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Animal welfare in extensive systems

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Animal welfare in extensive systems

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Editorial: Animal welfare in extensive systems

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Editorial on the Research Topic
Animal welfare in extensive systems

World livestock production is diverse and is supported by a wide range of systems, from intensive to extensive. Although most animal production originates from intensive systems, there are many regions in developed and developing countries where extensive systems are essential and play a role in ecosystems' integrity, social stability, cultural heritage preservation, and economic turnover. One of the most recent challenges for this system is to understand how animal welfare is impacted and how it may be assessed. To respond to this challenge, this Research Topic has put together a collection of research and review articles dedicated to a broad spectrum of topics related to animal health and welfare, as well as other features of extensive livestock production in different countries. These articles address questions about animal welfare in extensive systems, from different perspectives and for different species such as donkeys, goats, cattle, and water buffaloes.

Extensive livestock production is vital for many reasons in different continents. This aspect is discussed by Windsor in a review on the Australian reality, but with a clear message for other countries. This review emphasizes the need for continuing improvements in animal welfare to ensure social acceptance of animal-sourced food and fiber; a pertinent issue in the social context of strong opposition toward animal production. In addition, the need for sustainability of animal production is also highlighted in how it impacts the Australian economy. Severe drought periods subsequent to global climate changes have dramatically affected animal welfare in these systems, triggering the emergence of unforeseen disease severity (e.g., paratuberculosis) and overwhelming bushfires, causing an increase in mortality, morbidity, and suffering of extensive-farmed animals. This review suggests innovations for animal welfare surveillance and assessment that will improve the management of extensive farm animal welfare in Australia and will serve as a lesson globally.

Likewise, practices that will allow for the adaptation, or that will help mitigate the effects of climate change, ensuring a more sustainable production in Enugu State, Nigeria, are analyzed by Nwobodo et al. This work studies factors that significantly influenced the use of sustainable practices by 96 ruminant farmers. Access to veterinary services, monthly household, and annual income from ruminant production were considered the most important factors influencing the implementation of these practices.

Publications related to the health and welfare of donkeys are very much needed. The article by Deng et al. highlights farm demographics as well as the health and welfare issues of donkeys in Northeastern China. In this work, it is pointed out that 40% of the donkeys suffered from at least one health problem; the most common problems were colic, respiratory disorders, and skin conditions. The article also indicates that owners underestimated some of the most prevalent diseases in donkeys, which suggests that there is still room for improvement in health management, ensuring better welfare of donkeys in those regions.

The work presented by Nenadović et al. studies the impact of parasitological infections on the welfare of native goat breeds in extensive Serbian farming systems. Using the Animal Welfare Indicators (AWIN) protocol, correlations were found between infection by certain parasites and animal welfare-based indicators, such as poor hair coat condition and nasal discharge. Also working with goats, Battini et al. test the feasibility and reliability of the AWIN welfare assessment protocol for dairy goats in semi-extensive farming conditions. Inter-observer reliability analysis of different indicators showed a wide range of values, from excellent to insufficient. The results identified as insufficient were associated with differences in the background of the assessors and feasibility constraints, which is why there is a need for comprehensive training and validation of some group-level animal-based indicators, particularly those that evaluate daily activities' synchrony.

Aspects related to the behavior and welfare of cattle are addressed in three articles. Nakajima et al. show the importance of temperament trait changes in Japanese Black cows under grazing and confined conditions. The results show that grazing enhanced the cows' docility while being managed. On the other hand, Vicic et al. analyze the barriers facing non-replacement male calves in the Australian dairy industry. The main barriers identified were related to the cost and availability of feed, the additional cost of labor, and a lower economic return on the meat produced by this type of animal. Identifying these barriers represents a step toward non-replacement male calves being seen as a profitable commercial practice. Slayi et al. analyze grass species and their distribution patterns, and their effect on the behavior and weight gain of Nguni (NG) and Boran (BR) cattle, post-relocation to a novel environment. It was found that both breeds showed a reduction in weight gain and body condition in the first 3 weeks after moving, which was followed by adaptation to the novel environment conditions and stress reduction, with the recovery of their behavioral activities and weight gains.

Lastly, the article presented by Vilela et al. is related to thermolysis and skin microstructure dynamics in water buffaloes reared in a humid tropical climate. This work expands the knowledge on the heat tolerance capacity of Murrah buffaloes in tropical environments. It was shown that, despite the tolerance capacity of this species to heat stress, access to shade in buffalo rearing systems in tropical regions is essential.

In summary, the Research Topic of these articles will contribute to increasing the knowledge regarding the welfare of animals kept in more natural settings.

Author contributions

Both authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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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A Survey of Smallholder Farms Regarding Demographics, Health Care, and Management Factors of Donkeys in Northeastern China

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Essential information on the population dynamics and the health and welfare of Chinese donkeys is scarce. The objectives of this study were to describe the demographic characteristics, management and health care of a sample of donkeys under smallholder farm conditions of northeastern China. A cross-sectional survey of 731 randomly selected donkey owners on smallholder farms (1,658 donkeys) in 40 villages of northeastern China was conducted. Data on the composition and management of the donkeys and their routine health care were analyzed. The surveyed donkey population consisted of mostly (83.8%) jenny/filly donkeys with a mean age of 6.2 ± 5.0 years. Most (91.2%) of the farms kept 1–4 donkeys. The majority of donkeys were used for breeding and labor. Most (93.8%) of the farms did not have bedding, and their mean stable size was 17.7 ± 10.1 m^2. All of the animals were turned out for at least part of the year. The mean size of the turnout areas on the farms was 17.8 m^2. The condition of 12.5% of the donkeys was evaluated as "poor" with a body condition score of 1 on a scale of 5. More than one third (37.9%) of the donkeys had never been dewormed. Also, none of them were ever vaccinated or received dental care from a veterinarian. Their hoofs were trimmed once (45.9%) or twice (27.6%) a year. Forty percent of the donkeys were reported to suffer from at least one medical problem in the preceding year. The most common medical problems were colic, respiratory disorders and skin conditions. Owners seemed to underestimate some of the most prevalent diseases in donkeys, suggesting that their knowledge of the management of donkeys, including routine healthcare practices should be improved to ensure the health and welfare of donkeys in northeastern China.

Keywords: demographics, management, health care, survey, donkey, China

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INTRODUCTION

The latest estimate of the global donkey population is 50.45 million, of which 2.68 million are raised in China (1, 2). Donkeys comprise a key animal species that has made valuable contributions to our society. They have a long history of serving various purposes in China, such as milk, meat and hide production, and labor and recreation (3).

Previous studies have revealed demographic characteristics of donkeys, such as the size and composition of the endangered Miranda donkeys in Portugal (4, 5), the donkey population in

The Donkey Sanctuary of United Kingdom (UK) (6–8) and the working donkeys in developing countries (9, 10). These authors have described disproportionate or unstable distributions in age and gender among some groups of donkeys (9, 10) or the relatively long lifespans and good health of others (6, 7). Baseline demographic data on the general donkey population is essential to evaluate population dynamics (11), and the relevance and impact of outcomes of epidemiological control (12).

It is important to ensure appropriate management and routine care to maintain good health among donkeys. Different types of nutrition, stabling facilities, activity, environment, health care, and culture can influence the welfare, development of disease and average breeding life of horses and donkeys (7, 13–15). However, donkeys often suffer significant health problems owing to physiologic characteristics and stoical behavior which are often misinterpreted by owners (16). Donkeys often do not exhibit obvious clinical signs despite suffering from severe or even life-threatening conditions (17). The characteristics in behavior, physiology and health have been recognized by some donkey researchers, para-Governmental bodies and owners, who have developed management guidelines for disease prevention (18, 19) and applied improved management practices to control disease risks (15, 17).

More recently, the feasibility of establishing intensive donkey farming systems in China to supply the demand for hides has been investigated (11). More than 70% of donkeys in China are raised under extensive conditions on smallholder farms, which reflect the population dynamics of extensive donkey farming systems. Forty-five percent of all Chinese donkeys are raised, bred and used in northeastern China, an important donkey-breeding region, and most of them remain under smallholder farm conditions (2). Our recent study investigated the foaling-related parameters and dental disorders of jennies in this region (20, 21).

To the best of our knowledge, little information is known about the demographic characteristics, management and health care of donkeys in northeastern China. Hence, this cross-sectional study aimed to ascertain the demographic characteristics, management and health care of the extensively managed donkey population in northeastern China.

MATERIALS AND METHODS

Study Area

We conducted this study in Western Liaoning Province and the Eastern Inner Mongolia Autonomous Region, two adjacent zones with numerous hills in northeastern China, at an altitude ranging from 300 to 1,200 m above sea level, between coordinates 118°50'–122°26' E and 40°17'–43°01' N. This part of China has a semi-arid climate with an average monthly maximum temperature 24.1°C in July and minimum temperature −10.1°C in January. Meanwhile, the average monthly maximum precipitation is 129.8 mm in July and minimum precipitation is 1.8 mm in January. The mean relative humidity is 51%. The study area is 290 km from north to south and 300 km from east to west: 37,500 km^2 in total. The farmers practiced a mixed crop–livestock production systems, and used donkeys for breeding and light farm work. The donkey herds in the study area consisted of ∼0.8 million indigenous Liaoxi donkeys described in our previous study (20), which account for two-thirds of donkeys in northeastern China. Breeding was practiced in a relatively extensive system. Thirty to fifty jennies were bred by in-hand breeding with natural service using a shared jack, following oestrus, usually observed and detected by the jenny owners.

Study Methodology and Data Collection

A cross-sectional survey of 731 smallholder farms was conducted in 40 villages between March and July 2017 with face-to-face interviews of the donkey owners of each farm. The farms were selected using a stratified sampling method according to their geographical distribution (north, east and south areas). Among each area, the farms were randomly selected based on the density statistics of donkeys by local government. These farms were visited door to door, and owners were asked if they would like to take part in the survey, resulting in a convenience-based sample of owners who were willing to participate. The survey, which took ∼20 min to complete, was combined with a pre-tested, semi-structured questionnaire, which was developed using a modification of the tailored design method (22). The questionnaire contained sections on the demographics of the owner (age, gender, educational level, source of income and the number of years spent raising donkeys), the donkey (age, gender, herd size and use), general management practices (stabling, turnout and feeding) and health care measures and interventions (body condition scores, medical problems during the preceding year, deworming, vaccination, dental care and hoof trimming) (**Supplementary Table 1**). A trained enumerator measured the size of the stables and turnout areas and rated the body condition of the donkeys on a 5-point scale (1 = poor, 2 = moderate, 3 = ideal, 4 = fat, and 5 = obese), in accordance with the guidelines of The Donkey Sanctuary (23). Photographs of the donkeys were collected to confirm the identity of each one. The total sample size was ∼0.2% of the total number of households with donkeys based on the population census of 2016.

The data collection protocol was implemented with the approval of the Shenyang Agricultural University Animal Care and Use Committee (Permit no.: 201702018).

Statistical Analysis

The baseline survey data were entered into Microsoft Office Excel 2007 (Microsoft Corp., Redmond, WA), then exported to SPSS version 22.0 (IBM Corp., Armonk, NY) after checking it for possible mistakes, typing errors and outliers. Descriptive statistics, including frequencies, means, medians, ranges, percentages and standard deviations (SDs) of the variables were calculated. The results are expressed as mean or mean ± SD and 95% confidence intervals (CI). Normality was assessed using the Shapiro–Wilk test. Comparisons between groups were made using Pearson's chi-squared test for categorical variables. Statistical significance was set at $P < 0.05$.

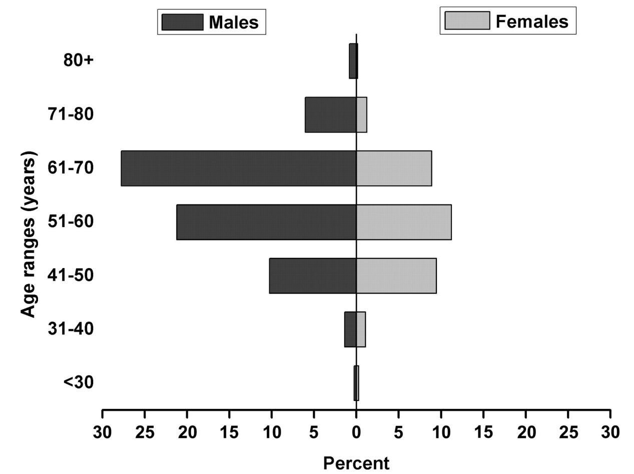

FIGURE 1 | Age and gender distributions of the donkey owners in Western Liaoning and Eastern Inner Mongolia of China ($n = 731$).

TABLE 1 | Respondents (Donkey owners)' number of years of education, source of income, and years raising donkeys in Western Liaoning and Eastern Inner Mongolia of China ($n = 731$).

Characteristic	Percentage (number) of respondents	95% CI
Education (years)		
0	13.7 (100)	11.2–16.2
<3	10.0 (73)	5.7–14.3
4–6	37.9 (277)	34.4–41.4
7–9	35.0 (256)	31.5–38.5
10–12	2.9 (21)	1.7–4.1
>12	0.5 (4)	0–1.0
Source of income		
Agricultural activity	48.6 (355)	45.0–52.2
Agricultural activity + Employment	51.4 (376)	47.8–55.0
Years raising donkeys		
<5	2.9 (21)	1.7–4.1
6–10	2.5 (18)	1.4–3.6
11–15	3.7 (27)	2.3–5.1
16–20	3.4 (25)	2.1–4.7
21–25	6.0 (44)	4.3–7.7
>25	81.5 (596)	78.7–84.3

CI, confidence interval.

RESULTS

Demographic Characteristics of the Respondents

A total of 731 donkey owners completed the survey. The age and gender distributions of the respondents are shown in **Figure 1**. Two-thirds of the respondents (67.7%, 95% CI: 64.3–71.1) were males and most of them (88.8%, 95% CI: 86.5–91.1) were 41–70 years old. The mean age of the respondents was 58.3 ± 9.5 years. The majority of respondents (96.6%) had ≤9 years of education, and nearly a half (48.6%) relied on agriculture as their all source of income. Most of them (81.5%) had raised donkeys for longer than 25 years (**Table 1**).

Age and Gender Distributions of the Donkeys

A total of 1,658 donkeys were included in the study. The herds mostly consisted of jenny/filly donkeys (83.8%, 95% CI: 82.0–85.6), jack/colt donkeys (15.9%, 95% CI: 14.1–17.7) and gelding donkeys (0.2%). The mean and median ages of the sample were 6.2 ± 5.0 years and 5 years (range: 0–23 years), respectively, and the largest and second largest number of age groups were ≤ 1 year (23.4%, 95% CI: 21.4–25.4) and 6–8 years (23.0%, 95% CI: 21.0–25.0), respectively. The number of females and males decreased dramatically after 1 years old. Among the 264 jack/colt donkeys, only 40 donkeys were over 3 years old and used for breeding, which account for a small percentage (2.4%) in this surveyed population (**Figure 2**).

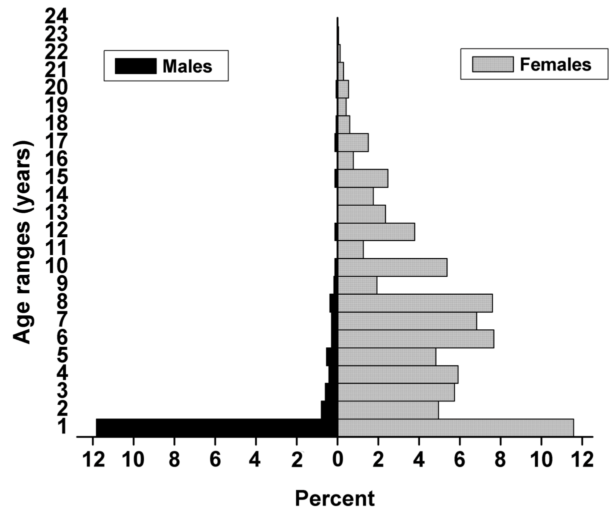

FIGURE 2 | Overall age distribution of the donkeys by gender in Western Liaoning and Eastern Inner Mongolia of China ($n = 1,658$).

Herd Size

The herd size of the donkeys across the 694 smallholder farms is presented in **Table 2**. The average farm size was 1.7 ± 1.3 ha and most (91.2%) of the farms kept 1–4 donkeys. The mean herd size and mean number of breeding jennies per farm were 2.5 ± 1.5 and 1.7 ± 0.9, respectively. The herd to farm ratio and breeding jennies to farm ratio were 2.2:1 and 1.6:1, respectively.

Uses of Donkeys

The majority of donkeys were used for breeding (79.7%), and a large proportion was used for work, such as driving (61.6%), packing (3.4%) and agricultural operations during drafts (69.1%). Only 11.4% of the donkeys were used for meat and hide production (**Table 3**).

TABLE 2 | Herd size of donkeys across 694* smallholder farms in Western Liaoning and Eastern Inner Mongolia of China.

Herd and farm size	Mean	Median	SD	Range
Farm size (ha)	1.7	1.3	1.3	0–12
Herd size	2.5	2	1.5	1–14
Breeding jennies size	1.7	1	0.9	0–10
Herd size/farm size (per ha)	2.2	1.5	2.1	0–22.5
Breeding jennies size/farm size (per ha)	1.6	1.2	1.6	0–22.5

SD, standard deviation; ha. hectare.
**Thirty-seven respondents did not provide the information.*

TABLE 3 | Uses of donkeys across smallholder farms in Western Liaoning and Eastern Inner Mongolia of China (n = 1,658).

Uses	Percentage (number) of donkeys*	95% CI
Breeding	79.7 (1322)	77.8–81.6
Draft	69.1 (1146)	66.9–71.3
Driving	61.6 (1022)	59.3–63.9
Production (meat and hide)	11.4 (189)	9.9–12.9
Packing	3.4 (56)	2.5–4.3
Not reported	0.8 (14)	0.4–1.2

CI, confidence interval.
**More than one answer was allowed.*

TABLE 4 | Characteristics of the stables across smallholder farms in Western Liaoning and Eastern Inner Mongolia of China (n = 731).

Stables	Percentage (number) of farms	95% CI
Type of structure		
Remodeled from old houses	44.7 (327)	41.1–48.3
Simple brick stable	40.8 (298)	37.2–44.4
Wood stable with an iron roof	14.0 (102)	11.5–16.5
No stable	0.5 (4)	0–1.0
Stable size (m^2)		
0	0.5 (4)	0–1.0
1–25	79.3 (580)	76.4–82.2
25–50	17.0 (124)	14.3–19.7
51–75	0.5 (4)	0–1.0
>75	0.3 (2)	−0.1 to 0.7
Not reported	2.3 (17)	1.2–3.4
Type of bedding		
No bedding	93.8 (686)	92.1–95.5
Straw	6.2 (45)	4.5–7.9

CI, confidence interval.

TABLE 5 | Types and sizes of turnout areas across smallholder farms in Western Liaoning and Eastern Inner Mongolia of China (n = 731).

Turnout areas	Percentage (number) of farms	95% CI
Type of turnout area		
Pole tied	39.0 (285)	35.5–42.5
Paddock without grass	24.2 (177)	21.1–27.3
Yard	8.3 (61)	6.3–10.3
Pasture	5.3 (39)	3.7–6.9
Not reported	23.1 (169)	20.0–26.2
Turnout area size (m^2)		
<25	53.9 (394)	50.3–57.5
25–50	16.4 (120)	13.7–19.1
51–75	4.8 (35)	3.3–6.3
>75	1.8 (13)	0.8–2.8
Not reported	23.1 (169)	20.0–26.2

CI, confidence interval.

Stable Management

Overall, 99.5% of the donkeys were routinely stabled at some time throughout the year. The types of stable structures and bedding varied (**Table 4**). Most of the stables were remodeled houses in which owners lived previously (44.7%) and simple brick stables (40.8%). Others were made of wood with an iron roof (14.0%).

The mean and median stable size per farm was 17.7 ± 10.1 m^2 and 14.8 m^2 (ranges 0–103 m^2), respectively. Among these farms, there were four (0.5%) kept their donkeys in the yard without any stable or shelter throughout the year. Most of the farms stabled the donkeys in areas measuring 1–25 m^2 (79.3%), followed by 25–50 m^2 (17.0%). Most of the farms did not have bedding (93.8%), and only a small number of donkeys had straw bedding (6.2%).

Turnout Management

The types and sizes of the turnout areas are summarized in **Table 5**. In the majority of turnout areas, a donkey was tied to a pole outside (39.0%), followed by a turnout to a paddock without grass (24.2%) and a walking area in a yard (8.3%). A small proportion of farms (5.3%) had a pasture with an access for donkeys.

The mean and median size of the turnout areas were 17.8 and 13.4 m^2 (ranges 1.7–145 m^2), respectively. Most of the donkeys were kept in small turnout areas measuring ≤50 m^2 (70.3%); only 1.8% of the farms kept donkeys in turnout areas larger than 75 m^2.

The durations of the turnouts varied by season (**Table 6**). The majority of donkeys (73.7–95.0%) were turned out during the day and stabled at night, throughout the year. A larger proportion was turned out 24 h/day without access to any stable during the summer (26.3%) and spring (22.6%), compared to the other seasons (0.8–16.6%, $P < 0.01$). A small proportion of donkeys were stabled 24 h/day during the winter (4.3%) and autumn (0.8%). Overall, the donkeys' median turnout duration was 10 h/day, although there was seasonal variation: a median of 12 h/day in the summer, 11 h/day in the spring, 10 h/day in the autumn and 8 h/day in the winter.

Dietary Management

Donkeys were mainly fed crop residue, such as millet straw (85.4%) and maize straw (83.1%), whereas 33.0% were fed hay

TABLE 6 | Stable and turnout management of donkeys across smallholder farms in Western Liaoning and Eastern Inner Mongolia of China throughout the year (n = 1,585).

	Percentage (number) of donkeys			
	Time of year			
Turnout	Sept–Nov	Dec–Feb	Mar–May	Jun–Aug
Night stabled, day turned out	81.4 (1290)^{b}	95.0 (1505)^{a}	77.4 (1226)^{bc}	73.7 (1168)^{cd}
Turned out 24 h/day	16.6 (263)^{b}	0.8 (12)^{c}	22.6 (359)^{a}	26.3 (417)^{a}
Stabled 24 h/day	2.0 (32)	4.3 (68)	0	0
Median time spent outside (h/day)	10	8	11	12

$^{a-d}$*Lowercase superscript letters indicate a significant difference (P < 0.01).*

(**Table 7**). More than half of the donkeys were offered forage twice daily (52.6%) and 32.2% were fed forage daily *ad libitum*.

Most of the donkeys' diets were supplemented with small amounts of homemade concentrates, including maize (89.2%), soybean meal (16.9%), sunflower seed meal (14.8%) and wheat bran (6.9%). Only 29.0% of the donkeys were given vitamin and mineral supplements, and 0.7% were fed commercial products. Three donkeys (0.2%) were fed without concentrates. Over half (51.1%) of the donkeys' diets were supplemented with concentrates twice daily, 24.7% were supplemented once daily and 23.5% were supplemented three times daily.

All the donkeys were provided with water daily, 5.5% automatic drinkers and 94.5% bucket. There were 72.3% donkeys could drink clean water, although remains with dirty water.

Body Condition Score

The BCS was ideal in the largest proportion of the donkeys (43.2%, 95% CI: 40.8–45.6), moderate in 27.7% of them (95% CI: 25.5–29.9), fat in 15.8% (95% CI: 14.0–17.6%), poor in 12.5% (95% CI: 10.9–14.1%) and obese in 0.8% (95% CI: 0.4–1.2%) of the donkeys.

Health Care

The provision of routine health care to the donkeys, such as deworming, vaccination, dental care and hoof trimming, were examined (**Table 8**). More than one third (37.9%) of the donkeys were not dewormed yearly. Nearly half (44.5%) were dewormed once per year and 12.4% were dewormed twice per year. None of the donkeys in this study had ever been vaccinated or received dental care from a veterinarian or a dental technician. A farrier performed hoof trimming of the animals without being shod. A total of 761 (45.9%) and 458 (27.6%) donkeys had their hoofs trimmed once and twice a year, respectively. Only 20 donkeys (1.2%) had their hoofs trimmed three times a year; however, 245 donkeys (14.8%) had never had their hoofs trimmed.

Common Medical Problems

The most common medical problems of the donkeys during the preceding year are presented in **Table 9**. Most (60.5%) of the donkeys did not suffer from any medical problems in the preceding year. Among those that did have medical problems, 13.5% had colic, 10.9% had respiratory disorders, 9.2% had skin disorders, 3.4% had lameness and 2.7% had oral/dental disorders, which were considered common issues.

TABLE 7 | Dietary management provided for donkeys across smallholder farms in Western Liaoning and Eastern Inner Mongolia of China (n = 1,658).

Feed type and feeding frequency	Percentage (number) of donkeys	95% CI
Type of forage feed*		
Millet straw	85.4 (1416)	83.7–87.1
Maize straw	83.1 (1378)	81.3–84.9
Hay	33.0 (547)	30.7–35.3
Alfalfa	0.7 (12)	0.3–1.1
Other	0.5 (8)	0.2–0.8
Not reported	0.4 (7)	0.1–0.7
Frequency of forage feeding daily		
1 time/d	4.1 (68)	3.2–5.1
2 times/d	52.6 (872)	50.2–55.0
3 times/d	10.7 (177)	9.2–12.2
Ad libitum	32.2 (534)	30.0–34.5
Not reported	0.4 (7)	0.1–0.7
Type of concentrate*		
Maize	89.2 (1479)	87.7–90.7
Soybean meal	16.9 (280)	15.1–18.7
Sunflower seed meal	14.8 (245)	13.1–16.5
Wheat bran	6.9 (114)	5.7–8.1
Mineral or vitamin supplements	29.0 (481)	26.8–31.2
Commercial products	0.7 (12)	0.3–1.1
Other	11.3 (187)	9.8–12.8
None	0.2 (3)	0–0.4
Not reported	0.4 (7)	0.1–0.7
Frequency of concentrate feeding daily		
1 time/d	24.7 (410)	22.6–26.8
2 times/d	51.1 (848)	48.7–53.5
3 times/d	23.5 (390)	21.5–25.5
None	0.2 (3)	0–0.4
Not reported	0.4 (7)	0.1–0.7
Water availability		
Automatic drinker	5.5 (92)	4.4–6.6
Bucket	94.5 (1566)	93.4–95.6
Cleanliness of water		
Clean	72.3 (1198)	70.2–74.5
Dirty	27.7 (460)	25.6–29.9

CI, confidence interval.
**More than one answer was allowed.*

DISCUSSION

The present study was designed to provide a description of the demographic characteristics, management and health care of a large cohort of donkeys under smallholder farm conditions in northeastern China. To the best of our knowledge, this is

TABLE 8 | Health care of donkeys across smallholder farms in Western Liaoning and Eastern Inner Mongolia of China ($n = 1,658$).

Type of healthcare	Percentage (number) of donkeys	95% CI
Frequency of deworming		
Never	37.9 (628)	35.6–40.2
Once per year	44.5 (738)	42.1–46.9
Twice per year	12.4 (205)	10.8–14.0
More often than every 4 months	0.4 (7)	0.1–0.7
Dewormed, depending on fecal examination	2.7 (45)	1.9–3.5
Not reported	2.1 (35)	1.4–2.8
Vaccinations		
Not vaccinated	100 (1658)	
Dental care		
Never by a veterinarian or dental technician	100 (1658)	
Frequency of hoof trimming		
Never	14.8 (245)	13.1–16.5
Once per year	45.9 (761)	43.5–48.3
Twice per year	27.6 (458)	25.4–29.8
Three times per year	1.2 (20)	0.7–1.7
Not reported	10.5 (174)	9.0–12.0

CI, confidence interval.

TABLE 9 | Common medical problems of donkeys across smallholder farms in Western Liaoning and Eastern Inner Mongolia of China ($n = 1,658$).

Medical problems	Percentage (number) of donkeys*	95% CI
None	60.5 (1003)	58.1–62.9
Colic	13.5 (224)	11.9–15.1
Respiratory disorders	10.9 (181)	9.4–12.4
Skin disorders	9.2 (153)	7.8–10.6
Lameness	3.4 (56)	2.5–4.3
Oral/dental disorders	2.7 (45)	1.9–3.5
Other+	4.4 (73)	3.4–5.4
Not reported	7.4 (123)	6.1–8.7

CI, confidence interval.

**More than one answer was allowed.*

+Other common medical problems including tetanus, intoxication, septic foals, colitis, body mass, and abortion from unknown origins.

the first comprehensive survey of donkey owners using face-to-face interviews and questionnaires to explore the status of a Chinese donkey population. Our analysis and evaluation of the data collected should be valuable in providing baseline reference information for future studies and improving the health and welfare of donkeys in China.

The farm sample for this cross-sectional survey was selected using a stratified sampling method by visiting the region and owners participated on a voluntary basis. However, the willingness of the owners to participate could be a bias toward the more caring and candid donkey owners. In our study, over 90% owners we visited were glad to participate in the survey; therefore, the sample could be representative of the population. The face-to-face method of obtaining data enhanced the respondents' participation and minimized nonresponses and errors, compared with previous surveys using only questionnaires related to donkeys (7) and horses (14, 24).

In this study, the majority of owners who raised donkeys were males, similar to the farmers who used working equines in the campesino hill-slope communities of central Mexico (25). The mean age of the owners was 58.3 ± 9.5 years, which was 7 years younger than that of the owners of the Miranda donkey herds in Portugal (5). The advanced age of the owners suggest a further decrease in the number of donkey herds in the near future (5). In terms of educational level, only a small proportion of owners had completed junior high school. Thus, lack of formal education could have affected the management and health care of their donkeys.

The mean age of the donkeys herein (6.2 ± 5.0 years) was similar to that of the market donkeys reported in central Ethiopia (26) and working donkeys on family farms in Albania (27). Nevertheless, they were significantly younger than the donkeys with a mean age >20 years in foster care (7), those raised in The Donkey Sanctuary in the UK (6) and those working in Zimbabwe (9). The mean age revealed a significantly younger and premature culling of donkeys in China, owing to the potential for nutritional stressors, improper health care and production use. This study's sample had a significantly higher proportion of female than male donkeys. Similar results were found in Nigeria (10) and Portugal (4), although an equal distribution of males and females was reported in Mexico (28). Furthermore, female donkeys younger than 1 year accounted for ∼12% of the current sample. To maintain the herd size, it would be reasonable for owners to have at least 25% pubescent females for their herds (5). In contrast to the results found in other countries, in this study a very small proportion (2.4%) of males was maintained in the herd as breeders and almost all males were sold during early weaning to the intensive feedlot for meat and hide purpose. Therefore, the low ratio of siring males could have resulted in the unequal contribution to the genetic pool of the population (5).

Some owners had not fully embraced the importance of the proper housing of donkeys. The use of a wooden shelter with an iron roof and no stable requires a critical evaluation because these housing features could be associated with stress caused by cold temperatures, especially in the early hours of the day in the winter (29, 30). The amount (93.8%) of donkeys stabled without bedding differed significantly from donkeys in The Donkey Sanctuary, where only 5.0% were reported to have no bedding (7). Fifty-five percent of facilities provided clean bedding to donkeys in Italy and UK (30). Lack of bedding could be detrimental to donkeys, which may suffer from cold-related stress (30). Straw is the most frequently (75%) used bedding type for donkeys (7). Horses on straw bedding have been found to spend three times longer in a lateral recumbent position overnight than they did on shavings, suggesting a beneficial health effect of straw (31).

Most of the donkeys in this study were stabled at night and turned out during the day, throughout the year. The mean stable size (17.7 ± 10.1 m^2) probably was inadequate considering the mean herd size per farm was 2.5 donkeys. One donkey should be stabled in a box ∼3.3 × 3.3 m (32) or provided with the suitable box according to the body size, to provide a healthy and safe environment. The mean size of the turnout areas in this study, were consistent with the recommended dimension of 7 m^2 per donkey for milk production (19). The duration of the daytime turnouts varied between seasons, consistent with previous studies of donkeys (7) and horses (14, 33, 34), indicating that time spent outside is likely to affect equine health and welfare (7) by providing opportunities to exercise (35), avoiding the risk of stereotypies (36) and reducing exposure to respiratory allergens within the stable environment (33). However, there was no regular turnout for grazing in this study; herein, pastures were scarce due to the semi-arid climate.

Consistent with the recommendations for feeding donkeys by The Donkey Sanctuary (37), all of them were provided with forage, the main source of high-fiber roughage, such as millet straw and maize straw, reflecting the types of products readily available in northeastern China. One third of the donkeys were fed with hay, which is the most frequently used forage feed for donkeys in The Donkey Sanctuary (7) and for horses in various countries (14, 33, 38, 39). In addition to the forage, most of the donkeys were fed some concentrates/supplementary feed; working donkeys may require supplementary feeds due to the increased energy requirement. Concentrates are required and should be low in cereal grain content in order to avoid related health problems (40). Nevertheless, owners' basic knowledge of donkey nutrition was inadequate (20), resulting in a diet with nutrient deficiencies and/or dietary excesses. Most of the donkeys in our study were taken to clean water by bucket during the day, while 15 and 20% donkeys were provided with dirty and very dirty water in 20 donkey facilities in Europe, respectively (30). The general rule is that donkeys should always be provided with free access to clean water throughout the day (41).

Body condition scoring is a useful tool for fine-tuning diets to a donkey's individual requirements (37). In the present study, most of the donkeys maintained an ideal BCS, as they were mainly involved in breeding and light farm work. Additionally, the condition of 12.5% of the donkeys was rated as poor and 27.7% as moderate, which was more than 0.36% (poor) and 6.85% (moderate) of donkeys in 20 donkey facilities in Europe, respectively (30). Working donkeys with a low BCS may have comorbid dental disease, liver disease, gait abnormalities and other health issues (42, 43). Thus, it is important to promote energy intake, dental care, parasites control and general health care throughout the day to improve a donkey's body condition. In contrast to the conditions in which 33.8% of the donkeys were overweight or obese, as described in The Donkey Sanctuary (7), only a few of the donkeys in our study could be considered overweight.

The frequency of preventive healthcare interventions, such as deworming, vaccination and dental and hoof care, was low in the current study, which was similar to the frequency reported for working donkeys in most developing countries (17, 44).

In this study, more than one third of the donkeys were never dewormed. Without anthelmintics, the average life of a donkey varies from 9 years in Ethiopia to 15 years in Mexico (45). Over half of the donkeys in this study were dewormed once or twice per year. A mean annual deworming frequency consisting of 2 treatments has beneficial effects on the performance (health, longevity, and ability to work) of donkeys. In Ethiopia, the most suitable time for deworming is at the end of the dry and wet seasons (45).

None of the donkeys in this study was reported to be vaccinated against any disease. To date, no domestic commercial vaccines are available for horses or donkeys in mainland China, except some commercial vaccines imported and used for sport horses. Infectious equine disease in donkeys has been found within China. For example, an equine influenza outbreak on a 300-head donkey farm in Shandong Province resulted in a 25% mortality rate (46), which was also reported to be a common occurrence. In addition, the increase in abortions due to *Salmonella abortus equi* was reported on a farm with over 1,000 jennies (47). Thus, donkeys should be included in vaccination programs promoted by the government to enhance herd immunity and reduce individual risk.

The 2.7% prevalence of dental disorders reported by the owners was based on their inadequate knowledge. However, all the respondents confirmed that none of the animals had received routine dental care by a veterinarian or dental technician. Large populations of adult donkeys with no dental care and moderate-to-severe dental disease are common in China (21). Major dental disorders in geriatric donkeys (48) could be prevented or managed more effectively if they received routine dental care from an early age. Poor dentition and infrequent dental treatment were associated with colic in donkeys (6, 49). The prevalence of dental disease in China may be underestimated, similar to The Donkey Sanctuary in the UK (7), given the few clinical signs exhibited by donkeys, the limited number of equine veterinarians and dental technicians and the lack of donkey owners' knowledge concerning specific dental problems. More recently, we revealed that the most common dental disorders of jennies were sharp enamel points, incisor diastemata and focal overgrowths in Liaoning Province of China (21); therefore, proper dental care should be provided to improve the welfare of donkeys in China.

