole oat production required 62 and 55% while oat/vetch mixtures production used 54 and 47% more TVC than Desho grass and Rhodes grass, respectively.

The GR and NR earned from the selling of food and forage crops during the second year of production are presented in Table 7. The result showed that the highest GR and NR were generated from perennial forage crops when compared to annual forage and food crops during the second year of production. The GR earned from Desho grass was 200, 168, and 92% more than sole oat, oat/vetch mixtures, and Rhodes grass, respectively. Similarly, Rhodes grass generated 56 and 40% more GR than sole oat and oat/vetch mixtures, respectively. The profit earned from the selling of wheat grain was 13% more than barley grain sold while barley straw sold earned 62% more profit than wheat straw sold. The GR generated from barley and wheat productions were 33 and 24%; and 18 and 11% more than sole oat and oat/vetch mixtures, respectively. The NR obtained from perennial forage crops was higher than food and annual forage crops. The NR obtained from Desho grass was the highest followed by Rhodes grass, oat/vetch mixtures, barley, sole oat while wheat generated the lowest profit. The Desho grass generated 308, 293, 287, and 232% while Rhodes grass generated 99, 92, 90, and 62% more NR than wheat, sole oat, barley, and oat/vetch mixtures, respectively. Furthermore, Desho grass produced 104% more NR than Rhodes grass while oat/vetch mixtures generated 23, 19, and 17% more NR than wheat, sole oat, and barley, respectively.

The BCR of food and forage crops during the second year of production is presented in Table 7. The result showed that the BCR of food and forage crops was greater than one indicating the production of these crops gives better profit in the study areas. The highest BCR was recorded for Desho grass followed by Rhodes grass, oat/vetch mixtures, sole oat, and wheat while the least was recorded for barley. The BCRs of desho grass were 7.8, 7.6, 4.9, and 4.1 times higher than barley, wheat, sole oat, and oat/vetch mixtures, respectively. Likewise, Rhodes grass made 3.9, 3.8, 2.4, and 2.1 times more BCR than barley, wheat, sole oat, and oat/vetch

TABLE 7 Partial budget analysis for food and forage crops in 2020 production year (Ethiopian birr).

Items	Sole oat	Oat/vetch	Desho	Rhodes	Wheat	Barley
Seed expense	2000	1680	0	0	6450	7200
Fertilizer expense	1350	1350	1616	1616	4991	3641
TIC (ETB) (A)	3350	3030	1616	1616	11441	10841
Land preparation expense	3481	3203	0	0	3412	3459
Expense for weeding	4649	4688	5481	5812	3247	4445
Expense for threshing	–	–	–	–	4167	5555
TLC (ETB) (B)	8129	7891	5481	5812	10825	13459
TVC (ETB) (C = A+B)	11479	10921	7097	7428	22266	24300
Income from forage	40734	45630	122202	63729	–	–
Income from seed	–	–	–	–	37935	33710
Income from straw	–	–	–	–	12555	20295
GR (ETB) (D)	40734	45630	122202	63729	50490	54005
NR (ETB) (D-C)	29255	34709	115105	56301	28224	29705
BCR (D/C)	3.6	4.2	17.2	8.6	2.3	2.2

TIC, Total input cost; TLC, Total labor cost; TVC, Total variable cost; GR, Gross revenue; NR, Net return; BCR, Benefit-cost ratio; ETB, Ethiopian birr; 1 kg DM Sole oat = 7.30 ETB; 1 kg DM Oat/vetch mixtures = 7.80 ETB; 1 kg DM Desho = 7.30 ETB; 1 kg DM Rhodes = 7.30 ETB; 1 kg wheat straw = 4.50 ETB; 1 kg barley straw = 5.50 ETB; 1 kg wheat grain = 30.0 ETB; 1 kg barley grain = 33.0 ETB; Labor cost for 8 hours = 50 ETB; January 31, 2021 exchange rate ($ 1 USD = Br. 39.4056).

mixtures, respectively. The BCRs of oat/vetch mixtures were 1.9, and 1.8 times higher than barley, and wheat, respectively. Furthermore, sole oat had 1.6 times higher BCR than the other tested food crops. For every one Ethiopian Birr (ETB) spent on the production of Desho grass, Rhodes grass, oat/vetch mixtures, sole oat, wheat, and barley, the farmers generated ETB of 17.22, 8.58, 4.18, 3.55, 2.27, and 2.22 returns or profits of 16.22, 7.58, 3.18, 2.55, 1.27, and 1.22 ETB on every one ETB spent for the production of the respective crops.

The partial budget analysis for food and forage crops combined over years is indicated in Table 8. The results showed that annual forage crops relatively required lower TIC followed by perennial forage crops and food crops. The mean TIC required for the production of food crops (wheat and barley) were increased by 95 and 253% over the mean of perennial (Desho and Rhodes grasses) and annual (oat and oat/vetch mixtures) forage crops, respectively. Similarly, the mean TLC were increased by 38 and 52% for food crops (wheat and barley) compared to the mean TLC of perennial and annual forage crops, respectively. The TVC required for production of sole oat, oat/vetch mixtures, Desho grass, and Rhodes grass were increased by 108, 112, 65, and 61% over barley, respectively. Similarly, 96, 100, 56, and 52% higher TVC over wheat were recorded for sole oat, oat/vetch mixtures, Desho grass, and Rhodes grass, respectively.

The GR and NR for food and forage crops which analyzed over years are indicated in Table 8. The highest mean GR was recorded for mean of food crops (wheat and barley) followed by perennial forage crops (Desho grass and Rhodes grass) while annual forage crops (sole oat and oat/vetch mixtures) gave the lowest. Accordingly, the mean GR for the mean of food crops were increased by 50 and 3% over the mean of annual and perennial forage crops, respectively. The mean GR for the mean of perennial forage crops was also increased by 46% over the mean of annual forage crops. The results revealed that the highest GR was obtained from Desho grass (93,083 ETB) followed by wheat (74,797 ETB), barley (72,129 ETB), oat/vetch mixtures (52,452 ETB), Rhodes grass (49,563 ETB) while sole oat (45, 207 ETB) gave the lowest. Similarly, the NB for Desho grass, wheat, barley, oat/vetch mixtures, Rhodes grass, and sole oat were 78,907, 52,728, 48,195, 41,430, 35,005, and 33973 ETB, respectively. The highest mean NB was recorded for the mean of perennial forage crops followed by food crops and annual forage crops. Thus, the mean NB for the mean of perennial forage crops were increased by 51 and 13% over the mean of annual forage crops and food crops, respectively. The mean of food crops gave 34% higher mean NB over the mean of annual forage crops.

The BCR of food and forage crops over years is presented in Table 8. The result showed that the highest BCR was recorded for perennial forage crops followed by annual forage crops and food crops. Among the crops, Desho grass gave the highest BCR followed by oat/vetch mixtures, sole oat, Rhodes grass, and wheat while barley gave the lowest. The BCR of Desho grass was 1.7, 1.4, 1.9, 1.9, and 2.2 times higher than sole oat, oat/vetch mixtures, Rhodes grass, wheat, and barley, respectively. Similarly, the BCR of oat/vetch mixtures was 1.2, 1.4, 1.4, and 1.6 times higher than sole oat, Rhodes grass, wheat, and barely, respectively. A unit of Ethiopian Birr (ETB) spent for the production of Desho grass, oat/vetch mixtures, sole oat, Rhodes grass, wheat, and barley, the farmers generated ETB of 6.6, 4.8, 4.0, 3.4, 3.4, and 3.0 gross returns or the net profit of 5.6, 3.8, 3.0, 2.4, 2.4, and 2.0 ETB on every unit ETB spent for the production of the respective crops.

TABLE 8 Partial budget analysis for food and forage crops combined over years (Ethiopian birr).

Items	Sole oat	Oat/vetch	Desho	Rhodes	Wheat	Barley
Seed expense	1900	1594	4000	2600	6225	6900
Fertilizer expense	1275	1275	2163	2163	4746	3471
TIC (ETB) (A)	3175	2869	6163	4763	10971	10371
Land preparation expense	3336	3203	1892	1885	3100	3453
Expense for weeding	4725	4951	6122	7911	3833	4556
Expense for threshing	-	-	-	-	4167	5555
TLC (ETB) (B)	8060	8154	8013	9795	11099	13563
TVC (ETB) (C = A+B)	11235	11022	14176	14558	22069	23934
Income from forage	45207	52452	93083	49563	-	-
Income from seed	-	-	-	-	59448	51685
Income from straw	-	-	-	-	15350	20444
GR (ETB) (D)	45207	52452	93083	49563	74797	72129
NR (ETB) (D-C)	33973	41430	78907	35005	52728	48195
BCR (D/C)	4.0	4.8	6.6	3.4	3.4	3.0

TIC, Total input cost; TLC, Total labor cost; TVC, Total variable cost; GR, Gross revenue; NR, Net return; BCR, Benefit-cost ratio; ETB, Ethiopian birr; 1 kg DM Sole oat = 7.10 ETB; 1 kg DM Oat/vetch mixtures = 7.60 ETB; 1 kg DM Desho = 7.10 ETB; 1 kg DM Rhodes = 7.10 ETB; 1 kg wheat straw = 4.35 ETB; 1 kg barley straw = 5.35 ETB; 1 kg wheat grain = 28.50 ETB; 1 kg barley grain = 31.50 ETB; Labor cost for 8 hours = 50 ETB; Mean exchange rate ($ 1 USD = Br. 35.7524).

4 Discussion

4.1 Yield performance of forage and food crops

The herbage dry matter accumulation of forage species didn't vary between locations. This might be related to the similarity in the amount and distribution of precipitation, soil characteristics, and the responsiveness of the crop to the environment. However, between the two years varied significantly, which resulted in differences in rainfall and temperature between the two production years. Environmental factors such as soil characteristics, moisture, and temperature have an impact on forage growth (Basford and Cooper, 1998; Gezahagn et al., 2017). The yielding ability of any crop is mainly controlled by the genetic makeup of a genotype, and it can also be affected by environmental factors (Shahzad et al., 2007). Genotype by environment interaction is the change in a cultivar's relative performance over environments, which results from the differential response of the cultivar to various edaphic, climatic, and biotic factors (Dixon et al., 1994; Gemechu, 2012). The yielding ability of genotypes is the result of their interaction with the environment. There is a strong influence of environmental factors during various stages of crop growth (Bull et al., 1992), thus genotypes differ widely in their response to environments (Romagosa et al., 1996). A genotype grown in different environments will frequently show significant fluctuations in yield performance (Muez et al., 2015).

The yield performance of different forage crops vary due to differences in genetic make-up and their differential response to the growing environments. The recent study done at Holetta indicated that Desho grass had greater herbage DM accumulation compared to other perennial forage species (Mulisa et al., 2021). The mean herbage DM accumulation observed in this study for Desho grass (Bimrew, 2016; Tekalegn et al., 2017; Denbela et al., 2020; Teshale et al., 2021); Rhodes grass (Brima, 2011; Allah and Bello, 2019); Oat/vetch mixtures (Getnet, 1999; Fantahun, 2016); and sole oat (Fekede, 2004; Getnet et al., 2004) were different compared to their respective DM yield reported by different scholars. This might be due to variation in varieties, soil characteristics, moisture, temperature, management, and their interactions.

The utilization of crop residues as an animal feed is a dominant aspect in the mixed crop-livestock production system. The straw yield of wheat in the current study was comparable with the finding of Annicchiarico et al. (2005) but the straw yield obtained from barley was slightly lower than the report of Seyoum et al. (2020). The variation in straw yield between the crops might be associated with differences in genetic makeup and their response to the growing environments. When genotypes grow in different environments, they respond differently due to the differential response of the genotypes to the environments.

The wheat grain yield observed in the current study was comparable with the findings of Annicchiarico et al. (2005) and Rahel and Fekadu (2016) and lower than the previous reports (Erkul et al., 2010; Li et al., 2013). Likewise, barley in this study showed comparable yield (Muhammad et al., 2017) and lower yield (Seyoum et al., 2020) compared to the previous findings. The variation might be related to differences in varieties, growing environments, and management conditions. The grain yield was higher at Holetta than Ejere this might be attributed to the variation of edaphic, climatic, and biotic factors between the testing locations or the differential response of the crops for the growing locations. The mean grain yield varied between the two production years in which a higher yield was produced during the first year of production due to better climatic conditions. The food crops and annual forage crops were re-sown on the same plot in the second year of production. This explicitly indicates that the growing conditions for food crops were less favorable in the cereal mono-cropping than in the crop rotation system. Similar observations were reported by different scholars (Lund, 1993; Soane et al., 2012; Ranjbar et al., 2015; Schlegel et al., 2018).

4.2 Nutritive value of herbage and straw yields

The CP, NDF, ADF, ADL contents, and IVDMD of Desho grass in the present study were higher than in other studies (Bimrew et al., 2017; Mulisa et al., 2021). The CP content of Desho grass was also higher than that reported in different studies (Denbela and Demerew, 2021; Teshale et al., 2021). The ash content of Desho grass in this study was higher than the previous findings (Bimrew et al., 2018; Denbela et al., 2020; Mulisa et al., 2021). The higher ash content in Desho grass could be an indication of high concentration of minerals. The metabolizable energy of Desho grass in the current study was also higher than in the previous study (Bimrew et al., 2017). The CP and ash contents of Rhodes grass reported in this study were higher than other studies (Arshad et al., 2016; Ihsan et al., 2018; Ashenafi et al., 2019). Moreover, the concentration of indigestible fiber or lignin content of this grass was lower than the previously reported value due to better IVDMD in the current study (Ashenafi et al., 2019). The average CP content of sole oat in this study was higher than the value previously reported for different oat varieties (Astatke, 1976; Fekede, 2004; Getnet et al., 2004). The CP and IVDMD observed in this study were higher due to lower fiber contents than the values reported for the same oat variety (Fekede et al., 2007). The CP, NDF, ADL contents of oat/vetch mixtures in the present study were lower while the ash and ADF contents were comparable and higher, respectively than the previous report (Fantahun, 2016). Getnet and Ledin (2001) reported a CP content of 8.6% for oat grown in mixture with vetch and harvested at the soft dough stage which is comparable with the present study.

The CP content of Desho grass, Rhodes grass, oat/vetch mixtures, and sole oat in the current study was higher than the critical level (7.0%) required for optimal rumen function and feed intake in ruminants (Van Soest, 1982). The roughages which have the CP contents ranged from 9.9–15.2; 6.6–9.1; and 3.0–6.1% were classified as high, medium, and low-quality roughages, respectively (Nsahlai et al., 1996). Based on these classifications, Desho and Rhodes grasses are high-quality roughages; sole oat and oat/vetch mixtures are medium quality roughages, and straws of wheat and barley are classified under low-quality roughages. The NDF concentrations of herbage and straws in the current study were above the critical level (55–60%) reported which resulted in lower voluntary intake and feed conversion efficiency (Shirley, 1986). The ADF and ADL concentrations of herbage in the current study lies within the range of 33.3–59.4, and 3.2–11.0%, respectively, as a result, classified under high-quality roughages (Nsahlai et al., 1996). The relatively low ADF and ADL contents in forage crops could indicate that improved forage crops have better digestibility than wheat and barley straws. Despite the considerable differences in IVDMD among the cultivated forage crops in this study, the IVDMD values in this study were lower than the level reported (Moore and Mott, 1973) and IVDMD values greater than 65% indicate high quality feed (Mugeriwa et al., 1973). The nutrient composition of forage crops generally varies depending on genotypic characteristics, environmental conditions, and harvesting stages of the plants (Pascual et al., 2000; Rotili et al., 2001). There is a significant increase in NDF, ADF, and ADL in plants with increased maturity (Kallenbach et al., 2002) and the dilution of CP also increases with increasing plant age (Gezahagn et al., 2014). Furthermore, the variations that existed in the chemical composition of wheat and barley straw realized in this study and those presented in other studies can be explained by the differences in varieties (Seyoum et al., 1998; Adugna et al., 2008), the proportion of botanical fractions (Agbagla-Dohnani et al., 2001), and growing (geographic, seasonal variations, climatic conditions, and soil characteristics) conditions (Mathison et al., 1999; Elseed et al., 2007; Bampidis and Christodulou, 2011).

4.3 Partial budget analysis for food and forage crops

Annual forage crops required lower total input cost (TIC) and total labor cost (TLC) than perennial forage and food crops during the first year of production. The TLC was generally higher than the TIC required for food and forage crops production. The highest total variable cost (TVC) was recorded for food crops (wheat and barley) followed by perennial forage crops (Desho and Rhodes grasses) while the lowest was for annual (sole oat and oat/vetch mixtures) forage crops. The highest TVC required for food crops was due to higher fertilizer cost and additional threshing cost compared to forage crops. The TVC required for annual forage crops was very low compared to food and perennial forage crops due to relatively lower demands of input and labor costs. In the second year of production, the TIC and TLC of food crops were the highest followed by annual forage crops while perennial forage crops required the lowest costs. There was no labor cost for land preparation for perennial forage crops (Desho and Rhodes grasses) in the second year of production, and as a result the TLC for these crops was the lowest compared to food crops and annual forage crops in the second year. The highest TVC was recorded for food crops followed by annual forage crops while perennial forage crops used the lowest cost. The lower TVC for perennial forage crops in the second year of production was due to nil seed and land preparation costs when compared with food crops and annual forage crops. In the combined across year analysis, the mean TIC, TLC, and TVC were higher for the mean of food crops followed by perennial forage crops and annual forage crops. The higher costs of TIC, TLC, and TVC for food crops could be due to higher input and labor costs required for food crops in both production years compared to annual and perennial forage crops. Moreover, the higher costs of TIC, TLC, and TVC for perennial forage crops compared to annual forage crops could be due to higher input and labor costs required particularly in the first year of production than the second year.

Income was generated from selling of forage as hay for forage crops and grain and straw for food crops. Though the grain price of barley was high compared to wheat, the income earned from barley was low due to the low productivity of the crop. However, the income generated from barley straw was higher than wheat straw because of its better nutritional quality (Girma et al., 1996) and its low availability on the market due to high utilization as sole and mixing it with wheat straw by farmers, and its low production and productivity compared to wheat in the study areas. The gross revenue (GR) obtained from food crops was higher than forage crops during the first year of production. The GR mainly depends on the yielding potential of the crops and the unit selling market price of the product. The unit selling market price of the product varies with the location of the markets (farm gate and open market), and seasons (cropping and harvesting) of the year. The price of feed at the farm gate and immediately after the harvesting period is lower than at the open market and dry and wet seasons of the year. Berhanu et al. (2009) also reported that the price of feed in Ethiopia is higher at open market compared to farm gate due to transportation cost and dry and wet seasons due to low supply of feed. Shortage of feed supply during dry and wet seasons is also reported in Ethiopia (Asaminew and Eyasu, 2009) which resulted in higher variations of feed cost over seasons. Spatial and seasonal variability of prices for feed resources is also reported in Ethiopia (Berhanu et al., 2009). The net return (NR) also depends on the amount of yield obtained, the unit selling market price of the product, and the cost of production. The benefit- cost-ratio (BCR) is used to determine whether or not a given activity is cost-effective. The highest BCR obtained from annual forage crop followed by food crops than perennial forage crops implies that annual forage and food crops were making more profit than perennial forage crops in the first year of production. The higher the BCR is the better the economic and management efficiency of the intervention.

The profitability of producing these different forage and grain crops differs if multi-year (second year) budget analysis is considered. The highest GR and NR were generated from perennial forage crops when compared to annual forage and food crops during the second year of production due to low input costs for perennial forage crops in the subsequent production years. The highest GR was recorded from Desho grass followed by Rhodes grass, barley, wheat, and oat/vetch mixtures while sole oat gave the lowest during the second year of production. Moreover, perennial forage crops produced the higher NR than food and annual forage crops. The BCR of perennial forage crops was the highest followed by annual forage crops while the least was observed from food crops indicating perennial and annual forage crops are more profitable than food crops in the second year of production. Generally, perennial forage crops are more profitable in the second year of production and thereafter due to their increased forage yield after the establishment year especially in the cooler highland areas of Ethiopia. Thus, maintaining the perennial forage crops for at least four to five years with optimum management conditions typically give better profit than annual forage and food crops due to their low production cost requirement and increasing biomass yield. The research results also indicated that the herbage DM accumulation in Desho grass is increased linearly up to fourth year of production (Mulisa et al., 2021; Gezahagn et al., 2023).

The combined analysis indicated that Desho grass generated the highest GR followed by wheat and barley due to higher herbage DM accumulation in Desho grass particularly in the second year of production and higher performance of grain and straw yields of food crops (wheat and barley) in the first year of production. The higher mean NR was recorded from the mean of perennial forage crops due to higher herbage DM accumulation, increase of feed price, and lower production costs in the second year of production compared to food and annual forage crops. The lower NR was obtained from food crops and annual forage crops due to lower yield and higher cost of production particularly in the second year of production compared to the first production year. The highest mean BCR was recorded for the mean of perennial forage crops followed by annual forage crops and food crops. This indicated that the perennial forage crops gave better profit across production years.

5 Conclusion

Variation in species, growing environment, and cropping season markedly affected the performance of forage and food crops in the study areas. In the current study, herbage DM accumulations of forage crops were affected by year, species, and the interaction effect of the year by species. The herbage DM accumulation of forage crops varied with production years resulting in a 26% DM yield advantage in the second year of production. Data from the two locations combined over years indicated that Desho grass had DM yield advantages of 104 and 88% over sole oat, and oat/vetch mixtures/Rhodes grass, respectively. In contrast, straw and grain yields of food crops were affected by differences in production years. The straw yield obtained from barley was generally higher while wheat produced better grain yield. In the combined over locations analysis, the yield advantages of 28% for straw yield and 133% for grain yield were observed in the first year of production due to better climatic and growing conditions compared to the second production year. The chemical composition and *in-vitro* dry matter digestibility of herbage and straw yields of forage and food crops varied for all measured parameters at each location and combined over locations. The CP and IVDMD of perennial forage crops were relatively better than that of annual forage crops and straw of food crops.

In recent years, the market for roughages is increasing due to the continuous reduction of grazing areas and expansion of commercial farms mainly in urban and peri-urban areas. Even though the market for the different feed types is increasing from time to time, there is no feed quality control and assurance mechanism in Ethiopia. Moreover, the poor feed marketing system, which is characterized by poor market information, and limited premium price for quality feed, is critically affecting the feed sector in Ethiopia. The economic performance of forage and food crops production indicated that the estimated gross revenue (GR), net return (NR), and benefit-cost ratio (BCR) varied between the two production years and combined across years. In the first year of production the estimated GR, NR, and BCR were the highest for food crops followed by annual forage crops while perennial forage crops produced the least profit. However, in the second year of production, perennial forage crops made the highest GR, NR, and BCR compared to food crops and annual forage crops. Therefore, based on the results of the current study, it can be concluded that cultivation of annual forage crops is profitable in the first year of production compared to perennial forage crops but the profit is much greater for perennial forage crops in the second year of production. The combined partial budget analysis across production years also indicated that the mean of perennial forage crops gave the highest NR compared to the mean of food crops and annual forage crops. This implies the better economic feasibility of perennial forage crops as they can be maintained using minimal management cost once they have been established. Moreover, both yield and profitability of perennial forages can be further increased via applications of management options such as irrigation which can result in multiple harvests and higher annual yield per unit area.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

Author contributions

GK: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Resources, Software, Validation, Visualization, Writing – original draft, Writing – review & editing. MF: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Resources, Software, Validation, Visualization, Writing – review & editing. FF: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – review & editing. GM: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Resources, Software, Validation, Visualization, Writing – review & editing. AK: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – review & editing. KM: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Resources, Software, Validation, Visualization, Writing – review & editing. MD: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – review & editing. DG: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – review & editing. MM: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – review & editing. MB: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – review & editing. KB: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – review & editing. JD: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – review & editing. AA: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The reviewer AM declared a past co-authorship with the author(s) GK, MF, FF, GM, KM, MD, DG to the handling editor.

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Epigenetic insights into creep-feeding: methylation profiling of *Longissimus thoracis* muscle at weaning in crossbred cattle

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Introduction: This study investigated the impact of creep-feeding supplementation on the genome methylation of the *Longissimus thoracis* (LT) muscle in crossbred beef cattle (*Bos taurus* × *Bos indicus*).

Methods: The experiment involved 48 uncastrated F1 Angus-Nellore males (half-siblings), which were divided into two groups: NCF – no creep-feeding (n = 24) and CF – creep-feeding (n = 24). After weaning at 210 days, all animals were feedlot finished for 180 days under identical conditions. LT muscle biopsies were collected at weaning for genomic DNA methylation analysis by reduced representation bisulfite sequencing (RRBS).

Results and discussion: The groups differed significantly (CF > NCF: $p < 0.05$) to weaning weight (243.57±5.70 vs. 228.92±5.07kg), backfat thickness (12.96±0.86 vs. 10.61±0.42mm), LT muscle marbling score (366.11±12.39 vs. 321.50±13.65), and LT intramuscular fat content (5.80±0.23 vs. 4.95±0.20%). The weights at the beginning of the experiment and at slaughter (390 days) did not differ significantly. Mean methylation levels were higher in CF with 0.18% more CpG, 0.04% CHG, and 0.03% CHH. We identified 974 regions with differential methylation (DMRs: > 25% and q < 0.05), which overlapped with 241 differentially methylated genes (DMGs). Among these genes, 108 were hypermethylated and 133 were hypomethylated in CF group. Notably, 39 of these DMGs were previously identified as differentially expressed genes (DEGs: log2 fold change [0.5]) in the same animal groups. Over-representation analysis highlighted epigenetic regulations related to muscle growth, PPAR signaling, adipogenesis, insulin response, and lipid metabolism. Key DMGs/DEGs included: *ACAA1, SORBS1, SMAD3, TRIM63, PRKCA, DNMT3A, RUNX1, NRG3*, and *SLC2A8*. These epigenetic changes improved the performance of supplemented animals up to weaning and enhanced meat quality traits, particularly higher intramuscular fat. The results provided insights into the intricate interplay between nutrition, epigenetics, gene expression and phenotypes in beef cattle production.

KEYWORDS

Bos taurus x Bos indicus, methylome, nutrigenomics, RRBS, supplementation

1 Introduction

Beef cattle farming in Brazil has employed different strategies to increase animal performance and the quality of the meat produced. Particularly interesting strategies include the use of crossbred *Bos taurus* × *Bos indicus* cattle and creep-feeding throughout the cow-calf phase. Angus × Nellore animals are used to produce tender meat with a higher intramuscular fat (IMF) content to add value to the product and to meet the demands of consumers who are willing to pay more for higher quality (Lopes et al., 2020). Creep-feeding consists of supplementing calves during the suckling phase with a grain-based diet rich in energy and protein to obtain heavier individuals at weaning. This approach helps reduce the feeding time during the phase when the carcass is finished for slaughter (Dantas et al., 2010; Scheffler et al., 2014).

Although acetate is one of the main acetyl group donors for IMF deposition, glucose derived from concentrate-rich supplements is also considered an important substrate (Smith and Crouse, 1984). The authors of that study highlighted that the adequate intake of concentrates during the lactation period, especially those rich in starch, favors the proliferation and growth of fat cells. This fact is the result of increased glucose metabolism, which can enhance marbling without increasing visceral fat, thus contributing to improving meat quality (Baldassini et al., 2021). Furthermore, Ferreira et al. (2020) proposed that higher levels of dietary crude protein increase the digestion and intestinal absorption of starch and promote an increase in plasma insulin and glucose which, in turn, increase the deposition of IMF by favoring the synthesis of fatty acid precursors. According to Stewart (2010), several studies have demonstrated that energy supplementation throughout the cow-calf phase can favor IMF deposition; however, to maintain this increase until slaughter, it is necessary to continue feeding cattle grain-based diets.

Animals are able respond to different environmental/nutritional factors, exhibiting phenotypic plasticity because of changes in gene expression patterns (Zhang, 2015). Thus, phenotypes can be modified by nutritional modulations, i.e., dietary exposure can have consequences for growth and for animal health (McKay and Mathers, 2011). New evidence is constantly emerging that nutritional stimuli can modify DNA methylation and thus affect gene expression and the phenotypes of individuals (Day et al., 2015; Farias et al., 2015; Farkas et al., 2015). DNA methylation is a biological process that involves the addition of methyl groups (CH_3) to the cytosine nucleotides of chromosomes. This process contributes to the epigenetic network that controls gene expression and, consequently, phenotypes (Zhang, 2015). Epigenetic marks in the genome, which can modify gene expression but not the DNA sequence, can be acquired and persist throughout an individual's life (Stenz et al., 2018). These marks can also be transmitted from cells of one generation to another (Reik et al., 2001).

Based on the above, the aim of this study was to evaluate for the first time the effects of creep-feeding supplementation on genome methylation and, consequently, on gene expression in the *Longissimus thoracis* (LT) muscle of crossbred F1 Angus x Nellore cattle at weaning, as well as on performance, carcass, and meat quality traits.

2 Materials and methods

2.1 Animals and experimental treatments

All procedures involving animals were approved by the Animal Use Ethics Committee of the School of Agricultural and Veterinary Sciences (FCAV), UNESP, Jaboticabal, São Paulo, Brazil (Protocol number 013689/19).

Forty-eight uncastrated (intact) crossbred F1 Angus-Nellore bulls born to the same Aberdeen Angus (*Bos taurus*) sire (half-siblings) and their mothers (Nellore – *Bos indicus*) with a body weight of 414 ± 45 kg were stratified according to the date and number of births (primiparous or multiparous) and distributed in two 20-hectare *Brachiaria* grass paddocks. Calves were randomly assigned to two treatments from 30 days of age until weaning at 210 days. The following treatments were applied: no creep-feeding (NCF, n = 24) and creep-feeding (CF, n = 24). In the creep-feeding system, calves were kept with their mothers in the same paddock but had exclusive and free access to a supplement (*ad libitum*) corresponding to approximately 1% of their body weight. The adjustment of the supplement amount provided per animal was made weekly based on performance prediction using a diet evaluation and formulation system. The supplement offered to CF animals contained 22% crude protein and 65% total digestible nutrients on a dry matter basis and consisted of ground corn

TABLE 1 Ingredients and chemical-bromatological composition of the feedlot diet.

Ingredients	(g/kg of DM)
Sugarcane bagasse	90
Tifton-85 hay	30
Ground corn	660.35
Soybean meal	175.3
Urea	3.80
Mineral-vitamin supplement	37.4
Chemical composition	**(g/kg of DM)**
Crude protein	153.4
Neutral detergent fiber	256.8
Ether extract	35.3

DM, dry matter.

(44.8%), soybean meal (40.4%), and mineral mix (14.8%). After weaning, the animals of both treatments were taken to the feedlot area where they remained for 180 days in collective covered pens (three animals/pen with 10 m^2 per animal). The two groups of animals received the same diet (Table 1), formulated with the maximum profit ration software RLM 3.3 (Lanna et al., 2011), using the ESALQ Tropicalized NRC system, *ad libitum*, divided into two equal parts (two times a day) at 8:00 am and 4:00 pm. The diet contained 12.6% forage and 87.4% corn-based concentrate.

2.2 Collection of muscle tissue and performance, carcass and meat quality traits

At weaning, LT muscle samples were collected by biopsy from 12 animals randomly selected from each of the two treatments. For biopsy performed at the level of the 13th rib, the thoracic region was shaved and a local anesthetic (lidocaine 2%, 4mg/kg) was administered subcutaneously. After cleaning the site, a 1-cm incision was made and a sterilized Bergstrom biopsy needle was used to obtain 1 g of muscle tissue, which was immediately stored in liquid nitrogen and later in an ultrafreezer at -80°C. These muscle samples were used to extract total RNA for analysis of differential gene expression and DNA for analysis of differentiation of genomic methylation.

During the experiment, the animals were weighed at the beginning of the cow-calf phase (initial body weight – BWi), at weaning (weaning weight – WW), and at the end of the feedlot period (final body weight – BWf). BWi and WW were used to calculate average daily weight gain 1 (ADG1: beginning of the cow-calf phase to weaning), and BWf and WW were used to calculate ADG2 (weaning to the end of the feedlot period).

After 180 days of confinement, the 48 animals were transported to a commercial slaughterhouse and slaughtered after fasting from solids for 16 h by stunning and sectioning of the jugular vein. The carcasses were identified, washed, and divided into two halves. The half-carcasses were weighed individually to obtain the hot carcass weight (HCW) and chilled in the cold room for approximately 24 h at 1°C. After chilling, the carcasses were removed from the room and weighed. After weighing, the LT muscle of the left half-carcass was separated from the remaining carcass and backfat thickness (BFT) and ribeye area (REA: between the 12th and 13th thoracic vertebrae) were measured before deboning.

During deboning, LT samples (portion of approximately 12 cm) were collected between the 12th and 13th ribs of the left half-carcass, vacuum packed, and transported to the laboratory under refrigeration. In the laboratory, the samples were cut into 2.54-cm thick steaks, vacuum packed, and aged for 7 and 14 days. After these periods, physicochemical quality traits were analyzed, including marbling score (MS), IMF content, and Warner-Bratzler shear force (WBSF7 and WBSF14).

Additional information about the carcass and meat quality analyses used in this study can be found in Ramírez-Zamudio et al. (2023).

2.3 Statistical analysis of weight, weight gain, carcass and meat data

Performance data and carcass and meat quality traits of the 48 animals (n = 24/group) [BWi (kg), WW (kg), ADG1 (kg), BWf (kg), ADG2 (kg), HCW (kg), REA (cm^2), BFT (mm), MS (score), IMF (%), and WBSF7 and WBSF14 (kg)] were analyzed regarding the presence of discrepant information, homogeneity of variances, and normality of residuals. The data were expressed as means and their respective standard errors. Means were compared between the two treatments by the *t*-test.

2.4 Analysis of differential gene expression

Differential gene expression was analyzed by RNA sequencing (RNA-Seq) in 12 individuals from each treatment (NCF vs. CF). RNA extraction, preparation of the cDNA libraries, and sequencing and mapping of reads to the bovine reference genome (*Bos taurus* – ARS-UCD1.3) were performed following protocols and parameters recommended by the international scientific community. Differentially expressed genes (DEGs) were identified using the method implemented in the edgeR v.3.40.0 package (Robinson et al., 2010) of the R software, which considers pairwise comparison and assumes that the count data follow a negative binomial distribution. Detailed information on the differential gene expression analysis used can be found in Ramírez-Zamudio et al. (2023).

2.5 Analysis of DNA methylation

2.5.1 Genomic DNA extraction

DNA was extracted from LT muscle samples (50 mg) using the DNeasy Blood & Tissue kit (Qiagen, USA) according to the manufacturer's instructions. The extracted DNA was quantified in a Qubit® 3.0 Fluorometer (Invitrogen, USA). DNA purity (260/280) and integrity were analyzed in a NanoDrop Lite microvolume spectrophotometer (Thermo Scientific, USA) and on 0.8% agarose gel, respectively. After dilution to the concentration necessary for methylation analysis (50 ng/μL), the DNA samples were stored in 1.7-mL minitubes at -20°C.

2.5.2 Library construction and reduced representation bisulfite sequencing

For analysis of differential DNA methylation, five DNA samples were randomly selected from the 12 animals of each treatment (NCF and CF) preliminarily submitted to RNA-Seq. The reduced representation bisulfite sequencing (RRBS) technique was applied using the Zymo-Seq RRBS Library kit (Zymo Research, USA) to sequence genome regions enriches for 5-methyl cytosines.

Digestion of genomic DNA aliquots, bisulfite conversions, and enrichment of the 10 libraries by PCR (one library/animal) were performed by the US company Zymo Research. The protocol consisted of digestion of 400 ng genomic DNA with 30 units of *Msp*I (New England Biolab, USA) at 37°C, a restriction enzyme with a CCGG recognition site, followed by end repair of the fragments of approximately 150 bp and addition of adapters.

The fragments were ligated to adapters containing 5-methyl cytosine instead of cytosine as specified by Illumina (USA). Adapter-tagged fragments ≥ 50 bp were recovered using DNA Clean & Concentrator™-5 (Zymo Research, USA) and then treated with bisulfite using the EZ DNA Methylation-Lightning™ kit (Zymo Research, USA). The resulting PCR products for enrichment of the libraries were again purified using DNA Clean & Concentrator™-5. Clustering and sequencing of the libraries of the DNA fragments were performed by Zymo (USA) using HiSeq2500 v4 2x100bp (Illumina, USA) according to the manufacturer's protocol on the NovaSeq 6000 platform (Illumina, USA).

2.5.3 Quality analysis of RRBS sequences and alignment to the reference genome

The FastQC 0.11.9 program (Andrews, 2010) was used to visualize the data obtained by RRBS, in which quality parameters were evaluated for the complete set of raw sequencing reads. Initial quality control was performed using TrimGalore v.0.6.4_dev (https://www.bioinformatics.babraham.ac.uk/projects/trim_galore/), in which adapters and unknown or low-quality bases were removed from the ends of the sequences.

The Bismark v.0.22.3 program (Krueger and Andrews, 2011) was used to align the generated sequences previously treated with bisulfite to the bovine reference genome (*Bos taurus* – ARS-UCD1.2) using the -N1 option, which allows a single alignment error. The reference genome was first transformed into a bisulfite-converted version, with C-to-T conversion on the top original strand and G-to-A conversion on the bottom strand, and finally indexed using Bowtie2 (Langmead and Salzberg, 2012). The sequences that produced the best unique alignments in the two processes (top and bottom original strand alignment) were compared to the normal genomic sequence to confirm the presence of a C in the reference sequence. The methylation status of all cytosine positions in the sequences was inferred using the methylation_extractor function of the Bismark program. The rate or level of methylation ranged from 0% to 100% at each site (nucleotide), considering that tissues frequently comprise different populations of cells that can harbor different methylation signals.