Hoof disease and hoof-related lameness is common in donkey populations worldwide. Donkeys are almost four times more likely to have sole abnormalities than are horses or mules (42). Lameness was reported in 3.4% of the donkeys during this study, which was significantly lower than the 27.2% prevalence of lame donkeys at The Donkey Sanctuary in the UK (50), 18.7% of the dairy donkeys' hoofs neglect in Italy (51), and 100% of the lame working draft donkeys in Pakistan (52). The prevalence of hoof disease in this study could be underestimated, owing to the donkey owners' lack of knowledge concerning the diagnosis of hoof disease. All the donkeys in this study were trimmed three or fewer times per year, which is significantly lower than the requirement that donkeys' hoofs be trimmed every 6–10 weeks (50). In this study, the percentage of donkeys reported to have never had hoof care was less than the 40% of donkeys reported in The Donkey Sanctuary in the UK (7).

A study in the UK reported that 59% of the donkeys suffered from at least one medical problem (7). However, only 39.5% of the donkeys were reported to have health issues in our study. The most common problems were colic and respiratory and skin disorders, which were more variable compared with studies of donkeys (7) and horses (14, 38, 39) in different countries. These differences may be due to the under-reporting of health issues in the present survey, which relied on the respondent's recall, compared to the medical records used by the veterinarians (34, 38). The traditional system of Chinese University education for army and working equine veterinarians has disintegrated (3); therefore, the existing equine veterinary services in mainland China are insufficient and lack routine data entries on donkeys into the medical records, owing to the small, scattered population of equine veterinarians with little or no formal education or training. Furthermore, it is possible that donkeys often do not exhibit obvious clinical signs when they experience disease. Thus, the recognition of some health problems by owners may be incorrect or inadequate, particularly chronic pain (17).

Colic is considered the third most important disease of horses in northern Britain (14), although it is ranked much lower on the list of common medical problems of donkeys in The Donkey Sanctuary in the UK (7). Our study revealed that the incidence of colic was 13.5%, making it the most common medical problem of the donkeys. All the cases were reported by the donkey owners; they were not diagnosed by veterinarians. The disease that caused the colic was unclear, considering the inadequate knowledge about its diagnosis. The incidence was higher than the incidence (3.9–5.9%) found in the donkeys in other studies (6, 49). It is more likely that the donkeys in this study stabled for half a day and were fed inappropriate concentrate feed or reduced water intake. Therefore, the colic that commonly occurred was similar to that reported in stabled horses (53).

CONCLUSION

The results of this study provide valuable baseline information on the demographics, management and health care of the donkey population in northeastern China. A significantly younger and premature culling of the donkeys and a low ratio of siring males probably had a dramatic impact on the donkey population of China. Inadequate knowledge and ineffective management affected the health and welfare of the donkeys. The general information obtained in this study permits exploration of the interaction between managerial factors and health to improve the welfare of donkeys and build an appropriate farming system of donkeys in the future.

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/s.

ETHICS STATEMENT

The animal study was reviewed and approved by Shenyang Agricultural University Animal Care and Use Committee.

AUTHOR CONTRIBUTIONS

LD performed the survey. SS, CT, and JL helped with data collection. YH and PX participated in data analysis. LD and JL edited the manuscript. All authors read and approved the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fvets.2021.626622/full#supplementary-material

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Progress With Livestock Welfare in Extensive Production Systems: Lessons From Australia

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The extensive livestock production industries are vital to the national economy of Australia. Continuing improvements to extensively-raised livestock welfare is desirable, necessary and in some situations mandatory, if the social license for animal sourced food and fiber production is to continue sustainably. However, meeting increasingly high welfare standards is challenging. The changing climate in this millennium, has seen the occurrence of two of the most severe drought periods on record in Australia, resulting in complex welfare issues arising from unforeseen disease, trade and environmental catastrophes. The onset of the first drought coincided with an uncontrolled epidemic of ovine paratuberculosis. It ended just prior to a temporary ban on live export of tropical cattle to Indonesia that induced a major market failure and led to severe morbidity and mortality on some beef properties. The second drought period progressed in severity and culminated in the most extreme bushfires recorded, causing unprecedented levels of mortality, morbidity and suffering in farmed animals and wildlife. Temperature extremes have also caused periodic heat-associated or cold-induced hyopthermia losses, requiring increased vigilance and careful management to reduce both temperature-induced stress during transport and the high ovine peri-parturient losses traditionally observed in extensive sheep farming. Several issues remain controversial, including surgical mulesing of wool sheep to manage flystrike, and the continuing live export trade of sheep and cattle. However, in reviewing the increasingly complex welfare challenges for the extensive livestock population industries that are export trade dependent and remain vulnerable to welfare activism, it appears progress has been made. These include development of prescribed livestock welfare Standards and Guidelines and the introduction of the Exporter Supply Chain Assurance System (ESCAS) to address export concerns. Further, the sheep mulesing crisis led to improved producer welfare attitudes and practices, including pain management during aversive husbandry procedures that is now occurring globally. Finally, innovations in animal welfare surveillance and assessment, are additional encouraging signs that suggest improving change management of extensive farm animal welfare is occurring that provides lessons well-beyond Australian shores.

Keywords: climate change, drought, heat, live export, pain management, welfare assessment

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INTRODUCTION

Livestock production accounts for ~40% of agricultural output in developed countries. Advanced genetics, feeding systems, pasture improvements, animal health prevention and controls including improved biosecurity, plus other animal production management technologies, have reduced requirements for livestock by about 20% yet doubled meat production in the last 40 years (1). With global meat production projected to increase another 19% by 2030 (2), improved adoption of emerging "best practice" technologies is required (3). As large ruminant production has now been associated with high outputs of greenhouse gas emissions (GHGe), it has been estimated that improved production efficiencies could potentially assist the global livestock sector to reduce GHGs by as much as 30% (1). Further, recent SARS (including Covid-19) and MERS outbreaks indicate that zoonotic pathogen spillover from livestock production (4) and wild animal populations is occurring, especially where wildlife biodiversity is high and land-use change is occurring (5). Further, there are increasing concerns of emerging food insecurity and safety (6) including antimicrobial resistance (AMR) from misuse of antibiotics, including in food production (7). Finally, with uncontrolled transboundary diseases emerging (e.g., African Swine Fever, Lumpy Skin Disease in SE Asia), it has been suggested that our food system is fragile, requiring radical change to build resilience and ensure our food supply is safer, fairer, and healthier for humans, animals and the environment in the future (8).

However, welfare challenges in livestock production are also considered a major threat to the sustainability of the production of animal-sourced foods (9). In Australia, it has been estimated that 95% of people view farm animal welfare to be a concern and 91% want at least some reform to address this (9). There exists a perceived gap between expectations and regulation, due to an increased focus on animals' level of sentience and related capabilities, with research indicating a fundamental community belief that animals are entitled to the protection of relevant rights and freedoms, closely aligning with activist sentiment (9). The public appears to have an increasing expectation for effective regulation to ensure highly transparent practices in livestock production (9), although achieving this in extensive production systems is challenging. Welfare challenges in extensive husbandry systems in Australia are recognized as mostly associated with: prolonged periods of food and/or water scarcity; climate extremes; high predation risk environments; issues of inadequate surveillance and management of diseases including the monitoring of ill animals with minimal availability of veterinary assistance for parturition, neonatal and other disorders; animal transportation; and biosecurity issues. A number of these challenges are likely to increase in complexity because of climate change. Increased understanding of these challenges and development of potential solutions requires strategic research, particularly for welfare assessment of farm animals in extensive systems and application of innovations that mitigate the impact of these challenges.

Australia has a particular interest in the introduction of new practices to mitigate welfare challenges of extensive livestock production. Extensive livestock production industries are vital to the national economy, and include 26.4 million beef cattle valued at AUD19.6 billion in 2019, and 70.6 million sheep valued at AUD6.6 billion for meat and AUD3.615 billion for wool (in 2019 and 2017, respectively) (10). Due to a number of welfare disaster events in the last two decades, Australian livestock producers are increasingly recognizing that continuing improvements to extensively-raised livestock welfare is desirable, necessary and in some situations mandatory, if the social license for animal sourced food production is to continue sustainably. Australia is a vast country with many marginal soil areas more suitable for extensive livestock grazing than intensive farming. Most sheep occur in the temperate zones of southern Australia, managed extensively in large flocks usually exceeding 3,000 individuals, especially the dominant Merino wool flocks. Some flocks are managed with or adjacent to *Bos taurus* beef cattle, with most enterprises successfully managing this proximity or cohabitation. However, the huge areas of tropical northern Australia are unsuitable for these animals. Livestock located there are mainly *Bos indicus* infused cattle that are raised under very extensive conditions, with climatic extremes, large distances and low management inputs (11). Meeting the increasingly high welfare standards expected is challenging, particularly in northern Australia where animals are rarely mustered more than once annually. Further, as demonstrated in the past two decades, extensive livestock welfare risks are increasing in association with the severity of climate change.

This millenium has seen the onset of two of the most severe drought periods on record in Australia (12–14). That were complicated by welfare issues arising from unforeseen disease, trade and environmental catastrophes. The onset of the first drought coincided with an uncontrolled epidemic of ovine paratuberculosis (15–18). The end of the drought coincided with a temporary ban on live export of tropical cattle to Indonesia that induced market failure, leading to mass morbidity and mortalities on some northern beef properties where extensive management of the animals is sometimes too remote for urgent remediation to prevent welfare issues (19, 20). The second drought period progressed in severity and culminated in the most extreme bushfires recorded, causing high levels of mortality, morbidity and suffering in farmed animals and wildlife (21–26). Temperature extremes also cause periodic heat-associated or cold-induced hyopthermia losses. Increased vigilance and careful management is required to reduce both temperature-induced stress during transport and the high ovine peri-parturient losses traditionally observed in extensive sheep farming. Further, an important issue affecting many extensively grazed properties is control of invasive animal pests causing challenging levels of livestock predation. Several issues remain particularly controversial, including surgical mulesing of wool sheep to manage flystrike, and the continuation of the live export trade of sheep and cattle.

This paper reviews the increasingly complex welfare challenges for the extensive livestock industries that are export trade dependent in Australia and are increasingly vulnerable to both welfare activism and the impacts of a changing climate (**Figure 1**). The review includes examples of improved

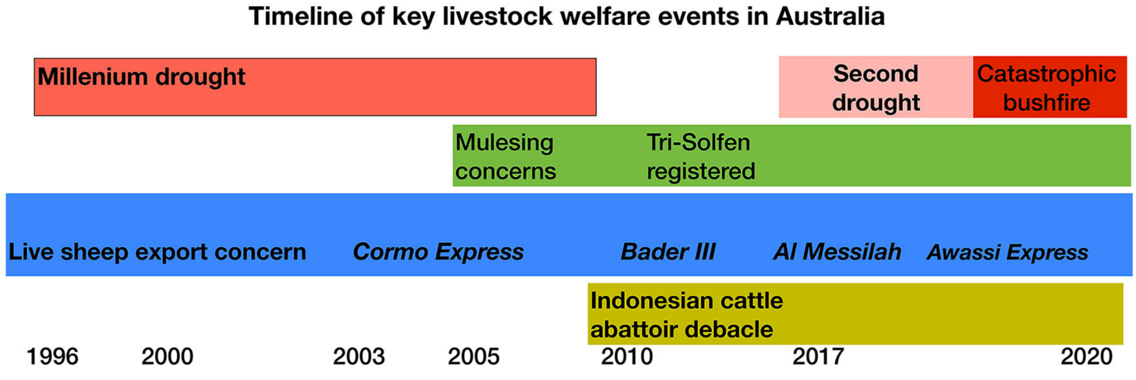

FIGURE 1 | Timeline of key recent livestock adverse welfare events in Australia.

producer welfare attitudes and practices through application of research innovations, including pain management during aversive husbandry procedures now occurring globally. Further, it describes new innovations in animal welfare assessment and surveillance and increasing adoption of prescribed welfare standards and guidelines to improve livestock welfare compliance. It concludes that these are encouraging signposts of improved change management of extensive farm animal welfare in Australia that provide lessons relevant to global considerations for the food security system.

THE CHANGING CLIMATE

Australia's climate has warmed on average by 1.44± 0.24°C since national records began in 1910, with an increase in the frequency of extreme heat events, accompanied by declines of ∼16% (April to October) and ∼20% (May to July) in rainfall, respectively, since 1970 (12, 13). Similarly, in the southeast of Australia, a decline of ∼12% (April to October) rainfall since the late 1990s, with decreasing streamflow at the majority of streamflow gauges across southern Australia since 1975, although rainfall and streamflow have increased across parts of northern Australia since the 1970s. These changes are recognized as increasing the impacts of temperature extremes, causing periodic heat-associated or cold-induced hyopthermia losses, plus increased vigilance and careful management required to reduce both temperature-induced and nutritional-deficiency stress during drought, plus the risk of extreme fire weather and the length of the fire season, across large parts of the country since the 1950s, especially in southern Australia (12, 13). This millenium has seen the onset of two of the most severe drought periods on record in Australia that were complicated by welfare issues arising from unforeseen disease, trade and environmental catastrophes.

The onset of the first and most prolonged so-called "millennium drought" occurred in the southeast of Australia from 1996 to 2010 (14), coinciding with emergence in the late 1990's of an uncontrolled epidemic of ovine paratuberculosis, or Johne's Disease (OJD), causing substantial on farm sheep losses, associated with mortalities exceeding 20% per annum on some farms (15, 16). Necropsy studies identified the mortalities as due to the combined impact of both OJD and drought (17, 18). Paratuberculosis caused about two thirds of the total estimated financial losses (17), with malnutrition accounting for 18% of the annual cost of all deaths among adult sheep (18). This indicated the importance of improving nutritional and disease management practices plus closer flock supervision to reduce the significant biological and financial impacts of OJD on sheep flocks during drought. Drought continues to negatively impact the welfare of both sheep and farming families in Australia, although the severe ovine morbidity associated with OJD and the widespread depression of sheep graziers following precipitous declines in values of affected farms due to OJD in the early millennium years, remains unprecedented (15–17).

In southern and eastern Australia, the recent droughts have been found to be the worst in the past 400 years and expectations are they will become more prevalent in the future (19). The second drought period in eastern Australia was from 2017 to 2020 and led to the entire state of New South Wales (NSW) and more than half of the neighboring state of Queensland to be declared drought-affected. Many described it as the worst drought in living memory, with numerous farmers choosing to cull their cattle due to welfare concerns (20). "Drought Planning" has been intensely promoted by the relevant state agricultural authorities in Australia for over three decades (21, 22). This process includes emergency response planning for "exceptional droughts" that encourage producers to be aware of the need for proactive drought "preparedness, prevention, response, and recovery," involving systematic: identification of enterprise risks; analysis of climate records; monitoring for trigger points; action planning for when triggers occur; financial analysis and building of cash, feed and water reserves; then regular review and updating of the drought plan. Despite these efforts, it appears that some welfare disasters appear almost impossible to manage effectively. These occur both during and immediately following the drought where there may be precipitous rain causing mass flooding events and even greater losses, both immediate and later disease-induced losses than were associated with the dry period of the drought (23, 24).

Unfortunately, an even more catastrophic post-drought event occurred during this period when in December 2019 through

January 2020, eastern Australia experienced its worst ever recorded extreme bushfire season. This culminated in more than 33 people killed, thousands of properties destroyed, at least 18 million hectares of once-green bushland becoming blackened and desolate, with billions of animals dead and injured (25). A new study is currently attempting to improve farmer bushfire preparedness by providing a comprehensive "Livestock Bushfire Preparation and Recovery Manual." The aim is for farmers to address the physical and financial effects of bushfires that could reduce stock and financial losses incurred by bushfire (26). The study is surveying herd and flock managers affected in the 2019–2020 fire season, assessing animal health and welfare issues, the effects on carcass damage and meat product quality and the financial strain on affected farmers. Further, patterns of burnt areas and livestock losses across landscapes is being assessed to identify a fire risk index that can determine the safest paddocks for stock to be placed in as part of their fire plan (26).

LIVESTOCK TRANSPORTATION WELFARE

The majority of Australian livestock producers and industry personnel, are well aware that to continue their access to domestic and overseas markets, they have important responsibilities for livestock welfare during the large distances required for the transport of animals by road, rail and vehicle onboard ships within and beyond Australia. This has led to the development of the national "Australian Animal Welfare Standards and Guidelines (S&G's) - Land Transport of Livestock" (27) to guide the processes from: the time that animals are mustered and assembled; their handling before and during loading; their journey duration and travel conditions with spelling periods and access to water; then the unloading and holding times. The Land Transport S&G's contain: (i) Objectives describing the intended outcome(s) for each section of the standards; (ii) Standards or minimum requirements that must be met under animal welfare law; and (iii) Guidelines for the recommended practices to achieve desirable animal welfare outcomes to guide and describe higher animal welfare outcomes compared to the minimum requirements of the Standards. This variation in acceptable practices reflects the vast differences in husbandry conditions between different agricultural regions, particularly in the extensive rangelands and tropical northern Australia where livestock farming is more often described as animal "harvesting." Here, the climatic extremes, large areas and distances within and between holdings (stations or farms) and low management inputs are necessary, ensuring that the extensive tropical cattle industry continues to face significant challenges to assure high standards of animal welfare (11).

However, it is the live export industry (LEI), where more than 2.7 million animals are shipped from Australian ports to nearly 20 countries around the globe annually, that faces the most scrutiny (28), with extensive research indicating that issues posed before, during and after live export results in the cumulative effects of combined stresses on the welfare of the animals (29). This has required the Australian Government assuming animal welfare responsibilities for export abattoirs and the live export trade, despite these issues being difficult to address through regulation and increasingly documented in the media, leading many Australians supporting an end to live export (30). Although welfare incidents in the sheep LEI had occurred sporadically prior to 2003, it was in that year that the "Cormo Express" captured international attention following refusal of entry of the ship and the 57,000 sheep on-board to Saudi Arabia following a claim that some had signs of a vesicular disease, presumably contagious ecthyma or scabby mouth from orf virus infection. The ship spent 2 months moving around the Persian Gulf, with animals exposed to high risk of heat stress, until the animals could be donated to the people of Eritrea (31). Then in 2011, an incident occurred that captured public attention on welfare in the cattle LEI, with the televised filming of disturbing slaughter practices of Australian cattle in Indonesian abattoirs. The exposed animal handling and operational techniques causing pain and injury, led the Federal Government to suspend the trade to Indonesia for a month. The sudden cessation of this AUD1.4 billion LEI of tropical beef cattle caused a precipitous market failure, with the domestic market unable to cope with the sheers numbers of cattle that were now in the supply chain. The impacts of this rapid yet controversial decision created difficult diplomatic, policy and industry issues, that are still debated (32–34) due to the negative impacts on economic returns, community attitudes and international socio-political relations.

The Australian Government in 2011, implemented the Exporter Supply Chain Assurance System (ESCAS) in an attempt to improve control and traceability throughout the LEI supply chain, requiring that transport, handling and slaughter complies with OIE welfare standards (35). However, in August 2012 an Australian ship carrying ∼22,000 sheep was blocked from unloading in Kuwait and Bahrain after local authorities also claimed that the animals had orf infection. The sheep had been at sea for 33 days and were left on board for almost a further 2 weeks until they were unloaded in Pakistan, where it was later reported that around 9,000 of the sheep had been killed on suspicion that they were diseased (36). Despite regular vaccination programs, it appears orf infection remains one of the numerous but important threats to the viability of small ruminant LEI's (37). Similar incidents have continued to recur and investigations reveal failures of monitoring and enforcement of ESCAS in destination countries, in both approved and non-approved facilities, most often revealed by the efforts of the various welfare agencies (38). The ongoing concerns have also led to a recent research review of 71 potential animal welfare indicators, categorized as animal, environmental and resource-based measures that would be appropriate for use throughout the LEI for feeder and slaughter livestock species in the 3 LEI sectors: (1) Australian facilities; (2) vessel; and (3) destination country facilities (39). The review identified 38 (sector 1), 35 (sector 2), and 26 (sector 3) measures currently being collected plus 20, 25, and 28 measures that are relevant to each LEI sector (sectors 1, 2, 3, respectively), and that could be developed and integrated into a future benchmarking system (39) should the LEI's continue, presumably as a transition industry until importing countries agree that processed meat is a preferred product to live animals.

PROGRESS WITH PAIN AND WOUND MANAGEMENT FOR AVERSIVE HUSBANDRY PROCEDURES

Perhaps as controversial as the LEI in extensive sheep welfare in Australia, is the "mulesing operation," a routine procedure with removal of skin from the breech and tail of lambs to create a bare area, providing lifetime prevention against myiasis (flystrike) in susceptible sheep (40). This mostly involves Merino lambs at high risk of the condition because of their breech conformation (wrinkle) that readily retains urine and feces and provides an attractive environment for deposition of the eggs of the sheep blowfly *Lucilia cuprina*. Following hatching, the blowfly larvae burrow deeply into adjacent tissues to the penetrating wounds in afflicted animals, rapidly causing the animal to become moribund because of blowfly strike and if untreated, death. Myiasis remains a serious cause of morbidity and mortality in Australian sheep despite long-term genetic improvement to reduce "blowfly-susceptibility" (41).

Until 2005, mulesing was performed without analgesia, resulting in welfare concerns for the lambs at and following surgery. Then a product designed to be readily used by producers, comprising a "stay and spray" approach for open wounds using a topical anesthetic formulation (TAF) to alleviate pain, plus components to minimize hemorrhage and provide antiseptic cover, was introduced (Tri-Solfen®, Medical Ethics, Australia). On application, it forms a long-lasting biocompatible barrier over the wound, creating its own intrinsic analgesic properties and acting as a slow-release carrier for the actives, including the two local anesthetics, lidocaine hydrochloride (5% w/w) and bupivacaine hydrochloride (0.5% w/w), in addition to the vasoconstrictor adrenaline acid tartrate (0.00451% w/w) and the antiseptic cetrimide (0.5% w/w). The combined synergies create prolonged analgesia extending to at least 24 h and well beyond the expected duration of the actives, plus enhanced healing of open wounds (41, 42). The TAF product has been researched extensively prior to and since it was registered for commercial use in 2012 and has been widely adopted by farmers in Australia, enabling the sale of wool classified as "PR" (pain relief) and improved welfare of sheep susceptible to flystrike during the extended period required until genetic alterations of Australian Merino sheep phenotypes can progress sufficiently to successfully address the risk of myiasis. It is estimated that 6–7 million lambs are treated annually, with well over 100 million sheep having now received treatment since the product was first registered.

This innovation has the potential to complement the various approaches to human wound debridement (43, 44). Further, TAF has been demonstrated to be safe and efficacious in managing pain and improving healing of acute surgical wounds incurred during surgical castration and tail docking of lambs (45, 46), surgical castration and dehorning of calves (47–49), and debridement of hoof lesions in cattle to reduce lameness (50). Effective pain relief at marking has been recognized as important in the northern beef industry. Herd musters commonly occur only annually, resulting in a broad range of ages of calves submitted to dehorning and castration, and variable degrees of restraint stress. Additional findings from recent studies with this product, include confirmation of rapid onset of surgical wound analgesia with positive welfare outcomes for an extended period, improved pain management when used with a non-steroidal anti-inflammatory drug (NSAID, especially meloxicam) or other products for pain relief (51–55), plus on occasions, improved productivity (56). More recently, the TAF has been successfully applied to lesions resulting from viral infection of the mucosa and epidermis, including Foot-and-Mouth Disease (57, 58).

The inclusion of an NSAID in the pain management research in animals is aimed at developing "best practice" options for multimodal pain management, where practical delivery of both the blockage of nociception and amelioration of wound sensitization is achieved. A method for oral delivery of NSAIDs (meloxicam as Ilium Buccalgesic®, Troy Laboratories, Australia) was developed in Australia and has been shown to be efficacious for procedures in both lambs and calves (52, 53). Although the widespread adoption by farmers of the addition of an oral NSAID to surgical procedures in sheep currently remains uncertain, the use of TAF accompanied by intramuscular injections of an NSAID, administered by beef farmers under veterinary advice for castration and dehorning, appears to be rapidly increasing in northern Australia. Recent research has confirmed both efficacy and productivity improvements with this multimodal approach when used in routine husbandry procedures on extensive beef cattle properties (56). In the Australian sheep industry, despite demonstration of the efficacy of an intra-scrotal and tail-docking wound spray of the TAF (45, 46), the convenient use of elastrator bands to cause ischaemic necrosis of the tail and scrotal tissues by producers, remains popular. An instrument recently developed (Numnuts®, Senesino Pty Ltd, Australia) that assists intravenous administration of lignocaine to the neck of the scrotum or tail, prior to application of the band(s), has been shown to reduce pain avoidance behaviors post-procedure (59). As with previous studies with the TAF, use of a multi-modal approach with an NSAID is likely to provide superior pain management.

PROGRESS WITH SURVEILLANCE, ASSESSMENT AND NEW TECHNOLOGIES

With the vast distances occurring on many Australian properties, addressing surveillance challenges for improved welfare in the extensive livestock industries has been recognized as an issue for many years. This is of particular concern with the demands and costs of managing endemic myiasis (*Lucilia cuprina*) and sheep lice (*Bovicola ovis*, *Lignognathus ovillus* and *Linognathus pedalis*) infestations in southern wool sheep flocks, and cattle tick (*Boophilus microplus*) and buffalo fly (*Haematobia irritans*) in the northern beef cattle herds. For both industries, the impacts of prolonged drought have proven to be extremely challenging, particularly with the recent example of the slow recognition of the moderate to high mortality rates emerging in adult sheep flocks with OJD that were only measurable when wool sheep were mustered for their annual shearing and required on-farm necropsy studies for definitive data (15–18). Similarly, the high background of lamb mortality in many flocks is not as well-recognized by producers as could be expected. This is because in many locations, dead lambs are rarely observed as they may be scavenged by wild canids and in some locations, feral pigs. High lamb mortality potentially reduces profitability and is increasingly perceived as an animal welfare issue for the sheep industry. Yet under extensive sheep production systems, especially in Merino flocks where the disturbance of lambing is to be avoided to minimize mis-mothering, data on mortality rates is usually only available when the flock is mustered for lamb marking and weaning. These interventions usually occurs ∼6–8 and 12–14 weeks after the commencement of lambing, respectively. A recent study of producer knowledge of lamb mortality rates, causes of lamb mortality, perceptions and practices that may contribute to lamb deaths, identified that ∼50% of producers estimated the mortality rate of lambs between birth and marking was 10%. This compared to published estimates of 20–25% (60). These perceptions impact negatively on the benefits of management strategies, including vaccination. Improved surveillance and extension services are necessary to ensure producers understand the causes of mortality and the key messages required. The generally low predation of live lambs in most cases and the high total costs to farm economics of lamb mortality from failures in disease prevention and management of climatic extremes (60), needs to be addressed.

Similarly, mismanagement of common cattle diseases is potentially a severe and prolonged animal welfare concern (61), with disease prevention almost invariably producing financial benefits that exceed the high costs of disease morbidity and mortality. As in extensive sheep flocks, in very extensive cattle systems, data on reproductive performance and mortality rate is usually only obtained at annual calf marking and weaning. Improving the accuracy of health and productivity records is an important area requiring improvement, with automated technology including drones to regularly visualize the herd, potentially enabling more effective syndromic disease surveillance. This approach remains an inadequately utilized tool that could greatly assist the recognition and diagnosis of welfare issues and disease (61) in extensive production systems. In tropical beef production, there is also the challenge of removing unrequired females from the herd in a suitable condition for sale. This requires that the ovarian function of these females is ablated, usually by spaying to prevent pregnancy and enable fattening (62, 63). The failure of a chemical spaying approach to be effective in the field and the delayed availability of immuno-spaying currently under investigation, has led to continuation of the reasonably common practice of surgical cattle spaying. This is now mostly performed by the Willis Dropped Ovary technique that has a low but recognized mortality rate.

Improving producer knowledge that the potential suffering of disease-affected animals is a welfare issue that needs to be avoided, is important, with timely and humane on-farm euthanasia required when necessary (61). Traditionally, a major driver for improved welfare has been the risk of prosecution via regulatory agencies with statutory responsibilities for ensuring animal welfare compliance. It is anticipated that improved awareness of the Australian Livestock Welfare S&G's could reduce this traditional reliance on regulatory action (27). The traditional reliance on enforcement of current animal welfare legislation in livestock systems in Australia is now being replaced by promotion of self-audits for accreditation schemes. This suggests that objective measurement of animal welfare by appropriate welfare assessment protocols is increasingly important in accompanying efforts to improve surveillance. The welfare relevance of animal health and the relative ease of recording has led to most approaches focusing on clinical measures and physical appearance, with inclusion of behavioral and mental state aspects of welfare suggested as requiring a more comprehensive approach (64). In extensive cow-calf operations, more research is required to develop robust and feasible indicators of positive welfare states for on-farm use. These include objective measures of behavior and the affective state of animals, enabling comparison and contrast of welfare implications of husbandry procedures that are versatile, relevant, reliable, affordable, and broadly acceptable by stakeholders (65). Qualitative Behavioral Assessment (QBA) is an integrated measure that describes behavior as a dynamic, expressive body language, enabling comparative, hypothesis-driven evaluation of various industry-relevant practices. Although most other welfare assessment methods record "problems" such as lameness or injury scores, QBA also captures positive aspects of animal welfare that occur when animals engage with their environment. As QBA is increasingly used in animal welfare assessments in Europe, it may have application in combination with other methods as a welfare assessment tool for the Australian livestock industries (65).

Continuous measurement and monitoring of the behavioral state of animals by using on-animal sensors to identify movements and locations that reflect the well-being of the animals, has potential for extensive livestock systems (66). With increasingly reliable animal welfare measures and decreasing costs of on-animal sensors, technology adoption will very likely increase, particularly if the value proposition for farm businesses and algorithm development, ensures validity and reliability (66). The application of new technologies to improve livestock management systems for improved animal welfare, should complement the learning abilities of the animals (67). Examination of virtual fencing identified that successful learning occurs when the animal perceives cues to be predictable (e.g., an audio warning always precedes a shock) and controllable (e.g., operant response to the audio cue prevents receiving the shock), with an acceptable management also ensuring that welfare is not compromised (67).

DISCUSSION AND CONCLUSION

Although animal welfare issues facing the extensive southern Australian sheep and beef cattle industries have some similarities to those faced by extensive livestock production industries in many other countries, management of external parasitism from myiasis and lice in wool sheep flocks and the impacts of prolonged drought have proven to be extremely challenging.

However, whilst the extensive tropical Australian beef cattle industry in northern Australia is also characterized by climatic extremes and external parasitism from cattle tick and buffalo fly, the large property sizes and distances requiring prolonged transport, plus the necessary low management inputs, ensure the industry still has significant challenges in meeting increasing standards for animal welfare. Issues remain with the mustering and moving of cattle, the timing and frequency of handling; transportation including live export, predation and aversive surgical husbandry procedures (11). With conversion of the northern herd to *Bos indicus* animals better adapted to the northern Australian environment, many of the previous livestock welfare problems have been ameliorated to some degree. Increasing implementation of management changes, including adoption of pain management for surgical procedures, improved planning for extended dry periods and drought, with wider use of supplementary feeding; and broader implementation of vaccination and weaner management programs, suggests dramatic improvement for large numbers of cattle is in progress. Research continues for less-invasive alternatives to cow spaying, and the calf marking procedure could be improved by increasing the adoption of polled genotypes to reduce dehorning plus the earlier castration of males, requiring more frequent mustering of herds.

The LEI's also continue to be problematic. It would appear that patience of many Australians is exhausted with the sheep LEI that sends temperate woolled animals on prolonged sea journeys to countries with harsh tropical environments. This presumably remains an industry in transition. With the exception of variable recipient country slaughter processes and despite some challenges with implementing ESCAS, the issues with the beef LEI, where tropically adapted animals experience far shorter journeys to neighboring tropical countries, appear more defensible. In northern Australia, a considerable number of livestock farming operations are geared for servicing the beef LEI and there is considerable resistance to cessation of this industry. With demand from importing countries likely to increasingly switch to processed meat as a preferred product to live animals, as refrigeration and supermarket sales become more established in regional developing countries, it is envisaged that eventually the northern beef LEI will transition to in-country processing and carcass exports.

Millions of global farm animals experience painful livestock management procedures annually and there is an increased requirement for producers to implement pain management protocols on farms, although in many countries, options for on-farm analgesia are limited (68). Whilst further research is needed on objective measurement of pain in food animals, the use of multimodal analgesia using local anesthetics and particularly TAF, with an NSAID and in particular meloxicam, are currently considered the best options for on-farm analgesia in Australia (44, 69). Further research on pain assessment and amelioration, including applications for inflammatory (57, 58) and neuropathic conditions are necessary to achieve best practice in livestock pain management (68, 69).

There are encouraging signs suggesting improving change management of extensive farm animal welfare is occurring in Australia. These include the adoption of prescribed livestock welfare Standards and Guidelines, introduction of ESCAS to address export concerns (70), improved producer welfare attitudes and practices including pain management during aversive husbandry procedures now occurring globally, plus new innovations in animal welfare surveillance and assessment. With exception of the continuation of the LEI, evidence suggests that a new paradigm has emerged on-farm, capable of sustainably addressing the complex welfare concerns arising in extensive livestock husbandry systems in Australia. Global consumers of extensively-raised livestock products likely need greater awareness of the quality of product raised under improving attitudes and practices of animal welfare on many Australian livestock farms. These lessons may provide valuable insights for producers in and advisors to, the extensive livestock industries in other countries.

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PW contributed the entirety of this review, although this work was dependent on a number of colleagues and collaborators as per acknowledgments featured as co-authors in the publications cited.

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Corrigendum: Progress With Livestock Welfare in Extensive Production Systems: Lessons From Australia

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In this original article, there was an error. The conflict of interest statement published was incorrect. The corrected conflict of interest statement is below.

The author reports no conflict of interest in this work. The extensive studies evaluating Tri-Solfen and other therapies for aversive animal-husbandry interventions that occurred prior to this review paper, were funded by an Australian Research Council Linkage Grant from the Australian government with financial contributions from Medical Ethics Australia and Bayer Animal Health Australia and the author has provided advice to these companies on the international use of pain management products. However, the content in this paper was not influenced by funding from either of these companies, nor did they have a role in the design, decision to publish, or preparation of the manuscript.

The author apologize for this error and state that this does not change the scientific conclusions of the article in any way. The original article has been updated.

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Welfare of Native Goat Breeds in Serbia—Emphasis on Parasitological Infections

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Native goat breeds in Serbia has been recognized as an important element of regional agrobiodiversity and play an important role in the safeguarding of cultural and traditional heritage. The aim of this study was to identify the main welfare issues likely to be encountered in extensive goat farming systems with an emphasis on parasitological infections. The study was conducted during the winter season on four small farms of native Balkan and Serbian white goats. For welfare assessment, animal-based indicators from AWIN protocol for goats were used. All fecal samples for parasites were qualitatively and quantitatively examined. The main welfare issues identified were poor hair coat condition (62.79%), dirty and light soiling hindquarters (31.40%), thin body condition score (26.74%), abscesses (19.78%), and udder asymmetry (18.60%). In addition, an important and prevalent welfare problem identified across all farms was parasite infection and weak significant ($p < 0.001$) correlation between certain parasites (Strongylidae, *Moniezia* spp., *Buxtonella sulcate*, and Protostrongylidae) and welfare indicators such as poor hair coat condition and nasal discharge. The results of this study provided the first overview and valuable insight into the impact of extensive systems on the welfare of native goats in the Balcan region.