2.5.4 Identification and characterization of cytosines, chromosome regions and differentially methylated genes

The metrics for cytosine methylation in the genome of the individuals analyzed were obtained with MethylDackel 0.5.0 (https://github.com/dpryan79/MethylDackel). This tool groups all cytosines into one of three sequence contexts – CpG, CHG and CHH, where H is the IUPAC ambiguity code for any nucleotide other than G.

To identify regions in the genome that are differentially methylated between NCF and CF individuals (differentially methylated regions – DMRs), i.e., regions that contain more than one methylated cytosine, the calculateDiffMeth function of the MethylKit 1.24.0 package was used (Akalin et al., 2012) in the R statistical environment (R Core Team: https://www.r-project.org) of the Bioconductor repository (https://bioconductor.org/packages/release/bioc/html/ methylKit.html). First, all cytosines were filtered for a minimum coverage of three reads and a maximum percentile of 99.9%. The methylKit function that normalizes coverage was applied using standard configurations. Next, the sliding window approach was used, with a window size of 1,000 bp and a step size of 500 bp. DMRs were defined when 1) the methylation difference was at least 25%; 2) the corrected *p*-value (*q*-value) was less than 0.05, and 3) sequence coverage was greater than 10-fold across all samples. Fisher's exact test was implemented to calculate *p*-values, which were subsequently adjusted to *q*-values using the SLIM method (Wang et al., 2011).

Genes with ensemble id's overlapping with DMRs shared between animals of the two groups were identified and termed differentially methylated genes (DMGs). The annotation of DMGs for identifying gene regions that harbor DMRs (promoter, transcription start site – TSS, gene body – exon and intron) was performed using the GenomationData package (version 3.8) through the methylKit library 1.24.0 (Akalin et al., 2012) of the R statistical software. The NCBI RefSeq annotation database file used was obtained from the UCSC Genome Browser. Promoters were defined 1,000 bp upstream and downstream of the gene's TSS following the methylKit pattern. Information on CpG islands (CpGi) and shores was obtained from the UCSC Genome Browser.

2.5.5 Relationship between DMGs and DEGs

To identify genes exhibiting modified expression at weaning due to methylation, the list of genes identified as DMGs was contrasted with the list of DEGs, considering the filters of a 25%

methylation difference and a $\log_2$ fold change (FC) of 0.5. In eukaryotes, CpGi are mainly located in gene promoter regions (regulatory regions), near the TSS. Methylation in these regions is associated with gene silencing; thus, in the absence of methylation of these islands, gene expression occurs (Oliveira et al., 2010; Portela and Esteller, 2010; Yuferov et al., 2010). CpG dinucleotides found outside these islands in regions known as “CpGi shores” are less methylated than the islands themselves (Tsankova et al., 2007; Robison and Nestler, 2011), but can also be associated with transcriptional inactivation. However, when methylation occurs in the body of genes (exons and introns), it may also be correlated with their expression (Doi et al., 2009; Irizarry et al., 2009; Portela and Esteller, 2010). CpGs in the gene body (exons/introns) and far from the TSS can inhibit or enhance gene expression (as long as the promoter is not methylated), depending on the genomic context. Methylation (CpG) of the gene body that occurs in a CpGi may be positively or negatively correlated with expression. On the other hand, if methylation does not occur in a CpGi, there will be a positive correlation with expression, i.e., increasing/decreasing methylation will lead to an increase/decrease in gene expression (Varley et al., 2013). These relationships were established and described by Enright et al (2003); Tost (2010), and Varley et al. (2013). Thus, the relationships found were classified as consistent (expected) or inconsistent (not expected), as shown in Figure 1.

2.5.6 Functional enrichment analysis

The set of genes identified as differentially methylated and differentially expressed – DMGs/DEGs – between NCF and CF individuals at weaning was used in over-representation analysis considering gene ontology (GO) terms (biological processes – BPs) and metabolic pathways (KEGG Pathway and WikiPathway databases). The WebGestalt tool, an online platform designed for functional analysis and interpretation of biological data (Wang et al., 2017), was used for this purpose. GO_BPs and pathways were enriched when $p < 0.05$.

3 Results

3.1 Performance, carcass and meat quality traits and differentially expressed genes at weaning

Regarding pre- and postweaning performance (Table 2), significant differences ($p < 0.05$) were observed between groups for WW and ADG1 during the cow-calf phase. For WW, CF animals (creep-feeding) were heavier than NCF animals (no creep-feeding) ($p < 0.05$). ADG1 was higher in CF compared to NCF ($p < 0.05$). No significant differences in BWi, BWf, ADG2, or HCW were observed between groups.

The meat quality and carcass traits are presented in Table 3. No significant differences between groups were observed for REA, WBSF7, or WBSF14. On the other hand, there were significant differences ($p < 0.05$) in BFT, IMF, and MS. For all three traits related to carcass fat deposition, CF animals (creep-feeding) had higher mean values than NCF animals (no creep-feeding).

A total of 947 differentially expressed genes between NCF and CF ($\log_2$ FC [0.5]; FDR 5%) were identified; of these, 443 were downregulated and 504 were upregulated in CF (cow-calf phase with creep-feeding). Detailed information on the results of DEG analysis can be found in Ramírez-Zamudio et al. (2023).

3.2 Quality of extracted genomic DNA, sequencing and alignment of reads

The genomic DNA extracted from LT muscle samples obtained by biopsy at weaning had adequate quality for RRBS. The 260/280 ratio ranged from 1.87 to 2.01 and the 260/230 ratio from 1.48 to 1.97. The DNA concentration ranged from 83.4 to 752 ng/μL and agarose gel electrophoresis showed high molecular weight DNA samples, with no signs of degradation.

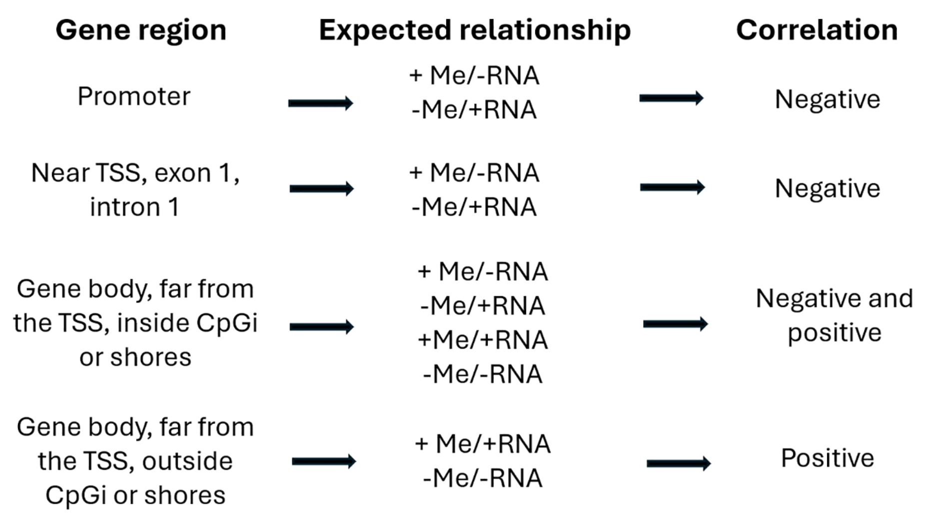

FIGURE 1
Expected relationships between the level of methylation in gene regions and expression in eukaryotic organisms.

TABLE 2 Means and standard errors of animal weights at the beginning of the cow-calf phase, at weaning and at the end of the feedlot period, average daily gain during the cow-calf and finishing periods, and hot carcass weight obtained for the two treatments.

	BWi (kg)	WW (kg)	ADG1 (kg)	BWf (kg)	ADG2 (kg)	HCW (kg)
NCF[1]	61.29 ± 2.41	228.92 ± 5.07[b]	0.93 ± 0.02[b]	484.64 ± 5.96	1.36 ± 0.02	269.22 ± 8.23
CF[1]	57.55 ± 2.61	243.57 ± 5.70[a]	1.03 ± 0.03[a]	491.85 ± 7.85	1.32 ± 0.03	273.62 ± 9.28

[1]NCF, no creep-feeding; CF, creep-feeding; BWi, initial body weight; WW, weaning weight; ADG1, average daily gain between WW and BWi; BWf, final body weight; ADG2, average daily gain between BWf and WW; HCW, hot carcass weight. [a, b]Different letters indicate significant differences ($p < 0.05$).

TABLE 3 Means and standard errors of carcass backfat thickness and ribeye area, fat percentage, marbling score, and shear force after 7 and 14 days of aging obtained for the two treatments.

	BFT (mm)	IMF (%)	MS	REA (cm^2)	WBSF7 (kg)	WBSF14 (kg)
NCF[1]	10.61 ± 0.42[b]	4.95 ± 0.20[b]	321.50 ± 13.65[b]	67.94 ± 1.16	4.52 ± 0.11	3.45 ± 0.11
CF[1]	12.96 ± 0.83[a]	5.80 ± 0.23[a]	366.11 ± 12.39[a]	65.50 ± 0.93	4.28 ± 0.12	3.42 ± 0.09

[1]NCF, no creep-feeding; CF, creep-feeding; BFT, backfat thickness; IMF, intramuscular fat percentage; MS, marbling score; REA, ribeye area; WBSF7, Warner-Bratzler shear force at 7 days post-mortem; WBSF14, Warner-Bratzler shear force at 14 days post-mortem. [a, b]Different letters indicate significant differences ($p < 0.05$).

Sequencing of the bisulfite-converted DNA samples/libraries generated an average of approximately 32 million raw reads for samples of the two experimental groups. Approximately 33% of the reads generated per sample showed unique alignment, i.e., they aligned with only one region of the reference genome. About 45% of the reads were mapped to two or more regions and about 22% could not be aligned to the reference genome (Figure 2).

The rate of bisulfite conversion of unmethylated cytosines exceeded 99% for CpG and non-CpG, indicating adequate bisulfitation of the samples.

Among reads mapped to a unique site in the genome, an average of seven million CpG cytosines were identified per sample, with a mean coverage of approximately 7X for CpG, as shown in Table 4.

3.3 Identification and characterization of differentially methylated regions

For animals in CF, the mean genome-wide methylation level was 4.25% of CpG, 0.54% of CHG (where H = A, T or C), and 0.46% of CHH. The values for NCF animals were 4.07% of CpG, 0.50% of CHG and 0.43% of CHH, resulting in a higher mean methylation level (0.18% of CpG, 0.04% of CHG, and 0.03% of CHH) in animals of the supplemented group during the cow-calf phase.

Based on the contrast established between the groups of animals, 974 regions that differed in methylation levels at weaning were identified (DMRs: > 25% and q-value < 0.05). In CF, 431 regions showed hypermethylation, corresponding to

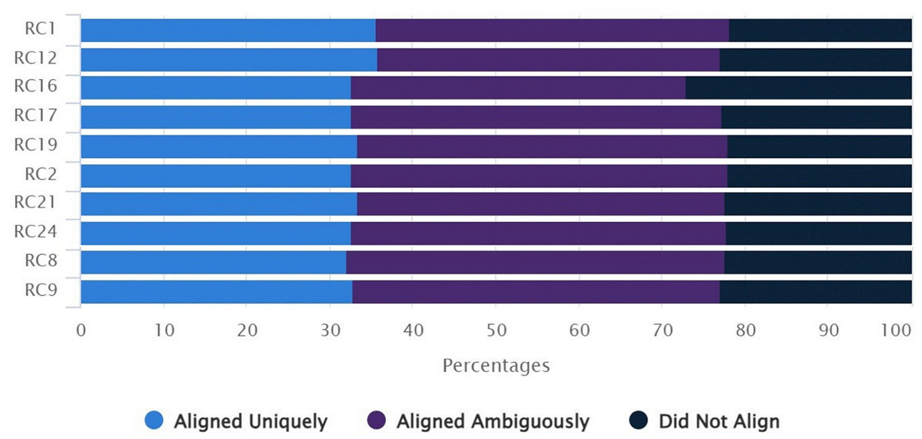

FIGURE 2
Percentage of reads generated by RRBS aligned or not to the bovine reference genome. RC1, RC2, RC8, RC9 and RC12: samples from NCF; RC16, RC17, RC19, RC21 and RC24: samples from CF. Blue bar: percentage of reads aligned to a unique region of the reference genome; purple bar: percentage of reads aligned to two or more regions of the reference genome; black bar: percentage of reads not aligned to regions of the reference genome.

TABLE 4 Data generated by reduced representation bisulfite sequencing.

Sample	No. of reads	% BS conversion (non-CpG)	% BS conversion (CpG)	Uniq. CpG	Avg. CpG coverage
RC1/NCF	38,689,684	99.4%	99.6%	7,440,987	10X
RC2/NCF	28,329,540	99.3%	99.5%	6,939,864	7X
RC8/NCF	30,538,031	99.4%	99.6%	6,946,079	7X
RC9/NCF	26,731,168	99.4%	99.7%	6,698,465	7X
RC12/NCF	37,295,594	99.3%	99.5%	7,724,811	9X
RC16/CF	25,249,436	99.3%	99.5%	6,778,697	6X
RC17/CF	37,041,812	99.3%	99.5%	7,373,151	8X
RC19/CF	33,405,004	99.3%	99.6%	7,129,808	8X
RC21/CF	32,170,424	99.3%	99.5%	7,198,074	8X
RC24/CF	27,669,506	99.3%	99.6%	6,915,952	7X

RC1, RC2, RC8, RC9 and RC12: samples from NCF; RC16, RC17, RC19, RC21 and RC24: samples from CF. % BS Conversion (non-CpG): percentage of successful bisulfite conversion of unmethylated cytosines (non-CpG), % BS Conversion (CpG): percentage of successful bisulfite conversion of methylated cytosines (CpG), Uniq. CpG: methylated cytosines exclusive of CpG, Avg. CpG coverage: average read coverage for CpGs.

44.25% of all regions identified, while 543 showed hypomethylation, corresponding to 55.75%. Among DMRs, 54% occurred in intergenic regions of the genome, while the remaining regions were distributed across promoter and gene body regions (Figure 3A). About 22% of the DMRs were in CpGi, while 25% were present in island shores (Figure 3B).

The 974 prospected DMRs overlapped with 241 genes which, as mentioned above, were called DMGs; among these genes, 108 were hypermethylated and 133 hypomethylated in CF. Of the 241 DMGs, 39 were correlated with DEGs, with a $\log_2$ FC $\geq$ 0.5 between the two experimental conditions. Part of the differentially expressed DMGs (DMGs/DEGs) exhibited more than one DMR, totaling 79 DMRs for 39 DMGs/DEGs (Table 5).

Twenty-one positively correlated DMGs/DEGs were identified; among these, nine [*AQP1*, *ATP2B2*, *CDK18*, *CLSTN2*, *CSF1R*, *CUX2*, *GNA12*, *HECTD2*, and *HERC1*] were hypermethylated and upregulated. Twelve genes [*ACAA1*, *CCDC146*, *FHL2*, *RALGPS1*, *RET*, *RFC2*, *RUNX1*, *SART1*, *SLC2A8*, *SORBS1*, *TNFRSF1B*, and *VWF*] exhibited hypomethylation associated with downregulation. On the other hand, 18 DMGs/DEGs showed a negative correlation. Among these, 10 [*ANXA9*, *DAPP1*, *FERMT3*, *IL6R*, *LAPTM5*, *NRG3*, *RBM47*, *SPI1*, *TP63*, and *TRIM63*] were identified as hypermethylated and downregulated, while eight [*COL27A1*, *CSDC2*, *DNMT3A*, *ENO3*, *GADL1*, *PRKCA*, *SMAD3*, and *UNC5B*] were characterized as hypomethylated and upregulated genes.

Thirty-three (85%) of the 39 DMGs/DEGs showed a methylation pattern compatible with the expression pattern (expected/consistent – according to Figure 1) and only six (15%) [*ANXA9*, *ENO3*, *FHL2*, *GADL1*, *IL6R*, and *TP63*] exhibited an unexpected/inconsistent pattern.

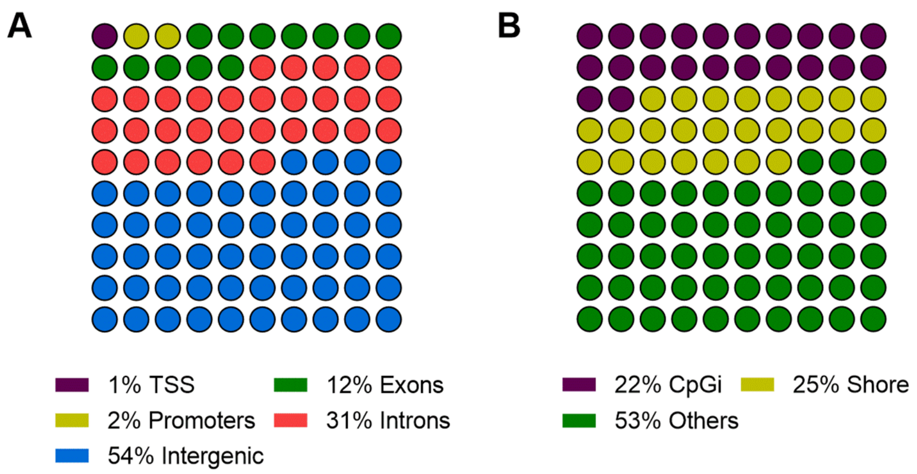

FIGURE 3
Proportion of DMRs among NCF and CF detected in different parts of the genome. **(A)** Overlap with intergenic and gene regions (promoter, transcription start site – TSS, exon and intron). **(B)** Overlap with CpG islands (CpGi), island shores, and others.

TABLE 5 Set of genes differentially methylated (DMGs) and differentially expressed (DEGs) between groups 1 and 2.

DMG/DEG	Gene Part	CpGi/shores	MeDiff	logFC.Rna	Correlation
ACAA1	exons_3	Yes	-25.670	-0.511	Positive
ACAA1	promoters_0	Yes	-25.670	-0.511	Positive
ACAA1	introns_2	Yes	-25.670	-0.511	Positive
ACAA1	introns_3	Yes	-25.670	-0.511	Positive
ANXA9	introns_7	No	25.287	-0.918	Negative
ANXA9	exons_9	No	25.287	-0.918	Negative
ANXA9	introns_8	No	25.287	-0.918	Negative
ANXA9	exons_8	No	25.287	-0.918	Negative
ANXA9	introns_9	No	25.287	-0.918	Negative
AQP1	introns_1	No	-30.879	0.728	Negative
AQP1	exons_1	No	26.541	0.728	Positive
AQP1	TSSes_0	No	26.541	0.728	Positive
AQP1	introns_1	No	26.541	0.728	Positive
AQP1	promoters_0	No	26.541	0.728	Positive
ATP2B2	introns_20	Yes	30.690	2.221	Positive
CCDC146	exons_3	No	-27.081	-0.685	Positive
CCDC146	introns_3	No	-27.081	-0.685	Positive
CDK18	introns_2	No	36.149	0.576	Positive
CDK18	promoters_0	No	36.149	0.576	Positive
CDK18	introns_3	No	36.149	0.576	Positive
CDK18	exons_2	No	36.149	0.576	Positive
CDK18	exons_3	No	36.149	0.576	Positive
CLSTN2	introns_8	No	25.210	1.093	Positive
COL27A1	introns_37	No	-34.545	0.550	Negative
CSDC2	exons_3	Yes	-25.559	1.036	Negative
CSDC2	introns_3	Yes	-25.559	1.036	Negative
CSDC2	introns_2	Yes	-25.559	1.036	Negative
CSF1R	exons_8	No	-25.725	-1.810	Positive
CSF1R	exons_9	No	-25.725	-1.810	Positive
CSF1R	introns_8	No	-25.725	-1.810	Positive
CSF1R	introns_9	No	-25.725	-1.810	Positive
CUX2	introns_3	No	25.526	1.249	Positive
DAPP1	introns_4	No	26.740	-0.531	Negative
DNMT3A	introns_1	No	-26.425	0.792	Negative
ENO3	introns_9	No	-39.722	0.839	Negative
ENO3	exons_8	No	-39.722	0.839	Negative
ENO3	introns_10	No	-39.722	0.839	Negative
ENO3	exons_10	No	-39.722	0.839	Negative

(Continued)

TABLE 5 Continued

DMG/DEG	Gene Part	CpGi/shores	MeDiff	logFC.Rna	Correlation
ENO3	introns_8	No	-39.722	0.839	Negative
ENO3	exons_9	No	-39.722	0.839	Negative
FERMT3	promoters_0	Yes	37.626	-0.744	Negative
FERMT3	introns_2	Yes	37.626	-0.744	Negative
FHL2	promoters_0	No	-37.952	-0.526	Positive
GADL1	introns_14	No	-33.765	3.082	Negative
GNA12	introns_1	No	25.339	0.840	Positive
HECTD2	introns_1	Yes	29.870	0.514	Positive
HERC1	introns_1	No	25.726	0.634	Positive
IL6R	introns_9	No	32.843	-2.065	Negative
IL6R	exons_10	No	32.843	-2.065	Negative
LAPTM5	introns_1	No	27.587	-0.583	Negative
NRG3	introns_1	No	25.066	-0.820	Negative
PRKCA	introns_2	No	-25.462	0.558	Negative
RALGPS1	introns_8	No	-25.842	-0.719	Positive
RBM47	introns_3	Yes	29.249	-0.504	Negative
RBM47	exons_3	Yes	29.249	-0.504	Negative
RBM47	introns_2	Yes	29.249	-0.504	Negative
RET	introns_1	No	-25.355	-1.746	Positive
RFC2	exons_2	No	-48.252	-0.520	Positive
RFC2	introns_2	No	-48.252	-0.520	Positive
RFC2	introns_1	No	-48.252	-0.520	Positive
RUNX1	introns_2	No	-28.744	-0.885	Positive
SART1	introns_12	Yes	-26.154	-0.585	Positive
SLC2A8	introns_9	No	-32.060	-1.383	Positive
SLC2A8	exons_10	No	-32.060	-1.383	Positive
SMAD3	introns_1	No	-27.604	1.013	Negative
SORBS1	introns_7	Yes	-31.747	-1.008	Positive
SPI1	TSSes_0	No	28.741	-0.533	Negative
SPI1	promoters_0	No	28.741	-0.533	Negative
SPI1	introns_1	No	28.741	-0.533	Negative
SPI1	exons_1	No	28.741	-0.533	Negative
SPI1	introns_1	No	25.330	-0.533	Negative
SPI1	promoters_0	No	25.330	-0.533	Negative
TNFRSF1B	introns_1	No	-29.221	-0.633	Positive
TP63	introns_3	No	28.317	-0.592	Negative
TRIM63	exons_2	No	27.249	-1.028	Negative

(Continued)

TABLE 5 Continued

DMG/DEG	Gene Part	CpGi/shores	MeDiff	logFC.Rna	Correlation
TRIM63	introns_2	No	27.249	-1.028	Negative
TRIM63	introns_1	No	27.249	-1.028	Negative
UNC5B	introns_1	No	-36.815	0.524	Negative
VWF	introns_2	No	-25.429	-0.536	Positive

DMG/DEG, differentially methylated and expressed genes; Gene Part, part of the gene where methylation occurred; CpGi/shores, CpG islands or shores; MeDiff, magnitude of differential methylation (%); logFC.Rna, magnitude of differential expression.

3.4 Functional enrichment analysis of DMGs/DEGs

Table 6 shows the BP-related GO terms enriched ($p < 0.05$) in the analysis involving the 39 DMGs/DEGs identified in animals of NCF and CF. Tables 7 and 8 list the enriched metabolic pathways ($p < 0.05$) using information from the KEGG and WikiPathways databases as a reference, respectively.

The higher WW, carcass fat coverage, and marbling (IMF and MS) observed in CF animals indicate that supplementation during

TABLE 6 Enriched biological processes (GO_BPs) considering genes differentially methylated and differentially expressed (DMGs/DEGs) between groups 1 and 2 at weaning.

Identification	Description	No. of genes	*p*-value	Genes
GO:0097191	Extrinsic apoptotic signaling pathway	5	<0.001	*IL6R*, *RET*, *SMAD3*, *TNFRSF1B*, *UNC5B*
GO:0051094	Positive regulation of developmental process	11	<0.001	*AQP1*, *CLSTN2*, *CUX2*, *IL6R*, *PRKCA*, *RET*, *RUNX1*, *SART1*, *SMAD3*, *TNFRSF1B*, *TP63*
GO:0048646	Anatomical structure formation involved in morphogenesis	10	<0.001	*AQP1*, *FHL2*, *HERC1*, *NRG3*, *PRKCA*, *RET*, *RUNX1*, *SMAD3*, *TP63*, *UNC5B*
GO:0045785	Positive regulation of cell adhesion	6	<0.001	*IL6R*, *PRKCA*, *RET*, *RUNX1*, *SART1*, *SMAD3*
GO:0044057	Regulation of system process	7	<0.001	*AQP1*, *ATP2B2*, *CUX2*, *PRKCA*, *SMAD3*, *TNFRSF1B*, *TRIM63*
GO:0043502	Regulation of muscle adaptation	4	<0.001	*PRKCA*, *SMAD3*, *TNFRSF1B*, *TRIM63*
GO:0043500	Muscle adaptation	4	<0.001	*PRKCA*, *SMAD3*, *TNFRSF1B*, *TRIM63*
GO:0035239	Tube morphogenesis	9	<0.001	*AQP1*, *CSF1R*, *PRKCA*, *RET*, *RUNX1*, *SMAD3*, *SPI1*, *TP63*, *UNC5B*
GO:0033993	Response to lipid	9	<0.001	*AQP1*, *DNMT3A*, *FHL2*, *PRKCA*, *RET*, *RUNX1*, *TNFRSF1B*, *TP63*, *TRIM63*
GO:0030225	Macrophage differentiation	3	<0.001	*CSF1R*, *PRKCA*, *SPI1*
GO:0022610	Biological adhesion	11	<0.001	*ANXA9*, *CLSTN2*, *FERMT3*, *IL6R*, *PRKCA*, *RET*, *RUNX1*, *SART1*, *SMAD3*, *SORBS1*, *VWF*
GO:0014897	Striated muscle hypertrophy	4	<0.001	*PRKCA*, *SMAD3*, *TNFRSF1B*, *TRIM63*
GO:0014896	Muscle hypertrophy	4	<0.001	*PRKCA*, *SMAD3*, *TNFRSF1B*, *TRIM63*
GO:0014743	Regulation of muscle hypertrophy	4	<0.001	*PRKCA*, *SMAD3*, *TNFRSF1B*, *TRIM63*
GO:0014741	Negative regulation of muscle hypertrophy	3	<0.001	*SMAD3*, *TNFRSF1B*, *TRIM63*
GO:0010614	Negative regulation of cardiac muscle hypertrophy	3	<0.001	*SMAD3*, *TNFRSF1B*, *TRIM63*
GO:0010611	Regulation of cardiac muscle hypertrophy	4	<0.001	*PRKCA*, *SMAD3*, *TNFRSF1B*, *TRIM63*
GO:0007155	Cell adhesion	11	<0.001	*ANXA9*, *CLSTN2*, *FERMT3*, *IL6R*, *PRKCA*, *RET*, *RUNX1*, *SART1*, *SMAD3*, *SORBS1*, *VWF*
GO:0003300	Cardiac muscle hypertrophy	4	<0.001	*PRKCA*, *SMAD3*, *TNFRSF1B*, *TRIM63*
GO:0001775	Cell activation	11	<0.001	*ACAA1*, *FERMT3*, *GNA12*, *IL6R*, *PRKCA*, *RUNX1*, *SART1*, *SMAD3*, *SPI1*, *TNFRSF1B*, *VWF*

TABLE 7 Enriched metabolic pathways (KEGG Pathways) considering genes differentially methylated and differentially expressed (DMGs/DEGs) between groups 1 and 2 at weaning.

Identification	Description	No. of genes	*p*-value	Genes
hsa05200	Pathways in cancer	8	0.001	*CSF1R, GNA12, IL6R, PRKCA, RET, RUNX1, SMAD3, SPI1*
hsa05221	Acute myeloid leukemia	3	0.002	*CSF1R, RUNX1, SPI1*
hsa04066	HIF-1 signaling pathway	3	0.006	*ENO3, IL6R, PRKCA*
hsa04659	Th17 cell differentiation	3	0.007	*IL6R, RUNX1, SMAD3*
hsa04380	Osteoclast differentiation	3	0.012	*CSF1R, FHL2, SPI1*
hsa04730	Long-term depression	2	0.021	*GNA12, PRKCA*
hsa04520	Adherens junction	2	0.029	*SMAD3, SORBS1*
hsa03320	PPAR signaling pathway	2	0.031	*ACAA1, SORBS1*
hsa05202	Transcriptional misregulation in cancer	3	0.032	*CSF1R, RUNX1, SPI1*
hsa05220	Chronic myeloid leukemia	2	0.033	*RUNX1, SMAD3*

the cow-calf phase favored muscle growth and development until weaning and lipid metabolism (adipogenesis and lipogenesis) until slaughter. Thus, BPs, metabolic pathways, and genes directly related to muscle development and fat deposition in the carcass were identified. The following BPs linked to muscle development can be highlighted: negative regulation of muscle hypertrophy (GO:0014741), regulation of muscle hypertrophy (GO:0014743), muscle hypertrophy (GO:0014896), striated muscle hypertrophy (GO:0014897), muscle adaptation (GO:0043500), and regulation of muscle adaptation (GO:0043502). These processes are related to the response of the muscle to different stimuli, not only for muscle growth but also for functional adaptations that improve the efficiency of this tissue. Another important process, response to lipid (GO:0033993), is related to the mechanisms of lipid metabolism. Regarding metabolic pathways, the following pathways can be highlighted as the most relevant: *PPAR signalin pathway* (hsa03320, WP3942) and *factors and pathways affecting insulin-like growth factor (IGF1)-Akt signaling* (WP3850).

Considering the enriched pathways and processes identified as the most important for the contrasting phenotypes between groups, the following DMGs/DEGs can be highlighted: *ACAA1*, *SORBS1*, *SMAD3*, *TRIM63*, *PRKCA*, *DNMT3A*, *FHL2*, *NRG3*, *RUNX1*, and *TP63*. The *FHL2* and *TP63* genes will not be included in the discussion since their methylation and expression patterns were incompatible with the expected. Although the *SLC2A8* DMG/DEG is not part of any enriched BP or pathway, this gene will be discussed due to its high affinity for glucose in skeletal muscle and adipose tissues.

4 Discussion

4.1 Effect of supplementation on genomic methylation

Although subtle, the difference in the global methylation pattern between NCF and CF calves indicates changes in the genome in

TABLE 8 Enriched metabolic pathways (WikiPathways) considering genes differentially methylated and differentially expressed (DMGs/DEGs) between groups 1 and 2 at weaning.

Identification	Description	No. of genes	*p*-value	Genes
WP4540	Pathways regulating hippo signaling	3	0.005	*CSF1R, PRKCA, SMAD3*
WP3850	Factors and pathways affecting insulin-like growth factor (IGF1)-Akt signaling	2	0.006	*SMAD3, TRIM63*
WP2848	Differentiation pathway	2	0.013	*CSF1R, IL6R*
WP3646	Hepatitis C and hepatocellular carcinoma	2	0.014	*IL6R, SMAD3*
WP4747	Netrin-UNC5B signaling pathway	2	0.015	*PRKCA, UNC5B*
WP2018	RANKL/RANK [receptor activator of NFKB (ligand)] signaling pathway	2	0.017	*FHL2, SPI1*
WP2849	Hematopoietic stem cell differentiation	2	0.022	*RUNX1, SPI1*
WP3945	TYROBP causal network	2	0.022	*RBM47, TNFRSF1B*
WP2324	AGE/RAGE pathway	2	0.024	*PRKCA, SMAD3*
WP3942	PPAR signaling pathway	2	0.025	*ACAA1, SORBS1*

response to creep-feeding (increased methylation in CF). Andrés et al. (2021) demonstrated that diet can influence DNA methylation patterns by analyzing global blood methylation patterns, biochemical profiles, and metabolomes of ewe lambs born to mothers subjected to early food restriction or fed ad libitum. Results revealed significant enrichment in functional categories related to cellular processes, phosphorylation, nervous system, immune response, and reproductive function in lambs born to mothers with food restriction, attributed to differences in gene methylation within these categories. Nutrition can influence genome methylation patterns by providing substrates necessary for adequate DNA methylation or cofactors that modulate the enzymatic activity of DNA methyl transferases (DNMTs). As a universal methyl donor for methyltransferases, S-adenosylmethionine (SAM) is synthesized in the methionine cycle from different dietary precursors (McKay and Mathers, 2011; Feil and Fraga, 2012). All of these precursors enter the methionine pathway at different points and contribute to the net synthesis of SAM. Thus, increased availability of SAM results in global DNA hypermethylation and vice versa (Zhang, 2015). In addition to the indirect regulation of DNA methylation patterns by SAM modulation, several other dietary compounds can directly influence the expression or activity of DNMTs (Mukherjee et al., 2015).

Del Corvo et al. (2021) demonstrated an increase in methylation of approximately 0.54% in CpG, 0.06% in CHG, and 0.02% in CHH in Nellore cattle recovered after a period of heat stress. Values in Angus were 67.7% CG, 0.3% CHG, and 0.2% CHH methylation in the experimental period and 67.14% CG, 0.3% CHG, and 0.22% CHH methylation in the post challenge period. Even if they do not reach significance on a genomic scale, these changes can lead to distinct patterns of gene expression and, consequently, to divergent phenotypes (Ribeiro, 1994; Miller and Grant, 2012). Furthermore, analyzing the bovine methylome, De Souza et al. (2022) identified epigenetic mechanisms associated with meat tenderness in the Nellore breed, as well as genes with differential methylation in contrasting groups for meat tenderness. Taken together, these studies demonstrate the need to investigate genes that show differential methylation in contrasting groups of animals, either due to differences in a particular phenotype of interest or as a result of exposure to different experimental treatments, as well as to elucidate the metabolic pathways affected by these changes. Given that supplementation affects production costs, a limitation of this research was the absence of testing various supplementation levels. This could potentially reveal similar outcomes with lower supplementation levels or even more intriguing results with higher levels.

4.2 Metabolic pathways related to muscle and adipose tissue development

As practical implications, this study demonstrated that energy-protein supplementation of F1 Angus x Nellore calves during the rearing phase at 1% of their live weight was sufficient to promote, through changes in DNA methylation patterns and gene expression, increased muscle and fat deposition in the carcass, traits of interest to the market.

The *insulin-like growth factor 1 (IGF1)-Akt signaling* pathway plays a crucial role in muscle growth, influencing both protein synthesis and degradation by activating the *mTOR pathway* (Schiaffino and Mammucari, 2011; Jia et al., 2014; Yoshida and Delafontaine, 2020) and by inhibiting GSK3B (Cross et al., 1994, Cross et al., 1995) and FoxO transcription factors, thereby preventing muscle atrophy (Sartori et al., 2021). Within this context, calves that received a daily supplement of 1% of their body weight containing 22% crude protein and 65% total digestible nutrients, as well as inclusion of 44.8% ground corn as a glucose source, responded with a higher body weight at weaning. This improvement could be attributed to the activation of the IGF1-Akt pathway; although not measured in this study, this activation was possibly induced by the elevation of IGF-1 and insulin, thus facilitating protein synthesis via mTOR activation. However, supplementation during the cow-calf phase did not result in significant differences in body weight (BWf and HCW) after finishing or REA, an indicator of muscularity assessed during slaughter, although the IGF1-Akt pathway was active at weaning. This lack of a difference may indicate a change in gain composition (Asher et al., 2018), specifically a reduction in the rate of lean tissue deposition and an earlier onset of fat accumulation (Gerrits et al., 1997) after the supplementation period. This hypothesis is supported by the increase in BFT and marbling (IMF and MS) in animals supplemented during the cow-calf phase, possibly due to activation of the *PPAR signaling pathway*.

The *PPAR signaling pathway* involves PPAR nuclear receptors, which act as key transcription factors in the regulation of lipid and glucose metabolism (Gross et al., 2017). These receptors respond to environmental stimuli and modulate the expression of genes related to lipid metabolism and energy homeostasis (Lamichane et al., 2018; Attianese and Desvergne, 2015). During adipogenesis, specific activation of PPARγ, a key transcription factor of PPARs that is highly expressed in mature adipocytes, is fundamental for the commitment of mesenchymal stem cells to preadipocytes and their eventual differentiation into mature adipocytes (Queiroz et al., 2009; Wu et al., 1999). The inclusion of long-chain fatty acids in the diet has been associated with higher expression of PPAR-related genes in cattle and humans (Bionaz et al., 2012; Sampath and Ntambi, 2004), highlighting the importance of dietary sources for the stimulation of this pathway during adipocyte differentiation. Unsaturated fatty acids such as linoleic and linolenic acids are found in oilseeds such as soybeans (Ladeira et al., 2020). Although these acids undergo ruminal biohydrogenation, a fraction can escape to the duodenum and be absorbed (Duckett and Gillis, 2010). The present results of daily supplementation of calves with 1% of their body weight of creep feed, with the inclusion of 40.4% of soybean meal in the concentrate, suggest that a fraction of the unsaturated fatty acids in the supplement can reach skeletal muscle. This fact may contribute to the upregulation of genes associated with the PPAR pathway, which would explain the greater fat deposition in CF animals. The synergistic interaction between the IGF1-Akt and PPAR signaling pathways thus indicates a complex metabolic mechanism that regulates growth and body composition.