Keywords: animal welfare, animal-based indicators, extensive systems, goats, parasites

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INTRODUCTION

In Serbia, at present, there is very little information as to the welfare of goats. Before the Second World War, in the Republic of Serbia, goat breeding had a significant place and was mostly represented as an extensive production, in the hilly, mountainous area (1). With the adoption of the Law on the Prohibition of Goat Breeding in 1954 (2), goat farming has become forbidden, which negatively influenced the overall size of the goat population in Serbia, as well as the presence of native goat breeds (1). The goat farming sector in Serbia has been rapidly developing during the last decades. Currently, in Serbia, there are 180,000 breeding goats (3). According to the Institute for Animal Husbandry's annual report, only 13 smallholder farms with a total of 429 native goat breeds are registered in Serbia today.

Native goat breeds in Serbia represent valuable and irreplaceable genetic resources and play an important role in the safeguarding of cultural and traditional heritage (1). There are two local goat breeds currently raised in Central and Eastern Serbia, Balkan goat and Serbian white goat, with

Balkan goat being the native breed and Serbian white basically being improved Balkan by crossing with Saanen bucks aimed at improving milk yield (1). Both of these breeds are very endurable that is easily adapted to modest conditions of care, housing, and nutrition, usually raised extensively in hilly, mountainous regions (1) and only during the winter season when there is provision of supplementary feeds at home in addition to grazing, then the production system is considered as semi-extensive.

Both breeds are used for combined production of both milk and meat, but for Balkan goat, the meat is the most important product (4), while Serbian white goat has higher milk production (1).

Extensive management systems allow animals to behave in a more natural way and express natural behaviors such as grazing, exploration, or exercise, which may be beneficial for their health (5, 6). These characteristics of extensive systems fit with one of the three conceptual frameworks used to assess animal welfare, "natural living," and also has clear links to similar concepts in the "five freedoms"—freedom to express normal behaviors and the "five domains"—behavioral or interactive restriction (7, 8). While the welfare of goats is largely positive when assessed according to natural living (e.g., providing animals with opportunities to play, make their own decisions, or to have positive social relationships) in extensive system may face a range of compromises to their well-being, but principally, these relate to nutritional stress, inadequate water supply, climatic extremes, parasitical diseases, lameness, and inappropriate managing (5, 9). Grazing goats are therefore exposed to a huge diversity of parasites since natural pastures are the main source of internal and external parasites (10). These parasites impact greatly on animal health, welfare, and productivity such as a considerable decline in weight gain, milk yield, and hair coat condition (10).

This paper aims to present the first outcomes of data collected in a sample of extensively reared native Balkan and Serbian white goat according to the AWIN protocol, and parasite data, as well as to identify the welfare problems that affect these animals.

MATERIALS AND METHODS

Farms and Management

The study was conducted in January 2021, on four small farms of native Balkan and Serbian white goat (**Figures 1A,B**). In **Table 1**, characterizations of the farms are shown. Farms are located in the hilly, mountainous regions of Central and Eastern Serbia (**Figure 2**). Serbia is a continental country in Southeastern Europe, in the central part of the Balkan Peninsula, between $41°53'$ and $46°11'$ N and $18°49'$ and $23°00'$ E. Due to the Pannonian Plain in the north, it is a part of Central Europe. Geographically and climatically, its southern part is a Mediterranean country. The Serbian climate is between a continental climate in the north, with cold dry winters, and warm, humid summers with well-distributed rainfall patterns, and a more Mediterranean climate in the south with hot, dry summers and autumns and average relatively cool and rainier winters with heavy mountain snowfall. January is the coldest month of the year in Serbia as the winter brings snow, heavy frost, and dense fog in many parts of the country. According to the Republic Hydrometeorological Service of Serbia, the average monthly air temperature for the period in January 2021 was in the range of from −0.7°C to 4.3°C (in mountain regions −5.2°C) with a total of 75–150 mm precipitation in Central and Eastern Serbia.

Animals from these areas were maintained under extensive management systems, where they foraged all day round in a fenced paddock during the day with minimum supplementation in the winter season (1 kg of a prepared meal of forage legumes + maize per animal). Water is provided from a natural spring, and shelter is provided by trees, shrubs, other vegetation, and artificial

FIGURE 1 | Serbian white **(A)** and Balkan goat **(B)**.

TABLE 1 | Characteristics of the examined farms.

Characteristics		Farms			
		I	II	III	IV
Goat status		Lactating	Lactating	Lactating	Non-lactating
Goat breed		Serbian white	Serbian white	Balkan	Balkan
Total goats/farm		15	36	10	60
Number of male breeder goat		1	1	1	1
Number of adult goats on farm		14	30	7	51
Number of evaluated goats (aged 2–10 years)		14	25	7	40
Number of pens		1	1	1	1
Pen dimension (m^2)		40	98	12	90
Stocking density (m^2/animal)		2.67	2.72	1.2	1.5
Bedding	Clean/dirty, wet	Clean	Clean	Dirty and wet	Clean
	Sufficient/insufficient	Sufficient	Sufficient	Insufficient	Insufficient
Type of water places		Bucket	Bucket	Bucket	Natural spring
Cleanness of water places		Clean	Clean	Dirty	Clean
Deworming in spring and autumn		Albendazole	Neositol (levamisole hydrochloride), ivermectin	Albendazole	No use

structures. During the winter season at night and the varying climatic extremes, goats are penned. In these areas, farmers use veterinary service (farm II), weak use (farm I and III), and do not use (farm IV) with goats not treated/dewormed (**Table 1**). An initial preventive planned deworming of animals against intestinal parasites was performed twice a year in spring and autumn (farms I, II, and IV).

Animals and Welfare Assessment

Since there was no specific protocol for extensively managed goats, the authors used animal-based indicators from AWIN welfare assessment protocol for sheep and goats (11) such as body condition score (BSC), hindquarters cleanness (12), hair coat condition, severe lameness, abscesses, udder asymmetry, oblivion, ocular and nasal discharge, latency to the first contact test and resource-based indicators such as bedding (sufficient/insufficient and clean/dirty, wet) and type and cleanness of water places. These indicators were selected because they address the main welfare concerns for goats, covering freedom from hunger, pain, injury, or disease. The assessment of the goats was conducted between 9 and 16 h by two assessors who were trained to use AWIN welfare protocol for sheep and goats. Welfare indicators are awarded with a score of 0 when welfare is good, a score of 1 is awarded when welfare has been poor and unacceptable, and a score of −1 is only awarded when the goat is thin.

The total number of goats on the farms was 131 (**Table 1**). We observed only adult goats aged 2–10 years, and a number of animals for assessment were selected (**Table 1**) according to the total number of animals on each farm (11).

Parasitological Examinations

In February 2021, parasitological examinations were performed at the Department of Parasitology, University of Belgrade Faculty of Veterinary Medicine, on fecal samples of native Balkan goat and Serbian white goat from four small farms. Coprological testing included both macroscopic and microscopic examinations of samples. Individual samples were collected from the same housing unit, regarding housing systems.

Sample Collections

The parasitological examination included the collection of goat feces, in the form of individual samples, which were put in PVC bags, and all the necessary information was labeled. Feces were sampled immediately after defecation. In order to avoid contamination of samples with pseudoplastic particles of plant and animal origin from the litter and the ground, sampling was performed from the upper segments of the excreted feces. The samples were stored in a handheld refrigerator at a temperature of +4°C and transported to the Parasitology Laboratory, where coprological diagnostics was performed within 24 h.

Macroscopic Examination

In the macroscopic examination, the formation, consistency, color, and odor of fecal samples were investigated. Any deviations in these parameters from the typical physiological characteristics of the feces of the goats were noted. The presence of impurities such as blood, pus, mucus, or undigested food was recorded as potential markers of certain pathological conditions of the gastrointestinal tract. Thereafter, the feces was carefully examined using tweezers, and any adult helminths and their parts were transferred to a Petri dish, rinsed in saline, and prepared for further analysis (13).

FIGURE 2 | Geographic position of farms included in the survey.

Microscopic Examination

Preparations for microscopic diagnostics were made by qualitative methods without concentration and with a concentration of parasitic elements.

Fecal Examination by Qualitative Method

Coprological examination was performed by qualitative methods without (Vajda method) and with a concentration of parasitic elements—flotation and sedimentation techniques (14).

Fecal Examination by Quantitative Method

Quantification of the obtained results was performed by the McMaster method (15), with a sensitivity of 50 eggs/oocysts per gram of feces to determine the helminth eggs/coccidia oocyst/ciliate cysts per gram outputs (16).

A saturated aqueous NaCl solution (>97%; Roth, Karlsruhe, Germany) was used to perform conventional flotation methods, which was prepared by mixing 210 g of NaCl with 1,000 ml of distilled water (specific gravity 1,200). The preparations made were observed under 100 magnifications for morphological identification of gastrointestinal (GI) parasitic eggs/oocysts/cysts, according to Soulsby (17).

Pulmonary strongylids were identified, based on the morphological characteristics of the first-stage larvae.

During the identification of the larvae, the presence of *Dictyocaulus filaria* was confirmed by the finding of the first-stage larvae with an anterior protoplasmic knob and black granular intestinal inclusions in the feces (18). The larvae of Protostrongylides are differentiated by their characteristic feature at the tip of their tail (19).

Statistical Analysis

Data were analyzed using GraphPad Prism software. Results were described by descriptive statistics (mean value and standard error) and as prevalence (the overall number of goats showing the measure regardless of severity). The distribution of the welfare indicators was tested by the Kolmogorov–Smirnov distribution fitting test, which showed a not normal distribution. The differences between welfare indicators were analyzed using the non-parametric Kruskal–Wallis test on the equality of the medians, adjusted for ties. When significant differences were found, Dunn–Bonferroni *post-hoc* test was performed. For animal-based parameters and different endoparasite infection and coinfections, the prevalence was calculated on a total number of goats, and the significant difference was determined by the Chi-square test.

Relationships between the different welfare indicators and endoparasites were examined by Spearman's rank correlation. For all correlation analyses, the absolute value of the Spearman's correlation coefficients assessed whether very weak ($0.0 \leq |rho| < 0.2$), weak ($0.2 \leq |rho| < 0.4$), moderate ($0.4 \leq |rho| < 0.6$), strong ($0.6 \leq |rho| < 0.8$), or very strong ($0.8 \leq |rho| \leq 1.0$) relationships existed as described by Campbell (20). Only those correlations significant at $p < 0.05$ are reported.

RESULTS

Welfare Assessment

Based on the results, significant differences ($p < 0.05$, $p < 0.001$) were observed at body condition score (thin and fat goats), hair coat condition, nasal discharge, severe lameness, and hindquarter cleanness between goats from different farms (**Table 2**). The most poor and unacceptable welfare indicators in goats were hair coat condition (62.79%, 54/86) with an average score of 0.63 ± 0.03, hindquarter cleanness (31.40%, 27/86, 0.31 ± 0.05), tin BCS (26.74%, 23/86, 0.41 ± 0.07), abscesses (19.78%, 17/86, 0.20 ± 0.04), and udder asymmetry (18.60%, 16/86, 0.19 ± 0.04) (**Tables 2**, **3**).

The average score of dirty hair in farm III (0.86 ± 0.14) and farm IV (0.40 ± 0.08) was significantly higher ($p < 0.001$) compared with farm II (**Table 3**).

Parasitological Examinations

In the examined feces of goats from four farms, nine endoparasites were identified in the form of coinfections—protozoa (Coccidia and *Buxtonella sulcata*), nematodes (Strongylidae, *Trichuris ovis*, *Capillaria* spp., *Dictyocaulus filaria*, and *Protostrongylidae*), cestodes (*Moniezia* spp.), and trematodes *Dicrocoelium lanceolatum* with a total prevalence of 100% (86/86) (**Table 4**). The most prevalent endoparasites in all farms observed was Coccidia (95.35%, 82/86) followed by Strongylidae (90.70%, 78/86) and Protostrongylidae (86.04%, 74/86).

In farm I, the most prevalent coinfections were *T. ovis*–*Moniezia* spp.–Coccidia–*Protostrongylidae* and Strongylidae–*T. ovis*–*Moniezia* spp.–Coccidia–*B. sulcata* with prevalence of 21.42% (3/14). Polyparasitism of Strongylidae–Coccidia–*Protostrongylidae* dominated in farm II (48%—12/25) and farm IV (40%—14/40), while on farm III prevailing coinfections were Strongylidae–*T. ovis*–*Moniezia* spp.–*Protostrongylidae* (28.57%—2/7) (**Table 5**). A significant difference ($p < 0.05$; $p < 0.001$) between four farms of extensively managed native goat breed was established in the prevalence of all coinfections except in quadruple infections of Strongylidae–*Moniezia* spp.–Coccidia–*Protostrongylidae* (**Table 5**).

In most fecal samples of goats, we detected a low degree of infection (<50–500 opg/epg) with coccidia, strongylidae, anoplocephalidae, and *T. ovis* (farms I, II, and III), coccidia and strongylidae (farm II), and *Capillaria* spp. (farm IV) (**Table 6**). Medium degree of infection (550–1,500 opg/epg) with coccidia was found in farm I (875 ± 25 opg), farm II (1,000 ± 22.60 opg), and farm III (733.3 ± 109.3 opg). The high degree of infection (>1,500 opg/epg) was with coccidian and was detected only in farm II (1,975 ± 141.70 opg) (**Table 6**).

Correlations Between Welfare Indicators and Endoparasites

Table 7 shows the significant correlations observed between the different welfare indicators and different endoparasites. There was a weak significant positive correlation between BCS and hair coat condition (rho = 0.28, $p < 0.001$) and a moderate positive correlation between bedding cleanness and dirty and light soiling hindquarters (rho = 0.35, $p < 0.001$) and bedding cleanness

TABLE 2 | Prevalence of welfare parameters in 86 individual goats examined in four farms in Serbia.

Animal-based indicators		Farms											
		I $N = 14$		II $N = 25$		III $N = 7$		IV $N = 40$		Total for all farms $N = 86$		χ^2	*p*
		n	%	*n*	%	*n*	%	*n*	%	*N*	%		
Body condition score (BCS)	Thin	8	57.14	7	28	2	28.57	6	15	23	26.74	8.32	0.03*
	Adequate	6	42.86	18	72	5	71.43	28	70	57	66.28	4.13	0.25
	Fat	0	0	0	0	0	0	6	15	6	15	7.42	0.05*
Hair coat condition		13	92.85	12	48	5	71.43	24	60	54	62.79	8.12	0.04*
Severe lameness		0	0	0	0	2	28.57	0	0	2	2.33	23.10	0.00***
Abscesses		5	35.71	3	12	1	14.29	8	20	17	19.78	3.33	0.34
Hindquarters cleanness		5	35.71	0	0	6	85.71	16	40	27	31.40	22.53	0.00***
Udder asymmetry		3	21.43	3	12	1	14.29	9	22.5	16	18.60	1.28	0.73
Nasal discharge		3	21.43	2	8	0	0	1	2.5	6	6.98	7.16	0.09
Ocular discharge		0	0	0	0	0	0	0	0	0	0	/	/
Oblivion		1	7.14	0	0	1	14.29	0	0	2	2.33	7.39	0.06

N, total number of samples; n, number of positive samples. ***$p < 0.001$; *$p < 0.05$.

TABLE 3 | Mean (±SEM) scores for animal-based welfare parameters in goats examined in four farms in Serbia.

Animal-based indicators	Farms					
	I	II	III	IV	Total for all farms	*p*
	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	
Body condition score (BCS)	0.57 ± 0.14	0.28 ± 0.09	0.29 ± 0.18	0.45 ± 0.12	0.41 ± 0.07	0.55
Hair coat condition	0.93 ± 0.07	0.48 ± 0.06	0.71 ± 0.18	0.60 ± 0.08	0.63 ± 0.03	0.13
Severe lameness	0	0	0.29 ± 0.18	0	0.02 ± 0.02	0.67
Abscesses	0.36 ± 0.13	0.12 ± 0.07	0.14 ± 0.14	0.20 ± 0.06	0.20 ± 0.04	0.66
Hindquarters cleanness	0.36 ± 0.13	0^{AB}	0.86 ± 0.14^{A}	0.40 ± 0.08^{B}	0.31 ± 0.05	0.00***
Udder asymmetry	0.21 ± 0.11	0.12 ± 0.07	0.14 ± 0.14	0.23 ± 0.07	0.19 ± 0.04	0.90
Nasal discharge	0.21 ± 0.11	0.08 ± 0.06	0	0.03 ± 0.02	0.06 ± 0.03	0.10
Ocular discharge	0	0	0	0	0	0
Oblivion	0.07 ± 0.07	0	0.14 ± 0.14	0	0.02 ± 0.02	0.92

****$p < 0.001$; $^{A,B}p < 0.001$.*

and severe lameness (rho = 0.51, $p < 0.001$). Likewise, a weak significant correlation between strongylids, anoplocephalids, *Buxtonella sulcata*, and hair coat condition (rho = 0.23, r = 0.21, rho = 0.25, respectively, $p < 0.05$) were observed (**Table 7**), and week significant negative correlation between protostrongilids and nasal discharge (rho = −0.28, $p < 0.001$).

DISCUSSION

Although we examined four farms and 86 extensively managed native goat breed in total, this study constitutes the first evaluation of the welfare of goats conducted in Serbia. The findings of this study are a sound basis for future research, providing valuable insight into the main welfare issues regarding extensive goat farming. Poor hair coat condition (62.79%), dirty and light soiling hindquarters (31.40%), thin BCS (26.74%), abscesses (19.78), and udder asymmetry (18.60%) showed high prevalence and should, therefore, be considered as major welfare problems.

In the present study, we observed a total of 66.28% of the goats were in adequate BCS, but the presence of the 26.74 and 15% thin and fat goats represent factors affecting welfare in those animals. These results can be ascribed to the fact that in extensive systems, goats due to seasonal variation and not timely grazing sometimes cope with long periods of grazing the forage with high fiber contents and low energy, and suffer chronic hunger (6). Even the food supplementation was provided by all farmers from the study; problems with inadequate body condition scores occurred. An additional problem with supplementation is that we can connect that some animals may be reluctant to eat the supplements if they are not accustomed to them, or there might be existing competition between animals (6). The importance

TABLE 4 | Prevalence of endoparasites in goats examined in four farms in Serbia.

Endoparasites	I (N = 14)		II (N = 25)		III (N = 7)		IV (N = 40)		Total (N = 86)		χ^2	p
	n	%	n	%	n	%	n	%	n	%		
S	9	64.29 (39.19–89.39)	25	100	5	71.43 (37.96–100)	39	97.50 (95.13–100)	78	90.70	9.41	***
T	10	71.43 (47.77–95.09)	0	0	4	57.14 (20.48–93.80)	15	37.50 (22.50–52.50)	29	33.72	23.6	***
M	10	71.43 (47.77–95.09)	0	0	6	85.71 (59.79–100)	16	40 (24.82–55.18)	32	37.21	29.01	***
C	14	100	25	100	5	71.43 (37.96–100)	39	97.50 (95.13–100)	82	95.35	53.31	***
C^1	0	0	0	0	0	0	6	15 (3.93–26.07)	6	6.98	7.14	*
D	2	14.29 (0–32.62)	4	16 (1.63–30.37)	0	0	9	22.50 (9.56–35.44)	15	17.44	2.32	0.50
BS	7	50 (23.81–76.19)	0	0	0	0	0	0	7	8.14	39.01	***
P	9	64.29 (39.19–89.39)	20	80 (64.32–95.68)	6	85.71 (59.79–100)	39	97.50 (95.13–100)	74	86.04	10.65	***
D^1	0	0	8	32	0	0	0	0	8	9.30	21.52	***

*N, total number of samples; n, number of positive samples; S, Strongylidae; C, Coccidia; T, Trichuris ovis; M, Moniezia spp.; C^1, Capillaria spp.; D, Dicrocoelium lanceolatum; D^1, Dictyocaulus filarial; B, Buxtonella sulcata; P, Protostrongylidae. ***$p < 0.001$; *$p < 0.05$.*

of dietary supplementation, especially protein supplementation, showed in numerous studies on resistance and resilience of sheep and goats to GI parasite infections, has been recently confirmed in a few studies (21, 22). According to Hoste et al. (23), GI parasitic infection is often equated to a nutritional disease because of the major negative impacts on total tract digestibility, diet intake, and the reorientation of nutrient use for the maintenance of tissue homeostasis. Ghosh et al. (24) reported that the nutritional status of goats is influenced by a number of factors such as feeding strategy and management, health (parasites, wasting disease, and viral or bacterial and metabolic diseases), age, social hierarchy, and goat status.

Among the etiological agents that affect the poor health status of goats, parasites are usually neglected, although they can lead to colossal morbidity and mortality of goats, which results in significant economic losses (25–27). In our study, the overall prevalence of endoparasites in goats from four farms was 100% and might represent a factor that affects body condition score. Although in our study we did not find that BCS and endoparasites correlated, according to many authors (28–33), endoparasites cause several subclinical effects such as hyperproteinemia, growth depression, reduction in milk yield, loss of appetite, and digestive inefficiency. Parasite infection negatively affects hosts by consuming host resources and directly damaging host tissues or indirectly by stimulating costly immune responses and by changing host movement, foraging, or social behaviors (34–36).

Polyparasitism in our study might be due to goat grazing activities on contaminated pastures, poor sanitation and management in farm III, unsystematic and inadequate deworming, or not treating in farm IV (**Table 1**).

We noted that the overall prevalence of coccidian oocysts in goats was 95.35%. Our results are similar to those reported in China—87.9% (37), Czech Republic—92.2% (38), Portugal—100% (39), and Slovakia—100% (40). The high prevalence of coccidian oocysts in studied animals might be linked with the poor hair coat condition since coccidia can invade and destroy intestinal cells of the hosts, and electrolyte loss exacerbates mineral deficiencies, and there is poor absorption of nutrients, and affected goats can show a rough hair coat, poor weight gain, and weakness (37, 41).

The current finding showed that 100% of the studied animals were positive with GI parasitic infection, predominated by coccidian oocysts and strongyloides eggs (90.70%) with a low degree of infections. These data indicates that all diagnosed endoparasitosis in extensively managed goats are mostly present in the subclinical form. As a consequence, infective agents mainly cause indirect economic damage to the extensively managed goat production and significantly affect the welfare of goats. Polyparasitism was found in all goats, which can compromise the immune system of the host increasing their susceptibility to other diseases or parasites (42). Similar surveys of parasites on goat farms have been conducted in other European and Asian countries. Eggs from one or more species of GI parasites were identified in 100% goats in Turkey (43), 95.90% in Slovakia (44), 87.95% in Nepal (45), and 96% in Northern Italy (46).

Another parasite, *Capillaria* spp. was currently reported to be a prevalence rate (6.98%) that was higher than reported in Italy (46), Nepal (45), Bangladesh (47), and Thailand (48) (lower than 2%). *Capillaria* spp. is critical in goats and shares a wide range of herbivores including man (49).

TABLE 5 | Prevalence of coinfection parasite infections in goats examined in four farms in Serbia.

Coinfections	Farms											
	I (*N* = 14)		II (*N* = 25)		III (*N* = 7)		IV (*N* = 40)		Total (*N* = 86)		χ^2	*p*
	n	%	*n*	%	*n*	%	*n*	%	*n*	%		
Double infections												
SC	0	0	5	20 (4.32–35.68)	0	0	0	0	**5**	**20**	12.95	***
MC	0	0	0	0	1	14.29 (0–40.22)	0	0	**1**	**1.16**	11.42	***
Triple infections												
TCB	2	14.29 (0–32.62)	0	0	0	0	0	0	**2**	**2.33**	10.53	*
SCP	2	14.29 (0–32.62)	12	48 (28.42–67.58)	0	0	14	35 (20.21–49.78)	**28**	**32.56**	8.33	*
MCP	0	0	0	0	1	14.29 (0–40.22)	0	0	**1**	**1.16**	11.42	***
Quadruple infections												
TMCP	3	21.42 (0–42.91)	0	0	0	0	0	0	**3**	**3.49**	15.98	***
SMCP	2	14.29 (0–32.62)	0	0	1	14.29 (0–40.22)	10	25 (11.58–38.42)	**13**	**15.12**	7.51	0.06
SCPD[1]	0	0	4	16 (1.63–30.37)	0	0	0	0	**4**	**4.65**	10.24	*
STMP	0	0	0	0	2	28.57 (0–62.04)	0	0	**2**	**2.33**	23.10	***
STCP	0	0	0	0	1	14.29 (0–40.22)	0	0	**1**	**1.16**	11.42	***
Fivefold infections												
STMCB	3	21.42 (0–42.91)	0	0	0	0	0	0	**3**	**3.49**	15.98	***
STMCP	0	0	0	0	1	14.29 (0–40.22)	0	0	**1**	**1.16**	11.42	***
SCDPD[1]	0	0	4	16 (1.63–30.37)	0	0	0	0	**4**	**4.65**	10.24	*
STCDP	0	0	0	0	0	0	9	22.5 (9.56–34.44)	**9**	**10.47**	11.56	***
Six-fold infections												
STMCC[1]P	0	0	0	0	0	0	7	17.5 (6.72–29.28)	**7**	**8.14**	8.76	*
Sevenfold infections												
STMCDBP	2	14.29 (0–32.62)	0	0	0	0	0	0	**2**	**2.33**	10.53	*
Total	**14**	**100**	**25**	**100**	**7**	**100**	**40**	**100**	**86**	**100**		

N, total number of samples; n, number of positive samples; S, Strongylidae; C, Coccidia; T, Trichuris ovis; M, Moniezia spp.; C[1], Capillaria spp.; D, Dicrocoelium lanceolatum; D[1], Dictyocaulus filarial; B, Buxtonella sulcata; P, –Protostrongylidae.

****p < 0.001; *p < 0.05.*

The current study identified the overall prevalence of 37.21% (*Moniezia* spp.), 33.72% (*T. ovis*), 17.44% (*D. lanceolatum*), and 8.14% (*B. sulcata*), significantly higher than those reported by other researchers (44, 46), and in agreement with results from Nepal (45).

According to authors from Ethiopia (50) and West Africa (51), the prevalence of moniesiosis in sheep is significantly higher compared with goats and more susceptible to parasite infection (52). Infection of *Moniezia* spp. in small ruminants was reported to cause severe pathogenic effects, viz. disturbance of gastrointestinal motility, secretion, diarrhea, and anemia along with reduced slaughter yield, increased water content, and reduction in protein and fat (53).

Dicrocoelium spp. hepatic infection is responsible for direct losses in sheep and goat production due to the discarding of parasitized livers and indirect losses through costs associated with anthelmintic treatments (53). It has been reported in Italy (54), Iran (55), India (56), Nepal (57), Malaysia (58), and

TABLE 6 | Quantitative assessment of fecal samples in in goats examined on four farms in Serbia.

Farms	Degree of infection (quantitative FEC method)		Endoparasites				
			Coccidia	**Strongylidae**	***Moniezia* spp.**	***Trichuris ovis***	***Capillaria* spp.**
I		**N**	**14**	**9**	**10**	**10**	**0**
	Low	*n*	12	9	10	10	0
		%	85.71	100	100	100	0
		Mean ± SEM	387.5 ± 28.29	106.3 ± 14.75	80 ± 15.28	53.33 ± 3.33	0
	Medium	*n*	2	0	0	0	0
		%	14.29	0	0	0	0
		Mean ± SEM	875 ± 25	0	0	0	0
II		***N***	**25**	**25**	**0**	**0**	**0**
	Low	*n*	11	25	0	0	0
		%	44	100	0	0	0
		Mean ± SEM	213.6 ± 7.04	28.60 ± 2.48	0	0	0
	Medium	*n*	6	0	0	0	0
		%	24	0	0	0	0
		Mean ± SEM	1,000 ± 22.60	0	0	0	0
	High	*n*	8	0	0	0	0
		%	32	0	0	0	0
		Mean ± SEM	1,975 ± 141.70	0	0	0	0
III		***N***	**5**	**5**	**6**	**4**	**0**
	Low	*n*	3	5	6	4	0
		%	60	100	100	100	0
		Mean ± SEM	225 ± 62.92	155 ± 66.22	51.25 ± 1.25	37.50 ± 5.12	0
	Medium	*N*	2	0	0	0	0
		%	40	0	0	0	0
		Mean ± SEM	733.3 ± 109.3	0	0	0	0
IV		***N***	**40**	**40**	**16**	**15**	**6**
	Low	*n*	40	40	16	15	6
		%	100	100	100	100	100
		Mean ± SEM	175 ± 16.11	152.6 ± 16.02	50.31 ± 0.31	37 ± 3.90	52.50 ± 2.50
Total		***N* (%)**	**84 (97.67)**	**79 (91.86)**	**32 (37.21)**	**29 (33.72)**	**6 (6.98)**

Low: <50–500 opg/epg; medium: 550–1,500 opg/epg; high: >1,500 opg/epg (opg/epg, number of oocysts/eggs calculated per 1 g of feces); N, total number of samples; n, number of positive samples.

Nigeria (59). According to Sharma et al. (53), dicrocoeliosis remained little known and underestimated since those infected are asymptomatic and masked by the presence of pathological effects of multiple parasitic infections in ruminants.

The current finding showed that lungworms, protostrongylide infection predominated (87.21%), which is in agreement with the results from Morocco (60), but differs from Ethiopia −13.4–53.6% (61). The finding of *D. filaria* (9.30%) is in agreement with those recorded by Paran et al. (62) who diagnosed the prevalence at 8.9%. In our study, we found six goats (6.98%) with nasal discharge and significant correlations with protostrongylidae. These results can be ascribed to the fact that the most common clinical sign of lungworms in sheep and goats are pyrexia, coughing, rapid shallow breathing, nasal discharge, and emaciation with retarded growth (63). Lungworm infections in goats are of considerable economic importance. The parasites cause chronic production losses as a result of reduced food conversion ratio (FCR) and weight gain (62). The variation and differences in the prevalence of lungworms of small ruminants in different areas might be associated with differences in nutritional status, level of immunity, management practice of the animal, rainfall, humidity, temperature, altitude differences (64), and season of examination on their respective study area (65).

Poor hair coat condition was found at a prevalence of around 62.79% (54/86) in all farms. This result is not in agreement with those recorded by Can et al. (66) who found that 20–25% of the

TABLE 7 | Spearman's rank correlations between the welfare indicators and different endoparasites.

		rho	*p*
Welfare indicators			
BCS	Hair coat condition	0.28	0.00***
Bedding cleanness	Hindquarter cleanness	0.35	0.00***
Bedding cleanness	Severe lameness	0.51	0.00***
Endoparasites vs. welfare indicators			
Strongylidae	Hair coat condition	−0.23	0.03*
Moniezia spp.	Hair coat condition	0.24	0.05*
Buxtonella sulcata	Hair coat condition	0.25	0.02*
Protostrongylidae	Nasal discharge	−0.28	0.00***

****p < 0.001; *p < 0.05.*

observed animals showed inadequate poor hair coat condition in intensive dairy goat farms. The available literature suggests that different factors may affect hair coat conditions in goats such as mineral deficiencies (41), lower BCS (67), and ectoparasite infestation (68). According to Battini et al. (67), this indicator can reflect a goat's nutritional and health status. In the present study, we found that BCS and certain endoparasites (Strongylidae, *Moniezia* spp. and *Buxtonella sulcata*) correlate weak with hair coat conditions. The finding was not in agreement with the report made by Battini et al. (67), who found no effect of GI parasitic infestation on hair coat condition due to low level of infestation and the no access to pasture that represents one of the main risk factors for gastrointestinal parasite infections (69). These results suggest that parasites are certainly one cause for a poor hair coat condition, although we cannot exclude that other factors may affect this indicator, such as cold temperatures during the winter season. Exposure to hot or cold environments can also be a welfare problem for extensive livestock (70, 71). In cold winters, energy requirements for maintenance are 20% greater (72). A study performed by Battini et al. (67) proved that goats with a rough hair coat were in a significantly poorer nutritional condition and health status compared with goats with a normal hair coat. These indicate that cold weather could indirectly affect poor hair coat conditions in goats with nutritional deficiencies. The previous study (73) have shown that prolonged exposure of goat to naturally occurring or artificially induced cold environments mobilized fatty acids together with the increased blood glucose, which could have been used in muscles for heat production.

In the present study, we noted dirty and light soiling hindquarters in 31.40% of the studied goats. This result is in agreement with those recorded by Can et al. (66) who found dirty hindquarter prevalence of 27.1%. The result from this study is highly related to immediate environmental conditions, stock attitudes of people, and care for animals (74). According to Bøe et al. (75), the most important characteristics of pen flooring for farm animals are considered to be thermal conductivity, softness, cleanliness, and slipperiness. Even if this welfare indicator was not included in the AWIN welfare protocol for goats (11), dirty hindquarters may reflect animal discomfort that affects the welfare of goats. Based on the results of Bøe et al. (75), the cleanliness of the floor influences animal preferences among others, while softness did not appear to be an important flooring characteristic for the goats. In this study, bedding and hindquarter cleanness were correlated. Also, the dirtiest hindquarters in goats were observed in farm III (85.71%) compared with other studied farms, reflecting poor management and cleaning routines (**Table 1**). These findings are unsurprising, as a range of factors, such as housing design and bedding type, affects the cleanliness of goats.

Damp and dirty environments lead to the spread of specific bacteria, which cause painful health problems such as lameness (76). In this study, bedding cleanness and severe lameness were correlated. Generally, the prevalence of obviously severe lameness in goats was very low (only two in farm III, 2.33%) which is in line with the studies of Anzuino et al. (77), Muri et al. (78), and Can et al. (66), which reported lameness prevalence of 3, 1.7, and 2.1%, respectively. Since lameness is a major welfare concern as it is a painful condition, it is important to identify and treat it (79).

The present study also describes the overall prevalence of abscesses (19.78%) and udder asymmetry (18.60%). The occurrence of external abscesses in the body is closely associated with caseous lymphadenitis in small ruminants (80), which is recognized as an endemic disease in many countries (81). According to Mattiello et al. (82), external abscesses may influence the health condition of the animals and behavioral changes. Udder asymmetry is a sign of chronic alteration that remains even after an udder has recovered from infection or injury (83).

The human–animal relationship represents the mutual perception of stockman and animals and is essential for good animal welfare (84). In the present study, the latency period to the first contact between goat and assessor was good on all studied farms. Regarding the studied animals, extensively managed sheep showed fear in relation to the first contact with assessors (85). According to Jackson and Hackett (86) dairy goats habituate faster with human presence and gentle handling with regard to sheep that receive only neutral or aversive contact with people in extensive systems, e.g., restraint, shearing, or medication administration. This appears to support the findings of Mattiello et al. (87) that ascribe the better and very close relationship between the stock person and the animals in small farms compared with large ones.

CONCLUSION

Although extensive systems of management provide appropriate physical living conditions (e.g., resting area, natural shelters from varying climatic extremes, and grazing area) where goats can express natural behavior, disadvantages in terms of animal welfare exist. Animal-based parameters provide information on the care of farmers for animals. These results demonstrated that the most common causes of further care were poor hair coat condition, dirty hindquarters, thin BCS, abscesses, and udder asymmetry, while other welfare problems are less represented such as severe lameness, oblivion, and nasal discharge. In addition, an important and prevalent problem in welfare identified across all farms was parasite infection. Nutritional deficiency and the probable scarcity of quality protein, together with sources of infection during stabling, characterize coccidiosis and *T. ovis* infections, which directly impact the quality of the hair coat and the body condition of the animals. Therefore, these findings suggest for a need of well-coordinated, sanitary monitoring of goat farms by field veterinarians and dissemination of knowledge to animal handlers and farmers to minimize the occurrence of infections. Overall, the issues identified in this study can be treated or mitigated by management practices. Also, it is recommended that protein supplementation be used, which leads to resistance and resilience of goats to GI parasite infections. While results in this study may be more representative of welfare problems in large-scale goat farms, the findings of this study are groundwork for future research, providing valuable insight into the main welfare issues likely to be encountered in extensive goat farming.

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 the research was not conducted directly on animals, we only observed animals and sampled from them (feces). In this way, the welfare of the examined individuals was not impaired. Written informed consent for participation was not obtained from the owners because we made an agreement over the phone that their farms would remain anonymous as we complied.

AUTHOR CONTRIBUTIONS

KN wrote the manuscript with input from all authors. KN and MV conceptualized and designed the study. TI and NJ acquired, analyzed, and interpreted the data. DB made critical revisions. All authors contributed to the article and approved the submitted version.

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Temperament Trait Changes in Japanese Black Cows Under Grazing and Confined Conditions

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The objective of the present study was to reveal the effects of grazing on the temperament traits of cows. Nine Japanese Black cows [344 ± 32 kg body weight (BW), 7.7 ± 3.0 year of age], which had various experiences, such as tethering, handling, and grazing, were used in this experiment. Five of the nine cows were grazed for 3 months on a 1.8-ha field composed of a sown pasture with forestland. The remaining cows were fed in confinement. On days 38, 52, 72, and 86 after the start of grazing, the temperament traits observed in various situations, such as moving to the body weight scale, weighing, handling, moving to the stock for blood sampling, holding in the stock, and obtaining a blood sample, were assessed with a visual analog scale (VAS: 1–10) or score (1–5). During weighing and handling, the intensity of resistance exhibited by the grazing cows, as evaluated by head movement, walking/stepping, tail flicking, rope tension, and overall movement, was lower than that exhibited by confined cows ($P < 0.05$). The resistance score exhibited by the grazing cows during blood sampling was also lower than that exhibited by confined cows ($P < 0.01$). These results suggest that grazing enhances docility in cows with various experiences in different situations encountered in daily management.

Keywords: confinement, docility, grazing, temperament trait, visual analog scale

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INTRODUCTION

In cattle, temperament is described as an animal's response to handling or forced movement by humans (1). Farmers use the term "temperament" to describe cattle behavior during handling. Temperament is one of the most important parameters in livestock production. It contributes to animal productivity and meat quality (2), animal welfare (3, 4), immunity (5, 6), and even the safety of those handling the animals (7). For example, cattle with excitable temperaments have lower average daily gain and higher mortality rates than those with calm temperaments (8, 9), suggesting that the temperament of cattle is a critical parameter for farmers because of the monetary impact.

Handling and rearing can affect cattle temperament. Cattle that are frequently handled tend to become more docile than those that are less handled (10); however, excessive handling could be detrimental in animal management with regard to human safety since these individuals develop no flight zone. In contrast, extensively managed beef cattle are relatively unfamiliar with humans (11). Less frequent human-animal interactions make cattle fearful, which can cause them to behave aggressively during handling (10). Social interactions between animals also affect their temperament (12). The presence of peers reduces the stress responses to fear-inducing situations in cattle (13). When peers are in sight, heifers display less behavior indicative of distress in response to a novel object (14). Cows engage in many more active social interactions with other individuals when they are grazed than when they are confined with tethering (15); thus, grazing could have a positive impact on temperament *via* social interactions.

Physical condition also relates to temperament. Calm and excitable cattle have different cortisol concentrations (16). Grazed cows have lower cortisol concentrations than confined cows (17). The change in stress susceptibility could change the behavior of cattle during handling and restraint. However, a report showed that calves kept indoors were calmer than calves kept outdoors (18), implying that grazing may adversely affect temperament. This inconsistency in the relationship between grazing and temperament traits may be attributable to the frequency of contact between humans and cattle (10). In addition, cattle's previous experiences can shape their future reactions to humans (10). For example, a negative experience such as poor handling and holding in a yard environment by the handler increases cattle reactivity (19), whereas a positive experience such as gentle handling by the handler reduces animal reactivity in future handling (20). Generally, the system used to rear Japanese Black beef cows requires frequent contact with humans during daily management practices. Japanese Black beef cows gain experience with handling, tethering and other types of interactions during the rearing process. Thus, the effects of grazing on the temperament of calves may not be the same as the effects of grazing on the temperament of adult dairy cows and beef cows that come into contact with humans on a daily basis. The visual analog scale (VAS) is a quantitative assessment with high intra- and inter-observer reliability. It is considered a reliable and practical assessment method for cattle temperament evaluation, although it is not generally superior to other methods (21).

The aim of this study was to reveal, mainly using the VAS, whether grazing affects the temperament of beef cows in various situations encountered in daily management practices, such as weighing, handling, and blood sampling.

MATERIALS AND METHODS

Animals, Housing, Grazing, and Diets

This study was conducted at the Minokamo livestock farm, Gifu Field Science Center, Gifu University (longitude 137°03′57″E; latitude 35°26′44″N), from June to August 2018. Nine Japanese Black cows (344 ± 32 kg body weight (BW), 7.7 ± 3.0 year, not lactating and not pregnant) with no clinical signs of disease and no external injury at the start of the experiment were used. All cows were housed in an 8 m × 7.3 m indoor pen and tethered to tie stalls in a closed barn for the first 2 weeks of the experiment. Each cow was tethered with a rope but was able to engage in social interactions with neighboring individuals. The pen had a concrete floor covered with sawdust bedding.

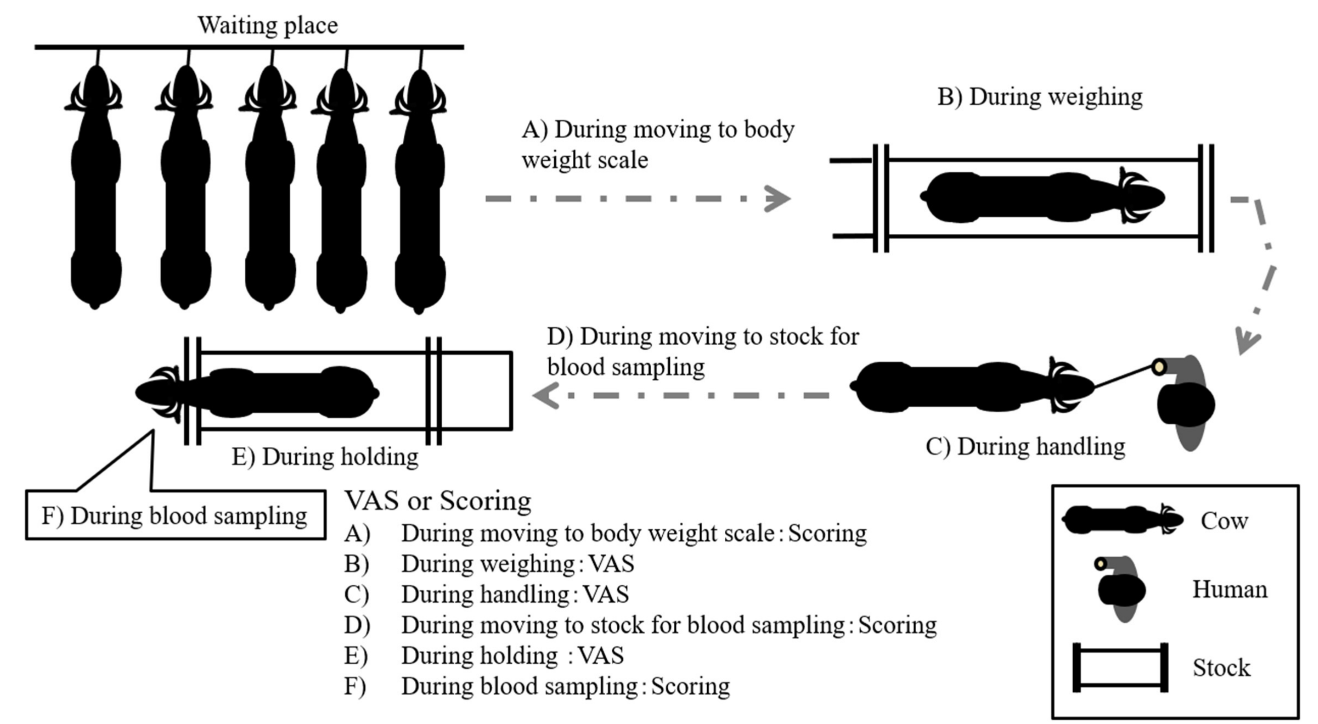

FIGURE 1 | Outline of the evaluation of temperament.

The cows were fed ∼5 kg/day Sudan grass hay, 1 kg/day wheat bran and 50 g/day calcium phosphate on an as-fed basis at 08:00 and 16:00 h according to the Japanese Feeding Standard for beef cattle (22). The cows had free access to water and mineral salt blocks. Then, five of the nine cows were rotated as a group between grazing on a 1.8-ha pasture composed of sown pasture, which was dominated by Italian rye grass [*Lolium multiflorum* (Lam.)], and a forestland for 3 months (**grazed cow: GC**). The dry matter (DM) content and grass height of the herbage in the sown grassland were 22.4% and 62.9 cm, respectively. The grazing area was divided into four paddocks, and the cows were rotated among the paddocks based on the availability of forage. The grazing cows remained outside all day and consumed only the herbage in the pasture, with access to a mineral salt block and water. The remaining cows were maintained under the confined conditions described above (**confined cow: CC**) for 3 months. The cows were allocated to the conditions so that average weight and age were matched as closely as possible between groups. In addition, all cows were old enough to have considerable experience with tethering, handling by humans and grazing in farm management. The frequency of daily monitoring was the same for the GCs and CCs, although the CCs also came into contact with farm staff when they were fed and when their pen was cleaned. The mean ambient temperature and humidity were 27.2 ± 5.1°C and 69.7 ± 19% during the experiment, respectively.

Temperament Trait Analysis

The average BW and age of GCs were 351 ± 30 kg and 10.0 ± 2.1 year, respectively, whereas those of CCs were 336 ± 37 kg and 9.8 ± 4.2 year, respectively. The age of all cows was over 6 year. The temperament traits of the cows were observed on days 0, 38, 52, 72, and 86 after the start of grazing. The recording of the temperament traits started at 08:00 h before feeding. The observational procedure was as follows: First, a handler moved each cow from a waiting place to a body weight scale using a handling rope (**Figure 1A**). The distance from the waiting place to the body weight scale was ∼15 m. The intensity of cow resistance during this movement was recorded and analyzed using the scoring system described below (**Table 2**). Then, the cows were weighed on the scale, and the intensity of cow resistance with regard to each individual behavior was recorded for 2 min [visual analog scale (VAS): 21] (**Figure 1B**). After weighing, the cow was held in one place by the handler for 2 min (**Figure 1C**). The distance from the body weight scale to the location for holding by the handler was ∼2 m. The length of the rope from the cow to the handler was kept at ∼1 m. The intensity of the cow's resistance with regard to each behavior while standing was recorded and analyzed with the VAS. Subsequently, the handler moved the cow into a stock to enable a blood sample to be drawn (**Figure 1D**). The distance from the location for holding by the handler to the stock used for blood sampling was ∼30 m. The intensity of the cow's resistance while

TABLE 1 | Definition of temperament traits and visual analog scale (VAS).

	Endpoints of VAS		Definition
Temperament trait	**Min (0)**	**Max (10)**	
Head movement	No movement	Head permanently moving/violent struggling	The head is displaced horizontally and/or vertically in relation to the median plane (23)
Tail flicking	No flicking	Constant flicking	Tail movement to the left or right of the center and back again, i.e., a tail movement from the left to the right side would count as two flicks (24)
Walking/stepping	No walking/stepping	Continuous walking/stepping	Two or more limbs are alternately raised and make contact with the ground again, with or without ground covered between movements
Rope tension[a]	Loose	Tightened	Evaluates whether the rope used to tie the cattle forms a curve (relaxed) or a straight line (tensed) (25)
Overall movement	calmness	Wild/Aggressive	

[a] *The tethering test only.*

TABLE 2 | Definition of temperament trait score.

Timing	**Temperament trait score**				
	1	**2**	**3**	**4**	**5**
During moving to body weight measurement scale During moving to stock for blood sampling	No resistance	Almost no resistance	A handler approaches and chases the individual from behind the cow	A handler pushes the individual hard from behind the cow "or" pulls a handling rope strongly from the front of the cow	A handler pushes the individual hard from behind the cow "and" pulls a handling rope strongly from the front of the cow
During blood sampling	No resistance	Slight resistance	Moderate resistance	Considerable resistance	Extreme resistance

TABLE 3 | Correlation (*r*) of visual analog scale (VAS) between two observers.

Temperament trait	Correlation (*r*)		
	Weighing	Tethering	Holding for blood sampling
Overall movement	0.86	0.79	0.74
Head movement	0.89	0.76	0.64
Tail flicking	0.75	0.72	0.53
Walking/stepping	0.88	0.89	0.71
Rope tension[a]	–	0.83	–

[a] *The tethering test only.*

moving to the stock was recorded and analyzed using the scoring system (**Table 2**). In the stock, the cow's behaviors were recorded for the first 2 min and analyzed with the VAS (**Figure 1E**). Finally, the intensity of the cow's resistance during blood sampling was recorded and analyzed using the scoring system (**Table 2**). The collected blood samples were used for further analysis (section Statistical analysis). During the behavioral test, the times of the behavioral test and waiting time per cow were ∼30 min and an hour and a half, respectively, per behavioral test.

The behaviors of all cows were recorded using two video cameras (GZ-MG575, Victor Co., Ltd., Yokohama, Japan) and were analyzed using a VAS (21) or scoring system (**Figure 1**). The VAS is a continuous horizontal scale. This assessment is used to measure the intensity of a behavior on a ten-centimeter scale in analog format (**Table 1**). The behaviors assessed with the VAS were overall movement, head movement, tail flicking, walking/stepping, and tension of the handling rope, as shown in **Table 1**. The inter- and intra-observer reliability of the VAS was confirmed by Vogt et al. (21). The intensity of resistance during the handling procedure was recorded using a scoring system (**Table 2**). This scoring system classified the degree of resistance into five stages from "no resistance [1]" to "intense resistance [5]." Scoring was conducted using a video clip to minimize scoring differences between observers. All video clips were analyzed by two observers. Observers were blinded as to which individual was in grazing or confinement when the behaviors of cows were analyzed by the VAS. The correlation between observers with regard to the VAS scores is also shown in **Table 3**.

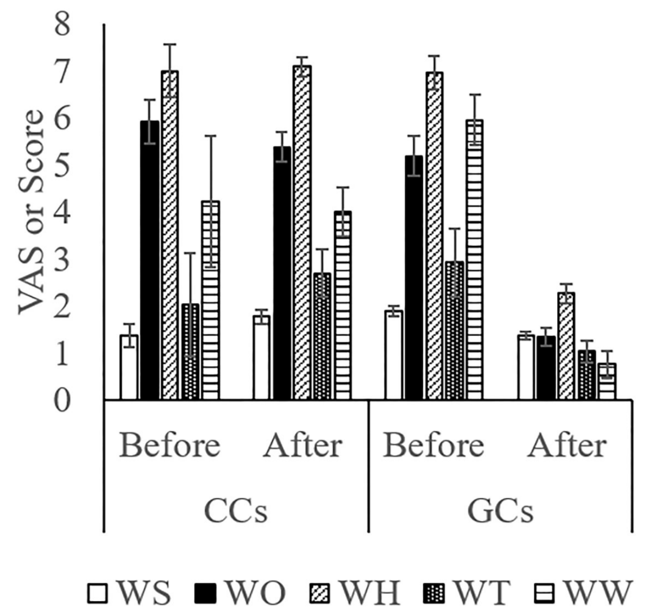

FIGURE 2 | Temperament while moving to the body weight scale and during weighing in GCs and CCs. The horizontal axis shows the temperament before and after the start of grazing. Data are presented as the means ± SEM. WS, The scoring of resistance during movement to the body weight scale; WO, The VAS score for overall movement; WH, The visual analog scale (VAS) score for head movement; WT, The VAS score for tail flicking; WW, The VAS score for walking/stepping.

Blood Analysis

Blood samples were collected from the jugular vein using a vacuum collection tube containing heparin (Venoject II vacuum blood collection tube, TERUMO Co., Ltd., Tokyo, Japan). Blood samples were centrifuged at 1,000 × g at 4°C for 10 min to collect the blood plasma. The plasma samples were stored at −80°C until cortisol analysis. The concentration of cortisol was determined using a commercial kit (Cortisol EIA Kit, Oxford Biomedical Research, Inc., MI, USA).

Statistical Analysis

We calculated the sample size using G*Power version 3.1.9.2 (two-way ANOVA with repeated measures, $\alpha = 0.05$, $(1–\beta) = 0.8$, University of Dusseldorf, Dusseldorf, Germany). Power analyses of temperament traits and blood parameters showed that appropriate power (0.8 or above) to detect differences in 11 of the 17 parameters could be obtained with a total sample size of nine or fewer animals. Considering the cost and availability of experimental cows, the sample size was determined based on the assumption of large effect sizes. The statistical unit in this experiment was the individual animal rather than the treatment group. This unit was chosen because the grazing period lasted 3 months, and it would have been difficult to create several replicates of the grazing treatment due to the limited pasture and herd sizes and the long study period. The adequacy of this approach was described by Connolly (26).

All data were analyzed statistically using the lmerTest package (27) in R software (version 3.0.2: R core team, 2013). Normality tests were conducted using the Shapiro-Wilk test before the analysis. Then, the data were analyzed using a generalized linear mixed model (GLMM) with repeated measurements according to the data distributions. The treatment (GC vs. CC), sampling day (days 0, 38, 52, 72, and 86) and their interaction were considered to be fixed effects, and an individual animal was considered to be a random effect. When the data matched normal distribution, we estimated degrees of freedom, *F*- and *p*-values using type III ANOVAs with Satterthwaite's approximation. While the data matched Poisson and binomial distribution, we used Type II Wald chisquare tests for calculating degree of freedom, Chi-square and *p*-values. Differences were considered

TABLE 4 | Statistical results of the temperament traits test of cows in each situation.

		DF			Num DF			Den DF			F value or Chi-square value			*P*-value		
Situation	**Item**	**S**	**T**	**T × S**	**S**	**T**	**T × S**	**S**	**T**	**T × S**	**S**	**T**	**T × S**	**S**	**T**	**T × S**
During moving to the body weight scale and during the weighing	During moving to body weight scale	3	1	3	–	–	–	–	–	–	17.6[a]	3.8[a]	3.2[a]	<0.05	0.05	0.36
	Head movement	–	–	–	3	1	3	21	7	21	1.5	130.0	1.0	0.26	<0.01	0.42
	Tail flicking	3	1	3	–	–	–	–	–	–	1802.6[a]	9.2[a]	252.4[a]	<0.01	<0.01	<0.01
	Walking/Stepping	–	–	–	3	1	3	21	7	21	2.0	26.7	1.2	0.15	<0.01	0.35
	Overall movement	–	–	–	3	1	3	21	7	21	4.6	84.3	2.4	0.01	<0.01	0.1
During handling	Head movement	–	–	–	3	1	3	21	7	21	0.7	57.5	1.0	0.55	<0.01	0.39
	Tail flicking	–	–	–	3	1	3	21	7	21	18.3	6.6	0.8	<0.01	<0.01	<0.01
	Walking / Stepping	–	–	–	3	1	3	21	7	21	2.0	88.2	0.1	0.14	<0.01	0.96
	Rope tension	–	–	–	3	1	3	21	7	21	1.8	30.1	0.2	0.17	<0.01	0.89
	Overall movement	–	–	–	3	1	3	21	7	21	4.7	68.8	0.3	0.01	<0.01	0.8
During moving to the stock for blood sampling, holding in the device, and during blood sampling	During moving to stock	3	1	3	–	–	–	–	–	–	31.5[a]	0[a]	25.2[a]	<0.01	0.93	<0.01
	Head movement	–	–	–	3	1	3	21	7	21	2.3	8.5	2.4	0.1	0.02	0.1
	Tail flicking	–	–	–	3	1	3	21	7	21	4.1	1.9	2.7	0.02	0.22	0.07
	Walking / Stepping	–	–	–	3	1	3	21	7	21	0.5	1.7	2.0	0.71	0.23	0.14
	Overall movement	–	–	–	3	1	3	21	7	21	12.6	3.7	3.6	<0.01	0.09	0.03
	During blood sampling	3	1	3	–	–	–	–	–	–	3.6[a]	9.2[a]	18.5[a]	0.31	<0.01	<0.01

DF, Degrees of freedom; Num DF, Numerator degrees of freedom; Den DF, Denominator degrees of freedom; T, Treatment: a significant difference between treatments (GC vs. CC); S, sampling day: a significant difference between sampling days (0, 38, 52, 72, and 86); T × S = treatment × sampling day interaction: a significant difference in the interaction of treatment × sampling day. [a]Chi-square value. Differences were considered significant at $P < 0.05$. A tendency toward significance was indicated by $0.05 < P < 0.1$.

significant at $P < 0.05$. Trends were identified at $0.05 < P < 0.1$.

RESULTS

Temperament Trait Analysis

The score while moving to the body weight scale (**Figure 1A**) was lower in GCs than in CCs after the start of grazing ($P = 0.05$: **Figure 2**; **Supplementary Figure 1**; **Table 4**). During weighing (**Figure 1B**), the VAS scores indicating resistance as expressed by overall movement, head movement, tail flicking, and walking/stepping were significantly lower in GCs than in CCs after the start of grazing (all behaviors: treatment: $P < 0.01$; **Figure 2**, **Supplementary Figure 1**, **Table 4**). During handling (**Figure 1C**), the VAS scores for resistance as expressed by head movement, tail flicking, walking/stepping, rope tension, and overall movement were significantly lower in GCs than in CCs after the start of grazing (all behaviors: treatment: $P < 0.01$; **Figure 3**; **Supplementary Figure 2**; **Table 4**). During movement to the stock for blood sampling (**Figure 1D**), there were no significant differences in resistance scores between GCs and CCs (treatment: $P = 0.93$). In the stock (**Figure 1E**), the VAS scores for overall movement (treatment: $P = 0.09$) and head movement (treatment: $P = 0.02$) were lower in GCs than in CCs (**Figure 4**; **Supplementary Figure 3**; **Table 4**). During blood sampling (**Figure 1F**), the resistance score was significantly lower in GCs than in CCs (treatment: $P < 0.01$: **Figure 4**; **Supplementary Figure 3**; **Table 4**).

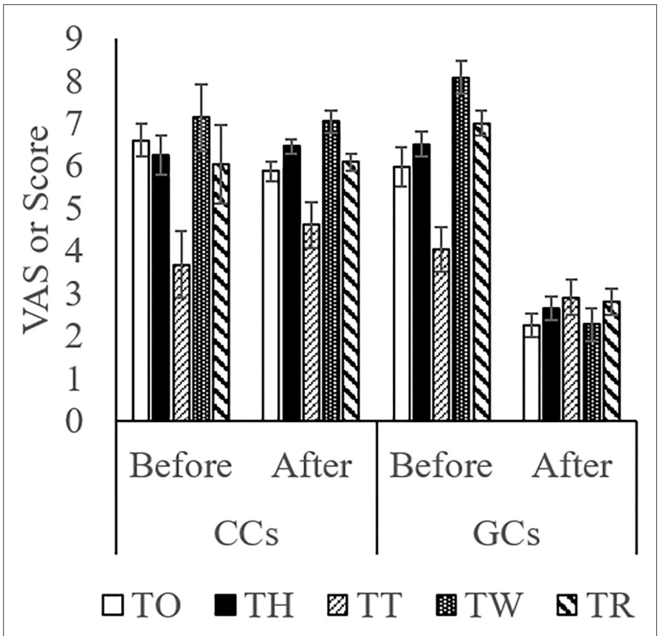

FIGURE 3 | Temperament during handling in GCs and CCs. The horizontal axis shows the temperament before and after the start of grazing. Data are presented as the means ± SEM. TO, The VAS score for overall movement; TH, The visual analog scale (VAS) score for head movement; TT, The VAS score for tail flicking; TW, The VAS score for walking/stepping; TR, The VAS score for rope tension.

Cortisol Analysis

No significant difference was found in the concentration of cortisol between the treatments, nor was the interaction between treatment and sampling day significant (treatment: $P = 0.41$; interaction: $P = 0.85$: **Figure 5**).

DISCUSSION

The GCs were more docile than the CCs in various management situations, including weighing, handling, and holding for blood sampling. The GCs were also calmer while moving to the body weight scale and during blood sampling. These results suggest that grazing has strong impacts on the temperament of cows. The social environment is linked to temperament (12). Grignard et al. (13) reported that the existence of social partners improves the tractability of calves during handling (23). Moreover, housing conditions influence animal affective state and cognitive bias (28). Horses tend to judge optimistically in ambiguous situations when a positive affective state prevails by accessing pasture and contacting conspecifics (29). In general, animals were pessimistic when in a negative affective state, whereas they were optimistic when in a positive affective state. In the present study, GCs engaged in more active social interactions with other individuals than CCs, as we previously reported (15). Thus, although the presence of social partners during the temperament test, including handling and restriction, was the same in both treatments (grazing vs. confinement), the increase in social interaction between GCs and accessing pasture might be one of the factors reinforcing calmness during handling and restriction.

Stress susceptibility is another factor leading to more aggressive behavior. Aggressive individuals have high cortisol concentrations (30). In the present study, the cortisol concentrations in CCs were in the range of 1.6–3.8 ng/ml whereas that in GCs were in the range of 1.7–2.9 ng/ml after the start of grazing, and no significant difference was detected in cortisol concentrations between the GCs and CCs. The cortisol concentrations of both treatments in the present study were close to the basal value of previous studies (31, 32). Thus, the cows in the present study might not be under intense stress. However, Higashiyama et al. (17) reported that the concentration of urinary cortisol increased 3.4-fold when grazing cattle were moved to a confined space, whereas when confined cattle were moved to a pasture, the concentration of cortisol did not increase. Urinary cortisol showed a similar pattern to plasma cortisol with an ∼0.5-h time lag (31). The increase in cortisol concentrations in those previous studies returned to baseline within hours to days, implying that it is necessary to evaluate the relative change of its concentration at short intervals. In addition, our previous study showed that grazing cows had higher antioxidant capacity than confined cows, implying that grazing cows are less susceptible to physiological stress (33). Thus, susceptibility to stress under different feeding conditions might still impact animal temperament, and further study is needed.

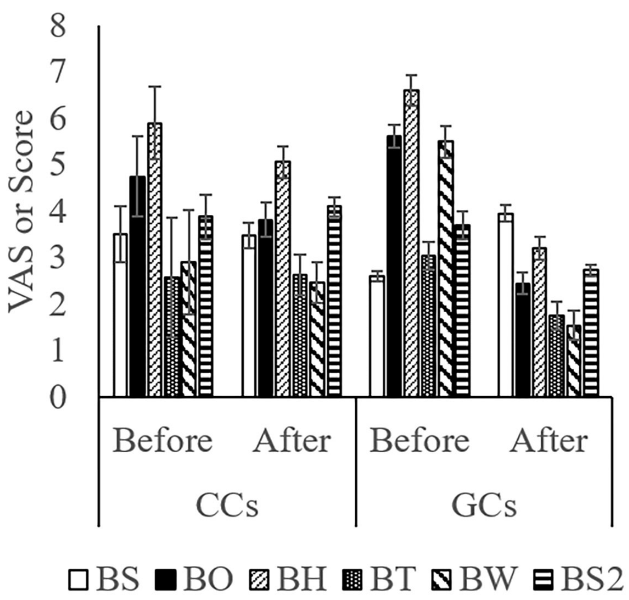

FIGURE 4 | Temperament traits while moving to the stock for blood sampling, while in the device, and during blood sampling in GCs and CCs. The horizontal axis shows the temperament before and after the start of grazing. Data are presented as the means ± SEM. BS, The score for resistance while moving to the stock for blood sampling; BH, The visual analog scale (VAS) score for head movement; BT, The VAS score for tail flicking; BW, The VAS score for walking/stepping; BO, The VAS score for overall movement; BS2, The score for resistance during blood sampling.

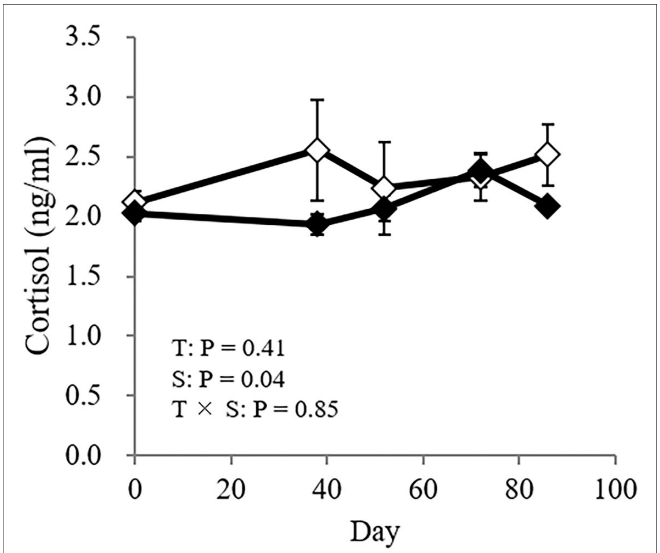

FIGURE 5 | The cortisol concentration in GCs (♦) and CCs (◇). The horizontal axis shows the day on which the blood sample for the cortisol analysis was obtained, and day 0 (0) represents the day the cattle were moved to a pasture. Data are presented as the means ± SEM. T, Treatment: a significant difference between treatments (GC vs. CC); S, sampling day: a significant difference between sampling days (0, 38, 52, 72, and 86); T × S = treatment × sampling day interaction: a significant difference in the interaction of treatment × sampling day. Differences were considered significant at $P < 0.05$. A tendency toward significance was indicated by $0.05 < P < 0.1$.

Experiences, including handling and transporting, affect temperament (34). Boivin et al. (18) reported that grazing caused a reduction in the expression of calm temperament traits in calves. The findings of the present study were inconsistent with the results of Boivin's study (18). However, the cows in the present study were adults that had experience with various management practices, including grazing, and were frequently handled by farm staff before this study. Moreover, age, breed, and genetics affect temperament (35). These traits of calves investigated in Boivin's study are different from those in the present study. Thus, the differences between the studies may induce different behavioral responses to grazing.

CONCLUSIONS

The present study showed that grazing was related to higher docility of cows in various management situations, such as weighing, handling, and blood sampling. Grazing may have contributed to mitigating the reaction to human-cattle interaction during handling and the reactions of cattle to restraint and painful operations. This is the first study to suggest a relationship between grazing and temperament in cows. Further study is needed to reveal the relationships between temperament traits and environmental factors such as social connections (29), stress conditions or ingestion of plants with antioxidants (33) while grazing.

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/s.

ETHICS STATEMENT

All animal experimental procedures were approved by the Committee for Animal Research and Welfare of Gifu University (#17140) and conducted in accordance with the guidelines for animal research and welfare of Gifu University.

AUTHOR CONTRIBUTIONS

NN designed the study, collected the data, conducted the statistical analysis, interpreted the data, and wrote and developed the manuscript. MY contributed to designing the study and interpreting the data and reviewed and developed the manuscript. HM conducted most of the data sampling and experimental work. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fvets.2021.705764/full#supplementary-material

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Feasibility and Reliability of the AWIN Welfare Assessment Protocol for Dairy Goats in Semi-extensive Farming Conditions

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The aim of this study was to test the feasibility and reliability of the Animal Welfare Indicators (AWIN) protocol for welfare assessment of dairy goats when applied to semi-extensive farming conditions. We recruited 13 farms located in the NW Italian Alps where three assessors individually and independently applied a modified version of the AWIN welfare assessment protocol for goats integrated with some indicators derived from the AWIN welfare assessment protocol for sheep. The applied protocol consisted of nine individual-level (body condition score, hair coat condition, abscesses, overgrown claws, udder asymmetry, fecal soiling, nasal discharge, ocular discharge, and improper disbudding) and seven group-level (severe lameness, Qualitative Behavior Assessment-QBA, thermal stress, oblivion, Familiar Human Approach Test-FHAT, synchrony at grazing, synchrony at resting) animal-based indicators. On most farms, the level of welfare was good. Many of the considered welfare problems (overgrown claws, fecal soiling, discharges, and thermal stress) were never recorded. However, oblivion, severe lameness, hair coat condition and abscesses were detected on some farms, with percentages ranging from 5 to 35%. The mean percentage of animals with normal body condition was 67.9 ± 5.7. The level of synchronization during resting was on average low (14.3 ± 7.2%). The application of the whole protocol required more than 4 h/farm and 3 min/goat. The inter-observer reliability varied from excellent (udder asymmetry, overgrown claws, discharges, synchrony at resting, use of shelter) to acceptable (abscesses, fecal soiling, and oblivion), but insufficient for hair coat condition, improper disbudding, synchrony at grazing, QBA. Differences in background of the assessors and feasibility constraints (i.e., use of binoculars in unfenced pastures, individual-level assessment conducted during the morning milking in narrow and dark pens, difficulties when using the scan and instantaneous sampling method due to the high number of animals that moved at the same time) can affect the reliability of data collection. Extensive training seems necessary for properly scoring animals when applying the QBA, whereas the

FHAT to evaluate the Human-Animal Relationship of goats at pasture seems promising but needs to be validated. Indicators that evaluate the synchrony of activities require to be validated to identify the best moment to perform the observations during the day.

Keywords: animal welfare indicators, *Capra hircus*, extensive husbandry systems, feasibility, inter-observer reliability

INTRODUCTION

Welfare assessment in extensive production systems has received a lower interest in research than in intensive husbandry systems (1). This is partly due to the belief that animals in open ranges live a more natural life, hence welfare issues are perceived as a minor risk (2). It is undeniable that farm animals at pasture can express a fuller behavioral repertoire, exercise during the day and enjoy the benefit of sun (3). However, the efforts to adapt to outdoor conditions can be costly, for example in terms of thermoregulatory activity and fulfillment of nutritional requirements. Therefore, extensive livestock systems require specific indicators in order to properly assess the welfare of animals, considering the variety of issues they may face (1). Furthermore, differently from intensive husbandry systems that are quite similar across Europe and industrialized countries, pasture-based systems present an extreme variability in relation to the environmental context, and therefore they require a wider range of indicators that encompass all the possible contexts of application (4).

There are at least three issues that need to be considered in case of welfare assessment in extensive conditions: (1) although valid indicators for welfare assessment in intensive/indoor husbandry systems are already available, many of them still need to be tested for validity under extensive conditions and, in some cases, new indicators need to be identified (5–7); (2) the feasibility of data collection may be compromised due to different management and environmental conditions in outdoor systems (e.g., adverse climatic conditions or difficulty to restrain animals for close examination); (3) data collection under these difficult conditions may affect the reliability of the results (e.g., the assessor may not be able to reach the animals for close inspection, and might be forced to use optical instruments for inspecting animals at distance). Therefore, in spite of the fact that feasibility traits of indicators and their reliability are fundamental pre-requisites that determine the effective application of a protocol (8), these last two issues present possible constraints when assessing animal welfare in open ranges.

Compared to other species [e.g. cattle, pigs; (9)], goats are more often raised in developing countries or marginal areas (Asia—especially China, India, Bangladesh, Pakistan, Africa, and Middle East), mainly in smallholder and mixed farming systems. Worldwide, goats are an important component of pastoralist herds. Even in Europe, goat farming is common in marginal areas, where other agricultural activities would be impracticable, and therefore is an important activity which limits the abandonment of such areas (10).

Even if there is a need for assessing the welfare of goats in extensive systems, the scarcity of the research on this topic (1) makes it difficult to find suitable indicators to develop a valid and comprehensive welfare assessment protocol. A recent review on welfare assessment of ruminants at pasture identified 33 animal-based indicators for cattle and 20 for small ruminants, namely sheep and goats (11). However, only three of these indicators were developed and tested specifically for goats in extensive conditions: Qualitative Behavior Assessment (12), Body Condition Score (13), and body weight (13).