4.3 Main DMGs/DEGs related to muscle hypertrophy, adipogenesis and lipogenesis

In the present study, hypermethylation and downregulation of the neuregulin-3 (*NRG3*) gene at weaning were observed in calves supplemented during the cow-calf phase. This gene encodes peptide ligands for transmembrane tyrosine kinase receptors, which regulate cell growth and differentiation, affecting different cell types (Zhang et al., 1997). In humans, variants of *NRG3* have been associated with the body mass index and biological processes that regulate body weight (Zhou et al., 2020).

Genes of the neuregulin family are important for the activation of skeletal muscle satellite cells, which are essential for repair processes (Gumà et al., 2010). Regarding muscle growth processes, satellite cells play a fundamental role in muscle fiber hypertrophy (Picard and Gagaoua, 2020). Although supplemented calves had higher WW, the downregulation of *NRG3*, as well as of RUNX family transcription factor 1 (*RUNX1*), in this case hypomethylated, may be a response to anticipated muscle hypertrophy. *RUNX1* is known to cooperate with *MyoD* during the early stages of myogenesis (Zhou et al., 2022). Therefore, the downregulation of *NRG3* and *RUNX1* suggests that supplementation during the cow-calf phase triggered increased satellite cell activity, leading to the early downregulation of genes crucial to the *anatomical structure formation involved in morphogenesis pathway*, which is mainly active during the early periods of development such as the embryonic and fetal stages.

This hypothesis is strengthened by the hypomethylation and upregulation of the *SMAD3* gene observed in calves supplemented during the cow-calf phase. This gene, which was enriched in the *factors and pathways affecting insulin-like growth factor (IGF1)-Akt signaling* pathway, plays an important role in the regulation of cellular responses to TGF-β (Shi et al., 2016). During fetal myogenesis, this gene is negatively regulated because of its repressive activity on myogenic regulatory factors such as those of the MyoD family (Liu et al., 2001). However, a study has shown that upregulation of *SMAD3* significantly increases the number of satellite cells by self-renewal during postnatal myogenesis in mice, which may contribute to muscle hypertrophy (Ge et al., 2011). Additionally, these authors demonstrated that inactivation of *SMAD3* in mice can upregulate *MuRF1* and the ubiquitin-proteasome system responsible for muscle atrophy. *Muscle ring finger 1*, also known as *TRIM63*, was hypermethylated and downregulated in the supplemented calves studied here. Suppression of the expression of this gene that integrates the *factors and pathways affecting insulin-like growth factor (IGF1)-Akt signaling* pathway promotes muscle hypertrophy (Peris-Moreno et al., 2021), which would explain the higher WW observed in supplemented calves.

Intramuscular fat, a qualitative meat trait of high commercial value, is influenced not only by intense cell proliferation activity during fetal life but also by a specific interval within the first 250 days after birth. During this period, known as the "marbling window", feeding a grain-rich diet specifically promotes the generation of new intramuscular adipocytes through hyperplasia, thereby establishing the sites for fat accumulation during the finishing phase (Du et al., 2013). This process paves the way for the activation of genes and other metabolic pathways associated with adipose tissue deposition. This hypothesis is strengthened by the observation of enrichment of the *PPAR signaling pathway*, accompanied by an increase in BFT and IMF at the end of the finishing period in supplemented calves. Genes of the PPAR family, particularly *PPARγ*, are key transcription factors that regulate preadipocyte differentiation and adipocyte metabolism (Duarte et al., 2013). Although the *PPARγ* gene was not directly demonstrated in this study, hypomethylation and upregulation of *DNMT3A* was observed in supplemented calves. Studies of subcutaneous adipose tissue in adult pigs revealed an increase in *DNMT3A* and *PPARγ* expression when compared to piglets (Qimuge et al., 2019). This increase in *DNMT3A* expression in adult animals suggests that the gene plays a fundamental role in the development and maturation of adipose tissue. Furthermore, the same authors found an increase in *DNMT3A* expression during the differentiation of intramuscular preadipocytes, indicating the involvement of this gene not only in the early development of adipose tissue but also in the maturation of adipocytes, a key process for the elevation of adipose tissue mass and for local metabolic regulation (Qimuge et al., 2019). The high expression of *DNMT3A* in supplemented calves observed at weaning may thus indicate that a high proportion of adipocytes were already mature and ready to start the intensive lipogenic process during the finishing period, which explains the greater marbling deposition in meat (IMF and MS) (Table 3).

The *SORBS1* gene, a strong candidate associated with higher IMF content in cattle (Sasaki et al., 2006) that integrates the *PPAR signaling pathway* in the present study, was hypomethylated and downregulated at weaning in supplemented calves. *SORBS1* is a member of the SORBS family and encodes proteins involved in insulin signaling and stimulation (Chang et al., 2016, Chang et al., 2018; Germain et al., 2015). In 3T3-L1 adipocytes, an established cell line commonly used to study adipocyte biology, *SORBS1* plays an important role in the regulation of glucose uptake (Baumann et al., 2000). This gene was also found in C2C12 muscle cells (model of muscle cell line); in addition, its downregulation is related to insulin resistance due to AMPK activation in this cell type but not in 3T3-L1 adipocytes (Aye et al., 2023; Yang et al., 2024). This finding suggests that the downregulation of *SORBS1* at weaning in supplemented calves is associated with a decrease in glucose utilization by muscle fibers, reducing competition between the latter and intramuscular adipocytes for this substrate for lipogenesis. The fact that skeletal muscle is a heterogeneous tissue that contains both muscle fibers and adipocytes (Ramírez-Zamudio et al., 2023) implies that the downregulation of *SORBS1* cannot be necessarily attributed exclusively to adipocytes. Additionally, hypomethylation and upregulation of the *PRKCA* gene were detected in supplemented calves. Although *PRKCA* acts as a negative regulator of insulin receptor signaling, it is important to highlight that insulin is known for promoting adipose cell differentiation, IMF deposition, and glucose oxidation in muscle cells (Lim et al., 2011; Hausman et al., 2009). The results of this study suggest a complex role of *PRKCA* in the metabolism of supplemented calves, indicating that its upregulation may alter the typical metabolic functions of insulin in unpredicted manners.

Insulin, when present in supraphysiological levels, can also induce adipogenic differentiation through the stimulation of IGF-1, a process known as transactivation (Fernyhough et al., 2007). These findings therefore reinforce our theory that muscle fibers of supplemented animals consume less glucose, increasing its availability for lipogenesis in adipocytes.

The reduced glucose utilization by muscle fibers in supplemented calves may also be supported by the observation of hypomethylation and downregulation of *SLC2A8* at weaning. The *SLC2A8* gene encodes GLUT8 transporters with high affinity for glucose in brain cells, as well as in skeletal muscle and adipose tissues (Gawlik et al., 2008). However, translocation of these transporters to the surface for insulin-stimulated glucose uptake has only been observed in murine muscle cells (Carayannopoulos et al., 2000) but not in adipocytes (Lisinski et al., 2001). Additionally, a study using cultured 3T3-L1 adipocytes suggested that GLUT8 expression is markedly increased during the phase of cell differentiation (Scheepers et al., 2001). This finding suggests that creep-feeding from day 30 of age until weaning of calves at 210 days, almost at the end of the "marbling window" (250 days) (Du et al., 2013), may have accelerated the differentiation of intramuscular adipose tissue cells when compared to the unsupplemented group. This anticipated adipocyte differentiation, facilitated by supplementation, may be associated with the downregulation of *SLC2A8*. This reflects complex metabolic dynamics involving various cell types and developmental stages within the muscle tissue that, in an integrated manner, affect glucose metabolism, energy homeostasis, and fat deposition.

The *PPAR signaling pathway* enriched in this study is essential for cell differentiation and lipid and carbohydrate metabolism (Feige et al., 2006). The *ACAA1* gene associated with the *PPAR signaling pathway* was found to be hypomethylated and downregulated in animals submitted to creep-feeding. This gene plays an essential role in the synthesis and regulation of enzymes involved in the beta-oxidation of long-chain fatty acids (Wanders et al., 2010). Studies on sheep (Wang et al., 2021), birds (Li et al., 2019) and pigs (Yao et al., 2021) generally reported that *ACAA1* expression is inversely associated with IMF deposition. These data are consistent with the results obtained here for supplemented calves, in which downregulation of *ACAA1* was correlated with greater deposition of marbling fat. Thus, our theory is that the downregulation of *ACAA1* in supplemented calves is due to the glucose-rich environment provided by the high dietary inclusion of corn (44.8%). Additionally, the decreased competition between muscle fibers and adipocytes for glucose, as demonstrated by regulation of the *SORBS1* and *PRKCA* genes, which are members of the *PPAR signaling pathway* and regulators of glucose metabolism in muscle fibers, reduces the beta-oxidation of fatty acids in adipocytes.

5 Conclusion

The results of the present study support the hypothesis that the implementation of creep-feeding supplementation during the cow-calf phase induces changes in DNA methylation and gene expression levels, modifying the regulation of biological processes and metabolic pathways related to muscle growth and fat deposition (adipogenesis and lipogenesis) in beef cattle. Epigenetic regulations of genes that play key roles in muscle growth and development, PPAR signaling, adipogenesis, insulin response, and lipid metabolism were identified. These biological changes help explain the phenotypes observed in supplemented animals in terms of both performance until weaning (higher weight and weight gain until weaning) and traits related to meat quality (higher marbling).

Data availability statement

All relevant data are within the paper. RNA-Seq (RNA sequencing) and RRBS (reduced representation bisulfite sequencing) data may be made available by contacting the corresponding author (rogerio.curi@unesp.br).

Ethics statement

The animal studies were approved by Animal Use Ethics Committee of the School of Agricultural and Veterinary Sciences (FCAV), UNESP, Jaboticabal, São Paulo, Brazil (certificate number 013689/19). The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent was obtained from the owners for the participation of their animals in this study.

Author contributions

LR: Investigation, Software, Writing – original draft. GR-Z: Investigation, Writing – review & editing. GP: Investigation, Writing – review & editing, Conceptualization, Data curation, Formal Analysis, Methodology, Software, Supervision, Validation, Visualization, Writing – original draft. JT: Data curation, Methodology, Writing – review & editing. LT: Investigation, Software, Writing – original draft. OM: Conceptualization, Data curation, Funding acquisition, Methodology, Resources, Writing – review & editing. LC: Conceptualization, Data curation, Methodology, Writing – review & editing, Formal Analysis. WB: Conceptualization, Formal Analysis, Methodology, Writing – review & editing, Visualization. RC: Conceptualization, Formal Analysis, Methodology, Visualization, Writing – review & editing, Data curation, Funding acquisition, Investigation, Project administration, Resources, Software, Supervision, Validation, Writing – original draft.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

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Evaluation of black soldier fly larvae oil as a feed ingredient for broiler chickens: effects on performance, carcass traits, meat characteristics, and blood parameters

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The aim of the present study was to evaluate the effects of replacing diet rice bran oil (RBO) with black soldier fly larva oil (BSFLO) on the growth performance, carcass traits, meat quality, and blood parameters of broiler chickens. At one day of age, a total of 180 male broiler chickens (Ross 308) were randomly allocated to 3 experimental groups (4 replicates and 15 birds/pen). To a basal control diet, either 50% or 75% of the RBO was replaced with BSFLO, respectively. The growth performance was monitored throughout the rearing period (divided into 3 periods: 1-10, 11-24, and 25-42 days). On days 24 and 42, Blood samples were taken from each treatment for hemato-biochemical index determination. At the termination period, 8 birds (two birds/pen) per group were slaughtered for carcass and meat quality measurement. Samples of the liver were submitted for fatty acid investigations. The results showed that the inclusion of 75% BSFLO in the broiler diet significantly increased FCR (Feed conversion ratio) in the finisher and overall periods. Interestingly, replacing 50% of RBO with BSFLO did not influence growth performance, carcass traits, and hematochemical parameters compared to 75% of BSFLO and control groups. The present study suggests that partially replacing RBO with 50% of BSFLO in broiler chicken diets has no adverse effects on growth performance, carcass-meat quality, or blood parameters.

KEYWORDS

insect oil, *Hermetia illucens* L., broiler chicken, productivity, blood profiles

1 Introduction

Insects are gaining traction as valuable additions to human diets in many countries (Borrelli et al., 2017). They also show potential substitutes for traditional sources of protein, such as fishmeal and soybean meal in livestock feed (Loponte et al., 2017; Bovera et al., 2018; Cutrignelli et al., 2018). The black soldier fly (*Hermetia illucens*) is a particularly attractive option. During its growth stage, this fly can efficiently convert large amounts of organic waste, which would otherwise be pollutants, into usable protein and fats for animal feed (Zheng et al., 2012; Li et al., 2016). Black soldier fly larvae (BSFL) boast a high nutrient content, containing up to 40% protein rich in essential amino acids, over 28% lipids, and essential minerals like calcium and phosphorus (Makkar et al., 2014; Wang and Shelomi, 2017).

Despite the nutritional advantages offered by insects as animal feed, concerns have emerged regarding potential hazards associated with their use. This is because insects raised on contaminated substrates may accumulate heavy metals and toxins, which could be passed on to the animals they are fed (Wang and Shelomi, 2017). However, insects have garnered attention as a promising alternative feed for monogastric animals due to their nutrient-rich composition and minimal environmental impact (Henry et al., 2015; Biasato et al., 2016; Overa et al., 2016). They boast low greenhouse gas and ammonia emissions, exhibit favorable feed conversion ratios as cold-blooded creatures, and require minimal water and soil for cultivation (van Huis, 2013; Makkar et al., 2014). Consumers appear increasingly receptive to products derived from these unconventional raw materials (Mancuso et al., 2016). Furthermore, insects can serve as bio-converters of food waste into animal feed, thus avoiding competition with humans for natural resources (Diener et al., 2011; Makkar et al., 2014).

Kierończyk et al. (2018) proposed that incorporating insect oils, such as those derived from *Tenebrio molitor* and *Zophobas morio*, into broiler chicken diets could effectively replace soybean oil (SBO) without compromising growth performance or nutrient digestibility. Additionally, Li et al. (2016) demonstrated that dietary black soldier fly larvae oil (BSFLO) led to increased deposition of omega-3 fatty acids in muscle tissues while reducing intraperitoneal fat accumulation in juvenile carp. BSFLO is particularly abundant in medium-chain fatty acids (MCFA) like lauric acid (C12:0), akin to coconut oil (CCO) (Li et al., 2016; Ushakova et al., 2016), which uniquely comprises approximately 50% lauric acid in its fatty acid composition (Dayrit, 2014). Wang et al. (2015) indicated that MCFA might contribute to abdominal fat reduction due to their preferential utilization for energy over long-chain saturated or unsaturated fatty acids. Furthermore, The MCFA, including lauric acid, exhibits antimicrobial properties against gut bacteria (Zeitz et al., 2015; Schiavone et al., 2017). Hence, it is possible to postulate that insect oils containing high levels of lauric acid may influence the growth performance and gut health of rapidly growing broiler chickens (Schiavone et al., 2018). The fatty acid composition of broiler diets has been found to influence that of meats and visceral organs, which are closely correlated with the storage and metabolism of fat (Taulescu et al., 2010; Khatun et al., 2018). Schiavone et al. (2017) observed that substituting SBO with BSFLO changed the fatty acid profile of broiler chickens. Nevertheless, a comprehensive understanding of the suitability of BSFLO as a poultry feed ingredient remains limited. Given the antimicrobial properties and capacity to modify body fatty acid composition associated with medium-chain fatty acids-rich insect oils, it is suggested that dietary BSFLO could enhance growth performance and gut health, influence the fatty acid composition of abdominal fat pads, and improve meat quality in broiler chickens. Previous studies have reported that dietary fat sources enhance broiler chickens' meat quality (Zeitz et al., 2015; Khatun et al., 2018).

In this study, we used two oil sources, including rice bran oil (RBO) and BSFLO, to see their effects on growth performance, carcass characteristics, meat quality, liver fatty acid composition, and serum parameters in broiler chickens.

2 Materials and methods

2.1 Ethics statement

This study was conducted following the ethics of animal experimentation recommendations of the National Research Council of Thailand. The research protocol was reviewed and approved by the Institutional Animal Care and Use Committee of Khon Kaen University, Thailand. (Record no. IACUC-KKU-60/66)

2.2 Source of BSFLO, experimental design, birds, and diets

The BSFLO was purchased from BSFLY Co. Ltd. (Udonthani, Thailand), which is a reputable supplier that prioritizes quality, sustainability, and adheres to strict regulatory standards. The fatty acid content of BSFLO and rice bran oil was quantitatively determined using the gas chromatographic method 996.06 (Association of Official Analytical Chemists, 1990) and was expressed as g/100g of the total fatty acid identified (Table 1).

The present study used a completely randomized design with three treatments and four replications. A total of 180 one-day-old, male-sex, Ross 308 broiler chicks (initial body weight 47.49 ± 0.04 g) were obtained from a commercial hatchery (Charoen Pokphand Group Co. Ltd., Nakhon Ratchasima, Thailand). Chickens were randomly allocated into one of three dietary treatments (4 replication pens per treatment, with 15 chicks per pen) for a 42-day study period. Dietary treatments were as follows: the control group was basal diet based on corn-soybean meal and RBO; the BSFLO groups replaced RBO with BSFLO at 50% and 75%. The treatment diets and nutrition values were formulated to meet or exceed the nutrition specification (2022) of Ross broiler chickens in three phases – starter (1-10 days), grower (11-24 days), and finisher (25-42 days; Table 2). All broilers had free access to feed and water during the experiment. The temperature of the chicken coop was maintained at 32°C at the age of the first crucial 7 days and then reduced by 1 to 2°C each week in open housing under tropical conditions, to ensure a comfortable and healthy environment for growing chicks.

TABLE 1 The fatty acid profiles of black soldier fly larvae oil (BSFLO) and rice bran oil (RBO).

Fatty acid compositions (g/100g)	BSFLO	RBO
Saturated fatty acid	45.83	24.99
Butyric acid (C4:0)	0.71	0.29
Lauric acid (C12:0)	12.79	0.02
Myristic acid (C14:0)	2.98	0.42
Pentadecanoic acid (C15:0)	0.12	0.02
Palmitic acid (C16:0)	22.81	20.70
Heptadecanoic acid (C17:0)	0.17	0.05
Stearic acid (C18:0)	6.02	2.29
Arachidic acid (C20:0)	0.19	0.91
Behenic acid (C22:0)	0.04	0.29
Unsaturated fatty acid	47.53	74.15
Myristoleic acid (C14:1)	0.05	ND
Palmitoleic acid (C16:1n7)	0.81	0.22
cis-10-Heptadecenoic acid (C17:1n10)	0.04	0.01
cis-9-Oleic acid (C18:1n9)	21.67	38.99
cis-11-Eicosenoic acid (C20:1n11)	0.06	0.38
cis-9,12-Linoleic acid (C18:2n6)	23.23	31.77
gamma-Linolenic acid (C18:3n6)	ND	0.08
alpha-Linolenic acid (C18:3n3)	0.89	1.05
cis-11,14-Eicosadienoic acid (C20:2n6)	0.72	1.10
cis-8,11,14-Eicosatrienoic acid (C20:3n6)	0.06	0.06
Arachidonic acid (C20:4n6)	ND	0.02
cis-5,8,11,14,17-Eicosapentaenoic acid (C20:5n3)	ND	0.47
Monounsaturated fatty acid (MUFA)	22.63	39.60
Polyunsaturated fatty acid (PUFA)	24.90	34.55
PUFA/MUFA	1.10	0.87
n-3	0.89	1.52
n-6	24.01	33.03
n-6/n-3	26.98	21.73

n-3, omega 3; n-6, omega 6; n-6/n-3, ratio of omega 6 per 3.

2.3 Sample collections and measurements

The broiler chickens were weighed, and their feed intake was recorded on days 1, 10, 24, and 42. Subsequently, the body weight gain (BWG), feed conversion ratio (FCR), survivor rate (SR), and productive index (PI; Equation 1) were calculated for each study period to comprehensively assess the growth performance.

$$PI = \{[BWG\,(kg) \times SR\,(\%)]/[Age \times FCR]\}\ x\ 100 \qquad (1)$$

At the end of days 24 and 42, four birds (one bird from each replicate pen) were randomly selected from each treatment, and 5 ml blood samples per bird were gently collected from the wing vein using a sterile syringe with a needle. The blood samples were then divided and transferred into the anticoagulant-free blood collecting tube 4 ml for analyzing blood serum biochemistry, i.e., alanine transaminase (ALT), aspartate transaminase (AST) alkaline phosphatase (ALP), glucose, albumin, globulin, total protein, triglyceride, cholesterol, low-density lipoprotein (LDL), and high-density lipoprotein (HDL). For serum biochemistry analysis, the blood samples were centrifuged at 1,500 g at 4°C for 15 min to separate the serum and stored at −20°C for analysis. The remaining 1 ml blood went into another tube that contained ethylene-diamine-tetra-acetic acid (EDTA) for plasma hematological analysis, i.e., complete blood count.

Upon completion of the 6-week experimental period, two birds per replication then proceeded to the euthanasia and slaughtering process. Prior to sample collection, the chicks were fasted for 12 h. Then, they were individually weighed, euthanized (by cervical dislocation), slaughtered and trimmed carcasses. The weight of each whole hot carcass was recorded. Following slaughter, the carcasses underwent immediate evisceration and subsequent sectioning. Visceral and edible meat organs were weighed and recorded to analyze carcass quality. The liver sample was immediately kept and stored at −20°C until the determination of fatty acid composition by gas chromatographic method 996.06 (25). The left side of the breast of each bird was pH-analyzed using a pH meter (CyberScan Ion 510, Eutech Instruments Pet. Ltd., Singapore), which was inserted directly into the breast muscle within 45 min and 24 h after disassembling the broiler. On the right side of each breast sample, meat color, drip loss, cooking loss, and texture profile were analyzed sequentially. About 45 min after the cut-apart, the meat was color-analyzed using the CIELAB system (Lightness: L^* value, Redness: a^* value, and Yellowness: b^* value), along with the Colorimeter (Chroma Meter CR-410 Series, Konica Minolta Sensing Inc., Tokyo, Japan), then the samples were then individually bagged in polyethylene and stored at 4°C for further evaluation of meat quality.

Afterward, the meat was weighed, encased in multiple layers of gauze cloth, and suspended at 4°C for 24 h. Following this, the meat was carefully patted dry with a paper towel and weighed again. Drip loss (Equation 2) was calculated as a percentage based on the difference in weight before (W1) and after suspension (W2). The meat was then cooked in water at 85°C until the core temperature of the thickest section of the meat reached 80°C. Once cooked, the meat was cooled in a running tap water bath and allowed to rest at 4°C for 2 hours before being weighed once more. Cooking loss (Equation 2) was determined as the difference in weight before (W1) and after cooking (W2).

$$\text{Drip loss or Cooking loss}\,(\%) = \{[W1 - W2]/W1\} \times 100 \qquad (2)$$

The cooked meat was subsequently sliced into cubes measuring 1 cm × 1 cm × 1 cm for texture profile analysis (TPA: Hardness), and into specimens measuring 1 cm × 2 cm × 1 cm for the shear force test. During the TPA and shear force analyses, the meat

TABLE 2 Formulation and nutritional composition of experimental diets (% dry matter).

Items	1-10 days			11-24 days			25-42 days		
	Control 0%	BSFLO 50%	BSFLO 75%	Control 0%	BSFLO 50%	BSFLO 75%	Control 0%	BSFLO 50%	BSFLO 75%
Ingredients (kg)									
Corn	47.80	47.00	47.00	51.20	50.00	50.00	56.50	56.70	57.50
Soybean meal (CP 45%)	30.00	26.25	26.25	26.50	22.50	20.00	21.00	17.80	16.00
Full-fat soybean (CP 36%)	15.30	20.00	20.00	14.80	20.00	22.50	15.00	18.00	19.00
RBO	2.30	1.15	0.58	3.00	1.50	0.90	3.00	1.50	0.75
BSFLO	0.00	1.15	1.72	0.00	1.50	2.10	0.00	1.50	2.25
Choline chloride	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Limestone	1.60	1.55	1.55	1.55	1.55	1.55	1.55	1.55	1.55
Dicalcium phosphate (P 21%)	1.80	1.75	1.75	1.75	1.75	1.75	1.75	1.75	1.75
Premix[a]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Salt	0.40	0.35	0.35	0.40	0.40	0.40	0.40	0.40	0.40
DL-methionine	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
L-lysine	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Calculated nutrient values (%)									
Metabolizable energy (kcal/kg)	3005.00	2978.00	2945.00	3078.00	3029.00	3017.00	3128.00	3071.00	3044.00
Crude protein	22.50	22.45	22.45	21.07	21.00	20.75	19.07	18.70	18.30
Crude fat	7.28	8.11	8.11	7.97	8.88	9.33	8.14	8.16	8.26
Crude fiber	3.43	3.52	3.52	3.23	3.34	3.36	2.97	2.95	2.95
Available phosphate	0.73	0.73	0.73	0.71	0.72	0.72	0.69	0.70	0.70
Calcium	1.24	1.21	1.21	1.20	1.19	1.19	1.18	1.17	1.17
Methionine	0.67	0.67	0.67	0.65	0.65	0.65	0.63	0.62	0.62
Lysine	1.43	1.43	1.43	1.33	1.34	1.33	1.19	1.18	1.15

BSFLO, black soldier fly larvae oil; RBO, rice bran oil.
[a]Provided per kilogram of diet: 4.80 MIU of vitamin A, 2.00 MIU of vitamin D3, 30,000 IU of vitamin E, 1.20 g of vitamin K3, 1.20 g of vitamin B1, 3.20 g of vitamin B2, 2.00 g of vitamin B6, 0.0064 g of vitamin B12, 24.00 g of niacin, 0.80 g of folic acid, 0.08 g of biotin, and 6.00 g of pantothenic acid. 40.00 g of Zn, 48.00 g of Mn, 16.00 g of Fe, 6.40 g of Cu, 0.50 g of I, 0.04 g of Co, and 0.12 g of Se. 0.20 g of antioxidant, 0.88 g of anticaking, and 1.00 kg of carrier.

underwent either double compression or perpendicular cutting against the alignment of muscle fibers, utilizing a texture analyzer (model TA.HDplusC, Stable Micro Systems, Godalming, UK) equipped with either a 50 mm cylindrical aluminum probe or a V-slot WB Blade, respectively. All textural parameters were automatically computed and recorded by the Exponent software (Stable Micro Systems, Godalming, UK).

2.4 Statistical analysis

The data were checked for normal distribution using the Shapiro-Wilk test and consistency of variance using Levene's test. Any outliers were eliminated. Outliers were identified using the interquartile range (IQR) method and were excluded from the analysis if they significantly deviated from the overall dataset and were deemed unrelated to the experimental conditions. Statistical evaluations were carried out utilizing one-way analysis of variance (ANOVA) and conducted using SAS version 9.1 software. The significance of group differences was assessed using Tukey's studentized range test at a significance level of $p < 0.05$. The statistical model is as follows:

$$Y_{ijk} = \mu + \alpha_i + \Sigma_{ij}$$

where Y_{ijk} = observation, μ = overall mean, α_i = BSFLO levels (i = 0, 50, and 75%), and Σ_{ij} = the experimental error.

3 Results

The study investigated the effect of BSFLO in broiler chicken diets on growth performance across different stages, as presented in

TABLE 3 The effect of inclusion of black soldier fly larvae oil (BSFLO) in broiler diets on performance.

Performances	Control	Levels of BSFLO		p-value	SEM
	0%	50%	75%		
IBW (g/b)	47.44	47.52	47.50	0.77	0.11
FBW (g/b)	3,029.32	2,889.01	2,894.82	0.18	78.59
Starter period (1-10 Days)					
BW (g/b)	273.38	290.70	301.25	0.15	13.00
BWG (g/b)	225.93	243.19	253.75	0.15	13.06
FI (g/b)	273.46	267.92	271.85	0.56	5.19
FCR	1.22	1.11	1.08	0.06	0.06
SR (%)	100.00	100.00	100.00	N/A	N/A
PI	186.52	221.75	237.65	0.13	22.87
Grower period (11-24 Days)					
BW (g/b)	890.52	892.37	893.68	0.96	15.83
BWG (g/b)	865.96	870.51	874.43	0.86	15.44
FI (g/b)	1,456.93	1,469.34	1,446.40	0.22	12.21
FCR	1.68	1.69	1.66	0.65	0.04
SR (%)	100.00	100.00	100.00	N/A	N/A
PI	214.48	215.10	220.48	0.73	8.27
Finisher period (25-42 Days)					
BW (g/b)	3,039.36	2,889.01	2,894.72	0.16	80.94
BWG (g/b)	2,138.85	1,996.65	2,000.04	0.12	70.63
FI (g/b)	3,528.20	3,229.60	3,596.80	0.19	160.67
FCR	1.65^b	1.62^b	1.79^a	0.04	0.06
SR (%)	81.67	88.33	88.33	0.35	0.25
PI	253.72	260.36	235.23	0.41	18.48
Overall period (1-42 Days)					
BWG (g/b)	2,981.93	2,841.50	2,847.22	0.18	78.64
FI (g/b)	5,182.00	4,966.90	5,288.00	0.22	174.06
FCR	1.74^b	1.75^b	1.85^a	0.05	0.04
SR (%)	81.67	88.33	88.33	0.35	5.05
PI	334.84	342.65	321.75	0.68	23.68

IBW, initial body weight; FBW, final body weight; FI, feed intake; FCR, feed conversion ratio; SR, Survivor rate; PI, productive index; NA, Not Applicable.
^{a,b}Different superscripts within a row indicate significant differences ($p < 0.05$).
SEM, Standard error of means.
N/A, Not Applicable.

Table 3. Results indicated that BSFLO inclusion at 50% and 75% did not significantly affect initial body weight (IBW), final body weight (FBW), body weight gain (BWG), feed intake (FI), survival rate (SR), and production index (PI) compared to the control group during the starter, grower, and finisher periods ($p > 0.05$). However, a significant difference in FCR was observed in the finisher period ($p < 0.05$). Additionally, when considering the overall 1-42 days period, BSFLO inclusion did not significantly difference in BWG, FI, SR, and PI ($p > 0.05$), but differences were observed in FCR ($p < 0.05$), with a lower ratio in the BSFLO50% and control groups compared to 75% BSFLO.

The blood serum parameters of the broiler are shown in Table 4. The data found that replacing soybean oil with BSFLO in broiler chicken diets may affect blood cell counts. On day 21 (D21), the 50% BSFLO was significantly different in hemoglobin and white blood cells (WBCs) count ($p < 0.05$), higher than in other groups, and lower lymphocytes ($p < 0.05$), similar to those in the control group. By day 42, most blood cell parameters showed no significant differences between the groups. However, eosinophils were slightly lower with 50% BSFLO compared to the control and 75% BSFLO groups ($p < 0.05$).

The effects of BSFLO on blood biochemistry in broiler chicken diets are shown in Table 5. At day 21, the 50% BSFLO showed the lowest aspartate transaminase (AST) and LDL (low-density lipoprotein) cholesterol levels significantly compared to the control group ($p < 0.05$). By day 42, The alanine transaminase (ALP) was significantly lower in both BSFLO groups ($p < 0.05$). Meanwhile, triglyceride was highly significant in 50% BSFLO than the other groups ($p < 0.05$). On the contrary, the dietary BSFLO inclusion did not significantly ($p > 0.05$) influence the liver enzyme (alanine transaminase; ALT) and serum protein (albumin, globulin, and total protein).

The carcass quality parameters are shown in Table 6. The data found that replacing rice bran oil with BSFLO in broiler chicken diets may have some effects on carcass yield. The dressing percentage was slightly lower with higher BSFLO inclusion ($p < 0.05$). There were no significant differences ($p > 0.05$) in the part of giblets, including the percentage of liver, heart, pancreas, spleen, and gizzard. However, broilers in BSFLO groups had higher abdominal fat percentage than those in control group ($p < 0.05$). Part of external organs, the percentage of the wing tended to increase when supplementing high levels of BSFLO ($p < 0.05$). Meanwhile, the thigh, breast, and drumstick percentages did not differ significantly among the treatments.

The results of the breast meat quality values when replacing with BSFLO to broiler chicken diets until termination at 42 days are shown in Table 7. There were no significant changes in breast meat pH at processing (Hour-1; $p > 0.05$) but a slight decrease at 24 hours (Hour-24; $p < 0.05$) with higher BSFLO levels. Alternatively, the meat color (L^*, a^*, and b^*), drip loss, cooking loss, shear force, and hardness were not significantly affected ($p > 0.05$).

The fatty acid profiles of the liver are presented in Table 8. Broilers fed diets with high levels of BSFLO (75%) had significantly higher ($p < 0.05$) levels of saturated fatty acids compared to the control or 50% BSFLO groups, particularly butyric acid (C4:0), palmitic acid (C16:0), and stearic acid (C18:0). Conversely, 50% BSFLO inclusion significantly decreased ($p < 0.05$) unsaturated fatty acids compared to the control and 75% BSFLO groups, leading to a notable decrease in palmitoleic acid (C16:1n7) and cis-9-oleic acid (C18:1n9c) levels. Moreover, polyunsaturated fatty acid (PUFA) levels were also lowered ($p < 0.05$) in both BSFLO groups, reducing linoleic acid (C18:2n6) and alpha-linolenic acid (C18:3n3) content. The total levels of PUFA, n-3, n-6, and n-9 were significantly higher ($p < 0.05$) in the control group compared to the BSFLO treatments.

TABLE 4 The effect of inclusion of black soldier fly larvae oil (BSFLO) in broiler diets on hematology.

Blood serum parameters	Control	Levels of BSFLO		p-value	SEM
	0%	50%	75%		
Days 21					
RBCs (x10^6 cells/mm^3)	2.14	2.34	2.09	0.28	0.09
Hemoglobin (g/dl.)	17.90^b	19.97^a	19.20^{ab}	0.04	0.40
Hematocrit (%)	26.67	30.00	27.00	0.06	0.62
WBCs (cells/μl)	4,066.67^b	9,400.00^a	5,500.00^b	0.01	546.71
Heterophils (%)	27.00	31.50	25.00	0.28	2.17
Basophil (%)	8.00	9.00	5.50	0.20	1.05
Eosinophils (%)	0.00	0.00	0.00	N/A	N/A
Lymphocytes (%)	64.00^{ab}	58.50^b	68.67^a	0.02	1.33
Monocytes (%)	1.00	1.00	1.00	N/A	N/A
H/L ratio	0.42	0.54	0.37	0.08	0.03
Days 42					
RBCs (x10^6 cells/mm^3)	2.35	2.30	2.35	0.76	0.06
Hemoglobin (g/dl.)	10.55	10.13	9.25	0.30	0.43
Hematocrit (%)	29.50	28.00	26.00	0.12	0.74
WBCs (cells/μl)	9,863.33	6,086.67	6,297.50	0.12	1,262.00
Heterophils (%)	32.00	19.33	28.00	0.26	4.95
Basophil (%)	3.00	2.67	2.67	0.88	0.54
Eosinophils (%)	2.50^a	1.00^b	2.00^{ab}	0.05	0.24
Lymphocytes (%)	61.67	76.00	66.33	0.17	4.72
Monocytes (%)	1.00	1.00	1.00	N/A	N/A
H/L ratio	0.52	0.28	0.42	0.21	0.8

RBCs, red blood cells; WBCs, white blood cells; NA, Not Applicable.
^{a,b}Different superscripts within a row indicate significant differences ($p < 0.05$).
SEM, Standard error of means.
N/A, Not Applicable.

TABLE 5 The effect of inclusion of black soldier fly larvae oil (BSFLO) in broiler diets on blood plasma biochemistry.

Blood plasma biochemistry	Control	Levels of BSFLO		p-value	SEM
	0%	50%	75%		
Days 21					
ALT (U/L)	5.33	6.00	4.00	0.30	0.84
AST (U/L)	213.50^a	198.33^b	223.00^a	0.01	3.11
ALP (U/L)	18,682.50	22,398.67	18,733.50	0.38	1754.04
Glucose (mg/dl)	227.00	215.33	229.33	0.15	4.41
Albumin (g/dl)	1.17	1.15	1.15	0.98	0.07
Globulin (g/dl)	1.87	1.65	1.45	0.15	0.11
Total protein	3.06	2.80	2.60	0.13	0.10
Triglyceride (mg/dl)	23.33	14.33	16.00	0.10	2.55
Cholesterol (mg/dl)	126.67	126.00	145.33	0.22	7.86
LDL (mg/dl)	26.50^a	21.33^b	25.33^a	0.01	0.62
HDL (mg/dl)	52.67	58.50	61.00	0.34	3.31
Days 42					
ALT (U/L)	6.00	5.50	5.00	0.90	0.20
AST (U/L)	436.67	345.00	341.67	0.07	25.91
ALP (U/L)	7,190.00^a	3,090.00^b	3,850.00^b	0.02	393.19
Glucose (mg/dl)	214.00	239.00	236.67	0.07	6.18
Albumin (g/dl)	1.35	1.20	1.33	0.09	0.03
Globulin (g/dl)	2.00	1.95	1.87	0.85	0.15
Total protein	3.35	3.15	3.20	0.78	0.17
Triglyceride (mg/dl)	33.00^b	49.00^a	34.00^b	<0.01	1.03
Cholesterol (mg/dl)	126.67	137.00	133.33	0.38	4.87
LDL (mg/dl)	28.67	33.00	24.33	0.14	2.87
HDL (mg/dl)	44.67	45.50	45.00	0.92	1.24

ALT, alanine transaminase; AST, aspartate transaminase; ALP, alkaline phosphatase; LDL, low-density lipoprotein; HDL, high-density lipoprotein.
^{a,b}Different superscripts within a row indicate significant differences ($p < 0.05$).
SEM, Standard error of means.

In contrast, broilers receiving the 75% BSFLO diet had a higher concentration of monounsaturated fatty acid (MUFA) and a lower PUFA/MUFA ratio ($p < 0.05$). Additionally, the n-6/n-3 ratio was significantly lower ($p < 0.05$) in both the 50% BSFLO and control groups.

4 Discussion

The main constituents of BSFL are proteins and lipids, with their composition varying depending on the substrate used for rearing. Typically, the BSFL contains around 40% protein and 30% lipid on a dry matter basis. While BSFL are primarily valued as a feed source due to their high protein content, their lipid content is often overlooked and considered a by-product in animal feed production. As a result, lipids or fatty acids derived from BSFL are frequently underutilized despite research suggesting their potential for alternative fat sources. The present experiment tests the BSFLO as a partial replacement in half and three-quarters of the RBO in the diet for growing chickens.

In the present study, the replacement of RBO with BSFLO for the broilers' diet did not significantly affect their growth performance, except FCR, which was identified as increasing in the finisher and overall phase when levels of BSFLO inclusion

TABLE 6 The effect of inclusion of black soldier fly larvae oil (BSFLO) in broiler diets on carcass yield.

Carcass compositions	Control	Levels of BSFLO		p-value	SEM
	0%	50%	75%		
Dressing percentage (%)	75.26[a]	74.29[b]	74.03[b]	0.03	0.23
Internal organ (%)					
Liver	1.78	1.78	1.76	0.97	0.04
Heart	0.37	0.39	0.41	0.10	0.01
Pancreas	0.18	0.19	0.19	0.75	<0.01
Spleen	0.16	0.17	0.20	0.46	0.02
Gizzard	1.14	1.15	1.10	0.33	0.02
Abdominal fat	1.03[b]	1.32[a]	1.16[ab]	0.02	0.05
External organ (%)					
Breast fillet	29.22	28.99	28.43	0.27	0.28
Breast inner fillet	5.50	5.59	5.63	0.59	0.07
Wing	10.49[ab]	10.32[b]	10.74[a]	0.03	0.09
Thigh	17.99	17.73	18.11	0.76	0.31
Drumstick	13.70	14.15	13.55	0.06	0.15

[a,b]Different superscripts within a row indicate significant differences ($p < 0.05$).
SEM, Standard error of means.

TABLE 7 The effect of inclusion of black soldier fly larvae oil (BSFLO) in broiler diets on meat quality.

Meat profiles	Control	Levels of BSFLO		p-value	SEM
	0%	50%	75%		
pH					
Hour-1	6.07	6.02	6.03	0.73	0.05
Hour-24	6.03[a]	5.93[ab]	5.85[b]	0.05	0.25
Color (CIELAB System)					
*L**	54.82	50.93	51.08	0.25	1.56
*a**	1.26	1.00	1.31	0.55	0.21
*b**	6.51	6.03	6.46	0.90	0.81
Drip loss (%)	3.94	4.28	2.62	0.34	0.81
Cooking loss (%)	19.29	15.76	17.49	0.17	1.25
Shear force (g/cm^2)	3,219.72	3,421.47	3,526.66	0.25	105.88
Hardness (g)	633.37	639.74	480.91	0.31	70.36

[a,b]Different superscripts within a row indicate significant differences ($p < 0.05$).
SEM, Standard error of means.

increased (75% BSFLO). The current study was not consistent with previous research, which also reported that the partial (50%) or total (100%) inclusion of BSFLO in a broiler diet had no significant impact on performance in all periods, as well as nutrient digestibility, intestinal morphological features, and even carcass and meat quality were not adversely affected by supplementation or addition of BSFLO to the diet (Schiavone et al., 2017; Kim et al., 2020; Dabbou et al., 2021). The disparity among all the studies could be attributed to variations in factors such as age, breed, trial conditions, management practices, dietary composition, and the wide variation in fatty acid compositions of BSFLO (Danieli et al., 2019; Gao et al., 2019; Kawasaki et al., 2019). Comparing the effect of three oil sources, i.e., BSFLO, coconut oil (CCO), and corn oil (CO) in broiler chickens, Kim et al. (2020) did not observe any impact on BW, BWG, or FI in chicks at 15 and 30 days old, they did report a noteworthy decrease in FCR for chicks fed CCO and BSFLO compared to the CO group. The authors suggest that this improvement in FCR is likely due to the MCFA abundant in insect oils, which can promote better nutrient digestion and absorption. According to our results, the inclusion of 75% BSFLO likely did not negatively affect the growth performance, especially the BW and FCR of starter and grower periods, compared to a middle level of BSFLO or control groups (Table 3).

The blood serum parameters of broiler chickens play a crucial role in assessing their health and physiological status. The findings presented in Table 4 indicate that replacing RBO with BSFLO in broiler diets exerts effects on some blood cell counts. Specifically, chickens fed with a diet containing 50% BSFLO exhibited higher levels of hemoglobin and WBCs counts on day 21, although increasing WBCs were identified for 50% BSFLO. This finding appears difficult to explain since none of the broilers showed any signs of physical distress. Meanwhile, at day 42, most blood cell parameters showed no significant differences among the groups, indicating potential adaptation or normalization of blood cell counts over time. Notably, eosinophil levels were slightly lower in chickens fed with 50% BSFLO compared to the control and 75% BSFLO groups. Nevertheless, all the hemato-chemical parameters analyzed for the broilers in the present study fell within the physiological reference intervals reported for chicken (Mat et al., 2022), thus suggesting that the BSFLO utilization did not negatively influence the health status or immune system of the broiler chickens. Moreover, The H/L ratio usually has been used for the measurement of distress conditions in chickens in which an increased H/L ratio may indicate that the animals suffered from infections, inflammation, or stress (Salamano et al., 2010; DeMarco et al., 2013; Pozzo et al., 2013). In the present study, the H/L ratio of chickens fed the experimental diets did not present any significant differences compared with the control group.

The activities of plasma ALT, AST, and ALP are generally related to liver damage, acting as biomarkers of liver health when they are damaged (Hyder et al., 2013). The blood biochemistry traits (Table 5) were significantly affected on AST and ALP by the dietary treatments, which showed lower value than the control group, but the value present trial fell and is still within the physiological normal ranges (Schiavone et al., 2018), suggesting that BSFLO may not cause negative effects on the hepatopancreas or liver health. The results of the present research agree with those of Schiavone et al. (2017) and Li et al. (2016), who showed that the use of BSFLO in substitution for soybean oil in broilers and juvenile Jian carp diets,

TABLE 8 The effect of inclusion of black soldier fly larvae oil (BSFLO) in broiler diets on fatty acid profile of liver.

Fatty acid composition	Control	Levels of BSFLO		*p*-value	SEM
	0%	50%	75%		
Saturated Fatty Acid	2.27[ab]	1.90[b]	2.93[a]	0.023	0.37
Buryric acid (C4:0)	0.03[b]	0.01[b]	0.24[a]	0.006	0.09
Myristic acid (C14:0)	0.01	0.01	0.03	0.131	0.01
Palmitic acid (C16:0)	0.95[ab]	0.75[b]	1.29[a]	0.012	0.19
Heptadecanoic acid (C17:0)	0.01	0.01	0.01	0.000	0.00
Stearic acid (C18:0)	0.91[b]	0.88[b]	1.09[a]	0.005	0.08
Arachidic acid (C20:0)	0.01	0.01	0.01	0.064	0.00
Behenic acid (C22:0)	0.02	0.02	0.02	0.100	0.00
Tricosanoic acid (C23:0)	0.33[a]	0.21[b]	0.24[b]	0.003	0.04
Unsaturated Fatty Acid	2.41[a]	1.51[b]	2.18[a]	0.021	0.33
Palmitoleic acid (C16:1n7)	0.05[a]	0.02[b]	0.06[a]	0.001	0.01
cis-9-Oleic acid (C18:1n9c)	0.97[a]	0.57[b]	1.08[a]	0.012	0.19
cis-11-Eicosenoic acid (C20:1n11)	0.02	0.01	0.02	0.065	0.00
Nervonic acid (C24:1n9)	0.01	0.01	0.01	0.100	0.00
cis-9,12-Linoleic acid (C18:2n6)	1.17[a]	0.79[b]	0.87[b]	0.009	0.14
gamma-Linolenic acid (C18:3n6)	0.02	ND	0.01	0.113	0.01
alpha-Linolenic acid (C18:3n3)	0.05[a]	0.02[b]	0.03[b]	0.001	0.01
cis-11,14-Eicosadienoic acid (C20:2n6)	0.02	0.02	0.02	0.100	0.00
cis-8,11,14-Eicosatrienoic acid (C20:3n6)	0.04	0.02	0.04	0.131	0.01
Arachidonic acid (C20:4n6)	0.01	0.01	0.01	0.100	0.00
cis-5,8,11,14,17-Eicosapentaenoic acid (C20:5n3)	0.02	0.02	0.01	0.065	0.00
cis-4,7,10,13,16,19-Docosahexaenoic acid (C22:6n3)	0.03	0.02	0.02	0.065	0.00
Monounsaturated fatty acid (MUFA)	1.05[a]	0.61[b]	1.17[a]	0.013	0.21
Polyunsaturated fatty acid (PUFA)	1.36[a]	0.90[b]	1.01[b]	0.011	0.17
PUFA/MUFA	1.30[ab]	1.48[a]	0.86[b]	0.014	0.22
n-3	0.09[a]	0.06[b]	0.06[b]	0.001	0.01
n-6	1.24[a]	0.83[b]	0.93[b]	0.009	0.15
n-9	1.24[a]	0.59[b]	1.09[a]	0.015	0.24
n-6/n-3	13.8[b]	13.8[b]	15.5[a]	0.043	0.69

[a,b]Different superscripts within a row indicate significant differences ($p < 0.05$).
SEM, Standard error of means.
ND, Not detected.

respectively, had no negative effects on the blood traits of the animals and confirmed the nutritional adequacy of these diets.

The replacement of RBO with BSFLO in broiler chickens did not negatively affect liver function indicators, as measured by AST and ALP activity, between birds fed diets containing different fat sources, consistent with a previous report (Taulescu et al., 2010). This suggests that BSFLO supplementation did not induce liver toxicity or impair liver function. However, the effects on plasma cholesterol were inconclusive. Meanwhile, medium-chain fatty acids are generally known to decrease triglycerides, total cholesterol, and LDL cholesterol, as well as increase HDL cholesterol in chickens (Wang et al., 2015; Khatibjoo et al., 2018). Our results did not show a consistent pattern; we found that triglyceride was higher by, on average, 18.8% in 75% BSFLO-fed chickens than in 50% BSFLO-fed ones (Table 5). However, the BSFLO did not significantly impact total and HDL cholesterol levels compared to chickens fed RBO. It is not clear how BSFLO affected cholesterol metabolism in chickens, necessitating further investigation. In line with our study, dietary BSFLO did not affect blood parameters in chickens, which aligns with previous studies where dietary BSFLO supplementation did not affect blood parameters in chickens (Schiavone et al., 2017), juvenile carp (Li et al., 2016), and rabbits (Gasco et al., 2019). Thus, BSFLO's influence on cholesterol metabolism appears to differ from RBO, although BSFLO is rich in MCFA. It should be kept in mind that RBO has a higher amount of linolenic acid than BSFLO, which is typically known to have a hypocholesterolemic effect (Lorenzo et al., 2014). It is likely that BSFLO would disturb or curb the MCFA–induced increase in plasma cholesterol. Thus, it may be likely that MCFA of BSFLO would disturb or curb–the induced increase in plasma cholesterol, similar to observations with CCO (Wang et al., 2015). Lekshmi Sheela et al. (2016) report that the coconut phyto-compounds, especially lauric acid, were found effective against HMG-CoA reductase enzyme which plays a central role in the synthesis of cholesterol.

None of the viscera was significantly impacted by dietary fat sources. The results of the present study agree with the findings of Schiavone et al. (2017), which reported that the partial or total replacement SBO with BSFLO in growing broiler diets did not affect the growth performance, the feed choice or the carcass traits. In the same context, Cullere et al. (2019) showed that defatted BSFL meal could be introduced into the diet for growing broiler quails at 10% and 15% inclusion levels, partially replacing conventional soybean meal and SBO, with no negative effects on productive performance, mortality or carcass traits. However, RBO partial replacement with both BSFLO groups increased abdominal fat percentages by 32% and 16%, respectively (Table 6). BSF oil is rich in saturated fatty acids (SFA) 1.8 times compared to SBO (Table 1), particularly lauric and myristic acids. In order that, the high SFA intake may promote lipogenesis (fat synthesis) and adipocyte hypertrophy (fat cell enlargement) in abdominal adipose tissue. As research has shown, the fatty acid profile of a broiler's diet directly influences the fatty acid composition of its meat and internal organs. This influence is closely linked to fat storage and metabolism within the animal (Taulescu et al., 2010; Khatun et al., 2018).

Different fats did not affect thigh and breast meat but affected wing yield traits. Meanwhile, the pH of the breast meat was decreased in chickens fed a diet containing BSFLO compared with those fed on SBO. Nonetheless, the negative correlation between the lightness and pH of fresh meat (Qiao et al., 2001) was not observed in this study (Table 7). Of interest is the BSFLO trend in medium-chain fatty acids vs. SBO, which has increased b^* values. Increased breast meat yellowness has been postulated with an increase in carotenoid contents (da Silva et al., 2017) or lipid contents (Zhao et al., 2018) in broiler chickens. Nonetheless, all observed values regarding pH, meat colors, and cooking loss of breast meat still fall within the acceptable range according to meat quality characteristics (Cullere et al., 2019). Further analysis of the nutrient profile, fatty acid composition, and antioxidant content of breast and leg meat could provide insights into the differences in meat quality observed between the various fat sources used in the diets.

The liver plays a central role in maintaining healthy fat balance (lipid homeostasis) through a complex network of precisely regulated biochemical pathways, signaling mechanisms, and cellular processes. Hepatocytes, the main functional cells of the liver, are responsible for these biochemical and metabolic functions, including lipid metabolism. In a healthy state, the liver processes large amounts of fatty acids daily while storing only a small portion as triglycerides. Omega-3 fatty acids, particularly docosahexaenoic acid (DHA) and its precursor docosapentaenoic acid (DPA), are critical factors reflecting changes in liver health (Schiavone et al., 2017). Notably, omega-3 fatty acids and their derivatives possess potent anti-inflammatory properties that help mitigate the metabolic effects of oxidative stress in the liver and other tissues (Dabbou et al., 2021; Schiavone et al., 2017). Additionally, maintaining a proper balance between different fatty acid types is crucial. This includes the n-6/n-3 and PUFA/MUFA ratios, which should not be excessively high. Our findings revealed that partially replacing refined RBO with BSFLO at 50% and 75% inclusion levels resulted in favorable n-6/n-3 (0.86) and PUFA/MUFA (1.38) ratios, respectively (Table 8).

In conclusion, the important differences in the dietary contents of lauric and myristic FAs with increasing BSFLO inclusion had no beneficial effect on amount of fat accumulated in the abdominal fat and also FCR in the final period. Despite this, the findings of this study suggest that BSFLO could partially replace RBO in chickens' diet at 50% without any adverse effects on growth performance, hematological parameters, serum biochemical indices, or carcass and meat quality. These findings suggest that BSFLO can be a suitable ingredient for poultry diets. Further research efforts are necessary to investigate the impact of BSFLO on the meat quality traits and fatty acid profile of broiler chickens in depth.

Data availability statement

The original contributions presented in the study are included in the article/supplementary materials, further inquiries can be directed to the corresponding author.

Ethics statement

The animal study was approved by the Institutional Animal Care and Use Committee of Khon Kaen University, Thailand. (Record no. IACUC-KKU-60/66). The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

NS: Investigation, Methodology, Validation, Writing – original draft, Writing – review & editing. PP: Conceptualization, Data curation, Formal analysis, Methodology, Writing – original draft, Writing – review & editing. WP: Conceptualization, Investigation, Methodology, Validation, Writing – original draft. NP: Investigation, Methodology, Validation, Writing – original draft. TS: Investigation, Validation, Writing – original draft. BT: Funding acquisition, Writing – review & editing. AC: Writing – review & editing. SW: Conceptualization, Data curation, Formal analysis, Funding acquisition, Methodology, Project administration, Resources, Supervision, Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Genetic parameters of milk yield and fertility traits in Moroccan Holsteins

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Relatively high milk-producing cows in Morocco are culled at an elevated rate due to poor fertility. This situation is due to genetic, environmental, and management-related factors. To remedy this situation or prevent an additional decline in reproductive performance, using locally produced replacement heifers with good fertility and yield traits was proposed as a solution. Identifying these animals requires estimating the genetic parameters of the relevant reproduction and production traits in order to develop a genetic evaluation. Three reproduction traits (number of inseminations per conception, success of first insemination, and days open) and 305-d milk yield were used in this study. Two datasets of 4,186 records (first parity cows) and 5,511 records (first and multi-parity cows) were used. The pedigree files for both datasets consisted of 8,758 and 9,935 animals, respectively. A threshold-linear model was used for the analyses. For the first parity, estimates of heritability for 305-day milk yield (MY), days open (DO), number of inseminations per conception (NIC), and success of first insemination (SFI) were 0.26 ± 0.04, 0.17 ± 0.04, 0.10 ± 0.03, and 0.10 ± 0.04, respectively. For multi-parity data, the estimates were 0.19 ± 0.03, 0.12 ± 0.02, 0.10 ± 0.02, and 0.09 ± 0.02 for MY, DO, NIC, and SFI, respectively. The genetic correlations between MY and reproduction traits were 0.15 ± 0.11, 0.38 ± 0.12, and -0.43 ± 0.11 for DO, NIC, and SFI respectively. Overall, the heritability estimates of fertility traits were low. The genetic correlations of DO, NIC, and SFI with MY were moderately negative allowing for further selection for milk production without an additional deterioration in reproductive performance. The relative impact of using high fertility bulls compared to low fertility bulls on the success of first insemination ranged between 1.2% and 6.3% depending on the production environment. Collectively, these results confirm the possibility of implementing a viable selection program based on an appropriate weighted selection index.

KEYWORDS

heritability, genetic correlations, milk yield, fertility, Moroccan Holstein

1 Introduction

Over the last two decades, milk production in Morocco has witnessed a significant increase largely due to the import of purebred heifers and semen of elite sires, the integration of artificial insemination, and the enhancement of on-farm data recording practices. Increased milk production and the multiple environmental and management-related obstacles have led to a deterioration in reproductive performance. This situation has increased the early involuntary culling resulting in a marked negative impact on dairy farms' profitability and sustainability (Boujenane, 2017). In fact, imported heifers rarely reach their third lactation in the Moroccan production environment (Sraïri and Baqasse, 2002). To remedy this situation or prevent additional decline in reproductive performance, locally produced replacement heifers with good fertility and yield traits was proposed as a solution. Identifying these animals requires estimating the genetic parameters of the relevant reproduction and production traits in order to develop a genetic evaluation.

The majority of fertility traits phenotypes suffer from a high noise level (Rodriguez et al., 2008) due to several reasons including inconsistent management practices, preferential treatment, and incomplete reporting. This has often resulted in low heritability estimates for these traits (Berry et al., 2019). Additionally, several reproductive traits are discrete (e.g., success of first insemination). Furthermore, these traits have moderate to high antagonistic genetic correlations with milk yield (Windig et al., 2006). Consequently, an accurate genetic evaluation for fertility and milk yield traits requires the availability of high-quality field data and an appropriate statistical model. Linear-threshold models are recommended for the joint analysis of fertility and production traits to maximize the amount of information and to accommodate discrete traits (Abdollahi-Arpanahi et al., 2013; Ayalew et al., 2017).

Three fertility traits (DO, NIC, and SFI) were systematically collected at the dairy herds used in this study. However, estimates of their heritabilities and correlations with milk yield are seldom available. This information is needed to assess the traits to include in a potential future genetic evaluation. The objectives of this study are to estimate the genetic parameters of 305-day milk yield, days open, number of inseminations per conception, and success of first insemination using an appropriate multivariate linear-threshold model in a Moroccan Holstein population and to assess the benefits of using semen from high fertility bulls on the SFI across different production environments.

2 Material and methods

2.1 Data

The data was collected at four herds owned by the Les Domaines Agricoles company. All herds are situated in two regions in north Morocco (Rabat-Salé-Kénitra, and Fez-Meknès). These regions are characterized by a Mediterranean climate with mild and humid winters and hot and dry summers. Cows were housed in open stalls and fed a total mixed ration twice a day. Artificial insemination (AI) was the sole breeding method utilized and it was administered by specialized AI technicians using frozen semen imported from Europe and North America. Semen quality is routinely assessed at artificial insemination (AI) centers for production and marketing purposes. However, at the farm level, while semen quality may be checked prior to purchase, it is neither quantified, assessed, nor recorded after thawing for use in artificial insemination procedures. Consequently, this critical parameter is excluded from data collection and subsequent analyses, despite its potential significant role and high correlation with multiple fertility traits in females, such as conception rate and non-return rate (Gebreyesus et al., 2021). However, the stringent assessment of the quality of semen used in AI will likely reduce the impact of male contribution on reproduction traits as supported by several studies (Averill et al., 2006; Miglior et al., 2017).

Estrus detection was conducted through visual inspection. The dataset consisted of 13,255 records collected on 7,928 cows between 2015 and 2023. The number of lactations per cow ranged between 1 and 5. Daily records including calving events, veterinary interventions, health issues, abortions, and dry-off dates were logged using herd management software.

Only the first 3 lactations were retained to reduce inconsistencies and non-random missingness. 305-d milk yield (MY), days open (DO), and number of inseminations per conception (NIC) were considered as continuous traits in the analysis, and success of first insemination (SFI) as a binary response. Parities with matching calving and abortion dates were removed. DO and NIC were considered missing when the conception date was missing, and no subsequent calving was reported. NIC and DO ranged between 1 and 17, and 20 and 574 days, respectively. Animals with first calving before 530 days of age were excluded. After editing, the final multi-parity data consisted of 7,600 records collected on 5,511 cows. The dataset for the first parity included 4,186. For the four-trait analysis, the pedigree file included 9,935 purebred Holstein animals, with cows born between 2006 and 2023. The majority of the animals (6,146 animals) have at least one parent known. The animals with records were born between 2016 and 2023. The average number of progenies was 4.05 and 1.16 for sires and dams, respectively. All inseminations were carried out using imported semen. The paternal grandsires were not available on all animals and the ages of the bulls were not documented. The average age of cows was 777, 1,219, and 1,655 days for first, second, and third parity, respectively.

2.2 Statistical model

A Bayesian multivariate linear-threshold model was used to jointly analyze four productive and reproductive traits for both first parity and multiparity cows. The linear model assumed at the liability scale included the fixed effects of herd (4 levels), calving month (12 classes), health status (2 levels), abortion status (3 levels), and two covariates (age at calving and days in milk at first insemination). Herd and calving month were included as separate factors and not as a contemporary group to prevent extreme-category problems (ECP) classes (all observations within a fixed effect class are either 0 or 1) for SFI. Health status was defined as healthy if no health issues (e.g., mastitis, metritis, and lameness), excluding abortion, occurred during lactation; otherwise, the cow was considered non-healthy regardless of the number of health problem episodes. Abortion status was coded 1 if no abortion occurred, 2 if one or more abortions occurred during the milk production period, and 3 if one or more abortions occurred during the dry period. In addition, the parity number was included as a fixed effect for the repeatability model.

At the liability scale, the following mixed linear model was used to analyze the data of the first three parities

$$\boldsymbol{y} = \mathbf{X}\boldsymbol{\beta} + \boldsymbol{Zu} + \mathbf{W}\boldsymbol{pe} + \boldsymbol{e} \tag{1}$$

where $y = (y_{MY}, y_{DO}, y_{NIC}, l)'$ is the vector of phenotypes for milk yield (y_{MY}), days open (y_{DO}), number of inseminations to conception (y_{NIC}), and the liabilities l for the binary response (success of first insemination). β, u, pe and $\boldsymbol{e}$ are the vectors of fixed, additive effects, random permanent environmental effects, and error terms. $\mathbf{X}$, $\mathbf{Z}$, $\mathbf{W}$ are known incidence matrices with the appropriate dimensions.

Conditionally on the model parameters, the joint distribution of the three continuous traits and the liability of the binary response were assumed to be normal

$$\begin{pmatrix} y_{MY} \\ y_{DO} \\ y_{NIC} \\ l \end{pmatrix} \sim N(\mathbf{X}\beta + \mathbf{Z}\boldsymbol{u} + \mathbf{W}pe,\ \mathbf{R_0}\otimes\mathbf{I})$$

where $R_0$is a 4x4 residual covariance matrix between the three continuous traits and the liability for the success of first insemination and I is the identity matrix with dimensions equal to the number of animals with data. Note that the last diagonal element of the residual covariance matrix is fixed to 1 to make the model identifiable (Gianola, 1982).

When using only the first parity data, the permanent effect and the parity number were dropped from the model presented in Equation 1. To finalize the Bayesian formulation, the following priors are assumed for the position parameters

$$p(\beta) \sim \mathrm{U}[-10^6,\ 10^6\ ]$$

$$\boldsymbol{u}|\mathbf{A},\mathbf{G} \sim \mathrm{N}(\mathbf{0},\ \mathbf{G}\otimes\mathbf{A})$$

$$\boldsymbol{p}|\mathbf{P} \sim \mathrm{N}(\mathbf{0},\ \mathbf{P}\otimes\mathbf{I})$$

where $\mathbf{G}$ and P are 4x4 genetic and permanent environmental covariance matrices, respectively. $\mathbf{A}$ is the additive relationship matrix.

For the genetic and permanent environment covariance matrices, scaled inverse Wishart distribution priors were assigned

$$\mathbf{G}\ |\boldsymbol{S_g},\ \vartheta_g \sim\ IW(\boldsymbol{S_g}\ ,\ \vartheta_g)$$

$$\mathbf{P}|\mathbf{S_p},\ \vartheta_p \sim\ IW(\mathbf{S_p},\ \vartheta_p)$$

where S_g and $\mathbf{S_p}$ are 4x4 covariance matrices and ϑ_g and ϑ_p are the degrees of belief *a priori* for the genetic permanent environmental covariances, respectively.

The residual covariance matrix $\mathbf{R}_0$, is not completely random due to the fixation of the fourth diagonal element (corresponding to the binary traits) to 1. Consequently, direct sampling of $\mathbf{R}_0$ is not feasible. To overcome the problem of sampling of the residual (co) variance, the methods described by Rekaya et al. (2013) were used.

Briefly, the model in [1] is multiplied by a matrix $\mathbf{D} = \ \mathbf{D}_0\otimes\mathbf{I}$ where $\mathbf{D}_0$ is a 4x4 diagonal matrix, and $\mathbf{I}$ is an identity matrix with the appropriate dimensions, yielding an equivalent model:

$$\boldsymbol{y}^* = \boldsymbol{X\beta}^* + \boldsymbol{Zu}^* + \boldsymbol{Wp}^* + \boldsymbol{e}^* \tag{2}$$

where $\boldsymbol{\beta}^* = (\boldsymbol{\beta}_1^*, \boldsymbol{\beta}_2^*, \boldsymbol{\beta}_3^*, \boldsymbol{\beta}_4^*)$, $\boldsymbol{u}^* = (\boldsymbol{u}_1^*, \boldsymbol{u}_2^*, \boldsymbol{u}_3^*, \boldsymbol{u}_4^*)$, $\boldsymbol{p}^* = (\boldsymbol{p}_1^*, \boldsymbol{p}_2^*, \boldsymbol{p}_3^*, \boldsymbol{p}_4^*)'$ with $\boldsymbol{\beta}_i^* = \boldsymbol{\beta}_i d_{ii}$, $\boldsymbol{u}_i^* = \boldsymbol{u}_i d_{ii}$, and $\boldsymbol{p}_i^* = \boldsymbol{p}_i d_{ii}$ are the vectors of fixed, additive, and permanent environmental effects for the trait i in the identifiable model in [1], respectively, and d_{ii} is the diagonal element i of the matrix $\mathbf{D}_0$. The resulting model in [2] is not identifiable because $\mathbf{D}$is unknown. The residual (co)variance matrix of the non-restricted model in [2] is given by:

$$var(\boldsymbol{e}^*) = \mathbf{DRD}' = \Sigma = \Sigma_\mathbf{0}$$

$$var(\boldsymbol{e}^*) = \mathbf{DRD}' = \Sigma = \Sigma_\mathbf{0}\otimes\mathbf{I} \tag{3}$$

where $\mathbf{R}$ is the original restricted residual (co)variance matrix in [1], and Σ_0 is a 4x4 residual (co)variance matrix of the non-restricted model in [2]. Thus, estimates of the restricted residual covariance matrix $\mathbf{R}$ could be easily obtained using Equation 3 and the non-restricted matrix Σ_0. The lack of restrictions in Σ enormously facilitates the Bayesian implementation via Markov Chain Monte Carlo (MCMC) methods. However, to obtain the parameters of the identifiable model in [1] from the draws of the non-identifiable parameters, the matrix D needs to be defined. The identifiable parameters, based on expressions in [2] and [3], can be retrieved as:

$$\boldsymbol{\beta}_i = \frac{1}{d_{ii}}\boldsymbol{\beta}_i^*; u_i = \frac{1}{d_{ii}}\boldsymbol{u}_i^*;\ \text{and}\ \boldsymbol{p}_i = \frac{1}{d_{ii}}\boldsymbol{p}_i^* \tag{4}$$

$$\boldsymbol{R} = \boldsymbol{D}^{-1}\Sigma\boldsymbol{D}^{-1};\ \text{and}\ \boldsymbol{R_0} = \boldsymbol{D_0}^{-1}\Sigma_\mathbf{0}\boldsymbol{D_0}^{-1} \tag{5}$$

Given that the diagonal element of $\mathbf{R}_0$ corresponding to the binary response is fixed to 1, the last diagonal element of the matrix $\mathbf{D}_0$ must be equal to the square root of the corresponding element in Σ_0, and the first 3 diagonal elements of $\mathbf{D}_0$ corresponding to the continuous traits are set equal to 1 as indicated in Rekaya et al. (2013) and Chang et al. (2017). A flat bounded prior was assumed for Σ_0.

2.3 Effects of the genetic component on the probability of success of first insemination

The success of the first insemination was scored as a binary response. Under the assumed model, the liability for animal i, conditionally on the model parameters is given by:

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$$l_i|\mu_i \sim N(\mu_i, 1) \quad (6)$$

where l_i is the liability for animal i, $\mu_i = x_i\beta + u_i + p_i$, and β, u_i and p_i are the vector of solutions for the fixed effects, the animal breeding value, and the permanent environmental effect, respectively. x_i is a known incidence vector relating the fixed effects to the liability.

The relationship between the observed ordered categorical response and the liabilities is given by:

$$c_j = \begin{cases} 1 \text{ if } l_i > T = 0 \\ 0 \text{ otherwise} \end{cases} \quad (7)$$

where $c_i = j$ is the binary response for animal i is equal to j $(j = 0, 1)$, and the threshold value (T) was assumed to be equal to zero.

Given the distribution in Equation 6 and the relationship in Equation 7, the probability of observing class $j = 1$ for animal i is given by:

$$pr(c_i = 1|\boldsymbol{\beta}, u_i, p_i, \sigma_e^2 = 1, T = 0) = 1 - \Phi(T = 0|\eta_i, \sigma_e^2 = 1) \quad (8)$$

where $\Phi(T = 0|\eta_i, \sigma_e^2 = 1)$ is the cumulative distribution function for the normal distribution with mean equal to η_i and variance equal to σ_e^2 evaluated at $T = 0$.

The impact of using the best (highest breeding value for SFI) versus the worst bull on the probability of success of first insemination was assessed across three production environments (bad: μ_i=-1; average= μ_i; and good: μ_i =+1).

TABLE 1 Least square means (LSM) and standard deviations for 305d-milk yield (305-MILK), days open (DO), number of inseminations per conception (NIC), and success of first insemination (SFI) across the first three paities.

Parity	Trait	Number of observations	LSM	Standard deviation
First	305-MILK, kg	4186	8759.0	1489.1
	DO, d		152.5	91.3
	NIC		3.7	2.9
	SFI		0.2	
Second	305-MILK, kg	2380	9838.0	1620.3
	DO, d		157.5	94.4
	NIC		4.0	3.6
	SFI		0.2	
Third	305-MILK, kg	1034	9965.0	1696.4
	DO, d		161.4	98.6
	NIC		4.3	3.9
	SFI		0.2	
Total		7600		

Gray shading indicates not applicable.

3 Results and discussion

3.1 Descriptive statistics for the traits

Table 1 presents a summary description of the four traits. Around 43% of the records were removed during the data editing for several reasons. Observation loss increased with the parity number. This trend can be attributed to the elevated culling rate and the dependence on imported heifers resulting in a substantial portion of cows failing to progress beyond their initial lactation due to fertility and health issues. The 305-d milk production increased with the parity and it was comparable to the results reported by Fahim et al. (2021). However, it was slightly higher than what has been reported by Ouarfli and Chehma (2021). Overall, the cows' reproductive performance across all parities were notably subpar. Across the three first parities, DO ranged between 153 to 161 days (Table 1). These results align closely with findings from Tunisia where M'Hamdi et al. (2009) reported a similar average DO interval of 151 days. Boujenane and Draga (2021), reported a shorter interval of 125 days, while El-Sherief et al. (2022) reported a much longer DO interval (173 days).

The NIC was relatively high across all three lactations, averaging around 4 inseminations per conception. Similar results were reported by Fahim et al. (2021) for Egyptian Holstein cows, and smaller estimates were reported by El-Sherief et al. (2022) and Boujenane and Draga (2021). It's worth noting that the number of inseminations per conception is directly linked to Days Open (DO), as both metrics reflect similar aspects of cow reproductive efficiency. Nevertheless, DO fails to account for the time elapsed between calving and first insemination, a gap addressed by the number of inseminations per conception (NIC) as suggested by Abdollahi-Arpanahi et al. (2013). However, DO is recommended for populations that lack a good recording scheme (González-Recio et al., 2006). Therefore, NIC emerges as a more comprehensive indicator of fertility. This comparison underscores the importance of considering multiple metrics to gain a holistic understanding of dairy cows' reproductive performance.

The success of the first insemination, a reflection of the pregnancy rate and closely linked to the non-return rate, was remarkably low across all parties, particularly in the first parity. This finding is unexpected, considering that the first insemination success rate typically tends to be higher for first parity cows compared to older ones. On top of the limited number of observations for older cows, one possible explanation for this discrepancy is the strong culling rate for cows failing the first 2 or 3 inseminations if they calved in fall or early winter. Thus, older cows are heavily selected for their ability to conceive early. The observed conception rate in this study appears to be lower than the 0.46 and 0.41 reported by Boujenane and Draga (2021) and El-Sherief et al. (2022), respectively.

3.2 Estimates of variance and genetic parameters

Table 2 presents the estimates of the posterior means (PM) and the posterior standard deviations (PSD) of heritabilities, genetic,

TABLE 2 Heritability estimates (diagonal), genetic correlations (above), and residual correlations (below) for 305d-milk yield (305-MY), days open (DO), number of inseminations per conception (NIC), and success of first insemination (SFI) in first parity.

	305-MILK, kg	DO, d	NIC	SFI
305-MILK, kg	0.26 ± 0.04	-0.11 ± 0.14	-0.23 ± 0.14	0.04 ± 0.10
DO, d	0.21 ± 0.04	0.17 ± 0.04	0.96 ± 0.019	-0.73 ± 0.08
NIC	0.21 ± 0.04	0.89 ± 0.006	0.10 ± 0.03	-0.80 ± 0.05
SFI	-0.32 ± 0.04	-0.67 ± 0.02	-0.81 ± 0.01	0.10 ± 0.04

and residual correlations for the four traits in the first parity. For fertility traits, heritability estimates were 0.17, 0.10, and 0.10 for DO, NIC, and SFI, respectively. The negative correlations of DO and NIC with milk production and the small correlations between SFI and the other traits should be noted. However, these results must be interpreted with caution given the wide high posterior density intervals associated with these estimates spanning from negative to positive values due to the limited size of the dataset and the sparseness of pedigree information.