In 2011–2015, an EU-funded project on Animal Welfare Indicators (AWIN) developed on-farm welfare assessment protocols for sheep, goats, horses, donkeys and turkeys, possibly using animal-based indicators (14, 15). Despite a common approach, each AWIN protocol has its own characteristics and target category depending on the species. To give an example, the sheep protocol is intended for adult ewes, both for milk and meat production, bred indoor and/or outdoor, whereas the goat protocol was only developed for adult dairy goats in intensive (defined as those in which goats are permanently kept indoors and diet is mainly composed of preserved forages and concentrate) or semi-intensive (similar to the intensive ones, but with occasional access to pasture) husbandry systems (16, 17). These systems differ from those that rely almost exclusively on natural resources for feeding, with no or limited access to housing structures (extensive systems) or from those that rely mainly on pasture, with limited use of feed supplements in periods of greatest need, and the presence of facilities for sheltering animals in case of need (semi-extensive systems).

Research on the assessment of animal welfare in goats kept in semi-extensive and extensive systems is relatively new. An attempt to compare the application of the AWIN protocol in semi-intensive and extensive husbandry systems was carried out in Brazil on meat goat does (18). Since the AWIN welfare assessment protocol was developed for intensive dairy goat farms, in that study the authors only retained few indicators from the original protocol, added some from the AWIN welfare assessment protocol for sheep, and some indicators were partly modified and/or proposed *ex novo*. Unfortunately, the research performed by Leite Oliveira et al. (18) does not clarify the process that led to the selection and/or exclusion of some indicators from the goat protocol, nor if the indicators extrapolated from the sheep protocol were reliable also for meat goats. However, this study provides useful information about the feasibility of such protocol under extensive farming conditions in Brazil. An adapted version of the AWIN welfare assessment protocol was applied to 41 farms housing double-purpose goats in Central Portugal (19, 20). The farms included in that study reflected the husbandry systems of Portuguese rural areas: goats were housed at night in sheds or stables underneath farmers' houses and taken to pasture almost every day, in mixed flocks together with sheep. The authors stated that the removal of few animal-based indicators (e.g., queueing at feeding and at drinking) and the addition of few resource- and management-based indicators to the original AWIN protocol increased the suitability of this protocol to the context, making it more feasible. The concurrent validity of some newly introduced indicators, such as the number of days at pasture, was verified based on its relationship with already validated animal-based indicators, such as the prevalence of overgrown claws. Although the reliability of the protocol was not specifically evaluated, the new indicators are supposed to be reliable, as they consist mainly of easy-to-collect resource and management information.

The aim of this research is to test the feasibility and reliability of a protocol for welfare assessment of dairy goats in semi-extensive systems, which are commonly found in the Italian Alps, using a modified version of the AWIN welfare assessment protocol for goats, integrated with some indicators derived from the sheep protocol.

MATERIALS AND METHODS

Farms

Goat farms were extracted from a database of 163 farms, provided by the ASL (Local Health Center) TO3 territory of Pinerolo-Collegno (Province of Turin, Piedmont, NW Italy). From the database, we selected farms presenting the following characteristics: (i) raising goats for dairy or dual purposes; (ii) making use of outdoor grazing in spring and autumn in proximity to the winter housing buildings; (iii) keeping goats only, with no coexistence with other domestic species; (iv) breeding prevalently Alpine and Valdostana breeds (and their crossbreeds, Alpine × Valdostana); (v) voluntary acceptance of the farmer. Only 13 farms satisfied all the inclusion criteria and were therefore included in the survey. None of the selected farms bred animals for dual purpose; hence, the assessment was only performed on dairy animals.

These farms housed the goats during the winter in indoor pens or, on three farms, in tie stalls. During spring and autumn, the animals were housed during the night and they were released in flat to medium/steep slope areas (ranging from 470 to 920 m a.s.l.) near the farms after the morning milking, giving them the opportunity to graze in meadows, but also to browse the surrounding bushy and woody areas. In some farms, only bushes and woods were available for foraging. In nine farms, woods were also used as shelters to protect the goats from wind, sun, and rain. When woods were not available, the goats had no shelters. On average the total area available for spring and autumn pasture was equal to 20,872.73 m^2, but large differences were found among the farms (min: 110 m^2; max: >100,000 m^2). The average available pasture area/goat was equal to 343.47 m^2 (SD: 502.82 m^2; min: 3.55 m^2; max: 1,470.60 m^2). The goats had access to pasture for 90–250 days/year for 4–12 h/day, except in one farm where they had permanent access to the outdoor grazing area. During summer, the goats were taken to alpine ranges from June to October for a total period of 90–180 days.

The total number of goats in our farm sample ranged from 12 to 77 with a mean (±SD) of 31.2 (±20.74) goats. Lactating goats ranged from 8 to 77 animals, with a mean (±SD) of 17.70 (±18.71) goats. The average age of lactating goats ranged from 24 to 78 months. Goats were in their mid-lactation stage and were milked twice a day. In 12 farms, the goats were manually milked, while one farm was provided with a mobile milking unit. Besides making use of fresh grass and bushes available in the grazing area, once or twice a day, during milking, eight farms provided supplementary feed consisting of hay, chestnuts, alfalfa, bran, whole or flaked barley, and whole or flaked corn. Three farms provided supplementary feed consisting of whole, flaked or mash corn, with or without mineral supplementation, in the winter period only. In two farms, no supplementary feed was delivered. Fresh and clean water was always available inside the barn. At pasture, water was available through streams (five farms) or watering tanks (four farms); water was not available at pasture in four farms. The distance of water from the pasture area ranged from 0 to 1,500 m.

Claw trimming occurred once a year in six farms, when necessary in five farms, every 6 months in one farm and every 4 months in another farm.

All the farms produced cheese from pure goat milk in small dairies adjacent to the farms. The cheeses were sold at the farm shop, at local markets or were destined to the small-scale organized distribution.

Assessors

In each farm, the welfare assessment protocol was tested in the period April–July 2019, during the spring grazing period. In order to test protocol reliability, the assessment was carried out by three assessors who had different background and level of experience with dairy goats. The three assessors were students of the MSc in Animal Science at the University of Turin (Italy). Assessor A also had a M.Sc. in Veterinary Science and in Biostatistics, worked as a veterinarian in the Public Health Service and had more than 10 years of experience with dairy goats. Assessors B and C had no specific experience with dairy goats. The three assessors received a common training before the beginning of the study, including both theoretical and practical sessions, and received the AWIN protocol (17) as training material. The training was given by two authors of the AWIN welfare assessment protocol for goats kept in intensive or semi-intensive production systems.

Data Collection

Farmers were contacted by telephone to illustrate the research and gather essential information about the farm routine, in order to identify the best time for welfare assessment, which depended mainly on milking time.

On each farm, the protocol was applied simultaneously and individually by the three assessors, without any kind of interaction among them. All the assessors were unknown to the farms. The assessments took place mostly under sunny weather (77%), with some cloudy days but never when raining. Visibility was always good. Wind was almost absent. Ambient temperatures ranged from 9 to 24°C, with an average of 18°C. Relative humidity ranged between 21 and 90%, with an average of 54%.

TABLE 1 | Indicators applied for refining the AWIN welfare assessment protocol for goats to semi-extensive farming conditions.

Indicator	Description	Origin[a]	Level[b]	Location[c]	Notes
Hair coat condition[1]	Goats with poor hair coat condition (described as: matted, rough, scurfy, uneven, shaggy hair coat, frequently longer than normal) are recorded	A	I	M	Converted to an individual-level indicator (formerly group-level indicator in the AWIN welfare assessment protocol for goats)
Body condition score[2]	BCS is visually assessed at the rear of individual goat, using a three-level scoring method	A	I	M	Retained as it is
Abscesses[1]	The presence of abscesses (ruptured or not) is recorded	A	I	M	Retained as it is from the 2nd level
Overgrown claws[1]	The presence of overgrown rear claws (exceeding the normal length and/or width leading to a loss of the common triangular profile) is visually assessed on individual goats	A	I	M	Retained as it is
Udder asymmetry [1]	The presence of one half of the udder that is at least 25% longer than the other is recorded	A	I	M	Retained as it is
Fecal soiling[1]	The presence of soft fecal matter below the tail head and on both sides of the tail is visually assessed on individual goats, as a sign of diarrhea	A	I	M	Retained as it is
Nasal discharge[1]	The presence of any mucous or purulent discharge (white or yellowish) from the nose is visually assessed on individual goats	A	I	M	Retained as it is
Ocular discharge[1]	The presence of clearly visible flow from one or two eyes is visually assessed on individual goats	A	I	M	Retained as it is
Improper disbudding[1]	Goats showing presence of residual horns (scurs) are recorded	A	I	M	Converted to an individual-level indicator (formerly group-level indicator in the AWIN welfare assessment protocol for goats)
Severe lameness[1]	Goats showing signs of severe lameness (based on abnormal gait, head nodding, spine curvature, kneeling) are recorded	A	G	T	Assessed when goats were brought to pasture
Qualitative Behavior Assessment (QBA)	The assessor integrates perceived details of behavior, posture and context into the summarization of an animal's style of behaving, or "body language", using a fixed list of descriptors. List of descriptors: aggressive, agitated, alert, bored, content, curious, fearful, frustrated, irritated, lively, relaxed, sociable, suffering	A	G	P	Retained as it is, but animals can be observed from only one observation point, with sessions lasting maximum 10 min
Synchrony at grazing	The number of goats grazing simultaneously is recorded, using an instantaneous and scan sampling method (60 min observation session, 30 min scan intervals)	N	G	P	Synchronization during grazing is usually evaluated using scan sampling method (21)
Thermal stress	The number of animals showing signs of heat or cold stress is recorded	A	G	P	The indicator was retained as it is, but it was collected using a scan sampling method (60 min observation session, 30 min scan intervals)
Oblivion	The number of oblivious goats is recorded. An oblivious goat is defined as an animal, which is physically or mentally isolated from the group	A	G	P	The indicator was retained as it is, but it was collected using a scan sampling method (60 min observation session, 30 min scan intervals)
Familiar human approach	The closest possible distance of approach from the farmer before an elicited flight response is recorded If no flight response is triggered (goats remain motionless at human approach) this is recorded as 0 m If the goats actively move toward (goats walk directly toward the stockperson) and interact (sniffing, nosing) with the stockperson, this is also recorded	N	G	P	(16)

(Continued)

TABLE 1 | Continued

Indicator	Description	Origin[a]	Level[b]	Location[c]	Notes
Synchrony at resting	The number of goats resting simultaneously is recorded, using an instantaneous and scan sampling method (60 min observation session, 30 min scan intervals)	N	G	P	Synchronization during resting is usually evaluated using scan sampling method (22)
Use of shelter	The number of goats resting simultaneously using a shelter is recorded, with instantaneous and scan sampling method (60 min observation session, 30 min scan intervals)	N	G	P	We considered the inclusion of this indicator not only as presence/absence of shelters [as in the AWIN welfare assessment protocol for sheep; (16)], but evaluating the effective use of shelters when present
Queuing at feeding	The number of goats queuing at the feed rack is counted during feeding time, using a scan sampling method during 15 min/observation (2 min/scan)	E			No feed delivered at the feeding rack during spring, but goats have access to pasture
Queuing at drinking	The number of goats queuing at the drinker is counted during feeding time, using a scan sampling method during 15 min/observation (2 min/scan)	E			No drinkers available
Kneeling at the feeding rack	The number of kneeling goats (front legs flexed, the rear up) is counted while they are at the feeding rack	E			No feed delivered at the feeding rack during spring
Latency to first contact test	The time elapsed from when the assessor stops in a pre-determined starting place in the pen and the contact with the first goat that nuzzles or touches any part of the assessor's body is recorded (max time: 300 s). After assessing the Latency to first contact test, the assessor leaves the pen before reentering to perform the Avoidance distance test	E			This test is not applicable outdoors
Bedding	Evaluation of the quantity and cleanliness of the bedding in the pen	E			This test is not applicable outdoors

When indicators are "Retained as it is" this is referred to the original AWIN welfare assessment protocol for goats. Further specifications are listed in the table.
[1] *Dichotomous categorical variable (absence = 0; presence = 1).*
[2] *Trichotomous categorical variable (very lean = −1; normal body condition = 0; very fat = 1).*
[a] *A, indicator retained from (17); N, new indicator, not originally present in (17); E, indicator from (17) which was excluded in the current protocol.*
[b] *I, individual level; G, group level.*
[c] *M, during the morning milking; T, during transfer from milking area to pasture area; P, at pasture.*

The protocol included 13 out of the 18 original indicators from the 2nd level AWIN welfare assessment protocol for goats (17), plus four new indicators. Some of the original indicators had to be adapted to the semi-extensive conditions (**Table 1**). The whole protocol applied in our study consisted of nine individual-level and seven group-level indicators. Some indicators of the AWIN protocol for goats kept in intensive or semi-intensive production systems were discarded, as they had no meaning or could not be applied in the context of extensive farming (**Table 1**). The main change from the original AWIN welfare assessment protocol for goats was the order of collection of the indicators that was modified to better adapt to the semi-extensive system, where animals are released outdoors in the morning after the milking routine (**Figure 1**). The animals were always milked in small pens inside the barn. Data collection started with individual-level assessment during the morning milking of the following indicators: body condition score, hair coat condition, abscesses, overgrown claws, udder asymmetry, fecal soiling, nasal discharge, ocular discharge, and improper disbudding. For these indicators, the same scoring—as detailed in the AWIN protocol for goats—was used (17). At the end of milking, the goats were brought outdoors by the farmer and allowed to graze pasture areas located near the farm. On the way to pasture (walking a distance of 300 m up to 1 km), severe lameness was recorded, based on the observation of abnormal gait, head nodding, spine curvature and kneeling (17). Due to management reasons, lactating goats were mixed with dry goats and yearlings at pasture; hence, the following group-level indicators were recorded both on lactating and non-lactating animals. The assessors first conducted the Qualitative Behavior Assessment (QBA), using the 13 descriptors detailed in the AWIN welfare assessment protocol for goats (17). QBA observations were always performed from one observation point for 10 min on the whole group of goats (12, 23). Then, using a scan sampling method (60 min observation period with 30 min scan intervals, i.e., three scans at time 0, 30, and 60 min), the assessors recorded the number of goats that

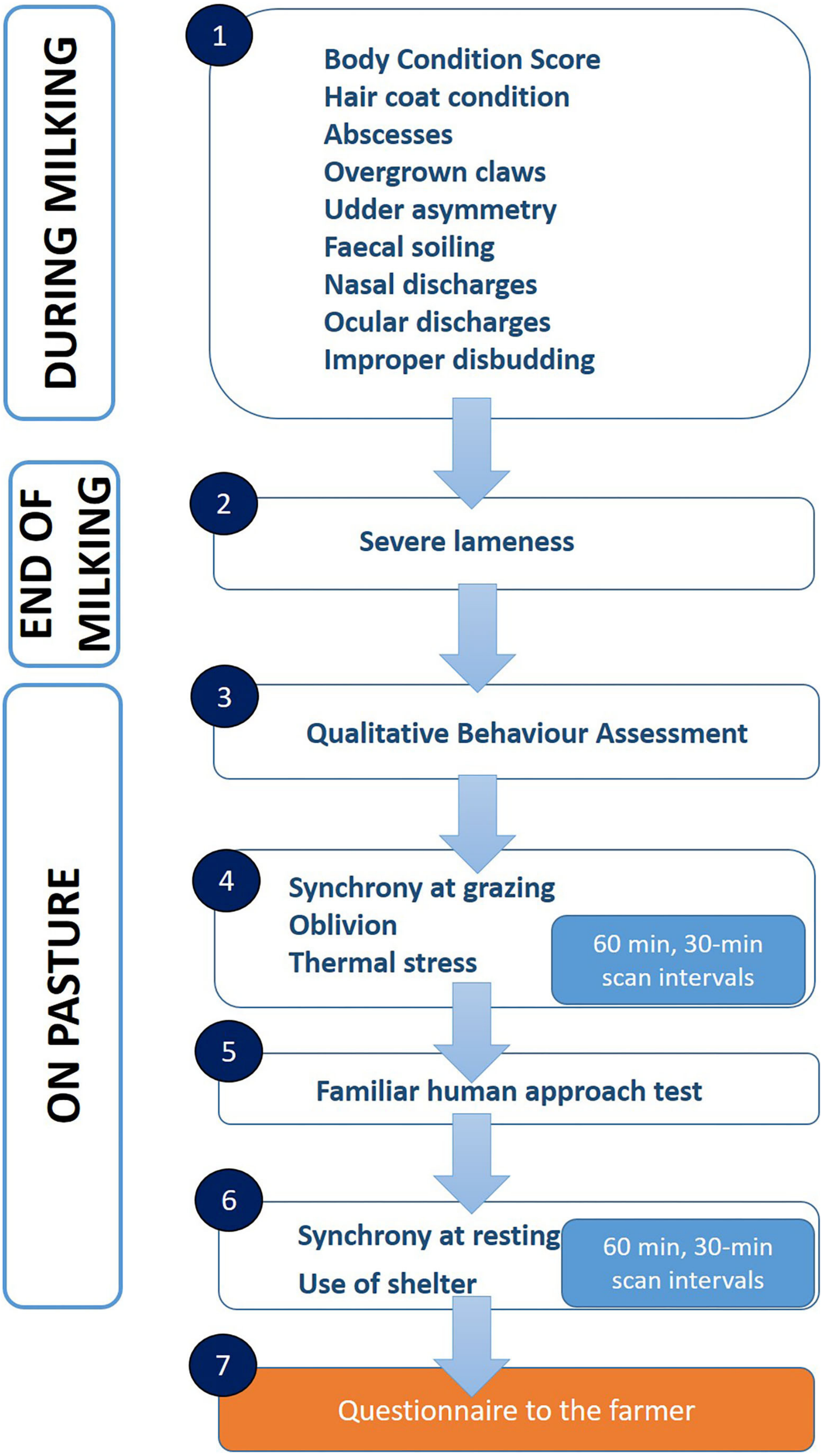

FIGURE 1 | Flowchart of data collection.

grazed simultaneously, the number of goats showing signs of thermal stress (shivering or panting), and the number of goats physically or mentally isolated from the group (oblivion) (17). Then, the quality of the human-animal relationship (HAR) was assessed by using a simplified Familiar Human Approach Test (FHAT), following the procedure described in the AWIN welfare assessment protocol for sheep, but in which only the reaction of goats toward the farmer was assessed (avoidance, contact, approach), whereas the distance expressed in meters was not assessed (16). This decision was made because this was a first attempt to apply a test, which has not been validated for goats yet. Assuming that approximately one and a half hour after milking goats start resting, possibly seeking for adequate shelters (24), a second scan session (60 min observation period with 30 min scan intervals) was then used to record the number of goats resting and the number of goats resting in a sheltered place (if present), specifying the type of shelter.

Assessor A evaluated all the 13 selected farms. Due to unforeseen circumstances, assessors B and C only assessed 12 farms. A minimum of two assessors per farm was always guaranteed.

Data Analysis

For individual-level indicators, absolute and percentage frequencies of animals without welfare problems were calculated. The prevalence of group-level indicators was calculated as the proportion of goats with absence of welfare problems out of the number of assessed goats. For indicators collected with the scan sampling method, we considered the proportion of goats in the scan with the highest number of animals synchronized during grazing and resting or presenting thermal stress or oblivion, out of the total number of goats. FHAT results were reported as the proportion of farms where goats avoided, accepted the contact or spontaneously approached the farmers out of the total number of farms. Principal Component Analysis (PCA, correlation matrix, no rotation) was used to explore results from the QBA. Data was analyzed using the statistic software IBM SPSS v. 26.0 for Windows (25).

The above-mentioned welfare outcomes are presented in the results referring to data collected by Assessor A. This choice is due not only to the fact that this was the only assessor who evaluated all the farms, but also because this assessor had more experience with goats than the others. Therefore, data collected by Assessor A were used as reference and compared with data collected by the other two observers in order to assess inter-observer reliability (IOR).

IOR was only calculated for the 11 farms where all the three assessors were present. For categorical data (individual-level indicators) IOR was calculated over all animals, regardless of farm, using the S index (26, 27). This index, selected in order to overcome the problem of the paradoxical behavior of Cohen's Kappa, considers the null hypothesis for which the agreement between pairs of observers can be considered as the result due to chance and can be calculated using the following equation:

$$S = \frac{p_o - \left(\frac{1}{M}\right)}{1 - \left(\frac{1}{M}\right)}$$

where p_o is the rate of observed concordance and M is the number of categories.

The percentage of concordance agreement was calculated for pairs of assessors against Assessor A (A vs. B and A vs. C) and for the three assessors together (A vs. B vs. C). The S index was only calculated for the three assessors together. For continuous data (group-level indicators), intra-class correlation (ICCs) coefficients were calculated (95% confidence intervals, based on absolute agreement, random effects type, mean-rating). According to Bateson and Martin (28), we adopted the following thresholds to evaluate the quality of reliability: <0.50 = poor; 0.50–0.75 = moderate; 0.76–0.90 = good; >0.90 = excellent.

For QBA, the IOR of Principal Component (PC) scores attributed by the three observers to each farm on the first two PCs was analyzed by using the Kendall Correlation Coefficient W. The results were interpreted according to Martin and Bateson (29, 30), where W: 0.0–0.2 = slight correlation; 0.2–0.4 = low correlation; 0.4–0.7 = moderate correlation; 0.7–0.9 = high correlation; 0.9–1.0 = very high correlation.

According to several authors (7, 29), a guideline for an acceptable threshold of correlation coefficients when assessing IOR might be set at ≥0.7. Even if the literature report different limits (28–30), our results will be discussed following this guideline.

At the end of the assessment, the assessors were asked to report the major constraints experienced during the application of the protocol.

RESULTS

The results of the application by Assessor A of the welfare assessment protocol for dairy goats in semi-extensive conditions are shown in **Table 2**, except for improper disbudding, severe lameness, and FHAT. The proportion of goats properly disbudded is not reported in **Table 2**, as this procedure was performed in one farm only (73.3% of goats properly disbudded). Severe lameness is not reported as only one goat showing this welfare problem was observed. As to the assessment of HAR quality, FHAT shows that in 61.5% of the farms the goats actively moved toward the farmers and interacted with them (sniffing, nosing). In one farm, the goats remained motionless at human approach, whereas in the remaining 30.8% of the farms the approaching farmer elicited a flight response.

Two descriptors (Bored and Frustrated) were scored 0 in all the farms by Assessor A; hence, they were removed from the analysis. PCA was performed on 11 out of 13 descriptors. The analysis identified four main PCs with eigenvalues >1 (4.624, 2.258, 1.753, and 1.080 for PC1, PC2, PC3, and PC4, respectively). The first two PCs together explained 62.57% of the total variance among the farms (PC1: 42.04%; PC2: 20.53%). **Figure 2** shows the distribution of the descriptor loadings and

TABLE 2 | Absence of welfare problems (mean ± SE; min–max) observed in 13 semi-extensive dairy goat farms, recorded during individual- and group-level assessment.

Individual-level assessment		Group-level assessment	
Indicator	**Mean% ± SE% (min%–max%)**	**Indicator**	**Mean% ± SE% (min%–max%)**
Normal body condition	67.9 ± 5.69 (25.0–100.0)	Absence of severe lameness	99.4 ± 0.50 (92.3–100.0)
Good hair coat	97.3 ± 1.99 (75.3–100.0)	Synchrony at grazing	92.5 ± 3.63 (60.5–100.0)
Absence of abscesses	88.1 ± 3.09 (65.0–100.0)	Thermal comfort	100.0 ± 0.00 (100.0–100.0)
Regular claws	100.0 ± 0.00 (100.0–100.0)	Absence of oblivious goats	99.3 ± 0.45 (94.9–100.0)
Symmetric udder	96.2 ± 1.44 (87.0–100.0)	Synchrony at resting	14.3 ± 7.22 (0.0–80.0)
Absence of fecal soiling	100.0 ± 0.00 (100.0–100.0)	Use of shelter (out of goats at resting)	95.1 ± 4.86 (56.3–100.0)
Absence of nasal discharge	100.0 ± 0.00 (100.0–100.0)		
Absence of ocular discharge	100.0 ± 0.00 (100.0–100.0)		

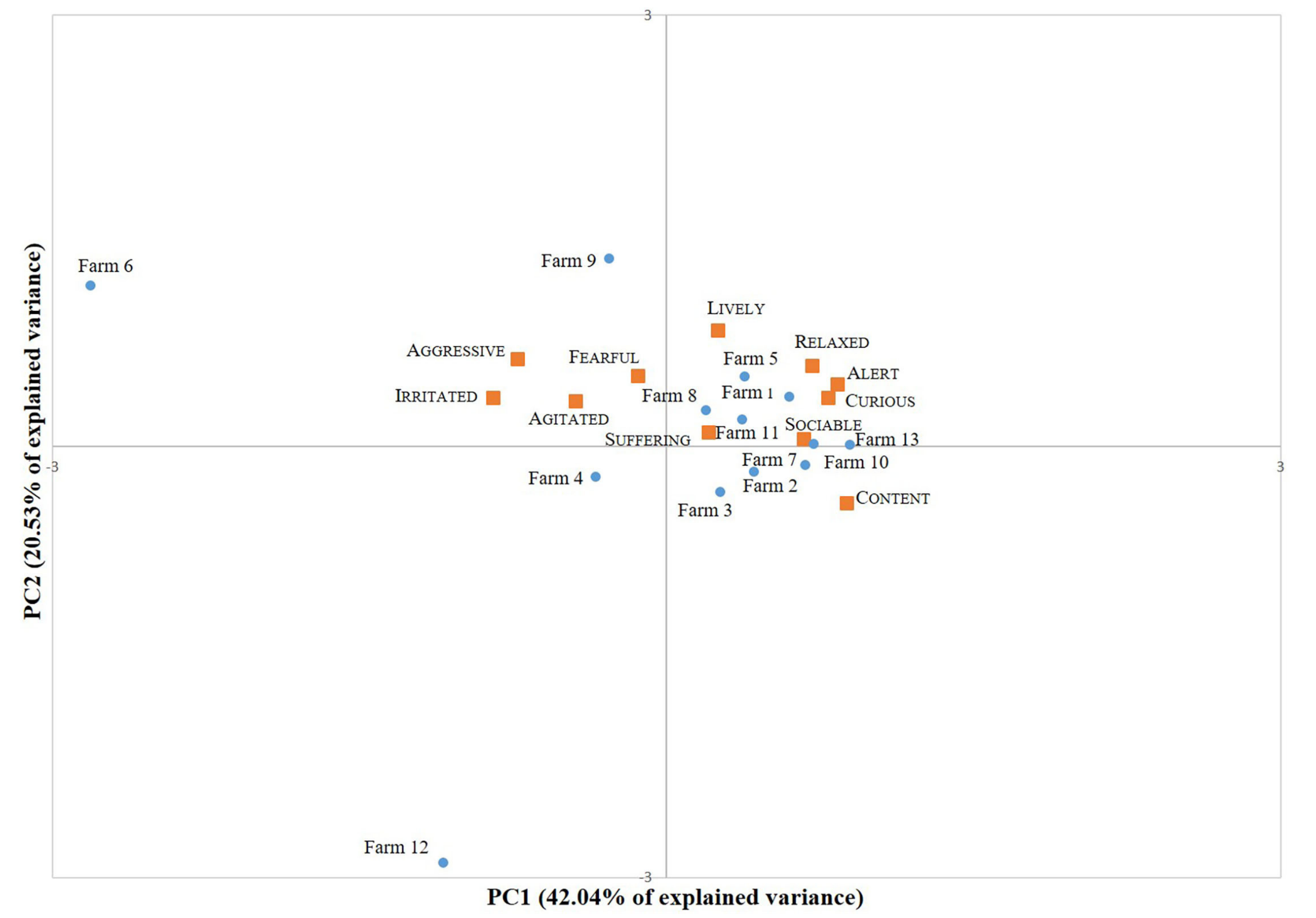

FIGURE 2 | Biplot showing the loadings of the descriptors (■) and the farm scores (●) on PC1 and PC2.

TABLE 3 | Inter-observer reliability for individual-level observations.

Indicator	Concordance agreement (A-B)		Concordance agreement (A-C)		Concordance agreement (A-B-C)		Inter-Observer Reliability (A-B-C)
	N	**%**	***N***	**%**	***N***	**%**	***S* index (LCL-UCL)**
Body condition score	153	78.4	214	70.1	137	75.2	S^{**} = 0.81 (0.77–0.85)
Hair coat condition	153	85.0	214	74.8	137	83.0	S^{*} = 0.66 (0.57–0.76)
Abscesses	152	94.7	197	83.2	129	90.7	S^{*} = 0.85 (0.80–0.90)
Overgrown claws	153	100	214	99.5	137	99.5	S^{*} = 1
Udder asymmetry	152	96.1	214	89.3	136	95.6	S^{*} = 0.91 (0.85–0.96)
Fecal soiling	152	86.8	213	88.7	135	92.1	S^{*} = 0.85 (0.78–0.92)
Nasal discharge	152	100	214	100	136	100	S^{*} = 1
Ocular discharge	152	100	214	100	136	100	S^{*} = 1
Improper disbudding	14	71.4	15	80.0	14	76.2	S^{*} = 0.56 (0.20–0.91)

N = sample size; % = percentage of agreement, LCL, Lower Confidence Level; UCL, Upper Confidence Level. S^{*} *= S weighted with linear weights;* S^{**} *= S weighted with square weights, Thresholds: <0.50 = poor; 0.50–0.75 = moderate; 0.76–0.90 = good; >0.90 = excellent. In bold, indicators where correlation coefficient is ≥0.7.*

TABLE 4 | Inter-observer reliability for group-level observations.

Indicator	Reliability ICC (95% CI)
Synchrony at grazing	0.64 (−0.44 < CI < 0.93)
Oblivion	0.74 (0.32 < CI < 0.92)
Thermal stress	N.D.
Synchrony at resting	0.94 (0.84 < CI < 0.98)
Use of shelter	1 (0.99 < CI < 1)

ICC, intraclass correlation coefficient; CI, confidence intervals; N.D., not determined. Thresholds: <0.50 = poor; 0.50–0.75 = moderate; 0.76–0.90 = good; >0.90 = excellent. In bold, indicators where correlation coefficient is ≥0.7.

of the farm scores along the first two PCs. Descriptors on PC1 (that commonly describes the valence of emotions) range from Content to Irritated, whereas descriptors on PC2 (that describes the arousal) range from Lively to Content.

The IOR calculated for individual-level observations is reported in **Table 3**. The reliability among assessors appears to be excellent for udder asymmetry, overgrown claws and nasal and ocular discharges, acceptable for abscesses and fecal soiling, but insufficient for hair coat condition and improper disbudding. For group-level indicators results are reported in **Table 4** and show that the IOR among assessors is excellent for synchrony at resting and use of shelter, whereas it is acceptable for oblivion (even if the lower limit of confidence interval only reached 0.32) and insufficient for synchrony at grazing.

The agreement among assessors for QBA was considered unacceptable for PC1, due to the rather low Kendall's *W* (0.597). The agreement on PC2 was acceptable (Kendall's *W*: PC2 = 0.750).

The collection of individual-level indicators took about 3 min/goat and the time required to perform the whole protocol was about 4 h/farm. The assessors reported the presence of some constraints that reduced the feasibility of both individual- and group-level indicators. For individual-level indicators, narrow pens and low-light conditions affected the quality of the assessment and extended the time needed for the inspection of the animals; for group-level indicators, open ranges, the presence of thick vegetation cover and the presence of guardian dogs were the major limitations.

DISCUSSION

This paper reports the results of the application of the AWIN welfare assessment protocol for goats in semi-extensive husbandry systems. The original protocol was partly modified to be adapted to the different context.

The assessment showed that in our farms some welfare problems were completely absent (overgrown claws, fecal soiling, discharges, and thermal stress) or almost absent (severe lameness, synchrony at grazing, and oblivion). However, a comment is necessary for hair coat condition and abscesses. The average situation for both these indicators was good also in comparison with Italian and Portuguese intensive farms (14, 31) and Brazilian meat farms (18). Nevertheless, in one farm a high prevalence of animals with poor hair coat condition was found (24.7%): this result is in line with intensive farms, but it is higher than the prevalence found in extensive meat goats in Brazil (12.12%) (18). It is important to underline that in our protocol the assessment of hair coat condition was performed on individual animals during milking and not as a group-level indicator with goats free to move in the pen as proposed by the AWIN protocol (17). The assessment of individual animals might be more accurate than the assessment in a group of animals; hence, we think that this change in data collection could have affected the prevalence of the indicator. As to the prevalence of abscesses, on average the situation is in line with abscesses found in Brazilian meat farms (9.84%), but in at least one of our farms a serious welfare problem is present, with 35% of animals with abscesses. Improper disbudding was actually a problem only in one farm, which was the only one to perform this practice. The high prevalence of this indicator in this farm (more than 1/4 of the animals) deserves attention, because residual horns (scurs) on the head of adult goats can press against the head or eye, causing lesions and pain.

Furthermore, scurs may be caught in fences and pen partitions, causing injuries and stress (17). As for hair coat condition, the assessment of improper disbudding was modified from group-level to individual-level indicator. Again, it is possible that the prevalence is affected by this change, making the assessment more accurate.

Potential welfare issues were highlighted regarding body condition and resting synchronization. The percentage of animals presenting a normal body condition was low (67.9%, but with a farm showing only 25% of animals with normal body condition) if compared to the results obtained in extensive double-purpose systems in Portugal [89.4%; (20)] and in intensive dairy systems in Portugal [77.7%; (31)] and in Italy [80.2%; (8)]. No direct comparison can be made with meat goats assessed by Leite Oliveira et al. (18), as the authors used a 5-point scale system, ranging from very thin to very fat. However, excluding the extremes (very thin = 14.5% and very fat = 2.1% of the animals), 83.4% of the animals in that study presented a normal body condition, in line with the other above-mentioned studies (8, 19, 20, 31). The low percentage of goats in normal body conditions in our farms was totally determined by the percentage of very thin animals. The presence of very thin animals is a problem also in Italian intensive farms (8) and in meat goats (18), but not in Portuguese intensive farms where the major problem is represented by very fat goats (31). The authors of the research performed in Portuguese extensive farms did not report the prevalence of too thin or too fat animals; hence, no further comparisons can be made (19, 20). The high prevalence of very thin goats in our farms could be explained by the fact that goats were in mid-lactation stage, when body reserves had not recovered from the losses occurred during the previous peak of lactation yet (32), aggravated by the absence of supplementary feed offered to goats in five farms during the grazing period. The risk for low energy intake is higher at pasture compared to indoor conditions as it is not always easy to fulfill the energy requirements of dairy animals only through natural forage resources (11). Additionally, animals at pasture move (horizontally and vertically) more than in indoor conditions and may be exposed to extreme weather conditions, thus spending more energy and requiring body fat mobilization (13).

The second indicator that poses some welfare concerns is synchrony at resting, which was very low in our study with only on average 14.3% of goats that lied down simultaneously, and even some farms where the animals never rested at the same time. When a sheltered area, such as a thick vegetation cover, was available, most of the goats (95.1%) used it for resting. According to Zobel et al. (24), hiding spaces (e.g., woods, caves), possibly in elevated areas, are important environmental features that allow goats to express their natural behavioral repertoire. The quality of sheltered areas may influence goats' resting behavior; probably those offered to the goats in our study were not sufficient to guarantee a high level of simultaneous resting. Furthermore, Negretti et al. (33) found that goats in an outdoor yard moved more, but rested less compared to housed goats. Hence, we could hypothesize that the low percentage of resting animals is due to a high exploratory behavior and need for movement in the outdoor environment before going back to the barns. The low observed number of resting goats might be due also to the presence of guardian dogs that elicited a strong fear reaction, and the presence of houseflies that bothered goats and prevented them from resting. Last, it is worth noting that the moment when goats were observed might not be optimal for resting observations. Findings from studies on feral goats would support the time we selected for performing the observations on resting synchronization. In fact, the diurnal activity of feral goats is characterized by feeding for >50% of daytime, mainly at dawn and dusk, and resting for >20% of daytime, mainly at midday, with a higher resting time from March to October, which decreases toward midwinter (34). Unfortunately, to our knowledge, no information is present in the literature regarding domestic goats at pasture. According to Stephan (35), animals can modify their activities during the day, to adapt to the surrounding environment. This might support the idea that goats at pasture graze until they are satiated, and may shift the time devoted to resting, performing it only in the barn after the evening milking. Furthermore, we may hypothesize that milking routine and other activities occurring in commercial farms, such as the distribution of supplementary feed, can affect the daytime activities of goats and, in this case, we may have missed the best timing for performing the observations on resting synchronization. In order to identify the best moment of the day to perform these observations, further research is needed to gather information about daytime activity budget and biorhythms of farmed goats. Therefore, in its present form, synchrony at resting is probably not suitable for inclusion in a welfare assessment protocol for goats, but certainly deserves further attention.

The AWIN welfare assessment protocol for goats (17) uses the Latency to first contact test to measure the quality of human-goat relationship. However, this test is not suitable for the assessment of the human-goat relationship when goats are at pasture. Hence, we used a different test, the FHAT, developed for the AWIN welfare assessment protocol for sheep, in order to check if it was suitable to evaluate the HAR quality in goats (16). However, since the validation of this test applied to goats is still pending, we used a simplified version, only considering the three possible reactions that goats could show, i.e., avoidance, contact and approach, and we did not record the distance expressed in meters when a flight response was elicited. The FHAT suggests that in most of the observed farms the relationship was positive, with goats voluntarily approaching the farmer in more than 60% of the farms. However, in more than 30% of the farms, the animals avoided any contact with the farmer. Caution should be used for the interpretation of these results since this test has not been validated for goats yet. Furthermore, comparisons with the results obtained in other extensively managed goats is difficult, due to differences in the procedures followed in other protocols for the evaluation of HAR. For example, in the Brazilian study on meat goats, an avoidance distance ranging from 57 to 239 cm was recorded, but it is unclear if only the avoidance distance was calculated, or if other possible reactions (approach, contact and avoidance) were assessed too (18).

QBA studies ground on contrasting expressive qualities where contexts are previously selected for their divergent characteristics (12). In our farm sample, farm characteristics are rather homogenous, and therefore the goats' expressive behavior on the farms showed a limited variation on the PCA plot, with only few exceptions. In most of the farms, the mood of the animals appeared to be positive, but the level of arousal cannot be clearly distinguished because Relaxed (low arousal) aligns with Lively and Aggressive (high arousal). An explanation for this can be the fact that Assessor A did not score any farm with Bored or Frustrated animals; hence, the evaluation is not complete and the QBA outcome was not very meaningful. QBA relies on the use of all the descriptors available in the list provided to the assessors: the absence of some descriptors influences the PCA plot, resulting in an uneven distribution of terms. In contrast with other studies [e.g., (36, 37)], QBA presented a low level of IOR, due to the insufficient agreement on PC1. QBA could be a feasible indicator to be used in semi-extensive systems (only 10 min of observations from one observation point), but our results suggest that the training provided during this trial was insufficient to obtain reliable results and that a more extensive training should be performed.

According to Kaufman and Rosenthal (38), IOR is frequently neglected in behavioral studies and, apart from studies on QBA (12), to our knowledge no studies have been conducted so far to investigate the IOR of welfare indicators collected on grazing goats. This issue is highlighted also by Richmond et al. (39) stating that the reliability of most of the physical and health indicators included in the AWIN welfare assessment protocol for sheep (16) was confirmed, but the majority of the behavioral indicators included in the same AWIN protocol (e.g., lying synchrony, human approach test) had not been tested for reliability before their inclusion in the protocol (39). Most of the indicators used in our research have been tested for reliability in intensive dairy goat farms in Portugal and Italy (40) and the results supported their inclusion in welfare assessment protocols for that specific context. However, these results cannot be automatically extended to semi-extensive conditions. Interestingly two of the indicators modified from the AWIN welfare assessment protocol (17), namely hair coat condition and improper disbudding that were originally collected as group-level indicator resulted in insufficient agreement among assessors when collected as individual-level indicator. This suggests the importance of training the assessors and testing IOR when some changes occur (e.g., context, data collection).

Most of the indicators collected on goats at pasture showed acceptable reliability; however, IOR was not sufficient for QBA (as already discussed) and synchrony at grazing. Investigating the reasons for this result, we identified some issues related to the background and training of the observers, and to feasibility constraints. Regarding background and training, assessor A had a sound experience with goats, whereas assessors B and C only had a limited experience. This may have affected the effectiveness of training that possibly did not bridge the gap among the assessors and in turn affected the results of the observations. Furthermore, the assessors reported several hurdles during the collection of the indicators that may have affected the IOR and reduced the feasibility of the protocol. The application of the whole protocol under semi-extensive conditions took more time than the application in intensive farming conditions. In fact, the average estimated time required in intensive conditions is 90 min/farm and 30–45 s/goat (17), whereas the application of the protocol in the present study required more than 4 h/farm and 3 min/goat for the individual assessment, and according to the assessors this was exhausting and time-consuming. When more than 15 lactating goats are present, according to the AWIN protocol the use of a sampling strategy for the individual-level assessment is recommended, where the sample size depends on the number of goats in the herd (14). However, in our study this strategy was not applied due to the small size of farms included in our research but, in the light of the results on the duration of the whole assessment, the recommendation to adopt a sampling strategy has to be kept in mind in the future in order to improve the feasibility of the assessment, especially in presence of large herds. This might allow reducing the time required for the application of the whole protocol, lasting possibly <2 h/farm and <5 min/animal. In fact, during a stakeholder consultation carried out within the AWIN project, farmers, veterinarians and technicians reported as acceptable for on-farm welfare evaluation a total time not exceeding 2 h and an individual assessment time of maximum 5 min per animal (8). The assessment of meat goat farms in Brazil ranged from 1 to 3 h, but the authors assumed that this time could increase with a greater number of animals (18). In this study a maximum of 50 goats were assessed in extensive systems. Furthermore, it has to be considered that the total time of application of the protocol may depend on the time needed to reach the grazing area that in some cases can be distant from the farm.

A further complication that affected the feasibility of our assessment, and in turn its reliability, is that observations performed when the animals were at pasture required the use of binoculars, in particular in unfenced pastures where animals could stray far away. If animals graze in areas with thick vegetation cover, observations can be difficult, as the vegetation reduces the visibility. As to visibility, the farms were visited on purpose only on good weather days, as we supposed that rain or fog could worsen the reliability of the results. Hence, we suggest checking the weather forecast before scheduling the farm visits.

In addition, the assessors reported difficulties to perform the individual assessment during milking in narrow and dark pens, especially because milking frequently occurred very early in the morning, under suboptimal lighting conditions. Hair coat condition, abscesses and udder asymmetry were considered the hardest indicators to be collected. A relatively low IOR was reached for improper disbudding in some cases (0.20), whereas a good IOR was obtained in other cases (0.91); the assessors did not report any specific constraints, probably because this indicator was only applicable on one farm. The assessors reported some difficulties to detect severely lame goats due to the different flooring on which the animals walked: in some cases, they walked on concrete floor, but in others, they reached the pasture on gravel roads with variable slopes. Different surfaces (e.g., hard or soft) and, in this case, also different slopes, may affect the reliability of the observation, as suggested by other authors (41). Although our original plan was to evaluate lameness on individual animals at the end of milking, while they were leaving the milking area, this turned out not to be feasible as, being milked in narrow pens, the animals did not have enough space to walk for a sufficient distance to be properly assessed. Therefore, we decided to evaluate it observing the group of animals while they were moving from the milking area to pastures. According to assessors B and C, the detection of severe lameness in large groups of goats moving together was not easy, but assessor A reported this as an optimal situation, as severely lame goats can easily be identified as they walk slower than the others. The absence of severely lame goats (and of overgrown claws) in our farms is in agreement with the results obtained in meat goats extensively raised in Brazil (18), suggesting a positive effect of pasture on the health of claws, as observed by de Morais (20). The beneficial effect of grazing for reducing lameness was observed also in cattle by several authors [e.g., (42, 43)]. The assessors also found difficulties in assessing the synchrony at grazing using the scan and instantaneous sampling method, due to the high number of animals that moved at the same time. The IOR among the three assessors was insufficient and further training seems necessary to make the collection of this indicator more reliable. Furthermore, the wide range of confidence intervals for this indicator ($-0.44 < CI < 0.93$) suggested that the reliability is likely to be affected by the group size, the environment (e.g., presence of woods) and the distance from the animals. For synchrony at resting very good IOR was reached, probably due to the low number of animals that lied down simultaneously and because they did not move when resting.

Further studies are needed to test the validity of the FHAT in goats, but all the assessors reported that this test is easy to be conducted with goats at pasture and the agreement among assessors was perfect for the three possible reactions of goats to the farmer. However, it is probably advisable to register the goat reaction toward a familiar human when the farmer really gathers the flock, in order not to affect the routine and management of the farm, as maybe goats would react differently if handled out of the normal routine. In any case, the validity of this indicator in goats still has to be confirmed and requires further consideration.

All the indicators used in this attempt to adapt an already existing welfare assessment protocol for goats are animal-based measures. However, no practical animal-based indicator was found to cover the "absence of prolonged thirst" criterion. This lack is common to most of the evaluations conducted at pasture (11). However, Morales et al. (44) used skin elasticity and enophthalmia as signs of dehydration in cattle kept in silvopastoral systems. Indicators of dehydration (e.g., skin tent test, capillary refill time, thirst index) are available for some species [e.g., calves (45), horses (46), camels (47)], but not for goats. Hence, further research is needed to identify suitable indicators to assess this criterion. Although farmers may not perceive thirst as a welfare issue on grazing animals because they eat fresh grass, prolonged thirst may represent a serious welfare problem. This is probably the case also in our farms, as 4 out of 13 farms did not provide water points during the grazing period, and this may represent a serious welfare problem, particularly during hot summers.

In conclusion, this research showed that most of the indicators selected to assess the welfare of goats in semi-extensive conditions could be applicable, even if most of them were originally developed for intensive conditions. For most indicators, an acceptable level of reliability was reached; however, further research is required in order to identify a complete set of robust indicators in this specific context. Finally, feasibility constraints should be taken carefully into account as they can affect the reliability of the evaluation. For example, the assessor may decide to collect the individual indicators during either morning or evening milking, choosing the moment when the light is higher, as a scarce illumination can negatively affect the results. Furthermore, it may be advisable to collect FHAT according to the farm routine, for example, when the farmer gathers the flock before entering the barn for the evening milking.

Specific research should be conducted on daily activities and biorhythms of farmed goats to select the best moment of observation for evaluating the synchrony during feeding and, in particular, during resting. This research highlighted the lack of animal-based indicators to assess the effect of prolonged thirst in semi-extensive conditions; hence, specific research is needed to fill in this gap.

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 according to the National Italian Law (D.L. 26/2014), no specific ethical approval was required, as no pain, suffering, distress or prolonged damage equivalent to or greater than that caused by the insertion of a needle was applied. Written informed consent was obtained from the owners for the participation of their animals in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

MG collected data and organized the database. MB and MG performed the statistical analysis. MB wrote the first draft of the manuscript. MR and SM wrote sections of the manuscript. LB supervised the work. All the authors contributed to conception and design of the study, manuscript revision, read, and approved the submitted version.

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Barriers to Utilizing Non-replacement Male Calves in the Australian Dairy Industry: A Qualitative Study

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Male non-replacement calves in dairy systems represent an underutilized economic resource for dairy producers worldwide. Despite this, increasing the practice of rearing non-replacement male calves has significant barriers both in on-farm adoption and practice. Poor neonatal rearing practices, higher levels of morbidity and mortality, and disaggregated production pathways with multiple points of handling, have all been described as barriers to adoption of surplus calf production. To identify the critical decision-determining challenges associated with broader adoption of raising non-replacement stock, and to investigate the whole-of-value chain issues faced by dairy producers to rear non-replacement male calves, we undertook a series of semi-structured interviews with Australian dairy producers to interrogate their key challenges. To achieve this, a constructivist grounded theory approach was used to inform the process of analysis of in-depth interviews with Australian dairy producers regarding their current practices and perceptions. Five major themes emerged from these conversations that were key barriers to on-farm non-replacement calf rearing in the producer group participants. These were: impacts of drought on cost and availability of feed for these calves and the whole herd; the management requirements of non-replacement male calves as an additional workload to that of their current operation; their attitudes and current practices to and surrounding euthanasia; perceived ease of supply-chain access for these calves, and their perceptions of the economic value of dairy-beef product as a return on investment. Understanding the barriers to adoption of non-replacement calf rearing, and addressing the value proposition for dairy beef, can assist increased uptake of non-replacement calf rearing. These findings will allow development of strategies to address these barriers, and extension of viable management strategies to increase adoption of profitable business practices surrounding non-replacement male calf production.

Keywords: bobby calves, non-replacement male calf, dairy, euthanasia (active voluntary), dairy beef production, beef value chain, producer perceptions

INTRODUCTION

There is currently a paucity of knowledge of the practices and management strategies, for animal growth pathways and available markets for non-replacement male calves in the Australian dairy industry. The term "bobby calf" is widely used in Australia as a description of a male calf under six weeks old that is unaccompanied by its dam and the perceived common practice for management of these animals is for them to be slaughtered at < 10 days of age on farm (1). Unlike beef calves that are reared by their dams, in order to maximize milk collection, dairy farmers must artificially rear male calves if they are going to be reared for sale, this representing a major on-farm investment in facilities, feed and time as calves are often housed individually (2) and require relatively high levels of neonatal health management (3–5). As such, and without clear pathways for sale, the economic viability of this practice is often questioned by the industry, making adoption of on-farm rearing a challenge.

There are several production challenges associated with the rearing of non-replacement male calves for beef. These include the need for a protected environment due to their relative immaturity compared to calves weaned under standard conditions who will spend several months with their dams (6). Early-separated dairy calves are more sensitive to climate and other environmental conditions due to their size and age (1) and thus require shelter to maximize their growth and minimize risk of disease. Internationally, the transport of young calves, heat or cold stress, and transit through sales yards have all been shown to cause increased risk of mortality in dairy calves, impacting on producer returns (4, 5, 7). Some specialized producers have established a specific market for rearing non-replacement male dairy calves, but these are not widespread or common. Other perceived deterrents internationally to non-replacement male calf production are perceptions around a lack of obvious saleable markets (8), the perception of the replacement calf as a "low value byproduct" (9) and the limited number of rearing facilities for non-replacement male calves available to take non-replacement male calves for rearing (10). These compounding issues have resulted in the production of non-replacement male calves being identified as a "health and welfare challenge" internationally (8) and a "wicked problem," namely a problem that is subject to real world constraints and with potentially multiple solutions, for the dairy industry in Australia (11).

Australia is in the minority of developed countries that still perceives the practice of slaughtering non-replacement male dairy calves as more profitable than rearing them for meat production. Despite the perceived practicality, this practice comes with significant welfare implications (9, 12). There are strong indications, both from industry, the public and consumers, that this practice is undesirable with the UK moving to ban this practice by 2023 (13). Globally, consumer opinion is driving practice change (14), with the general perception that calves should be productive as vealers, or slaughtered as mature cattle sold as "dairy beef" (15). Overcoming this issue has been describes as "inherently complex" due to evolving social culture, no ultimate defined solution, stakeholder expectations and producers achieving desired production goals (11). Therefore, understanding producer limitations and adoption of novel practices to manage male calves should present an economically viable option to retain these calves in the beef supply chain.

Although exact numbers of dairy-produced calves born in Australia are not known, recent figures suggest that approximately 400,000 non-replacement calves are processed each year in Australian abattoirs, with this number increasing from 2010 to the present (16). This number of non-replacement calves could represent a valuable proposition to dairy producers if they were to be utilized in an economically viable manner (17). To promote viable production practices for surplus male dairy calves, there is a need to define the barriers that are unique to the Australian dairy industry surrounding the adoption of non-replacement male-calf rearing and the generation of a profitable dairy-beef value chain.

To better understand the perceptions and challenges of Australian dairy producers in relation to adoption of rearing non-replacement calves for beef production we sought to investigate current producer experience of on-farm rearing strategies for non-replacement dairy calves and their associated challenges. Factors of interest included accessibility to markets for non-replacement male calves and dairy producer's perceptions surrounding dairy-beef products in relation to marketability and eating quality. A qualitative methodology with semi-structured interview questions was used to provide impromptu questions to suit the individual producer's responses. This allowed the researchers to capture the range and breadth of producer perceptions to current barriers to adoption of a viable dairy beef supply chain in Australia in the context of their individual enterprises.

METHODS AND MATERIALS

Ethical approval for the collection of original data from human participants for the interviews reported in this manuscript was provided by Charles Sturt University Human Research Ethics Committee (Protocol number: H19225). All participants gave informed consent to participate in these interviews. This work was carried out in full compliance with the National Statement on Ethical Conduct in Human Research (2007, updated 2018) and in accordance with the National Health and Medical Research Council Act (1992).

Methodological Framework

Semi-structured in-depth interviews were conducted with Australian dairy producers. Constructivist Grounded Theory (CGT) informed the research processes and analysis undertaken (18). In this study, the use of CGT as a methodology (19) allowed the researchers to explore, develop knowledge and focus on subjective experiences, perceptions and attitudes of participants concerning current issues associated with non-replacement male calves. The interactions, interpretations and understandings from the research allowed the researcher to deduct or build theory based on previous knowledge (19). This epistemology suggests that the researcher's existing knowledge, perceptions, and formal training in the field of dairy production influenced data collection and the interpretations of participant responses (18). The outcomes in turn reflect the researcher's ability to capture the experiences and opinions of producers through participant interactions. The results of the study are therefore a combination of the contribution of the researcher and the participants; the method is therefore able to capture outcomes that quantitative surveys can sometimes miss.

Sampling Strategy and Participants

Purposeful sampling was adopted to recruit current Australian dairy owners and/or managers over the age of 18 (20). This strategy identified information-rich participants that shared common attributes to provide in-depth knowledge that later formed central themes aligned with the objectives of the study. Aligned attitudes and opinions expressed in the interviews removed extreme variation among participant responses (20). Participant recruitment was achieved by distributing electronic flyers via dairy consultants, domain experts and dairy discussion groups. Few interviews were opportunistic and participant involvement was sought during a secondary study regarding dairy production. A total of 15 participants were interviewed. All participants owned or co-owned a dairy enterprise and were currently working in the enterprise at the time of the interviews (December 2019 to March 2020).

To address the scope of the research question, a saturation sampling technique was used to determine the number of interviews required to be undertaken (21). A point of saturation is reached in a qualitative study when no new insights or development of novel themes emerge from participant information, and data collection can terminate (22). Saturation was achieved after interviewing 13 participants which provided an information rich dataset enabling the research question to be addressed (20). No new insights were yielded, however a further two interviews were conducted to confirm this assumption.

Data Collection and Analysis

Fifteen face to face in-depth semi-structured interviews were conducted and audio-recorded by the first author. The interview questions focused on topics that explored past, present and emerging practices associated with rearing non-replacement male calves in dairy systems. A record of practice change (past, present and emerging) over time, allowed the researchers to assess the concurrent attitudes of producers surrounding their responsibility in relation to treatment and welfare of calves. It was of utmost importance to the study to interview owners and/or managers of dairy enterprises as they are the individuals who can implement the greatest changes within each production system. Identification of supply-chains for non-replacement male calves were accounted for through each participant's personal experience regarding the saleability of past male calves and expected future markets. The interview questions were designed to be presented in an open-ended manner. This approach was used to ensure unforeseen comments would be accounted for and subsequent questions could be tailored to each interview allowing overarching themes to remain central to the discussion formulated. Briefly, the interview questions covered the participant's involvement in the dairy industry, the scope of the dairy operation they managed and/or owned, calving management practices with a focus on non-replacement male calves, attitudes and practices toward euthanasia of non-replacement male calves, ideal management strategies of non-replacement male calves and how the strategies could be achieved, and opinions toward dairy beef products. A copy of the interview questions is available from the corresponding author upon request.

The researcher conducted each interview face to face, traveling to the location of each dairy enterprise where the participant resided. Convenience, ensuring participant confidentiality and reducing variation among data collection was a priority of the research team and is why the format of each interview was conducted in this manner. A $20 gift card was offered to each participant as compensation and a token of appreciation for engaging in the study and providing personal insight that contributed to the outcomes of the research.

Figure 1 depicts the process the researcher undertook to interview and examine the results and develop new findings. Audio recordings from each interview were de-identified and transcribed verbatim by the first author post interview. The author reflected after each interview and adapted and / or added questions accordingly to suit new emerging topics. Sections of audio where the researcher could not comprehend what the interviewee said were noted as inaudible. Each transcript was then proofread. This process lead the researcher to become familiar with the dataset and initiate the process of analysis. The data was hand coded line-by-line with gerunds to allow the researcher to study each fragment of the data and help define meaning, make comparisons and recognize emerging links within the data. Memo writing assisted with the development and reflection of early categories emerging in the data (18, 23). An electronic copy of the coded data was then created on a Microsoft Word document. This process was used to rearrange and segregate codes to establish analytical categorization through focus coding (18). To generate the development of categories and later central themes, a search of supporting evidence through the raw interview data was undertaken to negate or lead to connections between micro and macro levels of the significant themes established (18). The individuality of each participant's experience were linked here to drive novel findings and generated theory aligned to the research aims and questions.

RESULTS

A total of 15 participants were interviewed. The length of interviews ranged from 15 to 50 min with the average interview length of 22 min. All participants owned or co-owned a dairy enterprise and were currently working in the enterprise at the time of the interviews (December 2019 to March 2020). There were six female participants and nine male participants. All participants were located in south Eastern Australia, ten in the Riverina area of New South Wales and five were located in the Western Districts of Victoria. Herd sizes ranged from 160 to 800 head, with the majority of enterprises having a herd size of 200 to 400 milking cows.

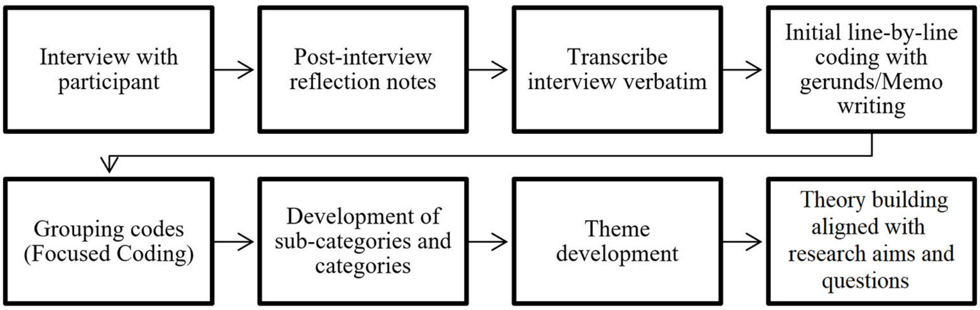

FIGURE 1 | Constructivist grounded theory data collection and analysis process.

Several major themes emerged: these focused on market sustainability, drought, and resourcing. All participants reported that drought had impacted all facets of their dairy operations, from daily production and management decisions to market access opportunities. All participants also identified they had access to one or more saleable markets for non-replacement male dairy calves in Australia, however, only half of the participants reported supply-chain access that was consistent and economically viable. The remaining participants reported that they frequently had to access opportunistic markets, the majority of which were non-profitable. This cohort of participants did not have a level of confidence in their rearing and sale processes, and commonly resorted to selling calves to the "bobby truck" (a colloquial term for the truck used to transport non-replacement male dairy calves to the saleyards or calf rearers), through the sale yards or social media outlets such as Facebook. Market access was not influenced by location of each dairy enterprise as challenges were seen across both NSW and VIC. All participants reported a range of barriers to rear non-replacement male calves in an economically viable manner including, but not limited to: "drought," "feed," "resources," "space," "land," "infrastructure," "time/labor," "cost/money," and "finding a market." Surprisingly, only two participants, reported the practice of euthanasia of non-replacement male calves on farm, but also stated that if a viable market was available, this route would take priority. For all participants euthanasia was not a preferred practice. These findings accentuate the need for producers to have access to profitable markets to sell non-replacement male calves, and to trust in those systems.

Primary Themes

Current knowledge, attitudes and practices associated with rearing non-replacement male calves in Australian dairy systems were identified through the subjective experiences of producers. These experiences generated five primary themes in the analysis: (1) impacts of drought, (2) management of non-replacement male calves, (3) euthanasia-related attitudes and practices, (4) supply-chain access, and (5) value of dairy-beef products.

Impacts of Drought

Statements regarding the impacts of drought on dairy production was prevalent throughout the interview process and as such, became a central topic of conversation. Nine participants reported drought conditions impacted production practices and management choices, which in turn affected all facets of their operation.

Poor seasonal conditions contributed to an increase in the time and labor allocated to monitoring animals, feed allocation, water management as well as presenting reduced market opportunities for all livestock. The additional operational costs required as a consequence of ongoing drought formed a large portion of the participants reflections.

> *Producer A: "We didn't calve many this year… consequence of being in 2 years of drought and were down to next to no water allocation… so the last 12 months the numbers have been dwindling down…"*

In contrast, major drought impacts did not affect six participants that had access to bore water, irrigated pasture and feed stockpiles. This cohort of participants were not as conscious of seasonal drought as these provisions were recognized to be alleviated.

> *Producer B: "… we store up as much fodder as we can we usually carry about 2 years worth of hay… we still have got enough feed in storage to carry us through but not pushing production…"*
>
> *Producers C: "… the droughts take a huge toll… we are a little bit protected here because we grow all our own feed… but still… usually we have surplus grain… so usually we have that income as well…"*

As such, location-specific impacts of drought were not observed. Within the same geographical region, participants who were better prepared for drought conditions expressed concern for other dairy producers who were not in such a fortunate position. These participants agreed that enduring previous droughts had forced innovation in their operations and promoted increased efficiencies within their production system to protect them against future economic risk associated with drought. Those participants that were more adaptable in their operating practices were at lower risk of encountering economic loss in the face of challenging climate conditions. The degree of focus on innovation differed, with some participants indicating a culture of innovation and others relying on traditional knowledge.

Producer A: "…trying to be as flexible in your management approach on the farm as you can be, and sustain it…it's not just a matter of this is the way I do it, oh hang on there's a drought, or I've got no water, ring up and buy in a stack of hay and grain…you know question everything you do… well is there a better way…even me sons coming up with new ideas and changing things around..."

Nine participants reported reduced profit margins and that these reductions were directly correlated with ongoing drought conditions. Factors predicating this economic cost included deteriorating land conditions leading to increased reliance on supplementary feeding and increased labor costs. Poorer quality feed resources resulted in decreased animal body condition scores that resulted in higher rates of morbidity and mortality on farm, reduced sale prices and decreased supply chain access for all on-farm animals, including non-replacement male calves. Notably, the difficulty in selling non-replacement calves and reduction in price at point of sale forced two participants to revert to euthanasia practices where they had been previously able to avoid doing so.

Producer D: "We never used to [euthanise] but we have had to because of the cost and then the sale yards and sometimes getting five dollars is not worth all that time and milk…"

Management of Non-replacement Male Calves

All participants reported that retained heifer calves received vaccinations and any other required veterinary prophylactic treatments as per standard production practices. Retained heifer calves received a consistent feed allocation that was inclusive of milk, *ad libitum* hay and concentrate in the form of grain or pellets. Shelter was also provided to reduce environmental stressors during the critical period of early weaning and growth. The majority of producers interviewed reported that retained heifer calves took priority over non-replacement male calves. Specifically, six producers noted that vaccination and treatment protocols, feed quality and shelter provided to heifer calves was not replicated for non-replacement male calves.

Producer E: "…if things get a bit tight…you know your guys [non-replacement male calves] are going to have to do it a bit harder than the heifers. Heifers will get first choice on where they go and they'll get grain and better hay…if I have to feed the steers grain, the hay it won't be as good…"

Four producers did identify that they felt it was their responsibility to treat all calves in a similar manner as a part of their "social license to operate" and to ensure that all animals were cared for in a "reasonable way." One producer had formal training as a veterinarian and justified this opinion with the explanation that the value of treating male calves maintained generally high health standards across the herd and increased treatment success rate among all calves, inclusive of the heifer calves that were a "long-term investment."

Producer C: "…from an experience point of view I have treated a lot of bull calves…it makes me much better at treating the heifers in the same position so I justified that way and I am much better looking after the heifers which you know are more valuable…I just don't like [not treating non-replacement male calves], it's still a life and it's got some value in it…I know that some farmers wouldn't be able to justify the cost of drugs and the extra time but that's my policy here…"

However, one participant suggested that veterinary treatment of male calves reduced their ability to pass these animals on to saleyards due to the need to comply with industry withholding periods as a justification for their different management strategies between male and female calves.

Producer D: "…we try and sell them straight away, like a week-old so we can't [send them to a sale yard] if they are treated and at the moment there is…no return for us to chase that market so we don't bother."

Producers that did not manage heifer and non-replacement male calves in a similar manner identified "time," "labor" and "costs" as the major barriers to this differential treatment. They reported that a lot of time is spent "off the books" to facilitate rearing male calves, implying a negative cost benefit to their operation. *One* participant offset the extra labor requirements through the use of a robotic feeding system. Some participants reported inefficient or inadequate physical infrastructure, and/or space restrictions as limiting their ability to rear male calves. In some cases, this was a direct effect of expansion and growth within the milking herd leading to less space to house and rear male calves. Inadequate housing due to space constraints and herd growth often resulted in male calves being housed in exposed pens and/or paddocks, resulting in increased mortality and morbidity rates.

Producer F: "…deciding to keep the bull calves was a big change because we probably…at the time we had enough room, the calf numbers were a lot lower because our cow numbers were at that low point…but now the numbers are getting big, the calving groups are getting big…that's why they're [non-replacement male calves] in those makeshift kind of pens.…we're currently working on building a calf shed because I think our main problem at the moment is [the non-replacement male] calves exposure to the weather…I think that's when the calves are most susceptible to getting sick. They're freezing cold during the night and then really hot through the day, or the rainy weather…"

The opposite was true of these producers with available grazing land that appeared to facilitate the rearing of male calves to steers with fewer on-farm limitations.

Producer B: "It works in quite well because we got a fair bit of hill country, about 900 acres of that. So once we get them off the bucket that's the most labor-intensive part of it…then you only drench them and vaccinate them once and you put them out the back and forget about them so labor-intensive is not there."

Euthanasia Attitudes and Practices

Participant opinions surrounding euthanasia varied, however, all participants agreed that euthanasia of non-replacement male calves should only occur as a last resort. This response included two participants who reported currently practicing euthanasia of non-replacement male calves. Twelve participants reported that they would never euthanise non-replacement male calves, even if it was non-profitable to rear them. These participants indicated strong feelings that the dairy industry must acknowledge non-replacement male calves as a part of every dairy production system and manage the production of male calves responsibly.

Producer G: "…part of our social license…is to make sure that [we are] caring for all our animals in a reasonable way."

Producer H: "…I see it's more unethical to be slaughtering calves like some people are doing on-farm than selling them to the abattoirs and using that product…whether you look at it from a commercial environment or social [point of view]…why are you killing something…that is perfectly healthy and perfectly edible…it would probably be more economically sensible for us to shoot that calf in the paddock and be done with it…it is economically costing us money sending it to an abattoir but I think that's better use of the resource…"

These participants expressed several terms with negative connotations when describing their feelings toward male calf euthanasia, such as "avoid," "frustrated," "hate," "not supportive," "refuse," "unethical" and "unpleasant." One participant reported previously practicing euthanasia and expressed high levels of personal frustration toward having to euthanize a healthy calf. Adverse effects on the producer's mental health and well-being were also reported in line with this practice. Specifically, one participant did not allow other farm employees to euthanize calves for this reason. They did, however, identify that formal training in euthanasia reduced the toll on their mental well-being as it provided confidence the practice was conducted in the most humane way.

Producer A: "It's very frustrating to [euthanise] because you've got a perfectly healthy calf, good fit decent size calf and he is good for nothing, and you go I'm shooting a damn good calf, why? … no one likes putting down good healthy calves so it wouldn't be good for staff's mental health either… before we were trained and got the captive bolt, I would leave stock for my dad to put down…I wasn't confident…when we did the captive bolt training and you're actually equipped to know exactly what you're doing, not just what you were taught by your dad or whatever…when you actually understand the science…it helps…"

Two participants, one who did euthanize and one who did not, reported that if it was not economical to rear male calves they considered it to be "acceptable" to euthanise non-replacement male calves. It was suggested that, in this instance, the milking herd should take priority for resource allocation. The participant who did practice euthanasia believed more negative impacts and stress was placed on non-replacement male calves sold through sale yards or not reared with optimal management strategies than the practice of humane euthanasia.

Producer D: "…well at the end of the day it is going to get euthanised isn't it…so I think rather than it getting sick…or going through the stress of the sale yard, I think is the most humane [to euthanise] …we've just got to be realistic about it, operating within our means…"

Producer B: "…I don't have an issue with it, we had to do it years ago…if there's not a market there, what you do with them…As long as it's done humanely it's not a problem, it's just something's gotta be done - it's a fact of life…"

"Dairy-Beef" Supply-Chain Access

Every participant reported the desire to access better markets for non-replacement male calves and to improve their production management practices but they also identified many constraints. These constraints presented an economic barrier to dairy producers and reduced their ability to make consciousness-based decisions while maintaining a profitable enterprise. This finding was in contrast to their treatment of replacement heifer (female) calves where their practices were highly consistent.

A visual representation of the co-relationships between the interview information surrounding producer market access/ supply chains for non-replacement male calves was compiled from their statements and is shown in **Figure 2**. Both dairy breeds, and beef x dairy first cross offspring were considered separately. Dairy breed producers identified three main production pathways for their non-replacement male calves, (1) calves that were sold to market at 7 days of age; (2) calves that were sold to markets at > 7 days of age; and (3) those that were euthanized. Those calves that were retained on farm longer than 7 days showed a greater number of finishing pathways than those sold very young. Beef first cross offspring only identified two pathways to sale: the calf rearer or retained on the home property.

The supply chains identified, and their relative profitability, varied between producers. Some producers reported inconsistent use of target supply chains (sale yards, vealer, steer market) for the sale of non-replacement male calves. This cohort of producers were holding onto their non-replacement male calves until market conditions improved (typically, prices to increase post drought) before committing to sale. These producers were therefore at higher risk of drought associated economic loss by this practice.

Producer A: "No specific market in mind at this point…at this stage if we break even that'll be about it, I don't expect to make any money on them at the moment, but going forward, better seasons, yes we hope to make a few bucks on them."