Estimates of heritability and repeatability using the first three parities data are presented in Table 3. The 305-day milk yield heritability estimate (0.19 ± 0.03) was comparable to the findings reported by Bakri et al. (2022). However, it was slightly lower than those reported in several studies conducted in arid and Mediterranean regions (Fahim et al., 2021; M'Hamdi et al., 2009; Ojango and Pollott, 2001; Sadek et al., 2021; Wahinya et al., 2020; Windig et al., 2006). Heritability estimates for fertility traits were low and within the range of reported estimates in the literature (Biffani et al., 2005; González-Recio and Alenda, 2005; Inchaisri et al., 2011; Jamrozik et al., 2005; M'Hamdi et al., 2009; Ojango and Pollott, 2001; Sadek et al., 2021; Wahinya et al., 2020) with a posterior mean of 0.12, 0.07, and 0.08 for DO, NIC, and SFI, respectively. Repeatability estimates (Table 3) ranged between moderate for milk yield (0.29) and low for fertility traits (0.08 to 0.014). Estimates of genetic correlations using the threshold-linear model indicated positive associations between 305-day milk yield, days open, and number of inseminations per conception, suggesting that high milk-producing cows often require more inseminations to conceive and exhibit prolonged days open. Conversely, a negative correlation was observed between the success of first insemination and all other traits, indicating that high milk-yield producers are more likely not to conceive soon after calving. This is likely due to high milk production around the peak of the lactation leading to a negative energy balance. The genetic correlations between SFI, DO, and NIC ranged between -0.97 and -0.89 (Table 4). The correlation between NIC and DO was high and positive (0.83). The residual correlations between the four traits were similar in trend and magnitude to those observed for the genetic correlations (Table 4) except for the correlation between 305-d milk yield and SFI which was substantially smaller (in absolute value) than the genetic correlation (-0.12 vs -0.43). Notably, the standard deviations associated with the estimates of genetic correlations between milk yield and fertility traits are relatively large indicating potential heterogeneity in managing cows based on their milk yield or/and reproductive performance. However, despite the small size of the dataset, most of the estimates of the genetic parameters are

TABLE 3 Heritability and repeatability estimates for 305d-milk yield (305-MY), days open (DO), number of inseminations per conception (NIC), and success of first insemination (SFI) using the first three parities.

Trait	Heritability	Repeatability
305-MILK, kg	0.19 ± 0.03	0.29 ± 0.02
DO, d	0.12 ± 0.02	0.14 ± 0.02
NIC	0.07 ± 0.02	0.08 ± 0.01
SFI	0.08 ± 0.02	0.09 ± 0.02

TABLE 4 Genetic (above the diagonal) and residual correlations (below diagonal) between 305d-milk yield (305-MILK), days open (DO), number of inseminations per conception (NIC), and success of first insemination (SFI).

	MILK, kg	DO, d	NIC	SFI
MILK, kg		0.15 ± 0.11	0.38 ± 0.12	-0.43 ± 0.11
DO, d	0.16 ± 0.03		0.83 ± 0.06	-0.89 ± 0.04
NIC	0.12 ± 0.03	0.81 ± 0.007		-0.97 ± 0.01
SFI	-0.12 ± 0.03	-0.86 ± 0.02	-0.99 ± 0.004	

Gray shading indicates cells where the correlation of a trait with itself is equal to 1. These values are not shown, as they are inherently perfect correlations and therefore not informative for the purposes of this analysis.

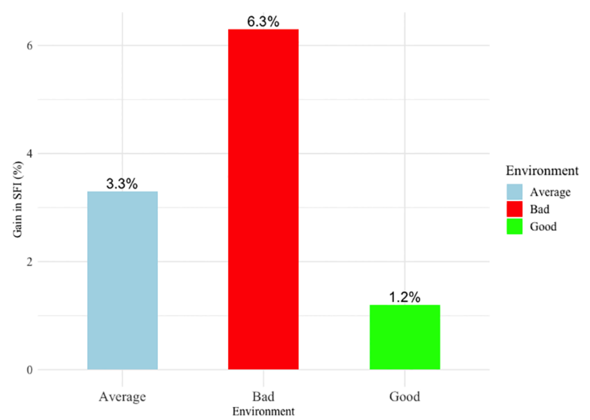

FIGURE 1
The relative gain in the success of the first insemination in a hypothetical scenario of using the best and the worst bulls across three different production environments.

reasonably accurate as reflected by the relatively small associated standard errors. This could be due in part to relatively better pedigree information.

Figure 1 presents the percentage gain in the success of first insemination (SFI) in the hypothetical case of using the best sire (based on estimated breeding values) compared to using the worst sire across three production environments (bad, average, and good). The data reveals a notable difference in impact depending on the environment type. In a bad production environment, the gain in SFI is the more substantial (6.3%) indicating that using a top sire for fertility traits can greatly enhance reproductive success under challenging conditions. Conversely, in a good production environment, the benefit of using a top sire will result only in a 1.2% increase in SFI suggesting that under favorable conditions, the choice of sire has limited impact in the SFI in concordance with previously reported results (Averill et al., 2006). In an average production environment, an intermediate benefit of 3.3% was observed. These findings highlight that the advantage of using sires with superior breeding values is most pronounced in suboptimal conditions, while in optimal settings, the relative gain diminishes.

In general, the results of this study align with previous research indicating the relatively low heritability of fertility traits in Holstein cows (Fahim et al., 2021; Inchaisri et al., 2011; M'Hamdi et al., 2009; Ojango and Pollott, 2001; Sadek et al., 2021; Wahinya et al., 2020). Additionally, our investigation reveals a strong positive correlation between days open and the number of inseminations per conception. This correlation suggests that cows experiencing prolonged intervals between calving and conception tend to require more inseminations to conceive. Notably, the repeatability of days open was higher than that of NIC. This can be attributed to the fact that the length of DO interval is more susceptible to management interventions, such as delaying the first insemination for high-yielding cows. In fact, it was argued against using days open as a trait for fertility evaluation, particularly in settings with comprehensive recording schemes and management practices (González-Recio et al., 2006). However, in contexts where such schemes are lacking, days open may still offer valuable insights into reproductive performance and management strategies (González-Recio et al., 2006). The success of first insemination is an important economic determinant, encompassing the cost of semen and the labor associated with monitoring heat cycles and conducting inseminations. Integrating SFI into the fertility index of a herd holds considerable merit, as it allows for a more comprehensive evaluation of reproductive performance and cost-effectiveness. Furthermore, the outcome of each insemination can be assessed as a longitudinal binary variable, as demonstrated by (Averill et al., 2006). This modeling approach incorporates all breeding information within a certain period, allowing for more precise estimations.

Accurate reproductive data is hard to collect especially under Moroccan dairy production and management conditions. Similarly, the pedigree information is often incomplete or inaccurate. Thus, stringing data pre-processing and editing is needed. This process often leads to loss of information resulting in relatively small datasets to carry out genetic analyses. Although the estimates of genetic parameters in this study seem to be accurate based on their associated standard errors, they should be interpreted with some caution. Overall, despite the limitations of this study, the results provide valuable insights while we accumulate additional data.

4 Conclusion

Female fertility is crucial for the profitability of dairy herds, particularly for Holstein cows. However, this relationship is often marred by an antagonistic correlation with milk yield. The moderate genetic correlations between milk yield and the three fertility traits considered in this study support the possibility to attenuate the deterioration or even improve dairy cows' fertility in Morocco without sacrificing the selection for higher milk yield. This could be achieved through a selection index that includes all relevant traits with their relative weights. Defining appropriate traits to include in selection indices is paramount. A particular emphasis should be put on traits directly impacting profitability, such as the number of inseminations per conception and the success rate at first insemination, which include information on veterinary, breeding, and labor costs. A fundamental hurdle in fertility trait analysis lies in the noisy field datasets and the non-normal distribution of some traits, notably the discrete nature of the success rate of first insemination.

Data availability statement

The data analyzed in this study is subject to the following licenses/restrictions: The data used in this study are not publicly available due to restrictions imposed by the company that provided the data. Consequently, we are unable to share the data as the company does not permit its dissemination. Requests to access these datasets should be directed to bouabidbadaoui@gmail.com.

Author contributions

NC: Conceptualization, Data curation, Formal analysis, Methodology, Writing – original draft, Writing – review & editing. BB: Conceptualization, Project administration, Resources, Supervision, Validation, Writing – review & editing. RR: Conceptualization, Formal analysis, Methodology, Project administration, Supervision, Validation, Writing – review & editing.

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Conflict of interest

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Breeding practices and selection criteria in Gudali cattle breed from Benin: implications for the design of a community-based breeding program

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Understanding farmers' breeding systems and preferred traits is crucial for establishing effective genetic improvement programs. This study investigated Gudali cattle breed selection, breeding objectives, and selection criteria in North-east Benin (Malanville and Tchaourou). We surveyed 120 Gudali cattle farmers using a structured questionnaire and conducted hierarchical clustering using R software. We identified the distribution of farmers based on herd composition and production systems. Farmers were grouped into four classes: Sedentary Purebreds (16.67%), Transhumant Purebreds (33.33%), Sedentary Mixed Breeds (34.17%), and Transhumant Mixed Breeds (15.83%), with average Gudali herd sizes ranging from 23.68 to 90.11 heads depending on the system. The overall average herd size was 42.67 $\pm$ 6.00 heads. The majority of farmers owned different breeds, including Borgou (26.67%), Yakana (26.67%), and Azawak (7.5%), with only 32.5% having Gudali only herds. Farmers chose Gudali cattle for their milk production, good growth and market value, with 96.67% prioritizing milk production. The main selection criterion was coat color in all breeding systems with respective indices of 0.59; 0.57; 0.54 and 0.47 respectively for sedentary purebred; sedentary mixed breed; transhumant purebred and transhumant mixed breed systems. Most mixed breed farmers (55.84%) cross Gudali with local breeds for better dairy performance. While only 3.33% of farmers were aware of community-based breeding programs (CBBP), there was strong interest (95%) in participating. Potential challenges such as access to feed and disease management were reported. The proposed mitigation strategies include establishing pasture areas and strengthening collaboration among stakeholders. Implementing CBBP programs by incorporating farmers' preferences and practices, could sustainably improve Gudali cattle productivity and resilience in Benin.

KEYWORDS

criteria, selection, cattle, CBBP, traditional knowledge, livestock improvement, West Africa

Introduction

Livestock plays important role in the economy of developing countries. Its contribution to GDP was estimated to about 44% for West African countries (Kamuanga et al., 2008). The West African region has more than 76 million heads of cattle, 279 million small ruminants, 564 million poultry, which makes it a quintessential breeding area par excellence (FAOSTAT, 2023). Livestock sector also represents a source of income and nutrition for the resourceless populations. For millions of people, it mainly represents insurance for postharvest risks, especially during lean periods (Lawal et al., 2018). In addition to its food and economic importance, livestock also has cultural importance and reflects the social status of the farmer.

Cattle breeding provides a large part of human food through dairy and meat production, which indirectly constitutes a source of savings for farmers (Chettoui and Boughena, 2020). However, Soukehal (2013) shows that livestock farming is subject to a multitude of constraints which hinder the development of dairy and meat production.

Traditional production systems, characterized by low productivity, dominate African livestock farming (Bello Abdul Sobour et al., 2021). In Benin, various national initiatives to improve milk production through crossbreeding local breeds with exotic ones have largely been unsuccessful (Doko et al., 2012; Youssao, 2015). The Zebu breed, particularly Gudali, offers a promising avenue for improving local cattle performance due to its robustness and productivity (Alladaye and Youssao, 2017; Assani, 2013; Boya et al., 2022). Despite its potential, research on the Gudali breed in Benin is limited, primarily focusing on morphological traits and performance (Assani et al., 2015; Assani and Alkoiret, 2014; Houessou et al., 2019). Located in Northeast Benin, the Gudali cattle are well adapted to harsh environmental conditions. However, there is a lack of studies on farmers' preferred traits, production objectives, and selection criteria. The first step in successful implementation of any breeding program is to characterize production systems and to identify farmer's specific preferred traits in relation to breeding objectives (Ouédraogo et al., 2020). Following the profound socio-economic changes that have affected both Gudali natural biotope and its genetic diversity, it is essential to evaluate the farmers' preferred traits, selection criteria of the breeding stock as well as the indigenous knowledge used for the breed improvement and conservation.

This study is unprecedented in its focus on the Gudali cattle breed in North-eastern Benin, addressing a significant gap in the literature by examining farmers' preferences and breeding strategies. The aims of this study were therefore to characterize the breeding practices, selection criteria and farmer's preferred traits for Gudali cattle breed in the North-Eastern part of Benin Republic in order to design community-based breeding strategies.

Materials and methods

Study area

Data collection took place in the municipalities of Malanville (GPS coordinates of 11° 51' 47.9988" North and 3° 23' 3.2892" East) and Tchaourou (GPS coordinates of 8° 53' 11.4" North, 2° 35' 51.1" East) in North-East Benin (Figure 1). The choice of the two municipalities is not only due to their large number of cattle herds but also due to their diversity in farming systems (Assani, 2013). In addition, livestock farming occupies a prominent place in the development of these municipalities. They have the main centers of development of the livestock sector.

Methodology

One hundred and twenty (120) cattle farmers with a total of 5144 heads of Gudali cattle breed were investigated from June to December 2022 using a questionnaire (semi-structured interview). The cattle farmers having at least one Gudali cattle breed in their herds were selected according to their availability and willingness to participate in the study. Snowball sampling was used to identify additional herders in the two municipalities (Assani, 2013; Dossa and Vanvanhossou, 2016; Worogo et al., 2023). The contents of the questionnaire included the reasons for the choice of the breed, the selection criteria they use, and their preferred selection traits in order of priority. The preferred selection traits were grouped by category for data analysis. The questionnaire was administered in the farmer's mother tongue with the help of translators.

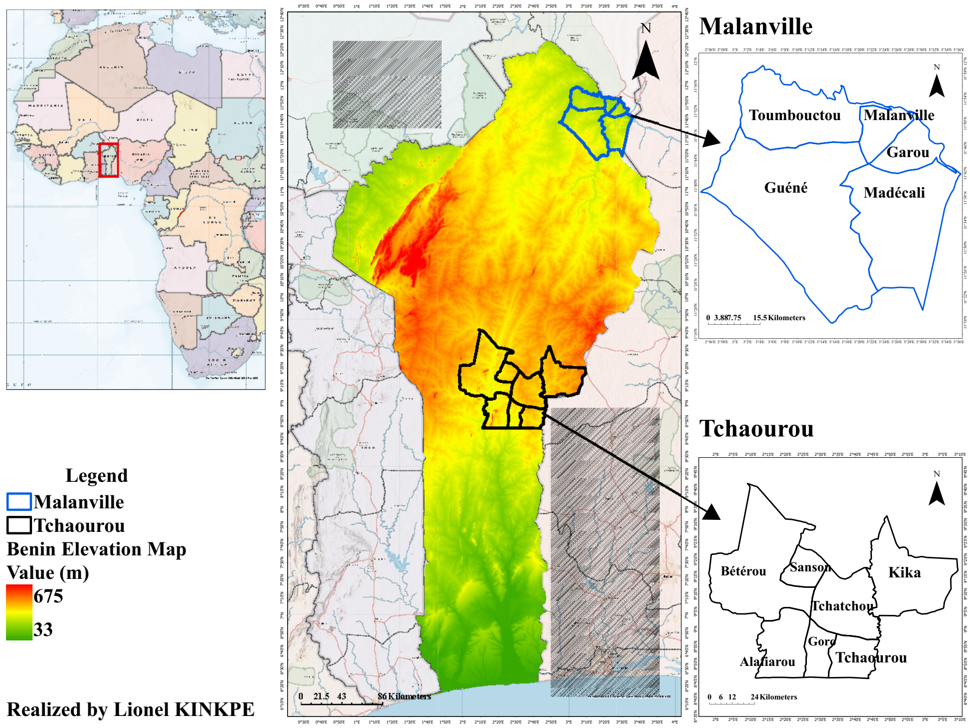

FIGURE 1
Map of Benin showing the study area.

The survey data was processed using an Excel spreadsheet, and corrected before being imported into the R version 4.2.0 software (R Core Team, 2020). An ascending hierarchical classification based on the characteristics of the farms on the most significant Factor Correspondence Analysis (FCA) components made it possible to identify and characterize the farms. The variables "main production", "breed preference, and reasons for the choice of farmers" were taken into account. For the ranking of trait preferences for bull breeds, the number of times and the order in which the trait was mentioned by individual cattle owners were considered to weigh the traits (ratio scale rating). The index of ranking I = Sum (3×rank1 + 2×rank2 + 1×rank3) for the individual trait/Sum (3×rank1 + 2×rank2 + 1×rank3) for the global traits was computed (Elias et al., 2018; Getachew et al., 2018; Zewdu et al., 2018) was calculated. The final trait ranking was made based on the traits rated on the ratio scale.

Results

Characteristics of breeding practices in Gudali cattle

The investigated cattle owners were not fairly distributed in the districts of the municipalities. In Malanville, they were mainly present in Garou and Madecali districts and in Tchaourou Center and Tchatchou districts in the municipality of Tchaourou. Not all the cattle owners interviewed were purely Gudali cattle farmers. The majority of them own other breeds of cattle such as Borgou (26.67%); Yakana (26.67%); Azawak (7.5%). The distribution of stock farmers according to herd composition (pure or mixed breeds) and the production system (Sedentary or Transhumant) allowed to group them in four classes: Sedentary Purebreds (16.67%); Transhumant Purebred (33.33%); Sedentary mixed breeds (34.17%) and Transhumant mixed breeds (15.83%). Only 32.5% (39 out of 120) had 100% Gudali herds. The average number of Gudali cattle in the investigated herds was 24.27 ± 3.63 and 23.68 ± 5.05 heads respectively for sedentary and transhumant mixed breeds. The farmers were from various ethnic groups, with a large majority of the Fulani ethnic group (78.33%), followed by the Fulani-Haoussa ethnic group (20.83%), originally from Nigeria (Table 1). The average herd size per household was 74.50 ± 9.4 for sedentary purebreds and 90.11 ± 30.34 for transhumant purebreds (Table 2).

The average age of the investigated farmers was 42 ± 1.16 years. This shows that the farmers have a certain experience in the field of breeding which they may pass on from generation to generation for the sustainability of the activity. Regarding education, a high percentage of the cattle owners (88.33%) had no formal education, highlighting the traditional nature of the cattle breeding practices. Few had attended primary school or coranic school, while technical education and secondary school attendance were minimal across the groups. In terms of animal origin, a significant proportion of cattle owners relied on legacy animals (55%), either inherited from their families or a combination of legacy and purchased animals (42.5%). Only a small portion depended solely on purchased animals. The group differences in animal origin were statistically significant, indicating varying practices in how herds are built and maintained among the different production systems.

TABLE 1 Socio-demographic characteristics of the different groups of cattle owners.

	Data Overall	Sed_mixed_breed; n=41	Sed_pure_breed; n=20	Trans_mixed_breed; n=40	Trans_pure_breed; n=19	P value
Ethnic group (%)						
Fulani	78.33	34.17	7.50	30.83	5.83	1.906E-09
Fulani_haoussa	20.83	0.00	8.33	2.50	10.00	
Zerma	0.83	0.00	0.83	0.00	0.00	
Education (%)						
None	88.33	26.67	16.67	29.17	15.83	0.3533
Ecol_coran	1.67	0.83	0.00	0.83	0.00	
Technical education	0.83	0.00	0.00	0.83	0.00	
National language	0.83	0.00	0.00	0.83	0.00	
Primary school	2.5	1.67	0.00	0.83	0.00	
Secondary school	5.83	5.00	0.00	0.83	0.00	
Origin of animals (%)						
Legacy	55	16.67	15.83	9.17	13.33	1.355E-06
Purchase	0.83	0.00	0.83	0.00	0.00	
Legacy_Purchase	42.5	16.67	0.00	24.17	1.67	
Legacy_Custody	1.67	0.83	0.00	0.00	0.83	
Other breeds						
Yes	32,5	34.17	0.00	33.33	0.00	< 2.2e-16
No	67.5	0.00	16.67	0.00	15.83	

Sed_mixed_breed, Sedentary mixed breeds; Sed_pure_breed, purebred sedentary; Trans_mixed_breed, Transhumant mixed breeds; Trans_ pure_ breed, purebred transhumant.

Farmer's reasons for choosing Gudali breed

The reasons reported by respondents for raising Gudali cattle breed varied across production systems and are presented in Figure 2. Most farmers in sedentary and transhumant systems raise the breed for dairy production. The milk is sold to generate daily income or is used for self-consumption to meet protein needs of households. In both production systems, some farmers believe they remain breeders in order to leave a legacy to the next generation. Thus, heritage (preserved heritage) is also one of the reasons why farmers keep the breed. For them, preserving traditions and knowledge associated with livestock farming is essential to ensure the sustainability of livestock systems and the well-being of

TABLE 2 Distribution of animal species.

	Generalized data	Sed_mixed_breed		Sed_pure_breed		Trans_mixed_breed		Trans_pure_breed	
	Mean ± SE	Mean ± SE	Beach	Mean ± SE	Beach	Mean ± SE	Beach	Mean ± SE	Beach
Goats	10.15 ± 0.95	11.88 ± 2.1	0-50	9.30 ± 1.75	0-25	9.63 ± 1.27	0-30	8.42 ± 2.32	0-35
Gudali	42.67 ± 6.00	24.27 ± 3.63	5-110	74.50 ± 9.4	6-200	23.68 ± 5.05	6-200	90.11 ± 30.34	17-633
Sheep	22.18 ± 3.59	19.80 ± 3.88	0-150	22.95 ± 2.64	6-50	25.55 ± 9.96	0-405	19.37 ± 2.09	0-35
Poultry	10.52 ± 1.15	11.34 ± 2.19	0-50	9.65 ± 2.11	0-30	10.98 ± 2.21	0-60	8.68 ± 2.08	0-25

SE, Standard Error; Sed_mixed_breed, sedentary mixed breeds; Sed_pure_breed, purebred sedentary; Trans_mixed_breed, transhumant mixed breeds; Trans_pure_breed, purebred transhumant.

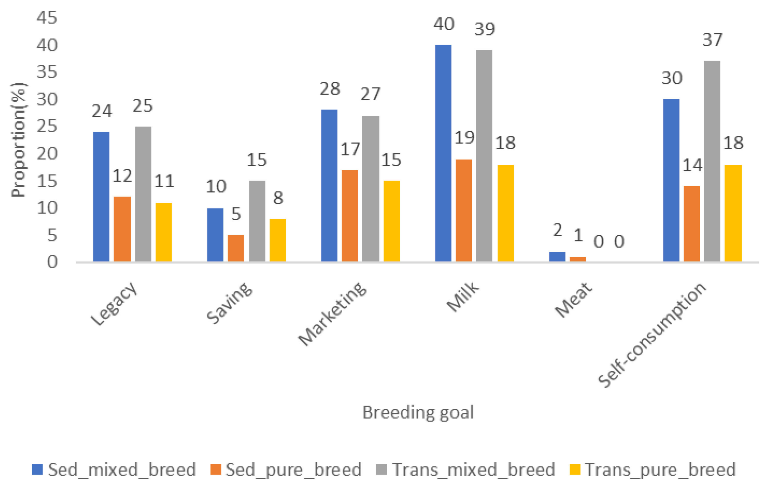

FIGURE 2
Purpose of keeping Gudali cattle breed.

rural communities. Most of the investigated farmers mentioned having Gudali cattle in other to sell calves, culled cows or even seriously ill animals. Cash for household needs including school fees as well as illness of a family member were also reported among the reasons for keeping Gudali cattle breed. Apart from the above reasons, breeders chose the Gudali cattle breed because of its milk production capacity, rapid growth, good carcass characteristics as well as good market value.

Selection criteria and husbandry practices

The selection criteria used by farmers are presented in Tables 3 and 4. There is a difference between selection criteria and their importance from one production system to another. The coat color was the most important criterion for the selection of breeding stock by farmers, followed by the milk yield. These farmers most often had animals with white coats because they believe that white color

TABLE 3 Index of selection criteria for sedentary cattle farmers in the study area.

	Sed_mixed_breed				Sed_pure_breed			
	Rank1	Rank2	Rank3	Hint	Rank1	Rank2	Rank3	Hint
Reason								
GM	0	2	4	0.05	1	0	1	0.04
RG	1	5	5	0.10	0	5	7	0.17
MP	22	0	1	0.39	17	0	0	0.50
Resistance	13	2	0	0.25	0	0	0	0.00
AfSEP	0	2	0	0.02	1	7	1	0.17
Quantitative Criteria								
HW	9	18	3	0.29	1	15	3	0.32
Milk	27	7	5	0.44	18	1	0	0.50
BL	2	12	14	0.20	0	4	10	0.16
Weight	3	3	0	0.07	1	0	0	0.03
Qualitative Criteria								
Coat_color	36	4	10	0.57	19	1	10	0.59
Form	4	25	1	0.29	1	16	0	0.30
Libido	0	1	1	0.01	0	0	2	0.02

Index = Sum of (3 × rank1 + 2 × rank2 + 1 × rank3) for the individual trait/Sum (3 × rank1 + 2 × rank2 + 1 × rank3) for all traits. The highest index value signifies the highest importance. Sed_mixed_breed, sedentary mixed breeds; Sed_pure_breed, purebred sedentary; GM, Good Meat; RG, Rapid Growth; MP, Milk Production; AfSEP, After-Sales Economic Profitability; HW, Height at the Withers; BL, Body Length.

TABLE 4 Index of selection criteria used by transhumant cattle farmers in the study area (continued).

	Trans mixed breed				Trans pure breed			
	Rank1	Rank2	Rank3	Hint	Rank1	Rank2	Rank3	Hint
Reason								
GM	0	1	0	0.01	0	0	2	0.02
RG	2	4	3	0.10	1	4	6	0.18
MP	17	1	0	0.30	17	0	0	0.53
Resistance	8	5	0	0.19	0	0	0	0.00
AfSEP	0	4	2	0.06	0	5	1	0.11
Quantitative Criteria								
HW	6	18	4	0.26	1	12	3	0.28
Milk	30	6	1	0.47	18	0	1	0.50
BL	3	12	15	0.22	0	4	10	0.17
Qualitative Criteria								
Coat_color	29	4	12	0.47	18	0	6	0.54
Form	9	24	2	0.34	1	9	5	0.23
Libido	0	1	4	0.03	0	0	0	0.00

Index = Sum of (3 × rank1 + 2 × rank2 1 × rank3) for the individual trait/Sum (3 × rank1 + 2 × rank2 + 1 × rank3) for all traits. The highest index value signifies the highest importance. Trans mixed breed, mixed breeds transhumant; Trans pure breed, purebred transhumant; GM, Good Meat; RG, Rapid Growth; MP, Milk Production; AfSEP, After-Sales Economic Profitability; HW, Height at the Withers; BL, Body Length

protects the animals against the attack of certain disease vectors. The format and height at the withers were the most important criteria in the sedentary mixed breeds production system. The Height at Withers was the only reported criterion in purebred sedentary animals' system (Table 3) while the animal conformation was the most reported criteria in transhumant production system (Table 4).

The survey revealed that farmers are used to keep a particular breed as a sire in their herd because they have inherited them (Heritage choice) and want to pass them on to the future generations. The libido and the state of the testicles in the male parents and the teats in the females were important criteria in the two breeding systems.

Main production of farmers

Table 5 presents the results of the Factorial Correspondence Analysis (FCA) on the matrix of variables of the main production of farmers. On the three dimensions that explain the distribution of the main production of the farmers by district, the first 2 axes of the analysis explain 89.78% of the total information. The factorial plane formed by these two axes is largely sufficient to explain the total variability of the data. Therefore, the variability associated with other components may be least significant.

TABLE 5 Results of correspondence factor analysis (CFA).

	Own value	Variance percentage	Cumulative percentage of variance
sum1	0.17	66.78	66.78
sum2	0.06	23.00	89.78
sum3	0.03	10.22	100

The projection (Figure 3) makes it possible to have two groups. The first group (66.67% of the samples) made up of pure-bred transhumant and sedentary farmers and mixed-race sedentary; it includes three districts (Alafiarou, Madécali and Tchaourou center) of the study area. The second group (33.33% of the sample) involved the rest of the arrondissements, which according to this figure are mixed-race transhumant herders. The figure shows that in the first group milk, meat and savings are the main productions of these farmers. On the other hand, prestige breeding is more observed in the second group.

Breed improvement and conservation methods

Four methods were reported by farmers: selection; castration; crossbreeding and conservation of the local breed (Table 6). Selection was practiced by around 15% of the farmers in each mode of breeding. The reasons for the selection by these farmers are among others the maintenance of the dairy performance of the cows; growth performance of young animals and maintaining the reproductive/production performance of breeding males. Castration is a method practiced by a minority of the investigated farmers. This method is more practiced by sedentary people of mixed breeds (5.83%) and transhumant people of mixed breeds (4.17%). The main reasons for castration were to control

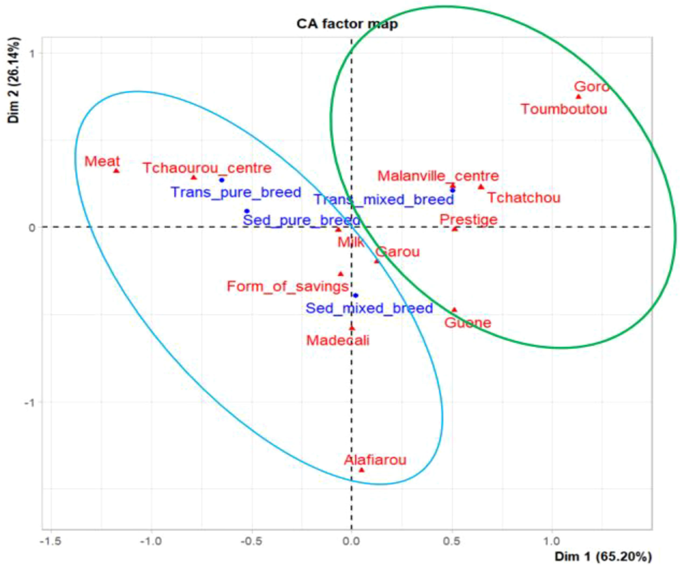

FIGURE 3
Correspondence factor analysis showing the relationships between significant variables.

mating, improve docility and avoid fighting. The practice of castration and the reasons for castration varied significantly from one production system to another. Crossbreeding is practiced by only sedentary and transhumant mixed breeds (55.84%) and justifies the reason for having different breeds in the herds. Crossbreeding is mainly practiced to improve the dairy performance of their herd and to benefit from transfer of disease resistance to the offsprings. Conservation of the pure local breed was practiced by almost all the farmers. The goal of this practice is most often " not to lose the breed" so that it is transmitted from generation to generation.

Farmer's level of knowledge on community based breeding programs

Among the investigated farmers, only 3.33% of them had at least heard of CBBPs, but none of them had been involved in a CBBP (Table 7). However, half of the farmers already belonged to a livestock farmers' association (Table 7). Three associations were listed by these farmers: UCOPER (Communal Union of Ruminant farmers and Professional), UDOPER (Departmental Union of Ruminant farmers and Professional) and APESS (Association for the Promotion of Livestock in the Sahel and Savannah). They are in these associations for different reasons. The majority adhere to UCOPER and UDOPER to benefit from veterinary care, technical supervision and practical breeding advice among others advantages. They belong to APESS for the purpose of defending their interests during conflicts and also to benefit from other advantages. Their Motto is "Stronger Together Forever". The remaining farmers do not belong to any associations for various reasons including mobility, lack of information, membership requirement which they consider restrictive, lack of trust and reluctance.

TABLE 6 Breed conservation and improvement methods (% of respondents).

	Selection		Castration of unselected males		crossing		Conservation of landrace	
	No	Yes	No	Yes	No	Yes	No	Yes
Sed_mixed_breed	19.17	15.00	28.33	5.83	7.50	26.67	15.00	19.17
Sed_pure_breed	0.83	15.83	15.83	0.83	16.67	0.00	5.00	11.67
Trans_mixed_breed	20.83	12.50	29.17	4.17	4.17	29.17	13.33	20.00
Trans_pure_breed	0.83	15.00	14.17	1.67	15.83	0.00	3.33	12.50
Total	41.66	58.33	87.5	12.5	44.17	55.84	36.66	63.34

Sed_mixed_breed, sedentary mixed breeds; Sed_pure_breed, purebred sedentary Trans_mixed_ breed, transhumant mixed breeds; Trans_ pure_ breed, purebred transhumant.

TABLE 7 Level of knowledge of CBBP by farmers (% of respondents).

	Association membership (%)		Level of knowledge of CBBP (%)		Having been involved in a CBBP program (%)
	No	Yes	No	Yes	No
Sed_mixed_breed	21.67	12.50	32.50	1.67	34.17
Sed_pure_breed	5.00	11.67	16.67	0.00	16.67
Trans_mixed_breed	17.50	15.83	31.67	1.67	33.33
Trans_pure_breed	5.83	10.00	15.83	0.00	15.83
Total	50.00	50.00	96.67	3.33	100.00

Potential benefits of CBBP and farmers' preferred traits to improve

Figures 4 and 5 show the possible benefits and traits of interest to be considered in a CBBP for Gudali cattle breed according to farmers. Increased production and technical support, and easy access to feed resources were the reported possible advantages of such a program. Their important traits of interest to be included in the CBBP included increased milk yield (94.17%), growth traits (64.17%), diseases resilience (40%) and prolificacy (35%).

Potential challenges in a Gudali CBBP and mitigation strategies

Tables 8 and 9 show respectively the potential challenges that could limit Gudali CBBP program as well as mitigation strategies according to farmers. As potential challenges, access to feed was mentioned by 98.33% of farmers followed by diseases (30%). The suggested mitigation strategies suggested by farmers were the establishment and installation of artificial pasture areas and routes reserved for cattle movements and managed by communities. All

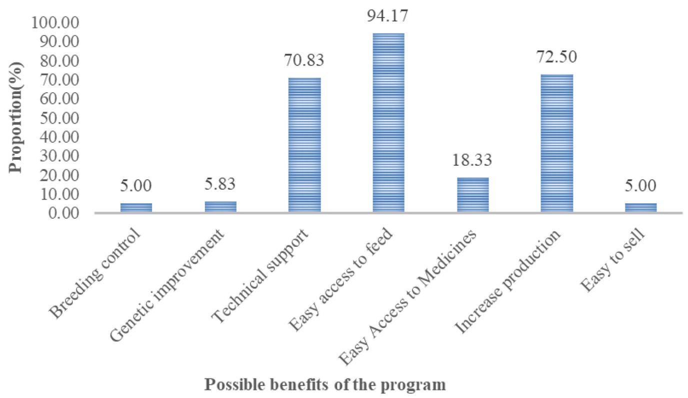

FIGURE 4
Distribution of program benefits.

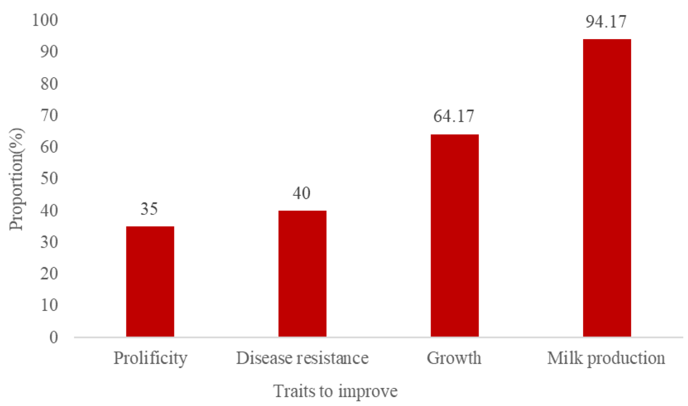

FIGURE 5
Farmers' preferred traits to be included in a Gudali CBBP.

TABLE 8 Potential challenges that could limit the smooth running of the CBBP program.

Challenges to overcome	Choices made	Workforce	Frequency (%)
Feed	No	2	1.67
	Yes	118	98.33
Sanitary (monitoring veterinarian)	No	115	95.83
	Yes	5	4.17
Diseases	No	84	70.00
	Yes	36	30.00
Water	No	106	88.33
	Yes	14	11.67
Instability and reluctance	No	114	95.00
	Yes	6	5.00

farmers mentioned that collaboration between various stakeholders of the CBBP program and existing farmer associations will be critical. Furthermore, all investigated farmers expressed high interest to be involved in a potential Gudali CBBP program in their region.

Discussion

The results show important variation in breeding practices, selection criteria, farmer's preferred traits of Gudali cattle breed in the North-Eastern part of Benin Republic. Furthermore, we investigated farmer's knowledge on a community-based breeding program, their interest to establish one for Gudali breed as well as the potential challenges and mitigation approaches. These results highlight broad points for discussion in the sections below.

Production objectives, selection criteria and breed preference

The main production objective of the farmers in the study area was milk. This milk was mainly intended for self-consumption and sales. Indeed, milk is mainly used for domestic consumption in Fulani households, but the surplus is sold on local markets, providing the main source of income for the women who mostly manage the milking (Hampshire, 2006). The sale of milk surplus by farmers has also been reported in Burkina Faso (Dossa and Vanvanhossou, 2016; Roessler, 2019) and use cattle manure to fertilize crops, earning money if necessary (Roessler, 2019). Similar studies on small ruminants and cattle have reported keeping animals for cash income, savings, wealth and social reasons (Duguma, 2010; Guangul, 2014). According to Dossa and Vanvanhossou (2016), milking was the main objective of Somba cattle farmers, although these animals were also used for agricultural work in the fields. According to Soro et al. (2015), Baoulé cattle are mainly raised in the " Lobi Country " in Côte d'Ivoire for ritual ceremonies, gifts and as security against the vicissitudes of life. In Mali, on the other hand, the main production objective was domestic meat consumption and social functions (Traoré et al., 2017). In southwest Burkina Faso, animals are raised for a variety of functions (Ouédraogo et al., 2020) as in West Africa with a difference in the order of importance (Ejlertsen et al., 2013; Traoré et al., 2017). Meat production constitutes the main objective of the majority of Lagune cattle breeders in Benin, in contrast to the breeding practices observed in our study. This choice aligns with the low milk production of the Lagune breed, making milking not economically viable (Adoligbe et al., 2020). The same authors emphasize, however, that some Lagune breeders are motivated by a deep passion for these animals and their particular temperament.

This study revealed that coat color and milk production are the most important criteria used in selection of Gudali cattle breed. Similarly, milk production, coat color and patterns were important aesthetic reasons for selection of Azawak cows in Niger (Belli et al., 2008). In addition, the majority of small-scale dairy farmers in Niamey (Niger) preferred the Azawak breed because of its milk performance (Belli et al., 2008). Likewise, small-scale dairy farmers in Jimma town (Ethiopia) preferred Holstein x indigenous zebu cows because of their high milk yield (Duguma and Janssens, 2016). In contrast, in southwestern Burkina Faso, milk productivity apart from cash income was the main trait (objective) of production (Ouédraogo et al., 2020). Moreover, in West Africa Tano et al. (2003) found that disease resistance was one of the most important traits among cattle keepers. For Roessler (2019), dairy performance was an important factor in the selection and trait preferences of cattle breeds in Ouagadougou (Burkina Faso).

Health traits are important but difficult to breed for in low-input systems with little pedigree record (Wuletaw et al., 2006). Breeders in this study reported that white coat color helps deter flies and reduces the risk of infestation, reflecting practical environmental adaptations. This aligns with studies showing the advantages of white coat color in tropical environments, such as improved heat dissipation and higher milk yields in Holstein cows (Anzures-Olvera et al., 2019; Maia et al., 2005; Theusme et al., 2023). However, it is important to note that while white coat color is prioritized in certain systems, it is not universally preferred across all production environments, as genetic, economic, and environmental considerations vary (Hall, 2012).

Thus, the selection of coat color, particularly white, in Gudali cattle is driven not only by aesthetic preference but also by practical

TABLE 9 Proposed solutions to the potential challenges.

Proposed solutions	Effective	Frequency (%)
Management Committee - Sustainability	28	23.33
Water	20	16.67
Contribution	29	24.17
Training- Awareness	14	11.67
Passage corridor-Grazing area	89	74.17
Good collaboration	120	100.00

environmental benefits, underscoring the complex nature of breeding decisions across different regions and systems. Criteria related to morphological traits such as horn shape and sheath were ranked low in our study. Our results corroborate the findings of previous studies showing their insignificance in Ethiopian Sheko cattle and in Central Africa Ankole cattle (Elias et al., 2018; Ndumu et al., 2008; and Wurzinger et al., 2006). These animal beauty traits may not be taken into account in community breeding programs with the communities concerned according to the results of studies carried out by Ouédraogo et al., 2020 in Burkina Faso. In addition, udders and testicles are reported as additional selection criteria for male and female farmers (Tadesse et al., 2005; Zewdu et al., 2018).

Cattle producers' breed and trait preferences and selection criteria were consistent with their primary production goals. The Gudali breed was particularly valued for its higher milk yield, large size/good growth rate, docility and higher market value (Roessler, 2019). Due to its good adaptation to local production conditions, the local Zebu Fulani was preferred over other breeds in a similar study conducted in Burkina Faso by (Roessler, 2019). Cattle farmers also considered this breed to be heirloom, meaning that farmers' attachment to this breed is from generation to generation. Body size/growth, breast milk production and behavioral traits were the most important farmers' preferred traits (ratio scale assessment) reported for selection of local Fulani zebu bulls (Roessler, 2019).

Other breeds of cattle such as Yakana, M'bororo, Borgou, and Azawak are preferred by some herders because of their disease resistance and relatively low feed requirements. These reasons are similar to those highlighted by Belli et al. (2008) on the Fulani zebu breed.

Animal performance enhancement techniques

The results of this study highlighted four methods (selection, castration, crossbreeding and conservation of the local breed) used by farmers to improve performance of their herds. Castration is a method observed more frequently in mixed sedentary and transhumant breeds. The main reasons for castration are to control mating, improve docility and prevent fighting. A study conducted in Burkina Faso by Mopaté et al. (2014) reported the systematic castration of males of other breeds mated with Baoulé cattle to avoid crossbreeding, which was not the case in our study. This practice varies significantly from one farmer to another.

The goals of some farmers in crossing other breeds with Gudali cattle, were to increase size, milk yield and growth performance. According to Traoré et al. (2015); Ahozonlin et al. (2019) and Ouédraogo et al. (2020), this traditional practice of breeders plays a crucial role in achieving their production objectives by strengthening the disease resistance of the offspring. The study by Orou Yorou et al., (2014) also highlighted crossbreeding and selection as methods for improving animal performance in Parakou.

Relevance of setting up a community-based selection program for Gudali cattle

Among the cattle farmers investigated, only 4 farmers or 3.33% (Table 7) had at least heard of CBBP but none of them benefited from any training in this regard. Furthermore, out of a total of 120 respondents, only 6 or 5% of these farmers consider that they do not belong to the program because of their instability (transhumance) or either their reluctance (Table 6) . At the end of this study, 114 (95%) farmers surveyed were available and agreed to the implementation of the CBBP program. Like Goudali cattle breeders in the study area, Somba cattle owners express a keen interest in participating in well-designed improvement programs, aimed at both optimizing production and preserving this breed of cattle native (Dossa and Vanvanhossou, 2016).

Community-based breeding (CBB) has a long history and has been used as an agricultural research tool since the 1970s (Omore et al., 2008). It is a breeding method that improves the performance of native breeds or varieties, while maintaining their adaptability and genetic diversity. It uses simple, low-cost methods (Haile et al., 2010) and strives to take into account farmers' needs, opinions, decisions and active participation (Haile et al., 2011; Mueller et al., 2015). The CBBP has been successfully implemented on small ruminants (sheep, goats) and llamas using smallholders' indigenous genetic resources in Latin American countries (Mueller et al., 2002; Wurzinger et al., 2013). This has also been successfully implemented in eastern and southern Africa in sheep and goats (Haile et al., 2014; Haile et al., 2023; Mueller et al., 2023). Digesa (2023) indicated improvements in the growth characteristics of sheep since the introduction of the selection program in Ethiopia. However, due to the widespread use of industrial breeding stock and the availability of assisted reproduction technologies, CBPP for cattle are very rare. All the cattle farmers surveyed were ready to be involved in a potential CBPP for Gudali cattle offering opportunities for sustainable genetic improvement and conservation of the breed in Benin.

Potential challenges to overcome in the implementation of CBBP for Gudali breeding

The implementation of a Community-Based Breeding Program (CBBP) for the Gudali breed may face several challenges that need to be carefully addressed. One of the key issues reported in CBBP is the definition of clear breeding objectives that align with the needs and priorities of the farmers involved (Ragot et al., 2018). Successful CBBPs depend on the willingness of communities to participate actively, and ensuring this engagement is essential for the program's success. It is important to assess community readiness and interest in collaborating on shared breeding goals, such as the exchange of breeding bulls and other resources (Zoma-Traoré et al., 2021).

Another significant challenge is the establishment of supportive institutions for critical CBBP components such as animal identification, data collection, and genetic evaluation (Mueller et al., 2015). Without these institutional supports, it becomes difficult to track breeding progress and ensure the preservation of genetic diversity. As highlighted in this investigation, farmers in remote areas often have limited access to veterinary services and technical assistance, which intensifies the difficulty of implementing these programs. The shortage of veterinary agents and lack of access to disease management tools in isolated regions can further reduce the effectiveness of the program (Mulatu et al., 2021).

Additionally, issues related to feed availability and grazing land due to environmental constraints have been reported as potential significant challenge for Gudali breeders. The absence of sufficient grazing areas often leads to poor nutrition, affecting the productivity and health of the livestock. Furthermore, as noted by Mueller et al. (2015), smallholder farmers often struggle with financial and material limitations that make it difficult to implement conventional breeding programs. The same challenges apply to CBBP initiatives if they are not adequately supported by local institutions and governments.

Although farmers often recognize the potential benefits of joining breeders' associations—such as increased income (Laborde et al., 2020) and greater sustainability of breeding programs (Gutu et al., 2015; Wollny, 2003) they are generally more hesitant when it comes to the practice of bull sharing. A study in Burkina Faso demonstrated that farmers who were members of associations or cooperatives had a more positive attitude towards the conservation of local cattle breeds than those who were not (Mopaté et al., 2014).

Based on these insights, we recommend the establishment of formally registered and recognized breeders' associations specifically for Gudali cattle. These organizations should be built on existing social structures to encourage greater acceptance among farmers (Wurzinger et al., 2011). Membership should include clearly defined rules, rights, and obligations (Wollny, 2003). Additionally, discussions within the association should emphasize the broader community benefits of bull sharing, such as improved herd genetics and economic advantages, to mitigate any negative attitudes.

Given the varying attitudes towards bull sharing and farmer collaboration, particularly among "Transhumant Fulani" farmers observed in this study, it is important to maintain continuous dialogue among stakeholders. This dialogue should explore collaborative options for preserving and improving Gudali cattle breeds. A successful community-based breeding intervention must rely on the active commitment of livestock keepers, while also respecting the decision of those who choose to opt out.

Finally, the lack of market access and low market value of Gudali livestock pose additional economic barriers. Inadequate market infrastructure may discourage farmers from investing in improved breeding practices, as they may not see immediate financial benefits. This underscores the importance of integrating market development with breeding programs to ensure that the economic incentives for participating farmers are clear and substantial. Addressing these challenges (defining breeding objectives, fostering community participation, establishing institutional support, and improving market access) will be crucial for the successful implementation of a CBBP for Gudali breeding. These elements are essential to ensure both the sustainability of the program and the long-term preservation and improvement of the Gudali breed.

Mitigation strategies in potential CBBP for Gudali breeding

Several recommendations have been made by several authors for successful implementation of CBBP (Bhuiyan et al., 2017; Haile et al., 2023; Ouédraogo et al., 2021). Thus, the success of any community-based selection program for Gudali cattle breed in the study environment should take into account the various challenges cited above. To this end, a participatory breeding program involving communities in the selection of breeding animals based on desired characteristics and local knowledge can lead to improved productivity and adaptation to local conditions (Getachew et al., 2018; Haile et al., 2023; Kosgey and Okeyo, 2007). This could involve establishing community selection groups and providing training on selection criteria and selection techniques (Mueller et al., 2015).

Regular capacity building through training for farmers on topics such as animal health, record keeping and breeding techniques can enable them to manage their herds more effectively and make informed breeding decisions (Boersema et al., 2023; Rhone et al., 2008). Empowering communities to collect and manage data about their livestock, such as performance records and pedigree information, can provide valuable information for selection and breeding decisions (Mueller et al., 2015). As mentioned by Aynalem et al. (2023), initiatives integrating income-generating activities into CBBP projects, such as ecotourism or the development of value-added products, can incentivize communities to invest in sustainable breeding practices and conserve the Gudali breed. Establishing partnerships with research institutes, government agencies and NGOs can facilitate access to resources, expertise and technology, and promote the sharing of knowledge and best practices for Gudali breeding (Sterling et al., 2017).

Conclusion

This study highlighted for the first time the production objectives, farmers' preferred traits and selection methods in four Gudali cattle production systems in North-East Benin. Transhumant and sedentary herders prefer Gudali cattle breed because of its good growth, higher milk yield and excellent market value. Despite the strong interest in community-based breeding programs (CBBP), farmers' knowledge of these programs remains limited, emphasizing the need for increased awareness, training, and capacity building. To ensure the sustainability of Gudali breeding, it is crucial to focus on participatory breeding programs, improving genetic diversity, and enhancing management practices through research and development. Additionally, promoting supportive policies and advocacy for community-based breeding programs, fostering strong collaborations between communities, stakeholders, and research institutions, along with supportive policies for CBBP, will be key to driving the long-term success of Gudali breeding initiatives. Furthermore, the study focusing on a specific region may limit its broader applicability. Further studies will be needed in other regions and production systems in the country.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

LZ: Writing – original draft, Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Resources, Software, Visualization. IH: Conceptualization, Funding acquisition, Project administration, Supervision, Validation, Writing – review & editing. AA: Conceptualization, Funding acquisition, Project administration, Supervision, Validation, Writing – review & editing. HW: Formal Analysis, Software, Validation, Visualization, Writing – review & editing. LK: Software, Writing – review & editing. KP: Formal Analysis, Writing – review & editing. IA: Supervision, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Sustainable utilization of wild fruits and respective tree byproducts as partial feed ingredients or supplements in livestock rations

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The long-term survival of livestock production systems depends critically on the sustainable usage of resource-efficient strategies, such as the supplementation or replacement of conventional feed ingredients (CFI) like maize and soybean with less expensive, more effective, and locally accessible sources. Wild fruits and their byproducts (WFBP) from multipurpose trees (e.g. leaves, seeds) offer a promising low-cost non-traditional alternative dietary source of energy, protein, vitamins, minerals, oxycaretenoids, and bioactive substances that function at a cellular level in livestock. According to research these non-CFI can enhance livestock voluntary intake, digestibility, and overall performance while alleviating the heavy reliance on CFI. Conversely, the use of these alternative feed resources (AFR) necessitates a thorough evaluation of their nutrient composition, antinutritional factors (ANF), and proper inclusion levels. At low appropriate inclusion levels in livestock rations, the availability of optimal crude energy, crude protein content, and availability of necessary amino acids (AA) in these AFR, can be taken advantage of in saving costs and reducing demands for soybean and maize, which are the indisputable CFI for both livestock and man. This review synthesizes the current research status of WFBP looking at their nutritional profiles (energy, protein, minerals, fiber etc.) and their impacts in livestock productivity. Furthermore, this review will address potential processing techniques to lessen their drawbacks like ANF or palatability. By highlighting these issues, this paper seeks to provide a guide for the sustainable incorporation of these AFR into livestock diets, striking a balance between nutritional effectiveness and economic feasibility.

KEYWORDS

alternative feedstuffs, byproducts, livestock, multipurpose trees, oilseeds, stockfeed, wild fruits

Highlights

- Global livestock production is facing one major challenge of ensuring adequate availability of nutritious feed.
- Due to the growing demand for meat and animal products, there is a need to design new alternative efficient feed systems.
- This review article details the possible use of wild fruits and their respective tree byproducts (WFBP) as partial or supplement stockfeed ingredients.
- The knowledge base of these potential alternative feedstuffs and their limitations are highlighted in this review.

1 Introduction

Wild fruits (WF) are a vital source of human and animal nutrition, vital for preserving and maintaining good health (Wadhwa et al., 2013; Chisoro et al., 2018). Additionally, they also contribute to the ecosystem in many areas by giving a range of wildlife species food and a habitant to live (Hassan et al., 2011). Lately, in animal nutrition, there has been a growing interest in the sustainable utilization of non-human edible WF trees output and their respective byproducts (WFBP) in livestock feed (Chisoro et al., 2018; Gürbüz and Özkan, 2018). Finding sustainable and affordable means or methods of feeding livestock has become increasingly crucial due to the rising demand for animal produce, especially in developing countries (FAO, 2011; World Economic Forum, 2019; Jalal et al., 2023). This is being exacerbated by the rising global population and the need for climate smart agriculture. WFBP can improve feed accessibility and sustainability while lowering dependency on resource-intensive feeds (like, soybean and maize) when used in animal feed (Paul, 2019; Chisoro and Nkukwana, 2020a; Balehegn et al., 2021; Msweli, 2021).

Furthermore, this lowers production costs and boost profitability, while helping farmers sustainability and promotion of the advancement of a more circular livestock-economy (Balehegn et al., 2021). Despite the potential benefits of the utilization of WFBP in livestock rations, there are practical considerations that need to be considered. In addition, the availability of WFBP can vary depending on season and location, which can create challenges for farmers trying to incorporate these materials in feed (Nkukwana, 2019; Luciano et al., 2020). Additionally, the appropriate processing techniques on WFBP need to be considered to reduce their non-nutritive components, especially ANF, mycotoxins, pathogens, toxins and a solid understanding of the proper inclusion levels in livestock diets. Even so, the crude protein (CP) content, lysine value, and essential sulfur-containing Amino Acids (AA) (cysteine and methionine) content of these alternative feed resources (AFR) needs to be considered. For their appropriate inclusion in livestock feed formulations at their modest inclusion levels for ideal livestock productivity (Rijsberman and Frank, 2016; Chakraborty and Gupta, 2017; Bhatti et al., 2018).

This review is going to discuss the various kinds and categories of WFBP that can be employed in livestock diets. Additionally, it is going to highlight their nutritional worth, potential advantages on animal performance and health, and the practical aspects of including these AFR in livestock diets. By highlighting the potential of WFBP as AFR, the goal is to add to the body of knowledge already available on sustainable livestock production systems. While giving a thorough evidence-based overview of the present state of research on WFBP, thereby supporting the notion on the sustainable use of these resources in livestock nutrition.

2 The livestock sector current global status

Globally, the livestock industry is very dynamic and is changing rapidly in developing nations due to the rising demand for animal products (World Economic Forum, 2019). While many production processes are becoming more efficient and environmentally sustainable, the market for livestock products is mostly stagnating in developed countries due to changes in dietary preferences and societal concerns. The demand for livestock products has historically changed due to changes in human population, income, and urbanization. This production response in various livestock systems has been correlated with advances in science, technology and increase in the number of animals (FAO, 2011). Competition for natural resources, notably land and water, competition between food and feed, and the requirement to function in a carbon-constrained economy are currently significantly influencing agriculture and will continue to have an increasing impact on the productivity of livestock (ILRI, 2012; UN, 2014; Stødkilde et al., 2023).

Considering that soybean meal (SBM) and maize are the most widely used food and feed ingredients, which are unsustainable, plant-based protein and energy sources for livestock nutrition, while also being the backbone of most worldwide production systems. Conventional protein crop production, soy production in particular, is closely associated with detrimental effects on the environment, such as deforestation and land degradation, the depletion of fossil fuels, atmospheric pollution, global warming, acidification and eutrophication, and harm to biodiversity (Chisoro et al., 2019; Pexas et al., 2023). Furthermore, significant financial investments are needed for the large-scale cultivation of protein crops, mostly related to labor, fossil fuels, land (such as rent), and synthetic and chemical inputs (such as fertilizers, insecticides, and herbicides) (Pexas et al., 2023).

Long market chains that employ at least 1.3 billion people worldwide and directly assist approximately 600 million poor smallholder farmers in developing countries are continuously being established in the livestock sector (Sattar et al., 2021). This sector is a crucial risk reduction approach for communities that are at risk, since livestock are crucial nutrient and traction sources for

Abbreviations: AA, Amino acids; AFR, Alternative feed resources; ANF, anti-nutritive factors; CP, Crude protein; CFI, conventional feed ingredients; SBM, soybean meal; LO, Lipid oxidation; WF, Wild fruits; WFBP, Wild fruits and respective byproducts.

smallholder systems' crop production. Worldwide, the consumption of livestock products makes up 17% of kilocalories consumed and 33% of the protein consumed. As shown in Figure 1, the production for beef, pork and chicken continued to increase from 2020 to 2023 despite the disruptions caused by the 2020 Covid-19 pandemic, the Russo-Ukraine war which had significant influences on global grain and soybean supply, and the 2022 global oil price fluctuations which had an impact on goods transportation and agriculture productivity (Glufke et al., 2020; Pexas et al., 2023; Rauw et al., 2023). However, there continue to be significant disparities between rich and poor countries (Thornton, 2014; Paul, 2019; Lukuyu et al., 2020).

This sector is expanding fast in emerging economies, and it accounts for about 33% of their agricultural GDP (Pexas et al., 2023). As shown in Figure 2, the estimated total consumption of meat in 2050 across the world in relation to 2019 consumption levels tends to imply that meat consumption will continue to increase globally. With pronounced increases in consumption being in East Asia, Latin America and Sub-Saharan Africa but with rather steady increases in Europe and North America according to the Falcon et al. (2022) report. Given that most demand is currently satisfied by domestic production and is expected to do so for the foreseeable future, the combination of the rising demand in developing nations and the rather stagnant demand in industrialized developed nations represents a significant opportunity for livestock farmers in developing nations (Thornton, 2014; UN, 2014; Balehegn et al., 2021).

In sub-Saharan Africa and South Asia, the food demand for animal products will almost quadruple, rising from around 200 kcal per person per day in 2000 to over 400 kcal per person per day in 2050 (Figure 2). From the early 1960s to the mid-2000s, livestock carcass weights increased by roughly 30% for chicken and beef cattle and about 20% for pigs (FAO, 2011; Donkor et al., 2021). For cows, gains in milk production per animal have been around 30%, roughly equal to increases in chicken egg production per animal over the same period (UN, 2014; Pexas et al., 2023). Large-scale forest conversions have taken place over the past 20 years in the Amazon Basin, Southeast Asia, and Central and West Africa. While the amount of forest land has increased because of the abandonment of agricultural land in the Eurasian boreal forest and other regions of Asia, North America, Latin America, and the Caribbean (FAO, 2011; ILRI, 2012). This presents an opportunity for the use of innovative methods or alternative feedstuffs to CFI in stockfeed to promote sustainable livestock production, in a way to avoid the continued forest conversions that are taking place in Latin America and Africa. Given the continued production of intensive CFI, particularly maize and soybean, which is highly unsustainable (Figures 3, 4). For sustainable livestock production and be in line with the UN sustainable Development Goals (SDGs) in mitigating climate change and greenhouse gases (GHG) (OECD/FAO, 2024).

3 The recent trends in the livestock feed industry

Conventional ingredients the likes of cereal grains (maize, wheat, barley, etc.), oilseed meals (soybean, rapeseed, fava beans, canola, etc.), and forages (hay, silage, etc.) dominate livestock diets (D'Mello, 2012; Patil and Patil, 2017). However, interest in AFR such as food byproducts, insects, wild fruit byproducts (WFBP),

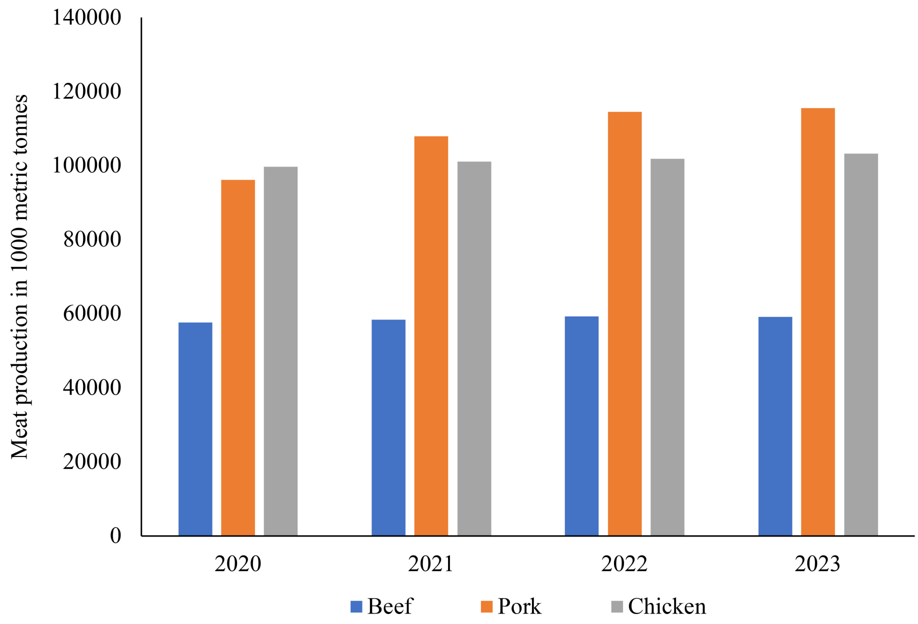

FIGURE 1
Global meat production trends from 2020 to 2023. Beef and pork are in 1 000 metric tons (Carcass weight equivalent) and chicken is in 1 000 metric tons (Ready to cook equivalent) source (USDA, 2024a), livestock and poultry: world markets and trade. As you can see there has been a steady increase in pork production with a rather constant production of chicken and beef since 2020.

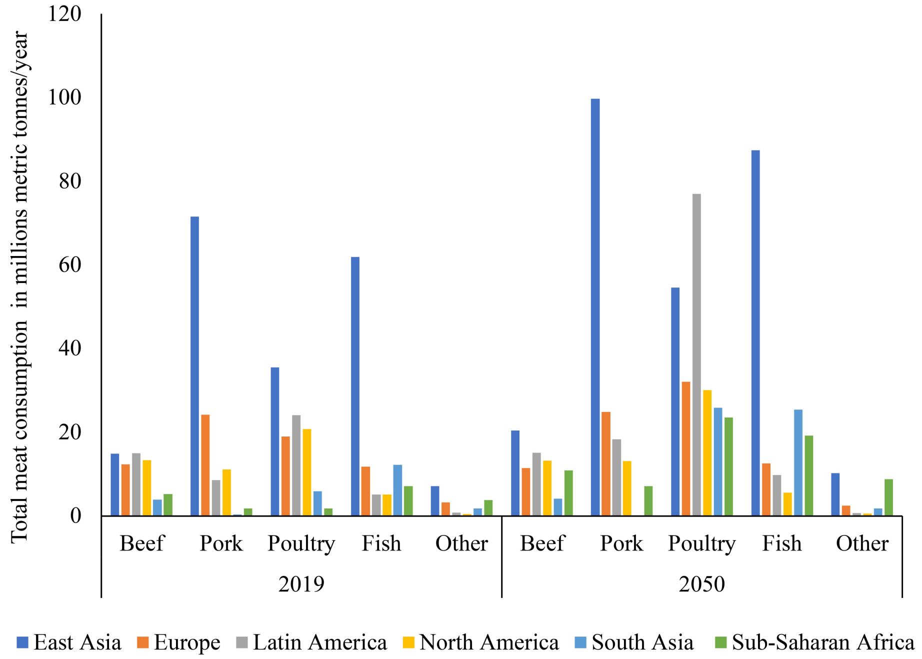

FIGURE 2
The graph shows the estimated total consumption of meat in 2050 across the world in relation to 2019 consumption levels. From this it implies that meat consumption will continue to increase in the coming years globally. Pronounced increases in consumption will be in East Asia, Latin America and Sub-Saharan Africa. Data sourced from the Falcon et al. (2022) report "Rethinking Global Food Demand for 2050".

algae, seaweeds and single cell proteins is growing due to sustainability issues and growing customer demand for organic and non-GMO products (Adamu et al., 2022; OECD/FAO, 2022; Chisoro et al., 2023). These substitutes offer reduced environmental footprints and lessen the heavy dependency on resource-intensive CFI (i.e. maize and soybean) as depicted by their increased need in production (Figures 3 and 4), especially in developing countries where CFI scarcity persists (Nyamukonda and Ndlovu, 2019; OECD/FAO, 2024).

Locally sourced, sustainable substitutes such as WFBP should be incorporated into stockfeed to lessen this dependency on imported protein feeds (such as grains and seeds). This change reduces supply-chain interruptions and contamination, two hazards associated with long-term storage and overseas shipping. For instance, Ukraine, a significant exporter of soybeans and sunflowers, has worsened storage and transportation circumstances due to the ongoing war, post-Covid trade obstacles, and energy constraints, resulting in damaged grains and feed insecurity (Pexas et al., 2023). The utilization of resources like WFBP reduces reliance on unstable international markets, while improving the livestock sector resilience and productivity when included in stockfeed formulations (Chisoro et al., 2023; Pexas et al., 2023).

Furthermore, the other increasing trend having this push is the advent of precision livestock farming, which maximizes animal health and output using technology and innovation. To track feed consumption and spot potential health risks earlier, sensors and data analysis are being used in livestock production (Islary et al., 2018; Adamu et al., 2022). The industry is already adjusting and adapting to these changes and innovations by creating cutting-edge solutions, like specialized feed formulas and fresh new substitutes or supplement ingredients. These adjustments are essential for addressing the industry's sustainability issues and meeting the demand for animal products globally (Banday et al., 2023; Jalal et al., 2023).

3.1 Factors currently affecting CFI usage in stockfeed

Despite advances in animal nutrition science, livestock in pastoral and mixed-production systems around the globe experience seasonal or ongoing nutritional stress, which compromises their wellbeing and productivity (Jalal et al., 2023). In both smallholder and commercial systems, inadequate nutrition continues to be a major productivity obstacle that is made worse by unstable supply networks, environmental stresses, and socioeconomic limitations. The following key influencing factors are making it more difficult in the utilization of CFI:

1. Price: Globally, maize and soybeans are the major ingredients in most livestock feed, but many regions, including Europe, Asia, and Africa, cannot continue to rely on their imports. The world's supply is dominated by major producers such as the U.S., China, Argentina, Brazil, Ukraine, and Russia, making importing countries susceptible to fluctuations in prices and disruptions in supply. For example, all of Malaysia's feed ingredients are imported, including fishmeal, SBM, and maize; the country imports 3 million tons of maize yearly from Argentina

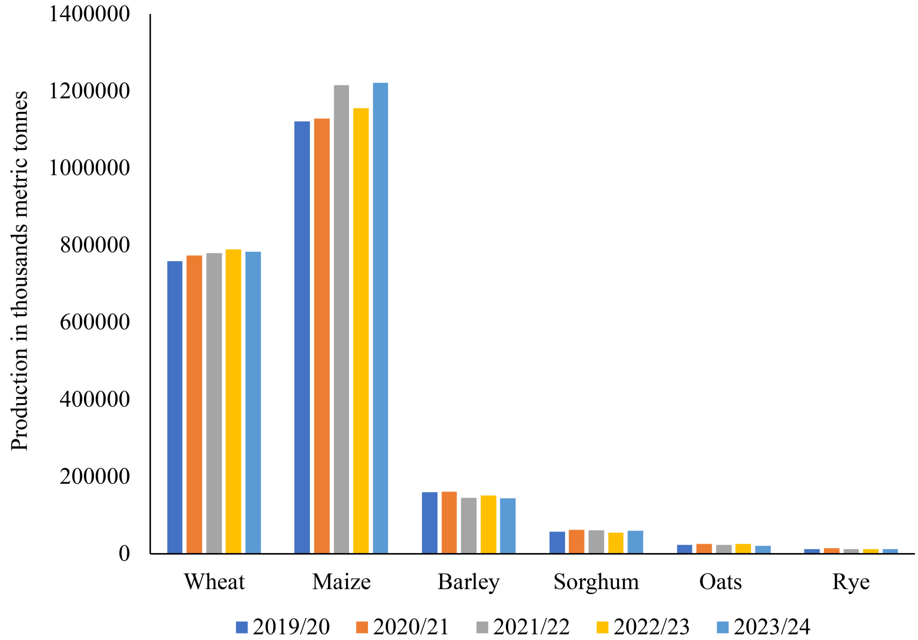

FIGURE 3
Global production trends of the commonly used grain CFI in animal and human nutrition as sourced from the (USDA, 2024b) report. Here we can see that the output of these resources was rather similar across 2019 to 2023. Implying that if these trends continue in the future there will be a great need for the use of alternative or supplementary feed ingredients to complement the demand and supply for these cereal ingredients in animal feed and for human consumption.

alone, at a cost of US$ 1.5 billion (Donkor et al., 2021). This reliance is prevalent in most areas that cannot supply their local needs for feed for people and animals. Moreover, the UN SDGs, which promote self-sufficient and sustainable agricultural systems, are at odds with this kind of dependence (Nasir et al., 2022). Price trends of CFI tend to fluctuate and have continued increasing globally and are heavily influenced by global trends the likes of the 2020-2022 Covid-19 pandemic, the ongoing Russo-Ukraine war and to some extend inflation and/or possible recessions in some nations (Figure 5).

2. Production: When comparing locally produced feed resources to imported CFI, life cycle assessments show clear environmental benefits. For instance, compared to importing a ton of soybeans from Brazil, with growing and using a ton of lucerne domestically in Europe can reduce land use by 63%, freshwater eutrophication by 97%, acidification by 98%, and global warming potential by up to 95% (Pexas et al., 2023). This emphasizes how local feed resources are more sustainable than imported CFI. The need to diversify feed protein sources is further underscored by climate change forecasts that indicate big yield losses for major soybean farmers, including an 86–92% drop in U.S. soybean yields by 2050. Global CFI growth rates will fall short considering the growing meat consumption demand (Figures 1, 2) and are unable to counteract the growing price volatility (Figure 5), according to recent production trends (2019–2023). To sustainably meet this anticipated demand for livestock products by 2050, these patterns highlight the vital necessity to incorporate AFR, such as locally farmed crops and WFBP (Aziz ur Rahman et al. 2019).

3. Availability: It is difficult for farmers to consistently supply livestock with high-quality feed since CFI are either limited or prone to seasonal shortages in many areas (Chisoro et al., 2019). Natural catastrophes, pandemics, and geopolitical conflicts are examples of global events that worsen shortages by upsetting supply systems and reducing livestock productivity. Economically, it is expensive and risky to import CFI, i.e. soybean meal (SBM), from far-off producers in South America, China, or the U.S. to support livestock systems in Europe, Asia, or Africa. Additionally, the price volatility for fossil fuels and erratic global trade dynamics exacerbates this (Chisoro et al., 2023; Jalal et al., 2023). In addition to destroying ecosystem services and releasing GHG into the environment, the production of CFI for stockfeed puts human food production in competition with it for freshwater resources and arable land (Pexas et al., 2023; Rauw et al., 2023).

4. Climate: The availability, quality, supply, and demand of CFI have all been severely impacted by global climate change. Reduced feed availability and extensive crop failures have resulted from extreme weather events including droughts and floods (Food and Agriculture Organization (FAO), 2018). For instance, in 2019 and 2023, Southern Africa was ravaged by Cyclones Idai, Kenneth, and Freddy, which destroyed infrastructure, livestock, and crops, making food shortages, poverty, and hunger worse (United Nations Development

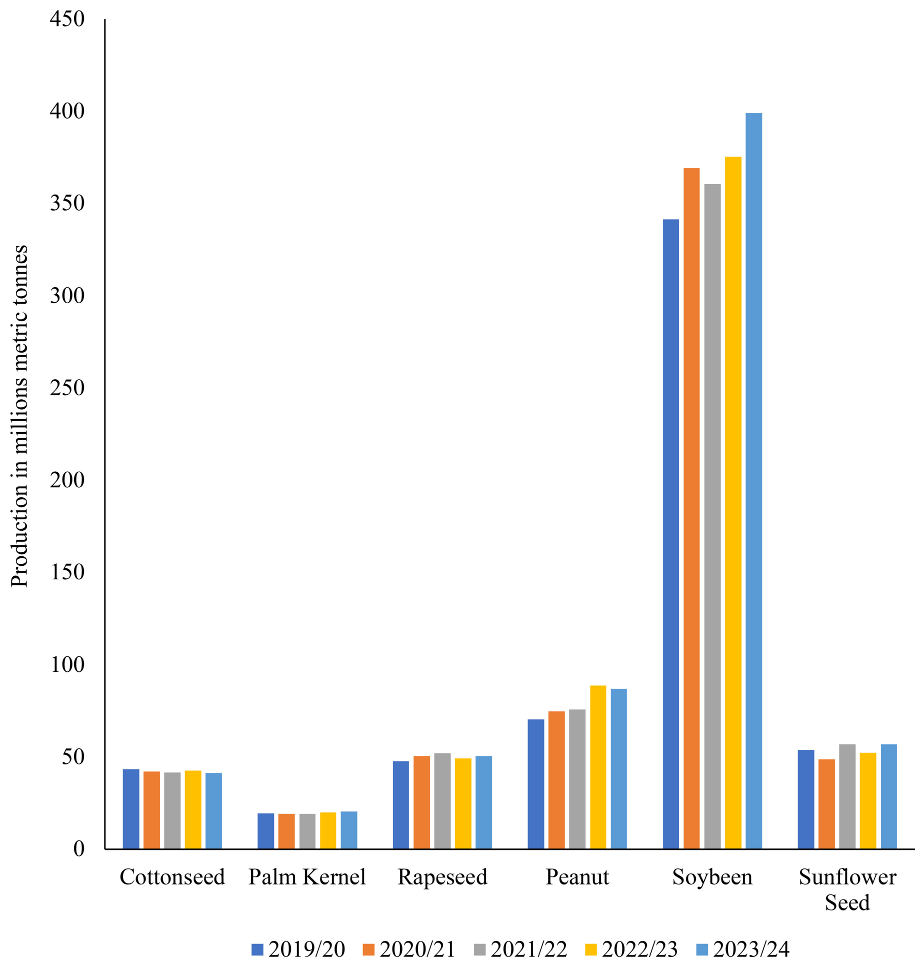

FIGURE 4
World production trends of the commonly used oilseeds CFI for stockfeed as sourced from the (USDA, 2024c) report. The trends here are like those noted on cereal CFIs, however, soybean showed significant increases in production especially in the 2022/23 and 2023/24 production seasons. As it is the most indisputable protein oilseed for animal and human nutrition, and it is a crop that is given the most significant attention by producers and governments globally.