In contrast, producers who had well-established sale options in place for non-replacement male calves, including pre-contracted sales to calf rearers and/or returning customers, reported successful sales and market access. They also appeared to have a positive outlook on the utility and viability of non-replacement

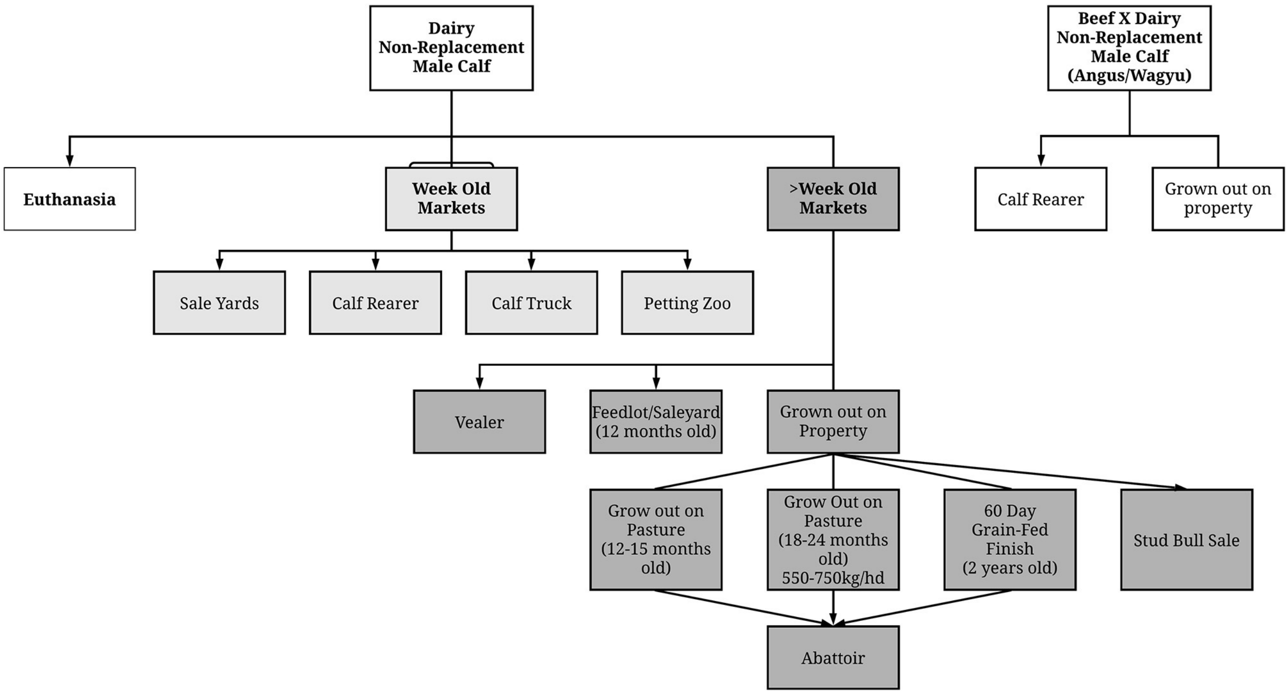

FIGURE 2 | Supply chain access for non-replacement male calves. Arrow (→) in diagram denotes end point and/or point of sale from the property of origin.

calf production and were more amenable to seeking alternative production solutions.

Breed Characteristics and Value of a "Dairy Beef" Product

Of the 15 producers interviewed, the majority (9/15) were not using other non-dairy breed genetics to increase likelihood of carcass yield in their male non-replacement offspring. For these producers, their focus was on dairy production and their sires were of primary dairy genetics: these included mixed crosses (Holstein x Jersey; Friesian x Jersey; Friesian x Ayrshire) with Brown Swiss, Montbeliarde and Swedish Red cattle all reported, or pure breed genetics (Friesian, Jersey or Holstein). The remaining six producers reported using beef breed genetics to improve carcass yield with Angus (4) being the predominant breed of choice, the others reporting use of Wagyu and BeefX bulls.

Participant views toward dairy-beef products only emerged as a point of interest during the fifth interview and therefore not all participants expressed an opinion on this topic. Within this sub-cohort of participants ($n = 11$), opinions varied regarding the value of a dairy-beef product. Although the dairy producers were not market experts, they also indicated their perceptions of consumer behavior toward dairy-beef. A latent theme suggested most participants did not believe that dairy-beef could target a premium market. The perceptions of this cohort of participants can be separated into two clear categories: those who thought dairy-beef could not be considered a premium product, and those who considered that there was a premium market for dairy-beef.

> *Producer G: "I don't know whether you would…advertise something as "dairy-beef"…you would want to do a lot of research on the name "dairy-beef" to see what connotations come up with it…you could be creating a can of worms for the people that might be euthanising…"*

Three participants believed that beef from dairy animals had similar or inferior meat and eating quality to other beef breeds. One participant conveyed doubt in the supply chain for dairy-produced beef carcasses explaining they currently experienced a price discount at slaughter for their carcasses, an industry implication that this represents a carcass of lesser eating quality. Interestingly, this participant also had a perception that dairy beef carcass and eating quality outcomes were equal to those of conventional beef animals despite the dairy carcass receiving a discount. Another participant agreed that dairy bred animals receive discount at slaughter but suggested that this was due to inferior pre-slaughter nutrition and that these animals would require grain-finishing to perform well.

> *Producer I: "…I finished some Friesian steers…on grain for the last 60 days so I got a pretty good price for them…they yielded [well]…but generally in Western Victoria you cop an absolute flogging for Friesians…in terms of you send them to the markets [and] you just get a massive discount even to send to slaughter. Unless you can really finish them off with grain you will get a massive discount, they will pay the lowest end that you can get…"*

Two participants explained their aim was to operate in a similar manner to match the standard they expect when purchasing food for themselves, indicating a high level of food consciousness. These participants were actively restructuring their businesses to suit markets in alignment with their own food preferences. The same producers said they would pay a premium for high-quality food items, including a dairy-beef product. However, they also suggested consumers generally do not think about the treatment of animals when purchasing food items and suggested there was a need to convey a positive animal welfare message for non-replacement male calves as a value chain opportunity. They identified that communication to the public around a dairy-beef product should include the dedication and good stewardship of dairy producers toward non-replacement male calves and their desire to rear them in the best possible manner. Drawing consumer awareness not only to product quality, but also to animal welfare and production, would be essential in creating a viable broad-spectrum dairy-beef brand and market.

Producer J: "I think you would sell the whole story so really it's going to be about... being clean and green, free range and all that sort of stuff... I'm the one who picks up the bulls... out of the paddock every day and I tell the cows I'm looking after them, they're in my care I have to look after them the best that I can... so I think you have to convey that, it's like a stewardship thing... "

DISCUSSION

This qualitative study explored the subjective experiences and attitudes described by dairy producers regarding the production challenges associated with the practice of rearing and growing out non-replacement male calves to sizable beef animals. Although the cohort was relatively small, the methodological approach ensured that identification of alternative views was unlikely due to saturation of responses. The authenticity of in-person interviews to establish the context in which the enterprises were operating was an approach that is more difficult to achieve by phone or internet-based surveys. Five themes characterized each participant's production/management decisions surrounding non-replacement calves: impacts of drought, management of non-replacement male, supply-chain access, euthanasia-related attitudes and practices and value of dairy-beef products.

Within these themes poor profit margins influenced many management choices made by dairy producers; this finding was similar to those observed in other studies internationally where producer concerns for a dairy beef supply chain lay within poor supply chain integration and lack of viable profit margins (8, 24). Dairy beef, through slaughter of cull cows as well as non-replacement calf production accounts for a small, but financially meaningful percentage of a dairy producers income, estimated in the U.S. to be between 5 and 15% of gross income, yet the product from these animals is often not identifiable to the consumer, and attracts downgrading for the producer (25, 26). In Europe, dairy beef production represents approximately one third of all beef produced in this region (27) and is widely accepted by European consumers. Current estimates indicated that approximately 2.8 million head of dairy cattle are processed annually in Australia, representing an important component of the beef and veal industry (28).

In this study, adoption of production of male non-replacement male calves as a viable product for their system were limited by labor, infrastructure, and other resources, resources that were more limited by drought seasons. One potential strategy to support development of an integrated supply chain for dairy beef has been reported by Irish producers when considering resourcing requirements, where grants from government bodies have been used to provide necessary additional infrastructure. This model could aid in preventing euthanasia of non-replacement male calves help to deliver the desired outcomes of the Australian Dairy Sustainability Framework toward reduction of euthanasia of non-replacement calves (24, 29).

Participants in our study reported monetary loss for the sale of non-replacement male calves was generally overcome by arrangement of pre-contracted sales to calf-rearers or other saleable markets (**Figure 2**). This assisted in giving these producers an economically viable route to market for these animals as oppose to participants who were waiting to see if market conditions improved to increased calf sale profit margins. Irish producers had concerns regarding price volatility and market uncertainty surrounding non-replacement male calves (24), so this represents a common challenge. By utilizing pre-contracted sales for non-replacement male calves, our study shows that this can support a pricing model that guarantees profitability and therefore mitigate risk for the dairy producer. This was also a favored option by Irish producers who preferred to send calves to a rearing facility with pre-contracted prices or retain ownership of calves of those calves in the rearing facility with a pre-contracted price negotiated prior to slaughter (24). This model could allow dairy producers at a national and international level to accommodate the extra labor requirements, facility usage, and grazing land capacity to support rearing of non-replacement animals, allowing more investment into the primary focus of their dairy production whilst supporting an integrated dairy beef supply chain.

Participants in our study reported that they would improve the conditions for rearing of non-replacement male calves if finance was not a potential barrier. This was similarly reported in a cohort of Irish producers where changes or improvement on farm to support a successful dairy beef integration system was the preferred option if finance was not a limiting factor (24). If grants and a more secure pricing structure was available for the sale of non-replacement male calves it could assist producers to invest in improved calf-rearing infrastructure and encourage them to put higher inputs into calf feeding protocols similar to those conditions described by participants for retained heifer calves. Improved feed quality and access to supported housing would assist male calves reaching target weights early and support them in their critical growth periods, improving calf mortality and morbidity rates (1). The interviews undertaken in this study also coincided with a period of sustained drought in south-eastern Australia. Drought was found to exacerbate the financial and practical requirements of non-replacement male calf production due to the cost of fodder, but participants also acknowledged that in a non-drought affected seasons it is currently difficult to make financially viable decisions due to a lack of obvious or easily accessible supply chain options.

Our findings showed that there was not one consistent supply chain preferred by producers interviewed in this study (**Figure 2**) even within the same geographical region. This suggests that development of a single dairy beef supply chain will be hard to achieve. Previous studies have suggested that multiple production models may be required to give sufficient options to dairy producers to invest in maintaining non-replacement calves for dairy-beef production (24). This may reflect that producers do not know how an integrated model will operate, or that sufficient options are not yet available. This represents a challenge to industry to determine the best dairy beef value chain model for industry adoption through targeted funding.

In our study, participants reported they did not think dairy beef should be marketed as a premium beef product due to "inferior" meat quality traits compared to other traditional beef breeds. This misconception or belief has also been reported in other studies (24, 30). Despite this perception amongst dairy producers, there is a growing body of work that suggests there is no difference between dairy and traditional British beef breeds in growth potential, lean meat yield, yield of prime cuts, and the quality of meat produced when grazed under similar conditions and slaughtered at the same chronological age or the same level of maturity (30). The reasons for this misperception of dairy beef as an inferior product needs to be further investigated. One possible reason could be due to the reported reduction in of quality feed inputs into early dairy-beef production systems from producers in our study, with the perception that this in turn will result in a poorer quality meat product. In contracts to a report by Maher et al. (24), who implied a lack of husbandry skills related to raising non-replacement calves, but rather a choice to treat male calves differently due to preconceived financial disadvantages of retaining these calves in the herd. Equally, perception of price discounts relating to a reduction in eating quality may also be feeding forward into these perceptions. Respondents in Maher et al. (24) also thought they were not receiving a fair price for the amount of labor and time needed for rearing non-replacement calves with this also limiting their desire to continue in production.

Six of the fifteen producers interviewed reported that they were using joining their dairy heifers with traditional beef sires to produce a better performing male non-replacement calf. The most hybrid common crosses were with Angus bulls. These cross-bred calves were seen to be more valuable in the marketplace and received better pricing at slaughter, either from farm or through calf rearers. In a study of Irish producers, Maher (24) reported that Angus, Limousin and Hereford were the sires that Irish dairy producers would consider using to generate dairy beef animals (24, 31). There is evidence to support the perception of Angus as a strong contender for best hybrid carcass production, as studies in New Zealand (31), the United States (32) and Australia (33) have reported that performance, carcass quality and eating quality of crossbred dairy calves sired by Angus bulls was improved compared to dairy sired animals.

Although generally offering reduced yield compared to other dairy breeds (34), Jersey beef has been identified as a particular niche product (32), showing quality traits related to marbling and palatability (35, 36). Interestingly, not many producers in this study utilized Jersey as a breed (only four of fifteen producers interviewed). In contrast to the evidence that the Jersey breed can produce a quality beef carcass (35), our cohort indicated that these male calves were extremely hard to sell, were of least value, and were therefore the commonly euthanized, similar to that reported by Irish producers (24). This suggests that greater education is required on the value proposition of different dairy beef crosses to ensure that the breeding market is informed of the evidence on the relationship between dairy beef genetic composition and carcass quality outcomes. This might improve uptake of more niche diary breeds as a viable genetic cross for high quality dairy beef production if a premium product market could be established.

One of the products of the interviews was that euthanasia of calves was a valid exploratory topic due to the variable access to market options for non-replacement male calves in Australia. Producer statements also identified a key novel finding regarding producer well-being related to experience of non-replacement calf euthanasia in the dairy industry. Euthanasia was recognized as a traumatic experience by some producers and for this reason they were not prepared to delegate this task to other employees. One previous study has identified the practice of euthanasia generating emotional strain in dairy producers (37) and chronic stress associated with euthanasia of animals in other animal professions such as the veterinary industry, has been shown to be related to increased rates of burn out (38). These finding suggests that the human impact of euthanasia of non-replacement dairy calves should also be considered as a key imperative for the generation of viable production pathways for these animals. Dairy producer well-being in relation to production practices is an area that should be further examined in future studies.

CONCLUSION

The authors believe this is the first report to examine Australian dairy farmers opinions and attitudes to production and management of non-replacement dairy calves and the dairy beef supply chain. The interviews conducted in this study showed that producers considered there are current challenges to rear non-replacement male calves and that there is a knowledge gap related to optimal practices needed to produce a beef carcass able to meet grid specifications for best return on investment. Finally, the personal impact of performing euthanasia was reported by producers, and was highlighted as a last resort, where other avenues for value-chain integration for non-replacement calves had failed. Clearly, producers are looking for options to maintain these animals as a viable income stream, and more options need to be available either on farm or through other production systems.

In response to these findings, the authors suggest that pricing structures and market stability are segments of the supply chain that could be improved to generate a viable dairy beef supply chain and create future market options for non-replacement calves and their retention in the system. This study will inform future quantitative research to expand on key areas including supply chain markets for non-replacement male calf in Australia and globally. There is also a need to further explore producer well-being related to euthanasia and management of non-replacement male calves in the Australian setting.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors on reasonable request.

ETHICS STATEMENT

This study was reviewed and approved by Charles Sturt University Human Research Ethics Committee (Protocol H19225). The participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

VV, AS, MC, and JQ conceived and designed the study. VV collected, compiled, analyzed the data, and wrote the first draft of the manuscript. All authors contributed to review of the manuscript and approve the submitted version.

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Determinants of Ruminant Farmers' Use of Sustainable Production Practices for Climate Change Adaptation and Mitigation in Enugu State, Nigeria

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A sustainable ruminant production system ensures economically viable livestock systems that meet the current and future demands of animal products as well as the environmental safety of current and future generations. The study analyzed the determinants of ruminant farmers' use of sustainable production practices for climate change adaptation and mitigation in Enugu State, Nigeria. Multistage sampling procedure was used to select ninety six (96) ruminant farmers that constituted the sample for the study. Semi-structured interview schedule with open ended questions was used in data collection. Data were analyzed using multiple regression and Pearson Moment Correlation statistics. Access to veterinary services ($t = 2.056$, $p = 0.044$), monthly household income ($t = 3.582$, $p = 0.001$) and annual income from ruminant production ($t = -2.635$, $p = 0.011$) were socio-economic factors that significantly influenced use of sustainable practices. The adjusted R- square implies that the three factors were able to explain 24% of variance in use of sustainable practices. There is a significant positive correlation ($r = 0.426$, $p = 0.000$) between knowledge level of farmers and their use of sustainable production practices. Schemes for financial inclusion such as payment for ecosystem services can spur farmers to adopt mitigation strategies. Improved climate change knowledge can enhance ruminant farmer's resilience to the increasing impacts of climate change.

Keywords: animal welfare, ruminant production, climate change, sustainable agriculture, adaptation and mitigation

INTRODUCTION

Since the Rio meeting of 1992, the world has committed to reducing greenhouse gases which cause climate change and to invest in processes that reduce the impact of climate change on lives and livelihoods of populations. Climate change adaptation and mitigation have therefore been on the front burner of scientific research, attracting international debates, and consensus. The emergent of numerous climate change research gave rise to interests in sustainability and with the Sustainable Development Goals coming on board, researchers and development practitioners have directed efforts to sustainable production. Ruminant production and climate change are strongly inter-dependent. The ruminant sector contributes to greenhouse gases (GHGs) emissions mainly through the emission of methane (CH_4), largely from enteric fermentation, nitrous oxide (N_2O) emission from manure and the use of nitrogenous fertilizers in growing feed, and carbondioxide (CO_2) from fossil fuel burning (1). Globally, ruminants contribute about 80 percent of livestock emissions of carbon dioxide (CO_2), 47 percent of Methane (CH_4), and 24 percent of Nitrous oxide (N_2O) emissions (2, 3). About 90% of livestock emissions are produced by ruminants alone through enteric fermentation (4). Enteric fermentation methane (CH_4), emitted from ruminants during digestion, is a main source of global methane emissions and is responsible for 25% of global methane emissions or 4% of overall anthropogenic GHG emissions (5).

As ruminant production increases worldwide due to increasing population, global increase in income and consequent increase in demand for animal protein, wastes from ruminant production is becoming a serious environmental concern. The expansion in production of ruminant animals in response to the increasing demand leads to resultant expanded quantities and areas of production. These have detrimental effects on the environment (6, 7). Obviously, in spite of their growing global importance, livestock are increasingly being held responsible for many adverse effects on the environments including climate change, loss of vegetation cover, reduced biodiversity, soil erosion and compaction, and excessive run-off often from overgrazing (8).

On the other hand, ruminants are adversely affected by the detrimental effects of extreme weather events. Climate change extremes and seasonal fluctuations in herbage quantity and quality affects the well-being of livestock leading to declines in production and reproduction efficiency (9). For instance, temperature affects most of the critical factors for ruminant production, such as water and feed availability, production, reproduction and health of animals (10). High temperatures predisposes ruminants to physiological stress and diseases. Also, high temperatures trigger the incidence of transmittable chronic respiratory diseases: *coryza*, salmonellosis and infectious *laryngotracheritis* (11). Thermal livestock stress decreases feed intake and efficiency of feed conversion, especially for livestock that are fed large amounts of high-quality feeds. In the case of cattle, feed intake reduction leads to a negative energy balance and reduced weight gain (10). Climate change affects feed availability and quality leading to nutrient deficiencies resulting in metabolic disease of varying nature. Mineral deficiencies results in anemia, retarded growth, and reproductive disorders in livestock. Also, the nutritional stress increases the case of pregnancy toxemia and neonatal death due to poor milk yield and resultant reduction in immunity with consequent proneness to many infectious diseases (12).

Vector-borne diseases are also highly influenced by climatic factors. Climate change result to an increased spread of existing vector-borne diseases and macro-parasites of animals as well as the emergence and spread of new diseases (13). According to Ashraf et al. (14), climate change exerts both direct and indirect influences on the transmission of vector borne diseases, affecting timing of outbreak or the intensity of an outbreak, establishing a temporal linkage and affecting geographical distribution, establishing a spatial linkage of many infectious diseases in animals. Prolonging of the warm season due to climate change may increase the number of cycles of infection possible within 1 year for warm- or cold-associated diseases, respectively. As climate change disrupts rainfall patterns, there are high risks of a number of infectious diseases of ruminants including zoonotic illnesses. For instance, under high humidity, the incidence of *helmenthosis* increases in ruminants (11). Also, prolonged period of no rainfall leads to drought spells which affects pasture availability, quantity and quality. Feed scarcity due to limited pasture leads to stress, immunosuppression and finally predispose ruminant animals to different diseases and death.

Ruminant production is very important to Nigeria's economy not only as source of animal protein but as source of livelihood for the rural poor farmers. In Southeast Nigeria, ruminants are specially raised as source of investment in which they could serve as source of income for household expenses, meat, and manure, used in social and religious ceremonies and as a source of insurance against crop failure (15) especially resulting from climate-related shocks. Sheep and goat accounts for majority of the ruminant production in Enugu State with the cattle being produced mainly for ceremonial activities. All members of the household including men, women and children are involved in the management of ruminant production in the area. Enugu State, ruminant production is faced with numerous challenges ranging from seasonal feed shortages, high mortality rate as a result of diseases and poor access to veterinary services, low reproduction and general sub-optimal management practices. Climate change further increases the already overwhelming risks facing ruminant production in the area.

The strong inter-dependence between ruminant animal production and climate change calls for concerted efforts toward sustainable production of ruminant animals. Hoving et al. (16) noted that sustainable intensification is critical to global production of animal protein and for the farmers and livelihoods that are dependent on livestock. Sustainable agriculture is an agriculture that must produce adequate amounts of high-quality food, protect its resources and be both environmentally safe and profitable (17). Sustainability rests on the principle that the needs of present generation should be met without compromising the ability of future generations to meet their own needs (18). Sustainable production practices are those practices that in as much as they increase production, does little or no harm to the environment. Therefore, the quest to increase ruminant production to meet growing demand should not be at the detriment of the environment. Ensuring economically viable livestock systems that meet the current and future demands of animal products as well as the environmental safety of current and future generations are the interests of a sustainable ruminant production system. Sustainable intensification of ruminant production can be achieved by improving animal health, welfare and production, without harming the environment (19). Hence, adaptation and mitigation strategies in ruminant production need to recognize the unique challenge to decrease absolute emissions, largely through reduced emissions intensity, while meeting the growing global demand for meat and animal products

(20). The FAO (2) noted that improving feeding practices and digestibility of diets, improving yields through genetics, feeding practices and animal health, reducing land use change from feed crop cultivation and pasture expansion, improving manure management, and improving the efficiency of feed crop production, are potential mitigation pathways in ruminant production. Hence, the following questions suffice: Do ruminant farmers use sustainable production practices? Therefore, the study sought to: identify sustainable production practices of ruminant farmers for climate change adaptation and mitigation, determine socio-economic factors influencing farmers' use of sustainable practices, determine the relationship between ruminant farmers' knowledge level and their use of sustainable production practices and examine the challenges encountered by ruminant farmers in using sustainable production practices.

THEORETICAL FRAMEWORK

The Theory of Planned Behavior (TPB) has been largely applied to the study of environmental science research as it can provide valuable implication not only in predicting and managing individual behavior, but also for increasing social and environmental sustainability (21). This paper is based on TPB) which stems from the Theory of Reasoned Action (TRA). The TRA posits that attitude and subjective norms are the determinants of intention, and that intention directly affects behavior to some extent (22). In the TPB, individual intention mainly depends on three determinants: attitude, subjective norms, and perceived behavioral control (21). The theory posits that behavioral intentions are influenced by the attitude about the likelihood that the behavior will have the expected outcome and the subjective evaluation of the risks and benefits of that outcome (23). The two theories are based on the premise that individuals make logical, reasoned decisions to engage in specific behaviors by evaluating the information available to them (24). The performance of a behavior is determined by the individual's intention to engage in it, which is influenced by the value the individual places on the behavior, the ease with which it can be done and the views of significant others and the perception that the behavior is within his/her control. This means that individuals will adopt a behavior which they think they can benefit from and which they have capacity to use within their own specific circumstances and which its adoption is supported by the members of their social system. Knowledge is a prerequisite for effective action (25). Li et al. (26) noted that factors which influence pro-environmental behavior of individuals include environmental knowledge, demographic factors, institutional factors, economic factors, social and cultural factors, motivation, and so on. Farmers' knowledge about a technology is often influenced by their access to information (27–30) which could come from extension, media and the farmers' social network (28, 31, 32). This knowledge influences farmers' evaluative capacity (30) which in turn influences farmers' views about the practices (perceptions) (33). Brokensha et al. (34) noted that farmers' perception, knowledge and practice influence how farming decisions are made. It follows therefore that if farmers have positive perception about sustainable production practices, they are likely to practice them. Their perception is however influenced by their knowledge of such practices. On the other hand, the socio-economic characteristics of a farmer determines whether or not he/she will adopt those practices. Carpenter et al. (35) and Prokopy et al. (30) identified farm and farmer characteristics as important factors enhancing a farmer's ability to adopt an innovation and considered it as a resilience capacity. Prokopy et al. (30), Baumgart-Getz et al. (27), and Li et al. (26) noted that important socio-economic variables influencing farmers' adoption decisions (including pro-environmental decisions) are: age, education (formal education and farmer training (extension), marital status, income, farming experience, tenure, social network, labor, place of residence, capital, information and so on. However, a short coming of this model is that is does not take care of other exogenous factors which could influence an individual's choice of using or not using a particular practice. This was taken care of in the study by adapting and modifying the TPB.

MATERIALS AND METHODS

The study was conducted in Enugu State, Nigeria. The State is located in the Southeast Geo-political Zone of the Country, lying between latitude 5° 56'N and 7°06'N, and longitude 6°53'E and 7°55'E (36). Multistage sampling procedure was used to select ninety six (96) ruminant farmers that were used for the study. At the first stage, two Agricultural zones (Nsukka and Awgu) were selected through purposive sampling technique from the six agricultural zones in the State. At the second stage, two blocks were selected from each zone using simple random technique giving a total of four blocks. The blocks were Nsukka and Igbo-Etiti (Nsukka zone), Awgu and Aninri (Awgu zone). At the third stage, two circles were selected from each block through simple random sampling technique to give a total of eight circles. The circles were Eziani and Obukpa from Nsukka Block, Ekwegbe, and Ozalla from Igbo-Etiti Block, Mgbowo, and Akwu from Awgu Block, Amorji, and Amokwe from Aninri Block. At stage four, from a list of ruminant farmers provided by the extension worker for each circle, 12 farmers each were selected through systematic random sampling technique. Thus, the total sample size for the study was ninety-six (96).

Semi-structured interview schedule with open-ended sections was used in data collection. To ensure face validity, the interview schedule was validated by three experts while a pre-test was done to ensure reliability of instrument. Written consent was presented and read out to the respondents.

(37) noted two ways of measuring sustainability indicators as: (a) practice-based indicators or action-oriented indicators, i.e., using information on farmers' practices or other causal variables (corresponding to most of pressure indicators), and (b) effect-based indicators or result-oriented indicators, i.e., based on an assessment of the effect at different stages of the cause-effect chain (from emission to impact indicators). For the purpose of this paper, sustainability was measured with practice-based indicators. The study used farmers' practices as a measure of their

use of sustainable production practices in ruminant production. Respondents indicated sustainable production practices (SPP) they used for climate change adaptation and mitigation. Each adaptation and mitigation practice used was scored one and a sum of the scores was generated for each respondent. Socio-economic variable were measured as sex (Male = 1, Female = 2), age (years), educational level (years), household size (number of individual in a household), access to extension (number of times in the past 1 year), access to veterinary service (number of times in past 1 year), years of farming experience (number of years), years of ruminant farming experience (number of years), access to credit facilities (Yes = 1, No = 0), annual household income (in naira), annual income form ruminant production (naira). Challenges encountered using sustainable practices were stated and rated on a four-point Likert-type scale of great extent (4), moderate extent (3), little extent (2), and No extent (1) with a mean/cut-off point of 2.5. Any variable with mean of 2.5 and above is accepted as a challenge faced by ruminant farmers in the use of SPP. All interview and discussions were done using local dialect. Data were analyzed using percentage and mean scores, and multiple regression. The socioeconomic factors influencing use of sustainable production practices were measured with a regression model as presented in the model below.

$$Y = \alpha + \beta_1X_1 + \beta_2X_2 + \beta_3X_3 + \beta_4X_4 + \beta_5X_5 + \beta_6X_6 + \beta_7X_7 + \beta_8X_{8.........} + \beta_{12}X_{12} + U$$

Where:

Y = Use of sustainable production practice (Number of sustainable production practices used by the ruminant farmer)

$\beta_{1-}\ \beta_{15}$ = Regression coefficient

X_1 = Age (years)

X_2 = Sex (Male = 1, Female = 0)

X_3 = Marital status [Married = 1 (Living with spouse), Not married (Not living with spouse) = 0]

X_4 = Educational level [Educated=1 (any form of formal education), not educated =0 (no formal education]

X_5 = Years of farming experience (years)

X_6 = Years of experience in ruminant production (years)

X_7 = Household size (number of people living under the same roof and having at least one meal per day together)

X_8 = Extension contact in the last 1 year (Yes = 1, otherwise = 0)

X_9 = Access to veterinary services in the last 1 year (Yes = 1, otherwise = 0)

X_{10} = Access to credit facilities in the last 1 year (Yes = 1, otherwise = 0)

X_{11} = Monthly household income (naira)

X_{12} = Annual income from ruminant production (naira)

U = Error team

Pearson moment correlation was used to determine relationship between knowledge level of ruminant farmers on climate change (KLRFCC) and their use of sustainable production practices (SPP). Knowledge statements were generated for each of causes, effects, and adaptation and mitigation measures to climate change. Respondents reacted to a set of thirty (34) KLRFCC test statements by indicating "True" or "False". Each correct answer was scored "1" while an incorrect answer was scored "0". A composite score was therefore generated for each respondent for knowledge on climate change and actual use of sustainable production practices (SPP). The relationship was measured at 0.05 probability level.

RESULTS

Climate Risks Faced by Ruminant Farmers

A high proportion of the respondents (88.5%) experienced reduced feed/pasture availability; and 94.8% indicated that climate change has resulted in increased price of grains and feed supplement as shown in **Table 1**. As uncertainties in onset and duration of rains increase as a result of climate change, the quantity and quality of pastures decline and farmers would resort to feed supplementation in order to cope. The competition for feed supplements will no doubt lead to high prices which means more financial pressure on the ruminant farmers. Also, 89.6% perceived increased livestock disease occurrence while 88.5% experienced increased mortality of animals due climate change. Higher risks of infection as a result of high temperatures could overwhelm the coping capacity of ruminant farmers leading to increased mortality of the animals. Similarly, 84.4% of the respondents indicated reduced growth rate and 81.3% perceived lower feed intake. Reduced growth rate is directly linked to low feed intake. When feed intake of animals are affected, major metabolic processes are retarded leading to poor growth rate, reduced milk production, low resistance to diseases and death may result. About 60.4% experienced heat stress on their animals and 54.2% experienced reduced water availability. High temperatures causes drought and heat stress on animals. These triggers physiological disorders and reduced activity in ruminants. This corroborates Malami and Tukur (38) that climate change has led to reduction on feed resources, loss in weight, increased mortality of young animals, increased heat load on the animals from cloudless skies for most part of the year, increased diseases and pest incidence in ruminant production. These effects will no doubt result to low production thereby impacting negatively on the farmers' income. Climate change is therefore hampering sustainable livestock production with the result that availability, accessibility and affordability of animal protein will be greatly affected.

Sustainable Production Practices of Farmers for Climate Change Adaptation and Mitigation

Results of the various adaptation options used by ruminant animal as contained in **Table 2** show that almost all (99.0%) of the respondents adapted to the effects of climate change by diversification with non-farming businesses. Ruminant farmers will better adapt to the often sudden and devastating effects of climate change like flooding by engaging in other income generating activities that are not highly dependent on weather events. Most (96.9%) of the respondents adapted by providing sunshade, and 95.8% adapted by ensuring adequate ventilation in the pens. Provision of sunshade and adequate ventilation of pens basically used as measures to reduce the effects of high temperatures which could predispose animals to heat stress and

TABLE 1 | Climate risks faced by ruminant farmers.

Risks factors	Frequency	Percentage
Heat stress on animals	58	60.4
Lower feed intake	78	81.3
Reduced growth rate	81	84.4
Reduced milk production	7	7.3
Reduced milk quality	4	4.2
Reduced feed/pasture availability	85	88.5
Reduced water availability	52	54.2
Reduced quality of pasture available	44	45.8
Increase livestock diseases occurrence	86	89.6
Increased mortality of animals	85	88.5
Reduced meat quality	4	4.2
Reduced fertility	27	28.1
Increase price of grain/feed supplement	91	94.8
Change in the distribution of pests	10	10.4
Cold stress	9	9.4

TABLE 2 | Sustainable production practices used by ruminant farmers for climate change adaptation and mitigation.

Adaptation options	Percentage scores	Mitigation options	Percentage scores
Reducing stocking density	**79.2**	Planting of trees around animal houses	22.9
Provision of sun shade	**96.9**	Reduced manure storage time	4.2
Adequate ventilation of pens	**95.8**	Providing bedding materials during cold	7.3
Use of resistant breeds	**84.4**		
Medication/treatment of animals	**63.5**	Using supplementary feeding	**83.3**
Diversification with non-farming businesses	**99.0**	Frequent removal of effluents	**94.8**
Diversification of livestock	**88.5**	Using rotational grazing system	24.0
Saving of animal feed (hay, straw, silage, etc.)	3.1	Intensive rearing of animals/home feeding	**87.5**
Feeding with higher proportion of concentrates	6.3	Provision of vegetative cover (grasses) around animal farm to reduce heat radiation from the soil	4.2
Provision of plenty fresh drinking water	**89.6**	Reduce temperature in manure storage	3.1
Use of local breeds resistant to prevailing climate conditions	**94.8**	Addition of essential oils to animal diet reduce emissions	4.2
Vaccination of animals	4.2	Diversification of animal feed	**90.6**
Diversification with crop farming	**92.7**	Reducing stocking density	**79.2**
Harvesting forage for ensiling at an early stage of maturity	3.1	–	–
Seasonal migration (movement) of animals	3.1	–	–
Cross breeding with resistant breeds	12.5	–	–

Bold values: Sustainable production practices used.

certain ill-health conditions. Most (94.8%) of the respondents also adapted by use of local breeds. Some local breeds are more resistant to extreme weather and have developed immunity to diseases prevalent in the local environment. The most common local breed present in the study area is the West African Dwarf (WAD) goat and sheep which are well-known to be resistant to trypanosomiasis prevalent in humid parts of the country. Also, 92.7% adapted by diversifying livestock production with crop farming. Diversification of farming enterprise helps farmers to withstand the potential economic losses associated with climate shocks. It increases the resilience of livelihoods to climate impacts (39).

Similarly, the majority (89.6%) of the respondents adapted by providing plenty drinking water for animals, 88.5% adapted by diversifying their livestock types, while 87.5% engaged in intensive rearing/home feeding of animals. Provision of plenty fresh drinking water can help cushion the effect of heat stress while diversification of livestock can help reduce the infestation and spread of weather- related illnesses that are more prevalent in a particular specie of animal (10). Also, 63.5% adapted by medication/treatment of animals. Regular treatment of diseases improves herd health and productivity thereby reducing death of animals and consequent economic loss resulting from climate change.

These results show that ruminant farmers are adapting to climate change by using various sustainable management practices. Improved management practices which do not increase harm done to the environment are advocated for sustainable ruminant production (40). These practice ensure that farmers continue to increase production to enhance profitability of their livelihood activities while constituting minimal or no harm to the environment.

Results show that respondents adopted mitigation options as indicated by 94.8% who engage in frequent removal of effluents, while 90.6% diversify animal feed and 83.3% used supplementary feeding. Deficiencies and metabolic diseases caused by feed scarcity and poor quality feed can be cushioned through feed diversification and use of supplements while removal of effluents can ameliorates the build-up of GHGs. About 87.5% engage in intensive rearing and 79.2% engage in reduction of stocking density which leads to lesser emissions of GHGs (41).

It is noteworthy however that a number of sustainable practices were yet to be embraced by a good number of

TABLE 3 | Socio-economic factors influencing respondents' use of sustainable practices.

Model	Unstandardized coefficients		Standardized coefficients		
	B	**Std error**	**Beta**	**T**	**Sig**
Constant	9.911	1.349		7.346	0.000
Age	0.046	0.031	0.309	1.493	0.141
Sex	−0.460	0.499	−0.122	−0.922	0.360
Marital status	−0.171	0.613	−0.033	−0.280	0.781
Educational level	0.758	0.584	0.173	1.298	0.199
Years of farming experience	−0.010	0.035	−0.069	−0.297	0.768
Years of experience in ruminant production	−0.015	0.030	−0.087	−0.502	0.618
Size of household	0.021	0.107	0.022	0.193	0.847
Extension contact	−3.586	1.966	−0.195	−1.823	0.073
Access to veterinary services	0.901	0.438	0.239	2.056	**0.044**
Access to credits facilities	0.367	0.949	0.044	0.387	0.700
Estimated monthly income	5.927-5	0.000	0.473	3.582	**0.001**
Annual income from ruminant production	−8.345-5	0.000	−0.382	−2.635	**0.011**

Dependent variable: number of sustainable production practices, $P = 0.05$, $R = 0.604$, $R^2 = 0.365$, Adjusted $R^2 = 0.236$. Bold values: Significant values.