Programme (UNDP), 2019; World Food Programme (WFP), 2019; United Nations Office for the Coordination of Humanitarian Affairs (OCHA), 2023). Similarly, the production and distribution of CFI have been significantly affected by the floods in Brazil and Sri Lanka in 2021, the floods in Pakistan in 2022, the frequent hurricanes in the U.S. and the earthquakes in Japan (OECD/FAO, 2022; 2024). These disastrous incidents show how susceptible CFI supply chains are susceptible to climate-related disturbances, highlighting the crucial need for AFR that can mitigate their supply-demand imbalances and are resilient to environmental events.

5. Infrastructure: The infrastructure required to maintain stable feed and agricultural systems, such as sufficient storage, processing facilities, and transportation networks, is lacking in many emerging countries. These restrictions affect feed manufacturing and animal production by making it more difficult for farmers to make or get high-quality feed components (Lalev et al., 2022; Chisoro et al., 2023). The difficulties faced by producers have been made worse by recent climate disasters as aforementioned that have damaged existing infrastructure (Donkor et al., 2021). Furthermore, Ukraine's agricultural infrastructure has been seriously devastated by the Russo-Ukrainian war, which has affected its productivity and its position as a major producer of sunflower, maize, and soybeans globally (Banday et al., 2023). To guarantee sustainable production, these infrastructure issues underscore the necessity for robust, locally suitable feed solutions.

These factors make it very difficult for farmers to use or access standard CFI. The use of locally accessible feed sources, like oilseeds of indigenous fruit bearing trees, WFBP, local forage crops, and

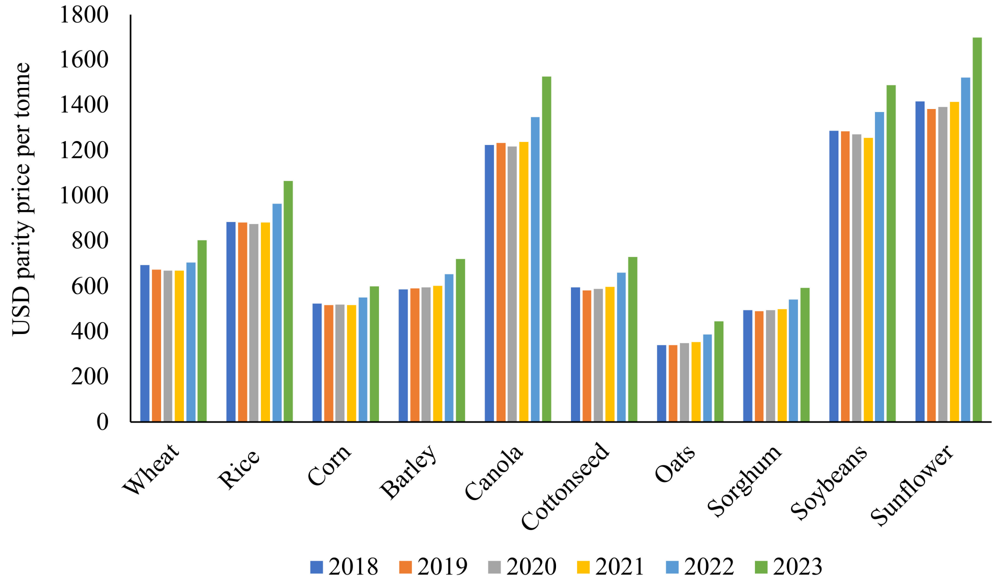

FIGURE 5
The parity price trends per ton of some of the most important CFI in livestock feed as sourced from the USDA (2019, 2020, 2021, 2022, 2023) reports. A parity price refers to a price at which the values of two assets or securities are equal. Prices that farmers receive in the local markets where they typically sell their produce are used to compute parity prices. Implying that, unless otherwise noted, the parity prices apply to the average of all classes and grades of the commodity as sold by all farmers' USDA (2023). From this we can see that the parity prices where rather similar from 2018 to 2021, but then significant increases of these prices were observed from 2021 onwards to 2023 probably due to the global events (i.e. Covid-19, Russo-Ukraine war, fuel price fluctuations and/or inflation).

crop leftovers, as well as the creation of substitute feed ingredients are becoming increasingly popular solutions to solving or mitigating some of these challenges (OECD/FAO, 2022; 2024).

4 Nutritional composition and potential uses in feed of WFBP

When fruits and vegetables are processed for human use, byproducts such oilseeds, seedcakes, pulp, peels, and pomace are produced (Mwale et al. 2008; Ogunlade and Omojola, 2021). These byproducts are good substitutes or supplements to CFI because they are nutrient-rich and include high amounts of CP, fiber, minerals, and vitamins (Hassan et al., 2011; Getachew et al., 2013). As evidenced by baobab seeds, WF trees yield nuts and fruits with distinct aroma and scents that improve livestock feed intake (FI) (Chisoro et al., 2018). In addition, to their nutritional importance, WF trees support environmental sustainability by offering vital ecosystem services such carbon sequestration and wildlife habitats (Rijsberman and Frank, 2016). Acorns (oak trees), wild cherry fruits, baobab seeds, marula seeds, moringa seeds and leaves, neem seeds, tamarind seeds and leaves, and wild grapevine seeds are a few examples of WFBP that have been researched for use in stockfeed (Chakraborty and Gupta, 2017; Chibwana et al., 2017). These resources provide a sustainable way to lessen dependency on CFI while diversifying the diets of livestock.

Table 1, details how WFBP are potential feedstuffs because of their high-quality fat, protein, vitamins, minerals, and energy content, comparable to the indispensable CFI (i.e. soybean and maize) (Sebola et al., 2021). With the best results at this inclusion level, Chisoro et al. (2018) found that adding baobab seed oilcake (BSOC) to broiler diets in place of 5% of soybean meal (SBM) enhanced growth performance and decreased feed costs. Similarly, Mazizi et al. (2019) showed that Japanese quail diets could be completely substituted with marula nut meal (MNM) in place of SBM without affecting FI or growth. Pig performance and meat oxidative stability were improved by Scerra et al. (2024), when 20% of the cereals (wheat bran and barley) were substituted with bergamot pulp meal (BPM) or olive leaf meal (OLM). In lamb diets, Gravador et al. (2015) found that substituting carob pulp meal (CPM) for 24–35% of barley preserved growth performance while raising the amount of polyunsaturated fatty acids (PUFA) in meat without compromising oxidative stability. Chingala et al. (2019) also discovered that, in comparison to steers fed SBM, feeding Malawi Zebu steers with baobab seed meal (BSM) and white thorn tree leaf meal (WTLM) enhanced microbial nitrogen supply, dry matter intake (DMI), and nutrient digestibility. Finally, gulf algae meal (GAM) derivatives improved immunological responses, performance, and reproductive features in laying Japanese quail, according to Rahmatnejad et al. (2024). Together, these studies demonstrate how WFBP can increase livestock output in a sustainable manner while lowering dependency on CFI.

Even though WFBP have potential as sustainable feed ingredients, their usefulness in livestock diets may be compromised by the presence of ANF like phytate, tannins, protease inhibitors (like trypsin inhibitors), oxalates, saponins, and alkaloids (Nkukwana et al., 2014; El-Deek et al., 2020). Unless they undergo specific processing, these substances decrease

TABLE 1 Proximate nutrient composition summary of a few examples of WFBP.

Parameter	Chemical composition, % DM					Essential Fats, % FA			Indispensable Amino Acids, g/16g N			Vital minerals, g/kg	
	DM	CP	CF	CFat	Ash	C 18:1	C 18:2	C 18:3	Lys	Met	Thr	Ca	P
Major Traditional Feed Ingredients													
Maize, yellow[a]	86.7	7.6	2.0	3.6	1.2	28.0	55.0	1.0	2.9	2.1	3.6	0.1	2.4
Soybean, raw[a]	89.7	36.3	8.6	19.6	0.5	22.0	54.0	8.0	6.2	1.4	3.9	2.2	5.0
Wild Fruits													
African baobab fruit, fresh[b]	27.8	15.1	23.4	4.0	11.7	-	-	-	-	-	-	11.9	3.0
African locust bean, aerial, fresh[b]	56.8	12.0	44.8	3.9	5.8	-	-	-	-	-	-	11.6	0.9
Breadfruit, dehydrated[b]	89.4	4.7	6.0	2.7	5.5	-	-	-	-	-	-	0.9	1.4
Carob, pod meal, without seeds[b]	85.3	5.1	9.0	0.5	3.6	-	-	-	3.8	1.4	5.1	4.9	0.9
Date palm, fruit[b]	84.0	3.7	6.1	2.3	3.7	-	-	-	2.7	1.5	3.4	3.9	2.7
Jackfruit, fresh[b]	35.3	12.9	8.1	1.8	5.1	-	-	-	-	-	-	-	-
Jujube, whole, fresh[b]	48.9	13.3	15.5	3.5	9.8	-	-	-	-	-	-	18.4	1.8
Marula fruit, aerial part, dry[b]	96.8	8.9	16.2	5.1	12.5	-	-	-	-	-	-	30.5	0.9
Moringa, pods[b]	10.7	17.6	33.5	3.5	9.7	-	-	-	-	-	-	2.7	5.4
Peach palm, whole fruit, dried[b]	87.6	7.0	7.3	11.3	2.3	43.7	12.9	2.9	3.7	1.6	3.5	2.4	3.9
Spanish lime, fruit[b]	-	10.1	10.8	1.3	2.7	-	-	-	-	-	-	-	-
Wild Fruit Tree Leaves													
Assyrian plum, aerial part, fresh[b]	38.0	13.9	20.2	5.7	14.1	-	-	-	-	-	-	25.0	2.1
Banyan, aerial part, fresh[b]	38.2	10.9	28.6	3.6	12.2	-	-	-	-	-	-	20.8	4.9
Baobab, fresh[b]	-	10.9	14.6	5.0	12.2	-	-	-	-	-	-	21.6	2.7
Flamboyant, leaves, fresh[b]	38.4	15.5	16.1	5.1	6.6	-	-	-	-	-	-	5.3	3.0
Indian laurel, areal part, fresh[b]	38.3	16.9	32.2	3.5	6.6	-	-	-	-	-	-	7.3	5.5
Luffa leaves[b]	74.5	16.0	12.0	2.8	6.0	-	-	-	-	-	-	-	-
Moringa, dried[b]	91.2	26.8	12.2	6.4	10.8	-	-	-	4.8	1.5	4.4	25.6	3.3
Moth bean, aerial part, fresh[b]	-	13.2	22.7	2.1	14.9	-	-	-	-	-	-	25.8	3.0
Neem, fresh[b]	33.5	16.6	16.8	3.7	12.0	-	-	-	-	-	-	18.3	2.5
Taro, aerial part, dried[b]	89.7	22.3	17.6	4.5	13.7	-	-	-	-	-	-	-	-
Sheanut leaves[b]	24.7	7.9	23.4	5.6	6.3	-	-	-	-	-	-	7.4	1.1
Tamarind[b]	37.1	11.8	23.3	5.9	7.6	-	-	-	5.9	1.3	4.8	18.7	2.1
Wild Fruit Seeds													
African baobab[c]	90.0	21.3	22.9	15.9	4.2	-	-	-	-	-	-	2.5	5.9
African locust bean[b]	-	31.1	10.8	18.5	5.6	-	-	-	6.7	0.6	3.3	3.7	2.5
Annatto, depigmented[b]	89.9	14.6	18.9	2.4	5.5	-	-	-	4.5	1.6	3.0	1.4	4.3
Colocynth[b]	95.0	13.0	40.8	24.9	5.2	12.3	60.1	0.3	3.7	2.5	3.7	2.4	2.5
Carob[b]	-	17.8	9.8	2.9	3.3	-	-	-	-	-	-	6.7	1.3
Chia[d]	-	16.5	30.2	40.2	4.8	10.5	20.4	56.8	1.0	0.6	0.7	63.1	86.0
Flamboyant[b]	89.8	20.0	12.9	6.8	6.8	4.9	37.1	7.6	3.3	1.1	4.1	1.7	4.6

(Continued)

TABLE 1 Continued

Parameter	Chemical composition, % DM					Essential Fats, % FA			Indispensable Amino Acids, g/16g N			Vital minerals, g/kg	
	DM	CP	CF	CFat	Ash	C 18:1	C 18:2	C 18:3	Lys	Met	Thr	Ca	P
Wild Fruit Seeds													
Guar[b]	92.4	28.0	6.9	3.7	5.0	-	-	-	4.0	1.4	2.8	-	–
Jatropha, defatted[b]	90.1	16.9	36.1	6.0	5.8	-	-	-	3.8	1.2	3.2	9.3	8.5
Monkey thorn[b]	97.3	31.2	-	9.3	1.4	-	-	-	-	-	-	6.3	1.4
Moth bean[b]	92.3	25.4	5.2	1.9	4.1	22.1	23.4	16.7	6.0	1.6	3.9	2.8	3.2
Red sour plum[e]	95.5	18.2	-	-	-	-	-	-	1.03	0.2	-	17.9	34.5
Sheanut kernels[b]	96.6	8.6	8.0	50.2	3.5	-	-	-	-	-	-	1.3	1.0
Silver leaf tree[e]	95.3	46.2	-	-	-	-	-	-	1.6	0.7	-	39.5	11.2
Tamarind[b]	91.0	15.4	26.4	7.0	2.7	-	-	-	6.5	1.2	3.2	3.0	1.7
Tamarug[b]	90.8	27.2	10.2	4.5	4.7	-	-	-	-	-	-	1.9	2.1
Wild Fruits Byproducts													
African baobab oil seed cake[b]	90.9	22.9	19.9	8.1	4.6	-	–	-	1.1	0.3	0.4	0.24	0.7
Bergamot pulp, ensiled by-product[f]	18.5	50.8	-	13.7	53.8	25.1	34.1	8.9	-	-	-	-	-
Date palm, pulp after extraction[b]	88.3	5.2	7.5	1.0	2.9	-	–	-	4.8	1.9	4.5	5.0	6.1
Guar meal[b]	95.0	42.0	12.7	5.3	5.8	-	–	-	4.1	1.1	3.0	7.0	2.1
Luffa oil meal[b]	90.3	42.3	2.2	15.1	9.8	-	–	-	-	-	-	-	-
Marula oil cake[b]	95.3	39.3	5.8	43.8	5.0	77.5	6.9	0.1	2.3	1.8	2.1	1.3	10.0
Moringa oil meal[b]	-	36.8	-	0.4	5.7	-	–	-	1.5	1.9	2.3	3.8	16.6
Neem seed and kernel cake[b]	95.4	37.3	13.8	6.3	15.0	-	–	-	-	-	-	8.8	7.2
Taro, tuber, dried[b]	89.5	8.1	6.1	1.0	4.0	-	–	-	4.3	2.3	3.9	4.8	2.6
Peach palm, fruit pulp, dried[b]	89.7	5.7	2.3	17.2	1.9	50.3	12.5	1.8	4.9	1.3	3.9	0.8	0.7
Sheanut cake, oil < 10%[b]	92.8	14.1	11.1	4.5	7.2	-	–	-	4.0	2.1	3.4	3.0	2.4
Tamarind hulls[b]	-	7.2	20.1	0.3	2.5	-	–	-	-	-	-	-	-

The – denotes that the value is not available.
[a]Information sourced from the CVB Feed Tables for 'Chemical composition and nutritional values for feedstuffs' (CVB, 2018).
[b]Information sourced from the Feedipedia website (Feedipedia, 2023).
[c]Information collected from the research study by Chisoro et al. (2018, 2019).
[d]Information collected from the research study by Kulczy´nski et al. (2019).
[e]Information taken from the review by Chisoro and Nkukwana (2020b).
[f]Information collected from the research study by Scerra et al. (2022).

palatability, hinder nutrient digestion, and restrict their potential as affordable substitutes for CFI (Nyamukonda and Ndlovu, 2019; Chisoro and Nkukwana, 2020b). It has been demonstrated, for example, that pretreatment of whole jujube with chemical or cold plasma reduces the synthesis of 5-hydroxymethylfurfural while increasing its total phenolic content and antioxidant capacity, hence increasing its functional value (Bao et al., 2021).

Similarly, adding exogenous enzymes (tannase, pectinase, and xylanase) to broiler diets enhanced nutrient availability and decreased the viscosity of ileal digesta in field bean-based feeds, proving the effectiveness of enzymatic detoxification (Abdulla et al., 2017). Additionally, processing methods can increase inclusion rates. For example, when treated, common vetch seeds (CVS) increased inclusion levels in broiler meals by up to 20% without affecting performance when compared to control diets (Sadeghi et al., 2011). These findings underscore the necessity of tailored processing techniques to optimize ANF mitigation and harness the full nutritional potential of WFBP (Kaczmarska et al. 2021). These feed ingredients offer a viable means of fostering sustainability in livestock production, even if additional research is required to grasp their full nutritional worth and potential advantages (Hassan et al., 2020). A summary of a few selected

WFBP proximate nutrient compositions is presented in Table 1, detailing their comparability with the main CFI (i.e. maize and soybeans). The table clearly displays how WFBPs contain relatively acceptable nutrient levels to be partial or supplement ingredients in stockfeed.

4.1 Factors that affect the full utilization of WFBP as feed supplements

As aforestated in the above section, these alternatives have factors that at present limit their full utilization (García-Moya et al., 2019). These limitations still require full understanding and innovative ways to counter them. While WFBP share physicochemical and antinutritional issues similar to CFI, critical gaps persist in translating mitigation strategies for the full utilization of WFBP. Through processing, supplementation, or genetic modification, decades of research on CFI have developed reliable methods to mitigate their drawbacks, including nutritional bioavailability, ANF, and digestibility. WFBP, in contrast, are still understudied and have little systematic data on practical ways to overcome their limitations. The following are key WFBP unresolved challenges in their full utilization:

1. Maturity: The fruit's ripeness level greatly influences its nutritional content and taste. For instance, fruits that are harvested before they are fully mature or ripe enough may be less tasty and contain lower nutritional value than those harvested when they are at their right stage of development (Nyamukonda and Ndlovu, 2019).
2. Processing: Processing techniques have a significant influence on the nutritional makeup, digestibility, and palatability of WFBP. Their chemical structure and nutritional profiles can be improved by processes like grinding, fermentation, or drying, which can increase or decrease their feed value. For example, research shows that drying WFBP may cause thermal degradation or leaching, which lowers concentrations of minerals, vitamins, and phenolic compounds (Chibwana et al., 2017). This necessitates optimized processing procedures to maximize nutrient retention and guarantee consistent quality in stockfeed applications as highlighted by this diversity.
3. Antinutritional elements (ANFs): Due to natural ANF such tannins, phytic acid, and oxalates, WFBP frequently have reduced palatability and bioavailability despite their high nutritional content. For instance, trypsin inhibitors reduce pancreatic enzyme activity, phytic acid chelates vital minerals (such as iron and zinc), and tannins bind to dietary proteins and inhibit proteolytic enzymes, all of which reduce nutrient digestibility (García-Moya et al., 2019). However, these difficulties can be lessened by using specific processing techniques before inclusion of WFBP in stockfeed formulations, such as fermentation, heat treatment, or enzymatic hydrolysis. The entire nutritional potential of WFBP as sustainable substitutes for CFI is unlocked by such interventions, which also improve palatability and neutralizes ANF.
4. Seasonal variation: Harvest timing has an impact on the nutritional makeup of WFBP, which shows notable seasonal variability. For example, the macronutrient, vitamin, and mineral profiles of fruits picked early in the season may be significantly different from those of fruits picked at maturity (Bhatti et al., 2018). When including WFBP into livestock rations, this intrinsic variability calls for thorough, regular nutrient analysis and flexible feed formulation techniques to guarantee constant dietary quality. In order to optimize the sustainable use of WFBP as dependable substitutes for CFI and to account for variations in nutrient density, such proactive approaches are essential.
5. Species variation: While some WF species are high in macronutrients like fiber, phenolic compounds, or antioxidants, others may have higher concentrations of micronutrients like vitamins or minerals (Adejumo and Machebe, 2018). Sensory features, such as unique aroma, also affect livestock acceptance, with some aroma's increasing or decreasing intake. These issues can be resolved by strategically using feed additives or enzymes to mask unwanted odors, improve palatability, and balance nutrient profiles. In this way, WFBP can be sustainably incorporated into livestock rations while reducing nutrient composition variability and sensory appeal.
6. Environmental elements: The nutritional value and palatability of WFBP are greatly influenced by environmental factors such as soil composition, rainfall patterns, and local climate. According to García-Moya et al. (2019), WFBP cultivated in nutrient-rich soils generally show a greater micronutrient density than those from deprived soils. Trees that have adapted to particular habitats are the source of many WFBP, including seeds and byproducts from native species including baobab, carob, tamarind, neem, marula, and sheanut. Consequently, their yields and availability are naturally erratic and dependent on regional climates (Chisoro and Nkukwana, 2020b). This reliance on the environment highlights the necessity of region-specific approaches to improve WFBP consumption in livestock feed and stabilize supply systems, guaranteeing their sustainability as sustainable substitutes for CFI (Wapi et al, 2013).
7. Technical aspects: There are several obstacles in the way of the sustainable incorporation of WFBP into livestock feed systems. Seasonal and unpredictable variations in supply, physicochemical characteristics including dustiness, high bulk density, and powdery texture, and supply chain inefficiencies in post-harvest management (collection, storage, and transportation) are some of the main obstacles (Pexas et al., 2023). Additional limitations stem from the requirement for costly processing procedures (such as dehulling, drying, mechanical, or thermal treatments) to improve safety and digestibility, as well as a lack of research

facilities to determine the optimum levels of nutrient inclusion in animal diets (Abdollahi and Ravindran, 2019; Rahmatnejad et al., 2024). Unlocking the full potential of WFBP as sustainable, regionally adaptable substitutes for CFI requires tackling these issues comprehensively i.e. through enhanced processing technologies, innovative logistics, and focused research and development.

Several unresolved issues continue to impact the nutritional quality and palatability of WFBP in livestock diets. To optimize their potential as sustainable substitutes for CFI, targeted strategies are needed, such as rigorous research to address ANF, optimized processing methods, and precise formulation protocols to determine optimum inclusion levels. Additionally, for WFBP to be successfully incorporated into livestock rations, three requirements must be met: (a) consistent availability (even if seasonal), (b) cost competitiveness in comparison to CFI, and (c) thorough characterization of their nutritional variability, digestibility, and metabolizable energy content (Hassan et al., 2020; Abdollahi and Ravindran, 2019).

Beyond these basics, improving the feeding value of WFBP necessitates a variety of strategies, including: (i) feed evaluation that prioritizes energy and digestible AA; (ii) diet formulations based on digestible rather than total AA; (iii) adding synthetic AA strategically to meet animal needs; (iv) using exogenous enzymes to improve nutrient utilization; and (v) customized processing techniques to reduce ANF and increase nutrient bioavailability (Abdollahi and Ravindran, 2019; Chisoro et al., 2023). When taken as a whole, these actions could increase the quantity of WFBP included in livestock diets while supporting the objectives of nutritional and economic sustainability.

5 Impact of incorporating WFBP in livestock nutrition

Population growth and changing dietary choices are expected to create a 70% increase in the demand for livestock-derived goods worldwide by 2050 (Food and Agriculture Organization (FAO), 2023; World Bank Group, 2021). To meet this demand, 30–50% more agricultural area may be needed if existing livestock production methods continue, which would put further strain on limited natural resources. Given that the livestock industry currently makes a substantial contribution to GHG emissions, land degradation, and biodiversity loss, such expansion runs the danger of making environmental degradation worse (Marchant-Forde and Boyle, 2020; Chisoro et al., 2023). To decouple livestock production development from ecological impact, it is imperative that sustainable alternatives be used, such as incorporating WFBP into stockfeed.

The incorporation of WFBP into livestock feed formulations offers the benefits of both economic efficiency and ecological protection. This avoids the need for more arable land and lowers the amount of land allocated for waste management, protecting local ecosystems by reusing materials like baobab seed oil cake (BSOC), tamarind leaf meal (TLM), sheanut seed meal (SSM), and mango seed meal (MNM), which are leftovers from processing WF. Crucially, because these materials are sourced from pre-existing agro-industrial waste streams, WFBP procurement avoids direct land-use competition and has a small land footprint. Their superiority over CFI is further demonstrated by environmental life-cycle assessments. With sustainable sourcing and processing methods, WFBP can reduce eutrophication potential by up to 83% and reduce GHG emissions, acidification, and other pollution indicators by up to 99% (Abdulla et al., 2017; Habibi et al., 2024).

Circular feed resource systems that emphasize regeneration and local sourcing can reduce input costs and increase productivity economically. Adoption of AFR allows for immediate cost savings by reducing dependency on synthetic fertilizers, irrigation, agrochemicals, and fossil fuels (all of which are major sources of cost in CFI production) (Hassan et al., 2020). Additionally, logistical costs are decreased via proximity-driven models for AFR, in which commodities are produced close to end-user markets or transit hubs. In addition to reducing emissions associated with transportation, this localization optimizes supply chains, reducing the costs associated with producing feed and maintaining livestock (Mbukwane et al., 2022). When combined, these tactics establish WFBP as essential elements of sustainable livestock systems that strike a balance between environmental preservation and financial sustainability.

Due to meat's high nutritional value (protein, vital fatty acids, and minerals) and gastronomic appeal, meat plays a crucial part in various diets around the world. However, the livestock sector is under increasing pressure from cascading disruptions as earlier stated, which have made supply-demand imbalances worse and made conventional meat production less predictable and more expensive (Omollo et al., 2020). However, despite production and religious restrictions in emerging countries, pork continues to dominate markets, with beef and poultry following closely behind (Figures 1, 2) (Egea et al., 2018; Wang et al., 2022; Xu et al., 2023). Notably, because of its versatility and lower resource thresholds, poultry continues to be a vital component of smallholder and subsistence systems in Africa, Asia, and Latin America (Jalal et al., 2023). To overcome limiting obstacles, use of AFR in stockfeed, such as WFBP, provides a practical way to boost output and maintain supply chains (Chisoro et al., 2018, 2023). Current research highlights WFBP's potential to reduce dependency on resource-intensive conventional diets by enhancing feed efficiency, disease resilience, and growth performance across species. Some case studies on the use of WFBP in ruminant, pig, and poultry systems are summarized in the sections that follow.

5.1 WFBP in poultry nutrition

One of the most indispensable sources of animal protein crucial for human growth and development is poultry meat. With an expected total meat consumption of 45.3 kg, current predictions predict that its share of the world's per capita meat consumption would increase from 13.8 kg (33%) to 17.2 kg (38%), by 2030 (Nkukwana, 2019; World Economic Forum, 2019). The output of chicken feed is anticipated to increase at a compound annual rate of 7.1% between 2018 and 2027, in tandem with this demand, reaching a total of about 890 million metric tons by the conclusion of this time frame (Abdollahi and Ravindran, 2019). But even in the most optimistic agricultural yield scenarios, CFI (i.e. maize and SBM), are unlikely to be able to meet these increasing demands. Since 60–70% of the costs of raising chickens are feed related, inclusion of AFR such as WFBP provides a dual benefit, they lessen dependency on CFI while lowering feed-related expenditures (Chisoro and Nkukwana, 2020b). These kinds of developments are essential for improving cost effectiveness, closing supply-demand gaps, and guaranteeing poultry systems' long-term scalability.

Various studies in poultry have shown pronounced benefits in the utilization of WFBP either as alternatives to antibiotics or supplements to maize and soybean. For example, Paul et al. (2022) showed that adding neem leaf extract (NLE) to Cobb 500 broiler feeds in place of enrofloxacin and ciprofloxacin greatly improved growth performance, final body weight (BW), and organ development. Similarly, broilers fed *Moringa oleifera* leaf meal (MOLM) in place of salinomycin and zinc bacitracin showed enhanced feed conversion ratios (FCR), BW growth, and gastrointestinal health (Nkukwana et al., 2014). This strategy was further supported by earlier research by Schiavone et al. (2008), which demonstrated that chestnut wood extract (CWE) may increase FI and BW while replacing synthetic DL-α-tocopherol acetate in broiler diets without sacrificing nutrient digestibility. These results support the urgent need for the poultry sector to lessen its reliance on antibiotics, which fuel antimicrobial resistance, a serious public health issue, especially in broiler systems where there is a higher danger of zoonotic transmission. Producers can address both production efficiency and antimicrobial stewardship by incorporating WFBP, such as NLE, MOLM, and CWE (Table 2).

5.2 WFBP in pig nutrition

As the most intensively produced type of meat and with the highest feed costs the use of AFR in pig production is very apparent. Numerous studies have been done in supplementing WFBP in pig diets with various promising results. For instance, developing pigs fed diets containing 15% sodium hydroxide-treated sheanut kernel cake (TSNKC) as a substitute for wheat bran showed equivalent growth performance and FI according to Oddoye et al. (2015). According to Magonka et al. (2018), growth parameters were maintained while feed costs were decreased when 5% sunflower cake (SFC) was substituted with baobab seed cake (BSC). The usefulness of WFBP was further confirmed by Mabena et al. (2022), who demonstrated that 15% amarula nut cake (ANC) may be used in place of SBM in the diets of growing pigs, producing comparable protein digestibility and FI while improving the color characteristics of the meat (redness, lightness). Beyond cost-efficiency, WFBP can improve meat quality, Scerra et al. (2022) observed that 15% exhausted bergamot pulp byproduct (EBPB) as a partial SBM substitute in fattening pigs increased docosapentaenoic acid and total n-3 polyunsaturated fatty acids (PUFA) in pork, enhancing its nutritional profile despite no differences in FI, final BW, or carcass yield.

5.3 WFBP in rabbit nutrition

In the context of global food security, the domesticated rabbit stands out as a notable potential livestock species, especially as the growing human population puts more strain on limited agricultural resources (McNitt et al., 2013). Rabbits show remarkable adaptation to high-roughage, low-grain rations while retaining robust growth and reproductive performance, in contrast to pig and poultry, which need grain-dependent diets (de Blas and Wiseman, 2010). According to recent research, grain-free diets high in fibrous plant matter can support rabbits' normal physiological development, providing a crucial edge as human and livestock competition for CFI intensifies (McNitt et al., 2013). Due to their ability to effectively turn unconventional feed sources (i.e. like crop residues, agroforestry byproducts, and wild vegetation) into premium meat, rabbits are positioned as a sustainable source of protein, especially in developing nations that are struggling with food scarcity and a lack of arable land (Mcafee et al., 2010). Additionally, the richness of native plants in these areas, which are frequently inappropriate for monogastric animals, complements rabbits' nutritional adaptability and lessens their dependency on feeds that require a lot of resources. Through the use of locally accessible WFBP in rabbit production, stakeholders can improve food security, reduce volatility of feed-costs, and promote circular agricultural systems; all of which are important goals for robust, just food networks.

Given that the world's population expected increase to 10 billion people by 2050, producing rabbits appears to be a viable way to meet this growing demand for food. The inclusion of WFBP in rabbit diets has two benefits: a) it lowers feed costs while improving growth and b) reproductive efficiency. For example, Ogbuewu et al. (2011) showed that neem leaf meal (NLM) enhanced female reproductive results without sacrificing growth or FI when 5% soybean meal (SBM) was substituted. Similarly, Henry et al. (2018) found that adding pawpaw leaf meal (PLM) to the meals of New Zealand White × Chinchilla crossbreed rabbits improved their reproductive success and follicle-stimulating hormone (FSH) levels without having an unfavorable effect on growth or other physiological balances. Adeyeye et al. (2021) also noted that replacing 10% SBM with kola nut pod-husk meal (KNPM) reduced feed costs and increased profitability while maintaining growth performance similar to control diets. While Adeniji et al. (2012) noted that a 60% addition of a 1:1 blend of groundnut cake (GNC) and MOLM to grower rabbit diets increased BW and reduced feed costs compared to the unsupplemented feeds. Taken together, these studies demonstrate the feasibility of WFBP as sustainable, affordable feed substitutes that maximize rabbit husbandry productivity (Table 2).

TABLE 2 Performance characteristics summary on monogastric animals fed varying inclusion levels of WFBP in their diets.

Species	Ingredient	Inclusion %	Control	Animal	Parameters	Effect	References
Pigs	*Moringa oleifera* leaf meal	0, 2.5, 5 or 7.5	Soybean	Finisher pigs	Feed intake Feed conversion ratio Body weight Meat quality	Highest 7.5 No effect No effect No effect	(Mukumbo et al., 2014)
	Treated Sheanut kernel cake	0, 10, 15 or 20	Wheat bran	Growing pigs	Weight gain Feed intake Feed conversion ratio	No effect No effect No effect	(Oddoye et al., 2015)
	Marula nut cake	0, 5, 10, 15 or 20	Soybean meal	Fattening pigs	Body weight Feed intake Feed conversion ratio Meat quality	Highest 5 No effect Highest 15 Lowest 10	(Mabena et al., 2022)
	Baobab seed cake	0, 5 or 10	Sunflower seed cake	Weaner pigs	Feed intake Body weight Feed conversion ratio	Highest T2 Lowest T3 No effect	(Magonka et al., 2018)
		Con and T_2	Cereal based diet	Fattening pigs	Final body weight Dry matter intake Feed conversion ratio Fatty acid intake	No effect No effect No effect Highest T_2	(Scerra et al., 2022)
Layers	*Moringa oleifera* whole seed meal	0, 1, 3, and 5	Gluten 20%	Hy-Line hens	Bird weight Feed intake Laying rate Egg weight	Lowest 5 Highest 0 Lowest 1 Lowest 5	(Mabusela et al., 2018)
	Oven-dried tamarind	0, 2, 4, 6, or 8	Wheat and rice polish	Hisex Brown, ISA Brown, Lohmann Brown, Starcross Brown, Babcock B-300, Starcross-579	Feed efficiency Feed intake Laying rate Egg weight York cholesterol Serum cholesterol	Highest 8 No effect Highest 2 No effect No effect No effect	(Chowdhury et al., 2005)
	[b]Guar meal (GM)	T_1, T_2, T_3 or T_4	Feed withdrawal group	Late-Phase laying hens	Bird weight reduction Days to 0 egg production Organ weights *Salmonella*	Highest T_1 Highest T_2 Lowest T_1 Highest T_1	(Gutierrez et al., 2008)

(Continued)

TABLE 2 Continued

Species	Ingredient	Inclusion %	Control	Animal	Parameters	Effect	References
					Enteritidis invasion		
	[c]Colocynth seed meal	T_1, T_2, T_3 or T_4	Basal diet	Hy-Line Brown layers	Egg number Egg weight Feed intake Stress response Immunological response	Highest T_2 Lowest T_3 Highest T_2 Highest T_2 Highest T_4	(Abbas et al., 2022)
Broilers	Baobab seed oilcake	0, 5, 10 and 15	Soybean	ROSS 308	Feed intake Feed conversion ratio Bird weight Feed costs	Lower 0 No effect Higher 0, 5 Higher 15	(Chisoro et al., 2018)
	[d]*Moringa oleifera* leaf meal	(T_1, C+), T_2, T_3, T_4, and (T_5, C-)	Salinomycin and zinc bacitracin	Cobb 500	Feed intake Feed conversion ratio Bird weight Apparent digestibility	No effect Highest T_5 Highest T_5 No effect	(Nkukwana et al., 2014)
	Marula nut meal	0, 25, 50, 75 and 100	Soybean	Japanese quail	Bird weight Feed intake Feed conversion ratio	Highest 25 Highest 75 Lowest 0	(Mazizi et al., 2019)
	Extract of chestnut wood	ENC0, ENC15, ENC20 and ENC25	DL-α-tocopherol acetate	Cobb 500	Feed intake Feed conversion ratio Bird weight Protein efficiency ratio Apparent digestibility	Highest ENC15 No effect Highest ENC20 No effect No effect	(Schiavone et al., 2008)
	[e]Neem leaf extract	NC, PC and NLE	Enrofloxacin and ciprofloxacin	Cobb 500	Bird weight Feed intake Feed conversion ratio Organ size Blood parameters Feed costs	Highest NLE Lowest NC Lowest NLE Highest NLE No effect Highest NLE	(Paul et al., 2020)
	[f]*Moringa oleifera* leaf meal	T_1, T_2, T_3, T_4 and T_5	Salinomycin and zinc bacitracin	Cobb 500	Bird weight Feed intake Feed conversion	No effect Highest T_5 Lowest T_3	(Nduku et al., 2021)

(Continued)

TABLE 2 Continued

Species	Ingredient	Inclusion %	Control	Animal	Parameters	Effect	References
					ratio Organ size Meat quality	No effect Highest T_2	
Rabbits	Kola nut pod-husk meal	0, 10; 20 and 30	Soybean	Chinchilla x NZ white cross	Body weight Feed intake Feed conversion ratio Blood parameters	Highest 0 No effect Lowest 0 No effect	(Adeyeye et al., 2021)
	[g]Pawpaw leaves	Control (T_1), T_2 and T_3	Concentrate feed	Chinchilla x NZ white cross	FSH (mIUmL-1) Progesterone (ngmL-I) Testosterone (ngmL-I) Estradiol(E2) (pgmL-I)	Highest T_3 No effect No effect No effect	(Henry et al., 2018)
	Neem leaf meal	0, 5, 10 and 15	Soybean	Chinchilla x NZ white cross	Paired oviductal weights Body weight Feed intake Feed conversion ratio Female reproductive tract	Highest 0 No effect No effect No effect Highest 5	(Ogbuewu et al., 2011)
	Microbially fermented cassava starch residue	0, 25, 50, 75 and 100	Maize	Weaner rabbits	Body weight Weight gain Feed intake Feed conversion ratio	Highest 50 Lowest 100 Lowest 0 Highest 100	(Oloruntola et al., 2018)
	Groundnut Cake with MOLM	0, 20, 40, 60, 80 and 100	Maize bran	Grower rabbits	Body weight Weight gain Feed intake Feed conversion Feed costs	Highest 60 Lowest 0 Lowest 100 Highest 0 Lowest 100	(Adeniji and Lawal, 2012)

[a]Only two experimental diets were used = Con, a cereal-based concentrate diet and T_2 the same diet with ensiled bergamot pulp partially replacing 15% dry matter of the Con diet.
[b]Treatments = T_1 (consisted of a feed withdrawal group), T_2 (full-fed groups combining standard laying hen feed with either 20% GM (20%GM)), T_3 (20% GM supplemented with 250,000 units/kg of β-mannanase (Hemicell; 20% + E)) and T_4 (full-fed standard laying diet group (nonmelted control)).
[c]Experimental groups = T_1 (non-stressed group provided with basal diet (non-stressed control group)), T_2 (non-stressed group provided with *Citrullus Colocynthis* seed supplementation (colocynth-seed-supplemented group)), T_3 (oxidative stress group injected with paraquat (PQ) and provided with basal diet (PQ-injected group)) and T_4 (oxidative stress group injected with paraquat and provided with *C. Colocynthis* seed supplementation (colocynth seed supplementation with PQ injected group)).
[d]Dietary treatments = T_1 (positive control (þC) with 668 g salinomycin and 500 g zinc bacitracin per kg of feed); T_2 ($MOLM_{low}$ (ML; 1, 3 and 5); T_3 ($MOLM_{medium}$ (MM; 3, 9 and 15 g); T_4 (MOLMhigh (MH; 5, 15 and 25 g) per kg of feed); and T_5 (a negative control (without supplementation)).
[e]NC = Negative control (basal diet without any additives), PC = positive control (basal diet with antibiotics, Vitamin B-complex, and amino-acid supplementation) and NLE = Neem leaf extracts treated group, basal diet supplemented with 0.1% aqua extract of neem leaves (20 ml of 5% NLE per liter drinking water).
[f]T_1 = 300 g/ton zinc bacitracin and 500 g/ton salinomycin; T_2 = 1000 g/ton MOLM; T_3 = probiotic (500 g/ton Enviva Pro); T_4 = organic acid (1000 g/ton Novyrate C) and T_5 = without additives.
[g]Dietary treatments = control (T_1) (concentrate and potato (*Ipomea batatas*) leaves), T_2 (concentrate and fresh pawpaw leaves) and T_3 (concentrate and wilted pawpaw leaves).

5.4 WFBP in beef nutrition

The inclusion of WFBP to beef cattle diets has shown interesting results, including nutritional and therapeutic benefits. For example, the leaves of tamarind (*Tamarindus indica*) and neem (*Azadirachta indica*), which are known across the world for their hepatoprotective, antibacterial, and antiprotozoal qualities, have been tested as natural anthelmintics. In contrast to their solo use (neem: 29.93%; tamarind: 42.67%), Rahaman et al. (2022) found that a 1:1 blend of neem and tamarind leaves had higher anthelmintic efficacy (68.15%), demonstrating synergistic potential in parasite treatment. Beyond their use in medicine, WFBP can be used as affordable alternatives to traditional feed antibiotics. Mlambo et al. (2011) found that replacing urea with marula seed cake (MSC) in weaner cattle rations yielded comparable growth rates, FI, and FCR, with no differences in average daily gain (ADG) between MSC-supplemented and control diets. This suggests MSC could reduce the reliance on synthetic nitrogen sources without compromising productivity. Similarly, Chingala et al. (2019) evaluated baobab seed meal (BSM) and white thorn tree leaf-meal (WTLM) as protein alternatives to soybean meal (SBM) in Malawi Zebu steers. Steers fed BSM diets outperformed their SBM-fed counterparts in terms of dry matter intake (DMI), final BW, and total tract nutrient digestibility (TND), all while displaying a higher microbial nitrogen supply. Overall, these studies demonstrate the dual utility of WFBP in beef production systems; as cost-effective substitutes for traditional protein or energy sources and as functional additives for health management (Table 3).

5.5 WFBP in dairy nutrition

Generally, the global dairy sector has been experiencing steady growth on both production and consumption (Gebreyowhans and Zegeye, 2019).The demand for dairy products has been increasing globally, driven by population increase, the rise in household income and the change in dietary preferences (International Livestock Research Institute (ILRI), 2016; World Bank Group, 2021). Global milk production peaked at 810 million metric tons in 2020, with China, India, and the U.S. as the top three producers (World Bank Group, 2021; USDA, 2022). At the same time, emerging nations have been the main drivers of this sectoral expansion; their dairy industries are growing at an average annual rate of 3.7% (World Economic Forum, 2019). Rising disposable incomes, urbanization, population increase, and dietary changes toward nutrient-rich foods are the main causes of this increase (International Livestock Research Institute (ILRI), 2016; Food and Agriculture Organization (FAO), 2019). The need to find sustainable feed resources, such as WFBP, to meet the growing demand while lowering dependency on CFI is highlighted by this rapid growth.

The need to implement climate-smart agriculture and reduce its environmental impact has prompted the dairy industry to embrace technology advancements and sustainability-driven methods more and more (Food and Agriculture Organization (FAO) 2019; Livestock Development Institute of Africa (LDIA), 2014; World Bank Group, 2021). However, this sectoral development and profitability are threatened by enduring issues such as fluctuating milk costs, competition from plant-based substitutes (such as soy, almond, oat, and rice-based products), and changing consumer trends (World Bank Group, 2021). Notwithstanding these challenges, there is a significant opportunity due to the ongoing growth of dairy output in emerging countries and the persistent desire from consumers for traditional dairy products.

Dairy rations that incorporate WFBP could improve sustainability, lower costs, and increase resistance to environmental and market stresses. Studies demonstrate their capacity to boost ruminal protein metabolism, milk yield (MY), and milk protein (MP) content in dairy calves without compromising FI or nutrient digestibility (Madzimure et al., 2011; Bashar et al., 2020). For example, Raj et al. (2016) discovered that adding karanja or neem seed cake to total mixed rations (TMR) in place of 30% of CFI increased MY while preserving MP levels and milk solids. Sesbania leaf meal (SSLM) increased MY in crossbred dairy cows without lowering FI (Gebreyowhans and Zegeye, 2019), while marula seed cake (MSC) replaced SBM in nursing cows to improve digestibility without changing MY (Mdziniso et al., 2016). Additionally, Bashar et al. (2020) found that adding moringa leaf meal (MOLM) gradually to diets based on silage increased milk output while maintaining digestibility and crude protein (CP) intake. The dairy industry can promote sustainable agriculture, lower GHG emissions associated with the production of conventional feed and conform to climate-smart practices by incorporating WFBP as partial substitutes or supplements (Table 3).

5.6 WFBP in sheep and goat nutrition

Small-scale ruminant production, especially that of sheep and goats, is essential to the economy of desert and rural areas of emerging nations and is expected to continue to be crucial to the world's meat supply. WFBP provide sustainable ways to improve meat quality and productivity in these systems. For example, Qwele et al. (2013) noted that Xhosa lop-eared goats fed either grass hay (GH), sunflower cake (SFC), or MOLM, discovered that supplementing with MOLM (200 g/day) increased the meat's antioxidant capacity, as shown by a lower lipid oxidation (LO) and a higher total phenolic content (10.62 ± 0.27 mg tannic acid equivalent/g). This enhanced the meat's color, chemical composition, and shelf-life stability over time. Similarly, Geron et al. (2015) showed that adding 15% tamarind pulp residue (RETP) to sheep diets preserved FI and BW while reducing feed expenses. More recently, Baleseng et al. (2023) found that in lamb fattening diets, morula kernel cake (MKC) can completely replace traditional protein sources like lucerne and sunflower meal (SFM) without affecting meat quality, FCR, final BW, DMI, or FI. This was ascribed by the study to MKC's balanced nutritional composition, which satisfied the growth and maintenance needs of lambs (Table 3).

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TABLE 3 Summary performance characteristics in ruminant livestock fed diets with varying inclusion levels of WFBP.

Animal	Ingredient	Inclusion %	Control	Animal/Breed	Parameters	Effect	References
Beef	[a]Marula seed cake	T_1, T_2 or T_3	Urea as a Nitrogen source	24-month dairy male weaners	Final live weight Weight gain Feed intake Feed conversion ratio	Highest T_2 Lowest T_1 Highest T_2 Lowest T_3	(Mlambo et al., 2011)
	[b]Neem and Tamarind leaf meal	T_1, T_2, T_3 or T_4	No supplementation	Indigenous beef cows	Fecal egg count Efficacy rate	Lowest T_4 Highest T_1	(Rahaman et al., 2022)
	[c]*Moringa oleifera* and Propolis leaf meal	CON, PRO, ML or MSC	Without supplementation	Rumen *in vitro* simulation study	Organic matter disappearance Crude protein disappearance Volatile fatty acid (VFA) profile Fermentation gases	No effect No effect Highest MSC Lowest PRO	(Mahmood et al., 2022)
	[d]Baobab seed meal and White thorn tree leaf-meal	Con, T_2 or T_3	Hay with Soybean	Malawi Zebu steers	Total tract nutrient digestibility Final body weight Feed conversion ratio Rumen microbial nitrogen	Highest Con Lowest T_3 Highest Con Highest T_2	(Chingala et al., 2019)
Dairy	[e]Moringa leaf meal fodder	T_0, T_1 or T_2	Napier silage and concentrate	Breed-1 (BCB-1) dairy cows	Crude protein intake Digestibility VFA profile Methane (CH_4) production Daily milk production	No effect No effect Highest T_2 No effect Lowest T_0	(Bashar et al., 2020)
	[f]Karanja cake and Neem seed cake	Con, dNC or dKC	Soybean meal	Crossbred dairy cows	Efficiency of milk production Milk yield Milk protein Total milk solids	No effect Highest dNC No effect No effect	(Raj et al., 2016)
	Baobab seed cake	0, 5, 10 or 15	Cottonseed cake	Guernsey cows	Feed intake Milk yield Milk protein Total milk solids	Highest 0 Lowest 15 Highest 15 Lowest 0	(Madzimure et al., 2011)
	[g]Marula seed cake	Con, SBM+MSC or MSC	Soybean meal	Lactating dairy cows	*In vitro* DM digestibility Apparent DM digestibility	No effect Highest MSC	(Mdziniso et al., 2016)
	Sesbania sesban leaves	0, 1.25 kg/d, 2 kg/d and 2.75 kg/d	No supplementation	Holstein Frisian x Zebu crossbred dairy cows	Total DM intake Feed conversion efficacy Apparent DM digestibility Daily milk yield	Highest 2 No effect Highest 2.75 Lowest 0	(Gebreyowhans and Zegeye, 2019)

(Continued)

TABLE 3 Continued

Animal	Ingredient	Inclusion %	Control	Animal/Breed	Parameters	Effect	References
	Ground flax seed	0, 5, 10, and 15	Corn and soybean meal	Organic-certified Jersey cows	Ruminal microbiota diversity Enteric CH_4 emissions Urinary purine derivatives	No effect No effect Highest T_2	(Resende et al., 2015)
Sheep and Goats	[h]*Moringa oleifera* leaf meal	T_1, T_2 or T_3	Grass hay and sunflower	Castrated Xhosa lop-eared goats	% Inhibition of $DPPH^+$ % Inhibition of $ABTS^+$ % Inhibition of lipid oxidation SOD activity (inhibition rate %)	Highest T_2 Highest T_2 Lowest T_1 Highest T_2	(Qwele et al., 2013)
	Extraction of Tamarind pulp	0, 5, 10 and 15	Soybean meal	Mongrel sheep	Body weight Organic matter intake Organic matter digestibility	No effect Highest 15 No effect	(Geron et al., 2015)
	[i]Morula kernel cake	T_1, T_2 or T_3	Sunflower seedcake	Finishing tswana lambs	Body weight Dry matter intake Apparent nutrient digestibility Nitrogen retention Hot carcass weight	Highest T_3 Highest T_1 Lowest T_3 Lowest T_3 No effect	(Baleseng et al., 2023)
	[j]Flax seed meal	T_1, T_2, T_3 or T_4	Soybean meal	Dorper × Small thin-tailed crossbred rams	Body weight Dry matter intake Feed efficiency Apparent digestibility	Highest T_2 No effect Lowest T_4 Highest T_3	(Hao et al., 2020)
	[k]Sesbania sesban and reed grass	Con, T_1 or T_2	Alfalfa	Farafra growing lambs	Body weight Dry matter intake Feed conversion rate Blood serum constituents Meat quality characteristics	Highest T_2 Highest T_2 Lowest T_2 Highest T_1 Lowest T_1	(Farghaly et al., 2022)
	[l]Neem leaf meal	T_1, T_2, T_3 or T_4	No supplementation	Anglo-Nubian male goats	Body weight Organic matter intake Organic matter digestibility Volatile fatty acids Nitrogen retention	Highest T_4 Lowest T_1 Highest T_4 Lowest T_2 Highest T_4	(Taethaisong et al., 2023)
	Sheanut meal	0, 5, 10, 15 or 20	Rhodes grass hay and maize bran	Growing sheep	Ruminal NH_3-N concentration Acetate concertation Butyrate concentration	Highest 5 Lowest 5 Highest 0 Highest 15 Lowest 10	(Amerit et al., 2023)

(Continued)

TABLE 3 Continued

Animal	Ingredient	Inclusion %	Control	Animal/Breed	Parameters	Effect	References
					Propionate concentrates Ruminal protozoan count		
	[m]Marula seed cake	T_1, T_2, T_3 or T_4	Grass hay (unsupplemented)	Nguni goats	Organic matter intake Organic matter digestibility Nitrogen intake Nitrogen retention	Highest T_1 Lowest T_2 Lowest T_2 Highest T_2	(Mlambo et al., 2011)

[a]Treatment (T_1) = commercial fattening ration (with urea as a N source) (CFR-U), T_2 = commercial fattening ration in which urea was wholly replaced with MSC (CFR-MSC) or T_3 = commercial fattening ration in which MSC and urea contributed equal amounts of N (CFR- MSC+U).

[b]Treatment (T_1) = Group A (control basal diet), T_2 = Group B (supplemented with grinded Neem leaves), T_3 = Group C (treated with grinded Tamarind leaves) and T_4 = (Group D treated with both grinded Neem leaves and Tamarind leaves).

[c]CON = control diet without supplementation, MSC = CON supplemented with moringa seed cake (MSC, containing 49% crude protein (CP)), ML = CON supplemented with moringa leaf powder (ML, containing 28% CP), or PRO = CON supplemented with raw propolis (PRO, 3% CP).

[d]Three treatments = Con, rangeland hay and maize bran with soybean meal; T_2, rangeland hay and maize bran with baobab seed meal (baobab diet); T_3, rangeland hay and maize bran with *V. polyacantha* leaf-meal (*Vachellia* diet) as the protein source.

[e]T_0 = control diet consisting of 1:1 dry matter (DM) of Napier silage and conventionally mixed concentrate, T_1 = control diet with concentrate replaced with 50% moringa feed or T_2 = control diet with 100% concentrate with moringa feed.

[f]Con = Control TMR (T1–Control) formulated using dried maize fodder (roughage source) and concentrate feed, dNC = used at 3.78% of TMR (T2-dNC) of 13.2% detoxified neem seed cake or dKC = 5.85% of TMR (T3–dKC) of soybean meal substituted with 18% detoxified karanja cake.

[g]Con = formulated diet containing soybean meal, SBM+MSC = formulated diet containing 50% Marula seed cake and 50% soybean meal (SBM) as a protein supplement; and MSM = formulated diet containing Marula seed cake as a protein supplement.

[h]T_1 = group 1 goats were supplemented with 200 g of dried *M. oleifera* leaves (MOL), T_2 = group 2 (positive control) supplemented with 170 g of sunflower seed cake (SC) per head per day and T_3 = group 3 (negative control) fed on grass hay (GH) ad libitum. DPPH & ABTS = free radical scavenging assay, SOD = Superoxide dismutase assay.

[i]T_1 (CD) = commercial diet, T_2 (MKCD) = morula kernel cake diet, and T_3 (MSCD) = morula seed cake diet.

[j]T_1 = 18% Soybean meal (SBM), T_2 = 12% SBM and 6% flax seed meal (FSM), T_3 = 6% SBM and 12% FSM and T_4 = 18% FSM.

[k]Con = fed as 60% concentrates and 40% fresh alfalfa, T_1 = fed as 60% concentrates plus 40% fresh Sesbania sesban and T_3 = fed as 60% concentrate plus 40% fresh reed grass.

[l]Treatment (T_1) = control, T_2 = control + 15% Propylene ethylene glycol (PEG) in the concentrate, T_3 = 6% Neem leaf (NL) meal in concentrate; and T_4 = 6% NL + 15% PEG in the concentrate.

[m]T_1 = 300 g/day of grass hay with no supplementation, T_2 = 300 g/day of grass hay in combination with marula seed cake (MSC), T_3 = 300 g/day of grass hay in combination with sunflower cake (SC) and T_4 = 300 g/day of grass hay in combination with soybean meal (SBM).

6 Antioxidant influence of the inclusion of WFBP in livestock feed

The nutritional value and sensory qualities of meat are significantly influenced by the content of the feed (Stelzleni and Johnson, 2008). Plant-derived natural antioxidants (PDNA), which are abundant in unsaturated fatty acids and bioactive compounds, such as essential oils, herbs, leaf meals, and seed extracts, have drawn more attention from researchers to improve the quality of meat (Cullere et al., 2013; Hoffman et al., 2014). Notably, the fatty acid profile, oxidative stability, and sensory qualities of meat are enhanced by the effective transfer of polyphenols and flavonoids, which are abundant in many PDNA sources, from feed to muscle tissue (Fu et al., 2022; Tang et al., 2023). While the bioavailability and metabolic fate of these compounds vary depending on their chemical structure and post-ingestion pathways, their role in mitigating LO and modulating volatile compound formation remains pivotal to preserving meat quality (Bellés et al., 2017; Estévez and Lorenzo, 2018). WFBP, which are particularly rich in phenolics and antioxidants, offer a sustainable means of enhancing these benefits. Incorporating WFBP into livestock rations can thus synergistically improve meat sensory parameters, such as flavor and shelf-life stability.

Furthermore, to enhance the quality of chicken, pork, and beef, it is customary to supplement with α-tocopherol (100–200 mg/kg feed). α-tocopherol is an essential part of the skeletal muscle's endogenous antioxidant defense and a major factor in the oxidative stability of biological membranes (Dalle Zotte et al., 2016; Tang et al., 2023). Supplementing with α-tocopherol has many well-documented benefits, such as protecting fresh meat from discoloration, promoting greater oxidative stability during storage, and reducing the oxidation of proteins, cholesterol, and PUFAs in processed meat products (Estévez and Lorenzo, 2018; Tang et al., 2023). Significantly, comparable results have been obtained with natural tocopherol-rich feedstuffs, including plant-derived materials (e.g., rooibos, tamarind, moringa, acorns, and olive leaf) and WFBP, which are in line with sustainable practices (Dalle Zotte et al., 2016; Wong et al., 2023; Wang et al., 2024). Whole plants, leaves, seeds, and their extracts are increasingly valued in livestock diets as multifunctional additives, acting as natural antioxidants, color stabilizers, and physiological enhancers (Qi et al. 2022; Tang et al., 2023). This highlights the feasibility of incorporating WFBP as partial feed ingredients or supplements, providing a dual benefit of enhancing meat quality and promoting resource-efficient, circular agricultural systems.

Due to their antibacterial and antioxidant qualities, WFBP show great promise as natural feed additives. For example, Jayawardana et al. (2015) showed that by lowering LO, MOLM improves oxidative stability in ghee, increasing shelf life. Similarly, Qwele et al. (2013) found that dietary MOLM supplementation reduced superoxide dismutase (SOD) activity and glutathione (GSH) depletion in goats while reducing LO in meat during storage. Gravador et al. (2015) discovered that carob pulp meal (CPM) preserved the oxidative stability of meat in ruminant diets by substituting 24–35% of barley without hastening deterioration. Scerra et al. (2022) further noted that adding 30% almond hulls (AH) to lamb diets enhanced meat oxidative stability (lowering LO in raw and cooked meat after 5 and 7 days of refrigeration, respectively) without affecting growth performance. In addition to terrestrial livestock, Hendam et al. (2024) noted how baobab fruit extract powder (BFP; 4 g/kg diet) benefits Nile tilapia by improving intestinal health, growth, antioxidant capacity, and serum immunity. Finally, Taylor et al. (2011) demonstrated that broiler diets containing volatile oil and dried rosemary leaves inhibited the production of off-flavor and thiobarbituric acid-reactive compounds (TBARS) in breast meat. Taken together, these studies highlight WFBP's dual function of enhancing meat quality and shelf life while supporting resource-efficient, sustainable animal production.

7 Potential WFBP ingredient processing techniques

To improve their safety, palatability, and digestibility for the best possible nutrient utilization by animals, feed ingredients must go through crucial preprocessing in the manufacturing of livestock feed to remove contaminants, ANF, mycotoxins, and pathogens (Kinyuru et al., 2018; Nyamukonda and Ndlovu, 2019). The reduction of ANF (i.e. naturally occurring compounds that impair nutrient bioavailability, FI, digestibility, and animal health) is essential for the successful integration of WFBP as partial ingredients or supplements in livestock rations (Jayawardana et al., 2015; Chisoro et al., 2023; Hendam et al., 2024). ANFs, which include tannins, alkaloids, and protease inhibitors, limit livestock productivity and product quality by interfering with the absorption of essential nutrients, such as minerals (like calcium and phosphorus), vitamins (like A, C, and E), and essential AA (like lysine and methionine) (Geron et al., 2015; Tang et al., 2023).

To lessen these impacts, traditional processing methods are used separately or in combination to break down ANF below critical toxicity thresholds, including soaking, fermentation, germination, steam sterilization, and dehulling (Bashar et al., 2020; Scerra et al., 2022; Rahaman et al., 2022). CFI processing already incorporates these techniques; for instance, cottonseeds are heat-treated to detoxify gossypol (Negi and Pant, 2017; Feedipedia, 2023), and soybeans are roasted to lower trypsin inhibitors and tannins (CVB, 2018). By using comparable techniques on WFBP, their potential as sustainable substitutes or supplements to CFI can be unlocked by improving their palatability, nutrient bioavailability, and safety. Specific processing strategies designed to maximize the use of WFBP in animal diets are examined in the sections that follow:

1. Sun drying: Before inclusion of WFBP to livestock rations, feed manufacturers use sun drying as a low-cost and widely used method to reduce moisture and ANF. This method works best for moisture-rich WFBP, like juicy fruits, leaf meals, and seeds, which often need to be dehydrated to improve digestibility, extend shelf life, and facilitate processing (Ramteke et al., 2019). Common uses include

drying azolla, duckweed, cassava peels, sweet potato foliage, and algae; materials that are susceptible to deterioration because of their high initial moisture content (Stein et al., 2016; Rojas and Stein, 2017; Widderich et al., 2022).

The final product's nutritional quality is greatly impacted by the drying process. For example, when Babiker et al. (2018) examined the effects of partial shade (PS) and direct sun (DS) drying on the leaves of *Moringa oleifera* and *M. peregrina*, they found different compositional results. Whereas DS-dried leaves showed increased antioxidant activity and overall phenolic content, PS-dried leaves maintained higher quantities of beta-carotene, ascorbic acid, minerals, and AA. These results highlight the need of choosing drying methods according to the feedstuff being dried, since DS improves oxidative stability while PS protects heat-sensitive vitamins. Such information is essential for maximizing the use of WFBP in feed compositions tailored to a given region.

2. Autoclaving: Like sun drying but more effective, autoclaving is a thermal processing method that uses heat, steam, and pressure to lower moisture and ANF in WFBP. Autoclaving is useful in cold or humid locations with little sunlight (such as parts of Europe), where typical sun drying is unfeasible, albeit being more expensive and energy intensive. This process improves nutrient bioavailability and shelf stability while hastening the dehydration of high-moisture products like fruit pulps or leaf meals. Its effectiveness in raising feed quality has been demonstrated by recent studies. For example, when common carp (*Cyprinus carpio*) were fed SBM, wheat bran, maize, and barley, Abdulazeez and Al-Tameemi (2023) showed that autoclaving greatly raised the apparent digestibility coefficients (ADCs) for dry matter, protein, and energy. Although fishmeal demonstrated little reactivity, autoclaved diets also increased growth rates and feed efficiency as compared to untreated controls. Similarly, Sung et al. (2022) assessed autoclaved poultry meal (APM) in broiler chickens and pigs, identifying metabolic responses unique to each species. The metabolizable energy (ME) of unautoclaved poultry meal (unAPM) was higher in pigs (4,792 kcal/kg DM) than in broilers (3,591 kcal/kg DM). While autoclaving for a longer period (134°C, up to 180 min) decreased ME values more sharply in pigs, indicating that broilers are better able to withstand extended processed APM. These results highlight the potential of using autoclaving to maximize WFBP utilization in feed systems, albeit with trade-offs.
3. Chemical or cold treatment: By reducing ANF and enhancing functional qualities, chemical and cold treatments are efficient ways to increase the nutritional value of WFBP. Because trypsin inhibitors are stable in acidic environments, Fernandez et al. (1993) noted that soaking faba beans in a 0.07% sodium bicarbonate ($NaHCO_3$) solution decreased trypsin inhibitor activity more efficiently than a 0.1% citric acid solution. Similarly, novel cold treatments like thermosonication and cold plasma have demonstrated promise in altering the structure of WFBP. According to Bao et al. (2021), pretreatments with chemicals, cold plasma, and thermosonication changed the morphology of the jujube's surface by forming pores and microcracks, which sped up moisture transport and cut drying periods by 18%, 12%, and 7%, respectively. Notably, chemical and cold plasma pretreatments decreased the formation of 5-hydroxymethylfurfural (a potential toxicant) by 25% and 15%, respectively, improving the safety and quality of dried jujube for feed applications. These treatments also improved nutrient retention; total phenolic content increased by 13%, 12%, and 6%, while antioxidant capacity (as determined by ferric reducing antioxidant power) increased by 13%, 11%, and 3%. These methods offer scalable, energy-efficient alternatives to conventional drying, especially for moisture-rich WFBP in areas lacking consistent solar exposure.
4. Soaking: For WFBP, soaking is an economical pretreatment that improves moisture content, palatability, and nutrient digestibility while lowering ANF including tannins and phytic acid (Gürbüz and Özkan, 2018). However, the type of plant, time, and soaking medium all affect how effective it is. For example, soaking lentil seeds in distilled water for 24 hrs decreased trypsin inhibitors by 58–66% (Batra et al., 1986), but soaking French bean (*Phaseolus vulgaris*) seeds in water for 18 hrs maintained 98–99% of trypsin inhibitor activity (Desphande and Cheryan, 1983). Recent developments emphasize the importance of targeted soaking solutions, Dhlakama et al. (2022) showed that treating baobab seeds with deionized water, 0.5% monovalent salts (NaCl, $NaHCO_3$), or divalent sodium salts ($Na_2S_2O_5$) significantly reduced bulk density, oxalate, and phytate content while raising the temperature at which they gelatinized, which are important improvements for feed processing and nutrient bioavailability. These results highlight the potential of soaking as a scalable technique to maximize WFBP for livestock diets, as long as the parameters are adjusted to the feedstuff's unique ANF profile and functional objectives.
5. Sprouting or Germination: This is a technique commonly used in hydroponic fodder systems (e.g., barley, wheat, and maize) that improves feed quality through biochemical modifications during seed germination (García-Moya et al., 2019). For example, sprouting decreased phytic-phosphorus levels in mung beans (*Phaseolus aureus*) and rice beans (*Vigna umbellata*) by 9.8% and 11.3%, respectively, in comparison to ungerminated seeds (Verma and Mehta, 1988). These biochemical changes highlight sprouting's potential to optimize WFBP for sustainable livestock feed formulations. Such decreases in phytic acid improve mineral absorption in livestock by reducing its chelating capacity (Khan et al., 1991; Sharma et al., 1996). Additionally, sprouting mitigates lectin-related

ANFs; germination decreased haemagglutinating activity in seeds by inactivating concanavalin A, as evidenced by a 49% reduction in jack bean (*Canavalia ensiformis*) after treatment (Esonu et al., 1998; Akande and Fabiyi, 2010).

6. Roasting and/or toasting: Thermal processing methods like roasting and toasting improve the quality of livestock feedstuffs by lowering ANF and increasing digestibility and palatability. These techniques work especially well with oilseed meals like cottonseed meal CSM) and SBM. For example, it has been demonstrated that roasting baobab (*Adansonia digitata*) seeds or seed cake improves FI and growth performance in sheep, layers, and broilers. This increase allows broiler diet inclusion levels to increase from 5% to 15% (Chisoro et al., 2018; Saulawa et al., 2014; Hassan et al., 2020b; Adeyeye et al., 2021). The nutritional content of baobab and jack bean (*Canavalia ensiformis*) seeds can also be increased by toasting them, enabling up to 20% of them to be included in poultry feeds (Akande and Fabiyi, 2010; Saulawa et al., 2014). Toasting by itself, however, might not be enough to reduce ANFs in some ingredients. Esonu et al. (1998) discovered that toasting jack beans did not considerably lower toxin levels, highlighting the necessity of combining thermal treatments with other techniques (such as soaking and fermentation) in order to optimize ANF reduction and safely increase of inclusion rates. According to these results, roasting and toasting are useful but context-dependent tactics that call for complementing processing methods to ensure the best possible feed safety and effectiveness in systems in livestock.
7. Fermentation: By using beneficial microorganisms to break down ANF, fermentation improves nutrient bioavailability, feed palatability, and digestibility in WFBP, stabilizes feed matrices, improves gut health, and reduces waste through the production of microbial metabolites (e.g., organic acids, enzymes). Research on baobab, tamarind, common vetch, and moringa seeds has shown that fermentation increases inclusion levels in livestock diets, increases dry matter digestibility, and improves FI in poultry and ruminants (Singh et al., 2007; Chisoro et al., 2017; Stein et al., 2016; Saulawa et al., 2014).

Fermentative bacteria, such as *Lactobacillus* and *Bacillus* species, play a critical role in biotransforming mycotoxins into non-toxic metabolites, improving microbial safety (Sugiharto and Ranjitkar, 2019). In addition to improving nutrition, fermentation also improves feed safety by reducing mycotoxin contamination. For instance, solid-state fermentation (SSF) reduced zearalenone (a Fusarium-derived mycotoxin) by 60–80% in cereal-based substrates (Yang et al., 2018), and Okeke et al. (2015) also observed similar detoxification in maize grains.

8. Enzymes: The inability of poultry to hydrolyze non-starch polysaccharides (NSPs) and ANF found in CFI like wheat, soybean, barley, and rapeseed has been addressed by exogenous enzyme supplementation, which improves nutrient digestibility, availability, and absorption in formulated feeds (Sugiharto and Ranjitkar, 2019). Although not a direct processing method, enzymes like amylase, phytase, and protease are essential feed additives that hydrolyze complex carbohydrates, phytic acid, and proteins, respectively, releasing bound nutrients (Kiarie et al., 2013; Rahman et al., 2021).

Enzyme supplementation can maximize nutrient release from fibrous matrices in WFBP added to livestock rations, negating the decreased digestibility frequently linked to non-traditional feed sources. For example, in diets high in WFBP, phytase increases the bioavailability of phosphorus, while proteases improve the absorption of AA, which are important factors in swine and poultry development performance (Abdollahi and Ravindran, 2019; Mbukwane et al., 2022). This approach lessens dependency on expensive, energy-intensive processing techniques while simultaneously increasing feed efficiency and promoting the sustainable use of WFBP.

The meticulous, context-specific application of techniques is essential to this process since different feed ingredients frequently call for customized combinations of techniques to maximize digestibility, nutritional availability, and palatability. Two separate stages of processing take place. To reduce ANF and improve nutritional utility, the first phase concentrates on ingredient-specific treatments (such as drying, fermentation, or detoxification etc.). For AFR, they must undergo stringent preprocessing to satisfy quality criteria as substitutes or supplements to CFI, this stage is especially crucial.

The secondary phase involves feed manufacturing steps: a) Grinding to increase surface area, disrupt ANF, and improve nutrient accessibility; b) Mixing to ensure uniform nutrient distribution; and c) Pelletizing/Extrusion to reduce waste and enhance digestibility by consolidating ingredients into consistent, nutrient-dense pellets. While secondary processing is standard in commercial feed production, the primary phase remains indispensable, especially for AFR. Given livestock producers' stringent demands for high-quality feed, meticulous preprocessing of WFBP is essential to ensure their viability in sustainable feed formulations.

8 Opportunities and future directions

This review highlighted a lot of points on the use of WFBP in livestock feed. This highlights a few additional points that must be considered for future full utilization of these resources in livestock feed as detailed below:

1. Value addition and commercialization: Examination of the potential for adding value to WFBP must be considered to increase their economic value and marketability. The appropriate processing methods as

aforementioned must be investigated and used according to the WFBP in question. To create products with added value for stockfeed, human use, and in other sectors. Entrepreneurship and the creation of small businesses for the production and sale of products based on these WFBP must be encouraged by local communities or the government (Chisoro et al., 2023).

2. Support for policies and regulations: Policies and rules that encourage the sustainable inclusion of WFBP in stockfeed must be promoted. Local communities and relevant government agencies must work together in the creation of such policies and standards for the collection, processing, and marketing of these resources. While promoting the inclusion of WFBP feed sources in national strategies and plans for continued livestock production (Chisoro and Nkukwana, 2020a; Nduku et al., 2021).
3. Research and development needs: Continued research on the proximate nutrient composition, bioactive substances, and potential health advantages of various WFBP through additional research (Chisoro et al., 2019). While also investigating how using feed made from these resources impacts animal performance, product quality, and environmental sustainability (Jalal et al., 2023). In addition, looking into how livestock diets can be improved, by considering the potential synergistic effects of mixing these AFR with other feed additives (antioxidants, probiotics, prebiotics, enzymes etc.). Further hands-on research and on-farm animal trials to determine whether it is feasible and acceptable to feed livestock with rations that contain these feedstuffs at various inclusion levels as shown in Tables 2 and 3.
4. Community engagement and capacity building: Engagement of local communities, farmers, and livestock producers in the sustainable use of these resources must be encouraged. By offering training and capacity-building programs on how to recognize, gather, prepare, and use WFBP as livestock feed ingredients (Mazizi et al., 2020). While also encouraging research teams, extension services, and regional communities to cooperate to transfer technologies and share information on the use and processing of these feed resources (Hassan et al., 2020).
5. Protection of the environment: Emphasize on the environmental advantages of using WFBP in feed, such as lowering demand on natural resources and fostering biodiversity preservation by local communities and governments. By speaking up in favor of conservation of WF-bearing trees and appropriate harvesting methods to assure their ongoing availability (Chisoro and Nkukwana, 2020b). While encouraging agroforestry systems that combine the production of livestock with the cultivation of WF trees, boosting the ecosystem's resilience and push UN SDGs (Chisoro et al., 2023).

It is essential to investigate the possibilities and prospects of incorporating WFBP into livestock feed to support sustainable livestock production systems. Producers can improve resource efficiency and lessen dependency on CFI by giving priority to research on scalable processing techniques, environmental effect reduction, and nutritional improvement. By implementing these tactics, the livestock sector will become more robust and ecologically balanced, maximizing the value of unused WFBP and bringing agricultural practices in line with international sustainability standards.

9 Conclusion

WFBP have a lot of potential to improve livestock production in a sustainable way, especially in developing nations where access to CFI is limited. In addition to resolving feed scarcity and lowering environmental footprints, these underutilized materials provide affordable, environmentally friendly CFI substitutes. However, more research is needed to determine species-specific nutritional profiles, safe inclusion levels, and commercial viability to optimize their incorporation into livestock diets. To guarantee constant quality and supply, practical issues including seasonal availability, sustainable harvesting methods, and post-harvest storage must also be methodically resolved.

Policy frameworks and institutional actions encouraging the sustainable use of WFBP could empower rural smallholder farmers, enhancing livelihoods and increasing socioeconomic resilience. This review underlines the dual benefits of WFBP adoption, improving sustainable agriculture through lower feed prices and ecological implications, while aiding rural development. Cooperation between governments, scientists, and international organizations is essential to achieving this promise. By making strategic investments in technology transfer, market connections, and context-specific research, livestock farmers will be able to efficiently utilize these resources, closing gaps in sustainability and food security.

Author contributions

PC: Conceptualization, Data curation, Writing – original draft, Writing – review & editing. BM: Conceptualization, Formal analysis, Investigation, Methodology, Writing – review & editing. IFJ: Formal analysis, Funding acquisition, Project administration, Supervision, Conceptualization, Investigation, Writing – review & editing, Validation. TN: Conceptualization, Investigation, Methodology, Validation, Project administration, Visualization, Writing – review & editing, Supervision. NA: Conceptualization, Methodology, Software, Validation, Writing – review & editing.

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Conflict of interest

Author PC is the Founder and CEO of Gwaimana Consolidated Pvt Ltd.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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