TABLE 4 | Correlation between knowledge level and number of sustainable practices used.

		Knowledge level	**Number of sustainable production practices used**
Knowledge level and use of SPP	Correlation coefficient	1	0.426
	Sig. (2-tailed)		0.000
	N	96	96

Source: Field data, 2018–2019.

ruminant farmers. A closer look at the results suggests that respondents were less engaged in mitigation practices. Planting trees around animal houses contributes to carbon sequestration as well as providing shading to animal houses in extreme weather conditions (10, 16), feeding with higher proportion of concentrates reduces methane release during enteric fermentation (42), addition of essential oils in feed reduces methane release during enteric fermentation (43, 44), reduced manure storage time lowers the emissions of nitrous oxide and methane by volatilization (10, 45), while reduced temperature in manure storage (manure cooling) reduces methane formation (46). The implication of the low use of the mitigation measures is that more harm will continue to be done on the environment by ruminant farmers in the area. On the other hand, the results reveal that proactive measures to possible effects of impending climate catastrophes were not widely practiced by the respondents. For instance, harvesting silage at early stage of maturity helps to ensure nutritious herbage (16, 42) even during drought. seasonal migration of animals helps farmers avoid impending climate risks like drought, flood, and disease epidemics (47), storing of animal feed can help ensure availability of enough quantity of feed in periods of scarcity such as drought, while immunization/vaccination of animals helps to prevent disease epidemic among herds which can be triggered by climate extremes (39). Harvesting of silage and storing of forage for use during drought periods could reduce migration and the consequent incessant farmer-herder conflicts which has resulted to unprecedented loss of lives and properties in the country.

Socio-Economic Factors Influencing Respondents' Use of Sustainable Practices

Table 3 shows the influence of socio-economic characteristics of respondents on their use of sustainable production practices. The overall result was significant ($F = 2.829$, $p = 0.004$), implying that socio-economic characteristics of ruminant farmers had significant influence on their use of sustainable production practices. The regression results show that among the socio-economic factors, access to veterinary services ($t = 2.056$, $p < 0.05$) had significant positive influence on use of sustainable production practices. This implies that ruminant farmers with access to veterinary services engaged more sustainable production practices than those without access. This could be attributed to the fact that veterinarians supply farmers with relevant advice on sustainable strategies used in dealing with the impacts of climate change on health of ruminant animals. Also, monthly household income had significant positive influence ($t = 3.582$, $p < 0.05$) on the use of sustainable production practices. Household income in this study refers to the totality of income generated (estimated monthly) by all members of a household including farm and non-farm incomes. This means that higher income status of households enhances the use of sustainable production practices. The result may stem from the fact that a number of sustainable production practice may require extra expenses and household members can willingly and easily offer financial assistance to the household member rearing ruminants. The implication of this is that relevant bodies such as development agencies, governments

TABLE 5 | Challenges to use of sustainable production practices in ruminant production.

Constraints	Mean	Std deviation
Lack of funds	**3.71**	0.78
High cost of feeds	2.41	1.25
High prevalence of animal diseases	**2.77**	0.83
Poor educational level of farmers	2.00	1.01
Failed government policies	1.70	0.95
Urbanization	1.14	0.57
Inadequate storage facilities	1.51	0.81
Glut when marketing during shock	1.27	0.62
Inadequate extension services	2.37	1.08
Inadequate manpower	2.20	1.15
Poor awareness on sustainable production practices	2.22	1.16
Water scarcity	1.70	0.81
Pressure on grazing lands	1.45	0.80
Lack of access to improved breeds	1.56	0.90
Theft	1.47	0.94
Inadequate modern farm input	1.56	0.89
Lack of good management skills	1.99	1.05
High cost of drugs	**2.68**	1.11
Transportation issues	1.39	0.64
Inadequate basic infrastructure	1.50	0.82
Land scarcity	2.52	1.24
Poor attitude to animal production	1.71	1.10
Cultural influence	1.03	0.18

Cut-off = 2.5. Bold values: equal to or greater than the cut-off point.

and financial institutions could support the use of sustainable production practices through programmes aimed at increasing the financial base of ruminant farmers. Terfa and William (48) stated that since rural farm households in sub-Saharan Africa are vulnerable to climate change as they have low financial base, it is crucial to examine how financial inclusion can enhance their resilience to the increasing impact of climate change.

On the other hand, results show that annual income from ruminant production had significant negative influence ($t = -2.635$, $p < 0.05$) on use of sustainable practices. This could mean that larger farms tend to engage in unsustainable practices than smaller farmers. According to Lin et al. (49), large scale livestock production increases the impact of livestock on GHGs emissions which includes a variety of production-related activities such as over grazing, enteric fermentation, feed-crop production with fertilizers and burning of fossil fuel through transportation of inputs, outputs and products. This result implies that ruminant farmers tend to overlook their production activities once their herd size gets larger. This is attributable to poor management arising from the financial, labor and other demands required in taking care of large herd size. Advocates of sustainable development could target larger ruminant farms in the provision of incentives for enhancing sustainable production of ruminant.

The R square value is the proportion of the variability in the use of sustainable production practices which was explained by the regression model. The adjusted R square is the estimate of r^2 for the population. Therefore, access to veterinary services, monthly income and income from ruminant production were able to explain 24% of the variance in the use of sustainable practices by respondents. The TPB posits that individuals will adopt a behavior which they think they can benefit from and which they have capacity to use within their own specific circumstances. This result support the theory in that it shows that the ruminant farmers can use those sustainable practices for which they have financial capacity to adopt.

Relationship Between Respondents' Knowledge Level on Climate Change (KLCC) and Their Use of Sustainable Production Practices (SPP)

Table 4 shows a Pearson Moment correlation between ruminant farmers' knowledge level on climate change (KLCC) and their use of sustainable production practices (SPP). Results show that there is a significant positive correlation ($r = 0.426$, $p < 0.05$) between KLCC and their use of SPP. This means that higher knowledge level of ruminant farmers on climate change enhances use of sustainable production practices. Nwobodo and Agwu (18) had noted that climate change knowledge level of farmers needed to be improved in order to foster effective adaptation and mitigation as continuous neglect of cognitive adaptive capacity of individual actors on climate change will undermine efforts of attaining the goals of current and future adaptation strategies. The implication of this is that climate change communicative interventions such as trainings and symposiums should target ruminant farmers to boost their knowledge and enhance their use of sustainable practices. Such interventions could focus more on activities that enhance mitigation by reducing greenhouse gas emissions thereby reducing catastrophic events resulting from climate change. This will promote long term resilience of ruminant farming systems. Pretty and Bharucha (50) suggest improvement of farmers' knowledge and capacity through the use of farmer field schools, videos and modern information communication technologies.

Challenges to Use of Sustainable Production Practices

The challenges (**Table 5**) farmers encounter in using sustainable practice in ruminant production were: lack of funds ($M = 3.71$, SD = 0.78), land scarcity ($M = 2.52$, SD = 1.24), high prevalence of animal diseases ($M = 2.77$, SD = 0.83), and high cost of drugs ($M = 2.68$, SD = 1.11). Land scarcity and lack of funds hinders expansion of ruminant production enterprise. Farmers are limited in the number of animals they can keep because of insufficient fund and limited land area. Farmers in the area could only construct small pens within the available land which can only contain limited number of animals. This will restrict the spaces available and could subject the animal to overcrowding. Respondents narrated how livestock diseases such as tryponosomiasis, mastitis, brucellosis, foot and mouth disease, ecto- parasites and endo-parasites are reported had constrained their ruminant production enterprise. They stated that high cost of drugs and veterinary services to attend to sick animals is very high leaving many of the farmers without regular access veterinary services. Offor et al. (51) had noted that insufficient fund and disease incidence were the major constraints identified by small ruminant farmers. The increased incidences of diseases outbreak resulting from climate change without regular access to veterinary services leads to high mortality which translates to huge economic losses to ruminant farmers.

The standard deviations from almost all the results were less than zero (0), except for high cost of drugs. This suggests that respondents had convergent view on the challenges they faced in using sustainable production practices. However, respondents had little bit of divergent view on high cost of drug. This could mean that high cost of drug is relative to the income level of each of the respondents. Most of the ruminant farmers were poor and would find it costly to afford basic drugs for their animals. Having identified diseases outbreak as a major constraint, the farmers would not have complained about the cost of drugs if they were high income earners.

DISCUSSION

Ruminant farmers make use of sustainable production practices. However, they made more use of adaptation practices and little of mitigation practices. They may have engaged more in adaptation because those measures are more or less *ad hoc*, offering immediate relief to the effects of climate change. This is attributable to their low scale of production which does not encourage long term investments especially in practices that do not yield immediate results. Also, farmers may not be inclined to adopting mitigation measures if they do not see a tangible link between such measures and farm productivity and/or household food security. Wollenberg et al. (52) argue that smallholder farmers in developing countries prioritize immediate benefits and are more likely to adopt mitigation measures if they perceive co-benefits or outcomes in using such measure in enhancing productivity and improved household food security. Ruminant farmers could be encouraged to adopt mitigation strategies through schemes such as payment for ecosystem services, increased prices for sustainable low carbon impact products. Agricultural development agencies and governments could mainstream mitigation of climate change through such policies that offer incentives to ruminant farmers who adopt mitigation measures. Although the number of sustainable productive practices used appears to be limited in terms of explaining the use of SPP. However, the challenges to use throws explained other factors that could constrain or enhance use. Lack of funds, high prevalence of animal diseases, high cost of drugs and land scarcity were the challenges faced by farmers in using sustainable production practices. Having access to veterinary services, estimated monthly income were the socio-economic factors that positively influence farmers use of sustainable practices while annual income from ruminant production negatively influence farmers use of sustainable practices.

CONCLUSION

Relevant policies that promote financial inclusion such as improved access to credits, loans and grants will help ruminant farmers build resilience while minimizing their contributions to climate change. The significant positive correlation between knowledge level on climate change and use of SPP implies that more knowledge of climate change issues could translate to better adaptation to and mitigation of climate change. Therefore, **a**gricultural extension agencies should prioritize adaptation and mitigation in its tool kit. Famer-to-farmer extension should be encouraged in order to offer ruminant farmers more opportunities to learn from fellow farmers who could act as climate change vanguards by development agencies. More sophisticated adaptation and mitigation strategies could be introduced to farmers in the area. Governments could make provisions for veterinary services at a subsidized rate for farmers.

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/s.

ETHICS STATEMENT

The study involving human participants was reviewed and approved by the Department of Agricultural Extension, University of Nigeria, Nsukka. Written informed consent of the participants was received during the field survey.

AUTHOR CONTRIBUTIONS

CN conceived and conceptualized the study and wrote the draft of the manuscript. BN wrote the background, collected the data, and wrote part of the manuscript. JI scrutinized and improved the manuscript. VO and RO read and corrected the background and methodology. All authors contributed to the article and approved the submitted version.

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Dynamics of Thermolysis and Skin Microstructure in Water Buffaloes Reared in Humid Tropical Climate—A Microscopic and Thermographic Study

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The thermolytic capacity test is used to assess the adaptability of animals to existing environmental conditions. However, there is insufficient information on the relationship between histomorphometry and adaptability of buffaloes. Thus, this study aimed to assess the use of thermolysis pathways by buffaloes reared in a hot and humid environment so as to understand the relationships between environment, skin morphological characteristics, and heat storage, as well as the intensity and proportionality of use of its ways of dissipating heat to maintain homeothermy. The heat tolerance test, associated with the evaluations *via* infrared thermography, was applied to 10 female Murrah buffaloes and tegument histomorphometry was carried out. The animals exhibited very high heat tolerance with an average of 9.66 ± 0.21 and used thermal polypnea as the main heat dissipation pathway. Their mean skin thickness was 6.03 ± 1.16 mm and the active sweat and sebaceous gland tissue were $1.57 \pm 0.38\%$ and $1.08 \pm 0.39\%$, respectively. The buffaloes exhibited a positive correlation between eyeball temperature and internal body temperature ($r = 0.84523$, $p < 0.0001$) and a negative correlation between respiratory rate and skin thickness ($r = -0.73371$, $p = 0.0157$). The high thermolytic capacity in shade conditions confirms the importance of access to shade in buffalo rearing systems in tropical regions.

Keywords: acclimatization, *Bubalus bubalis*, heat stress, histology, infrared thermography, thermoregulation, precision livestock farming

INTRODUCTION

Domestic buffaloes (*Bubalus bubalis*) are animals that inhabit different ecosystems (1). The worldwide buffalo population is estimated at approximately 208 million animals, 62% of which reared in the intertropical zone, distributed across 37 countries located in Asia (61.42%), Americas (1.16%), Africa (0.002%), and Oceania (0.002%) (2). There is also a considerable buffalo population that, despite being reared in subtropical or temperate climates, has been stricken with more intense and more frequent heat loads (3). In all these countries, one of the most important roles of buffaloes is, undoubtedly, milk production, significantly contributing to food safety, in addition to being efficient as draft power (2, 4). Thus, the buffalo is a model of multipurpose bovid which serves larger-scale productions as well as plays an essential role in the assets and economy of smallholders in several countries in Asia and Latin America, such as the Philippines, Thailand, Brazil, and Mexico (5–8). One of the most notable characteristics of buffaloes is their adaptative capacity (9). However, despite their adaptability to different environmental conditions, the water buffaloes have morphological peculiarities that impact the regulation of their body temperature (BT) (10, 11), making them more susceptible to heat stress, particularly when submitted to environmental conditions of high temperature and relative humidity and/or intense direct solar radiation (12).

The skin is the largest organ in the body and has a role of thermoregulation, defense, and protection of the organism (13). Buffaloes have thick skin, with a prominent stratum corneum, that can be twice as thick as the skin of bovines (11 μm vs. 5 μm) (14, 15). Besides very pigmented skin, the high melanin concentration provides them increased protection against ultraviolet radiation (10). Moreover, the epidermis of buffaloes extends through numerous papillae, which influences the distribution of blood vessels and favors the vasodilation process (16). However, the water buffaloes have a lower number of hair follicles (HF) on their body surfaces when compared with Zebu cattle (135–145 vs. 3.000 follicles.cm^{-2}) (17) and this reduced the layer of reflecting fur on the epidermis makes them more susceptible to visible and infrared radiations, which are more absorbed and transmitted due to the black color of the epidermis (18, 19). Their hairs are relatively long and thick and connect to the skin associated with sweat and sebaceous glands (20). The density of sweat glands is low, and those glands are of the type apocrine and merocrine in a simple saccular and spiral tubular format, located deep into the reticular dermis and surrounded by blood vessels and nerve fibers (20). The sebaceous glands of buffaloes are simple or compound alveolar located in the reticular layer of the dermis and surround the entire HF, secreting sebum in a holocrine way (21).

Heat stress is antagonistic to animal welfare and leads to economic losses resulting from the reduction in productive and reproductive performance, besides increasing buffalo morbidity and mortality (22). The recent studies have shown the negative effects of heat stress on dairy buffaloes (23–25), with significant changes in milk composition and industrial yield such as reduced contents of fat, protein, lactose, and total solids (26–29). In addition, the negative effects of heat stress have been reported on conception rates, which decrease when temperature-humidity index values are above 80 (30–32), and on semen quality, with decreased percentage of live spermatozoa and increased abnormal sperm population (33–36). Heat stress also impairs the immune response, affecting the gene expression of cytokines and their receptors (37, 38). To mitigate the deleterious effects of heat stress in water buffaloes and provide them greater thermal comfort, establishing environmental management strategies (39) as well as the identification and selection of more heat-tolerant animals are equally important (40, 41).

A thermotolerant animal is able to maintain its homeothermy even under high environmental heat loads (29). Several measures have been proposed for phenotyping thermotolerant animals, which, in general, include functional evaluations based on the monitoring of physiological variables related to body thermoregulation, including internal temperature, respiratory and heart rates, and blood parameters. Among the blood or serum parameters commonly assessed are hematocrit, hemogram, hemoglobin concentration, oxidative stress markers, and the concentration of glucocorticoid and thyroid hormones and of heat shock proteins (42, 43). In turn, body surface temperature (ST) monitored by infrared thermography has been used as an indicator of heat production since this technique allows assessing the amount of thermal energy emitted by longwave radiation on a surface (19). Implementing a program for the identification and selection of thermotolerant animals requires evaluation strategies that can be adopted easily and with low cost. In this sense, the heat tolerance test (HTT) has been used to assess the adaptability of water buffaloes to hot environments (44), a test that requires relatively little time and can be performed on-farm (45). However, there are no studies associating buffalo thermolytic response (capacity of dissipating heat through peripheral vasodilation, sweating, and thermal polypnea) in this test with microscopic anatomic tegument characteristics, which is a gap in knowledge seen as the buffalo tegument system has features that completely distinguish it from other domestic animal species. Although some morphological particularities of buffaloes are known, there is a little fundamental knowledge on the histomorphometric characteristics of the skin of those animals and their relationship with thermolytic adaptive capacity. Therefore, this study is proposed aiming at expanding the knowledge and understanding of buffalo thermoregulating mechanisms and thermal transfer mechanisms between the animal and the environment. The aims of the study are as follows: To (i) assess the thermolytic responses of buffaloes submitted to thermal challenge in a humid tropical environment and (ii) determine the relationships between the thermal environment, cutaneous morphological characteristics, and heat storage (HS), as well as the intensity and proportionality of the use of heat dissipations pathways to maintain homeothermy.

MATERIALS AND METHODS

Site, Climate, and Period

The experiment was conducted at the Biotechnology Center of Animal Reproduction—CEBRAN of the Federal University of Pará, in Castanhal, PA, Brazil (01°30'48"S and 47°94'23"W, 41-m altitude). The local climate subtype was humid tropical, Afi (Köppen), characterized by well-distributed annual rainfall, with a rainier period from January to June and a less rainy period from July to December. The mean annual air temperature was 26.6°C (min. 23.0°C; max. 32.0°C). The mean annual relative humidity was 83% and the cumulative annual rainfall was 2,900 mm (46). The study was conducted between November and December.

Animals and Handling

Ten female Murrah buffaloes previously reared under the same environmental and handling conditions were used. Prior to the beginning of the study, the animals underwent clinical evaluation, diagnostic tests, and a quarantine period to ensure proper health condition. At the beginning of the experiment, the animals were 18 ± 0.7 months of age (18–20 months), with mean weight of 336.3 ± 38.2 kg (264–398 kg) and body condition score of 3.0 ± 0.4 (2.5–4.0) (scale from 1–5) (47). The buffaloes were kept in a 0.2-ha pen with *Brachiaria decumbens* pasture and natural shade (90 m^2 per animal). The animals were fed daily (between 8 a.m. and 4 p.m.) supplementary feed made up of elephant grass (*Pennisetum purpureum*) silage and concentrate based on wheat bran, in addition to mineral mixtures. Access to the automated drinking trough was *ad libitum*.

Heat Storage and Cumulative Heat Storage

The buffaloes were individually weighed on an electronic scale and the body weight was used to calculate HS (in $W \cdot m^{-2}$) and cumulative heat storage (CHS) ($W \cdot m^{-2}\ h^{-1}$) on the days of data collection, according to methodology described by McGovern and Bruce (48) [Eqs. (1) and (2), respectively].

$$\Delta RT = ((3600 \times HS \times A))/((Bw \times cb)) \quad (1)$$

where ΔRT is the differences between rectal temperatures at different hours; HS is the HT ($W \cdot m^{-2}$); A is the animal surface (m^2) calculated by the equation: $A = 0.13 \times Bw^{0.556}$ (where Bw is the body weight in kg) and cb is the specific heat of the animal (3.4 $KJ \cdot kg^{-1}\ K^{-1}$).

$$CHS = \sum_{12}^{3} HS \quad (2)$$

where CHS is the cumulative heat storage ($W \cdot m^{-2}.h^{-1}$) calculated the by sum of the storage heat in different time intervals (from 12 p.m. to 3 p.m.), with the zero value being considered at 12 p.m.

Heat Tolerance Test

To determine the individual thermolysis capacity, the heat tolerance test (HTT) described by Baccari Junior et al. (44) and modified by Titto et al. (45, 49) was applied. Over a 15-days period, the test was applied on 4 non-consecutive days, which exhibited specific and predetermined meteorological characteristics of cloudless sky, no rainfall or wind gusts, and minimum black globe temperature of 45°C in full sunlight. During the application of the test, the animals were kept standing to avoid heat exchange with the soil *via* conduction, and remained in food and water fast so as to not change HS (45).

For the test, the buffaloes were conducted to a corral with holding pens and rough concrete floor, 4-m ceiling, and roofing with ceramic tiles, which provided 162 m^2 of shade, with access to an adjacent open area of 120 m^2 with dirt floor, with no drinking or feeding troughs, and delimited by a fence with six smooth wires, where the animals were exposed to direct solar radiation.

According to the protocol of the HTT by Baccari Junior et al. (44), modified by Titto et al. (45, 49) to compare the rates of heat acquisition and dissipation, the buffaloes were kept under shade condition for 2 h, from 10 a.m. to 12 p.m. (Period 0). The physiological variables recorded in this period represented the baseline individual reference values. Next, the animals were exposed to solar radiation for 1 h, from 12 p.m. to 1 p.m. (Period 1), the period of heat challenge. After that, the animals were returned to the shade, where they were kept for 2 h, from 1 p.m. to 2 p.m. (Period 2) and from 2 p.m. to 3 p.m. (Period 3), representing the period for tolerance recovery of the animals after the heat challenge. By the end of Periods 1, 2, and 3, the physiological variables of internal body temperature, respiratory rate, and body ST were recorded (see description in the Section, Physiological variables). All buffaloes were assessed on the same days as the test.

Next, the heat tolerance index (HTI) was calculated based on the equation that takes into account the mean internal body temperatures in Periods 0 and 3 [Eq.(3)].

$$HTI = 10 - (BT3 - BT0) \quad (3)$$

where HTI is the heat tolerance index, which is calculated by the difference between the internal BT obtained at the end of Period 3 (BT3) and the internal BT obtained at the end of Period 0 (BT0).

The result of the HTI varies on a scale from 0 to 10, which represents the capacity of the animals to dissipate the heat absorbed during exposure to sunlight. The closer the result is to 10, the more tolerant the animal (44). According to Titto et al. (45), an animal is classified as very sensitive to heat stress when its HTI is below 7.5; low heat tolerance when its HTI is between 7.51 and 8.2; medium heat tolerance with HTI between 8.21 and 8.9; high heat tolerance with HTI between 8.91 and 9.5; and very high heat tolerance when its HTI is equal to or higher than 9.51.

Physiological Variables

The comparison of the rates of heat acquisition and dissipation was based on alterations of internal BT (°C), respiratory rate (RR) ($mov \cdot min^{-1}$), surface temperature (ST) (°C), and on the calculation of the respective HS in each experimental period. RR was recorded *via* inspection and counting of thoracic movements for 1 min with the aid of a stopwatch and was recorded before the animals entered the holding pen for verification of the other physiological variables. The BT was measured by clinical transrectal thermometry using a digital thermometer (Termomed, Incoterm, Brazil) with the measuring range, 32.0–42.0°C, resolution of 0.1°C, maximum error of ± 0.2°C, and a self-checking system.

The ST by Infrared Thermography

The ST was assessed by infrared thermography using a portable thermographic camera (FLIR E-5, Oregon, USA) operating in the spectral range of 7.5–13 μm, with detector of 160 × 120 pixels, thermal sensitivity of <100 mK, field of view of 45° × 34°, and an automatic calibration. The thermographic images were always recorded on the right-side antimere of the animals, with 0.98

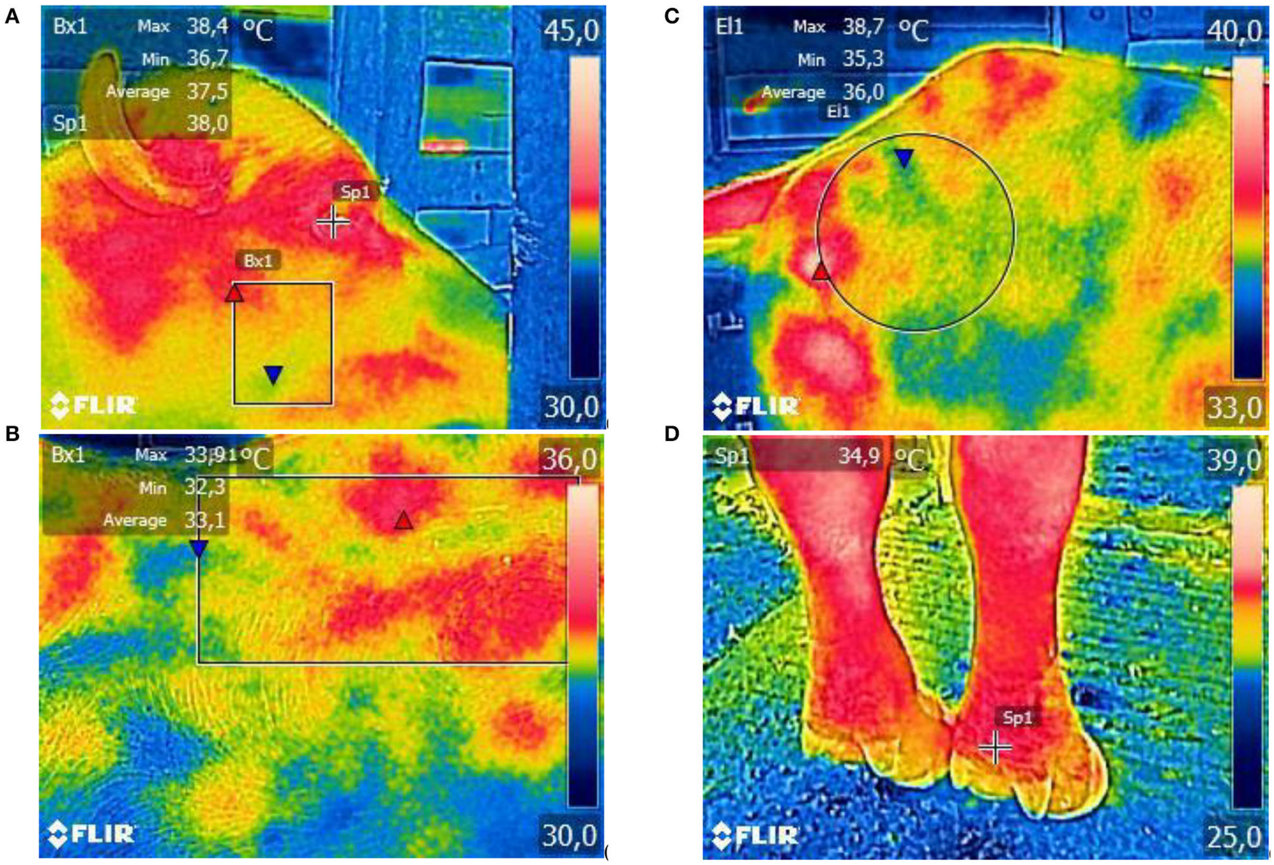

FIGURE 1 | Illustrative images of thermographic images taken to assess STs of the eyeball and cheek **(A)**, trunk **(B)**, rump **(C)**, and forelimb **(D)** of adult female buffaloes. Images parametrized for the rainbow color palette and automatic thermal scale.

emissivity adopted according to indications by Brcko et al. (50). All thermograms were analyzed in the software FLIR Tools (FLIR tools, RRID:SCR_016330).

The STs of interest (**Figure 1**) were eyeball surface temperature (EST) (°C), resulting from the hotspot recorded in the eyeball region (51); cheek surface temperature (CST) (°C), determined by the mean temperature of the rectangular area between the region of the zygomatic and jaw bones (50); trunk surface temperature (TST) (°C), determined by the mean temperatures of the rectangular area in the dorsal region between the sternal ribs (50); rump surface temperature (RST) (°C), determined by the mean temperature of the circular area between the ileum and ischium (50); and forelimb surface temperature (FST) (°C), measured in the region of the ergot, above the phalanx (50). In addition, the compound surface temperature (CompST) (°C) was calculated, a derivative variable expressed by the arithmetic mean of the STs in the other body regions assessed, as proposed by Sevegnani et al. (52).

Biometeorological Monitoring and Black Globe Temperature and Humidity Index

During the experiment, the biometeorological variables of dry-bulb temperature (DBT; °C), relative humidity (RH) (%), and black globe temperature (°C) were permanently monitored and recorded in 10-min intervals using electronic devices placed in the handling corral, in the environments where the animals underwent the HTT, in full sunlight, and in the shade. The variables were monitored using a HOBO U12-012 datalogger (Onset, Brazil) (HOBOware Pro, RRID:SCR_021915) installed at a height of 1.60 m from the ground. The BGHI was later calculated as described by Buffington et al. (53), determined by Eq. (4).

$$BGHI = T_{bg} + \left(0.36 \times T_{dp}\right) + 41.5 \tag{4}$$

where T_{bg} is the black globe temperature (°C) and T_{dp} is the dew point temperature (°C).

According to Baêta and Souza (54), BGHI values up to 74 indicate a comfort condition for the animals; from 74 to 78, an alert situation; from 79 to 84, a danger situation; and above 84, an emergency situation.

The biometeorological data recorded during the HTT are presented in **Figure 2**.

Skin Histomorphometry

Thirty days after the end of the HTT, the skin microbiopsy was performed in all animals for histomorphometric analyses. The

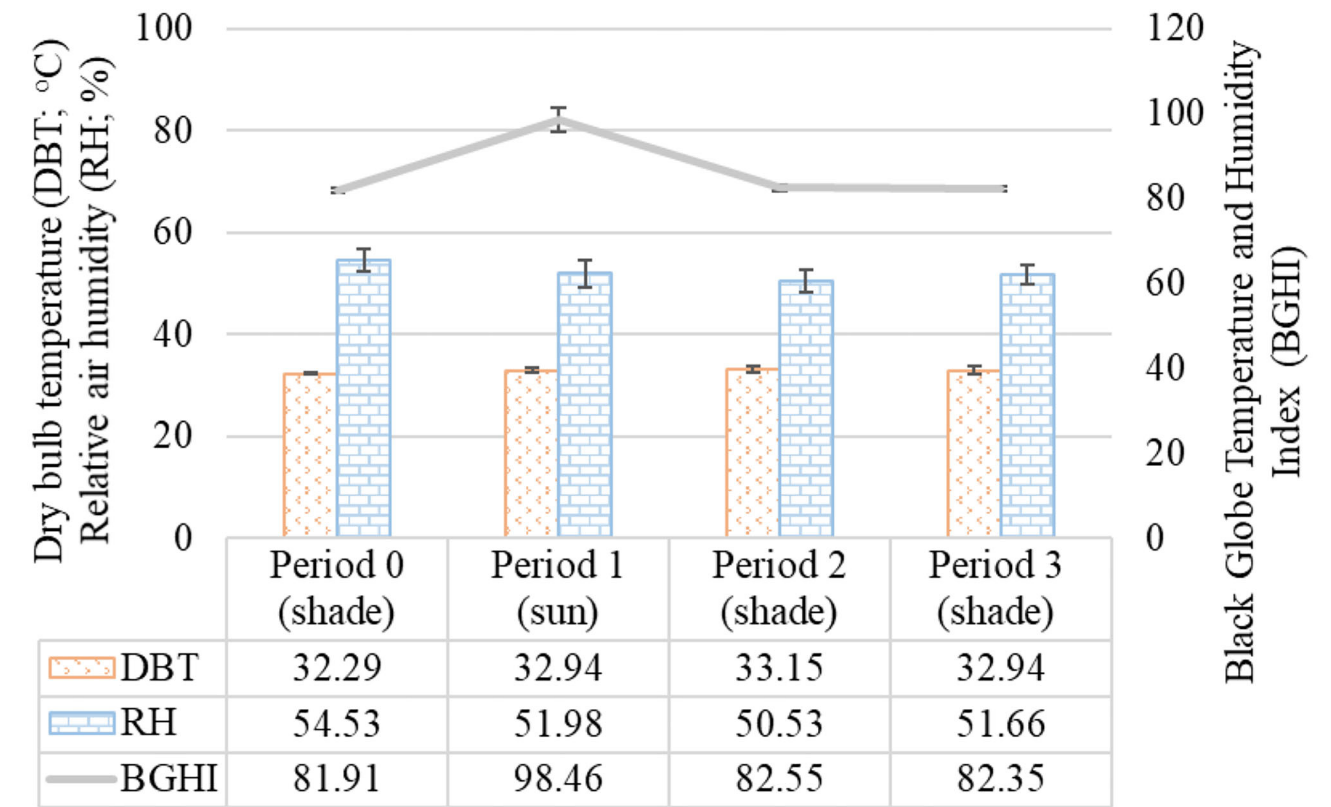

FIGURE 2 | Mean and standard deviation of the biometeorological variables recorded in different observation periods during the HTT carried out in adult female buffaloes in humid tropical climate. Period 0 (shade): From 10 a.m. to 12 p.m.; Period 1 (sun): From 12 p.m. to 1 p.m.; Period 2 (shade): From 1 p.m. to 2 p.m.; Period 3 (shade): From 2 p.m. to 3 p.m.

microbiopsy was performed on the right-side antimere at the mean height of the back, in the region of the 12th intercostal space. The buffaloes were in water and food fast for 12 h and the microsurgical procedures were performed after physical restraining the animal and local skin anesthesia as described by Kahwage (55).

The samples were fixed in 10% formalin solution for 48 h and then underwent an 8 h cycle in an automated tissue processor (OMA DM-40, OMA Metalúrgica, Brazil) for dehydration and clarification. The samples were then included in paraffin blocks and processed histologically, with 4-μm thick cuts parallel and perpendicular to the skin surface made. Next, the samples were mounted on glass slides, stained using Masson's trichrome method (56), and assessed in light-field optical microscopy (Leica DME, Leica Microsystems, Germany) (Leica Microsystems, RRID:SCR_008960). The assessment was carried out with total magnification of 40× for the parallel cuts and 100× for the perpendicular ones. The images were digitized and stored in a database for later analysis using the software Image J (ImageJ, RRID:SCR_003070) (57).

In the digitized images of the parallel cuts, the following parameters were assessed: (i) The number of HF; and (ii) hair density (HD) (follicles·mm^{-2}). In the digitized images of the perpendicular cuts, the following parameters were assessed: (i) Thickness of skin (TS) (mm); (ii) sweat gland area (ASwG) (μm^2), which corresponds to the mean value of the area of each sweat gland; (iii) sebaceous gland area (ASG) (μm^2), which corresponds to the mean value of the area of each sebaceous gland; (iv) sweat gland area by surface (ASwG_surface) (%), which corresponds to the percentage of active sweat gland tissue in the sample; (v) sebaceous gland area by surface (ASG_surface) (%), which corresponds to the percentage of active sebaceous gland tissue in the sample; and (vi) height of sweat gland (HSwG) (μm) at four spots of the gland epithelium (58). The TS was determined after the mounting of composite images (**Figure 3**) using the software Adobe Photoshop CS5, with the standard of 30% overlap adopted between images to create homogeneous mosaics. Five composite images were analyzed per animal with three skin thickness measurements per image at three distinct anatomic spots. Skin thickness was given by the linear distance measured on the surface of the epidermis until the smooth musculature, with the mean value of each sample being adopted.

Statistical Analysis

The response variables were approached using different statistical models according to the characteristic of each variable. Initially, a descriptive analysis of the data was made for the biometeorological and physiological variables. Data normality was evaluated by Shapiro–Wilk and/or Kolmogorov–Smirnov tests and the equality of variances was evaluated by Levene's

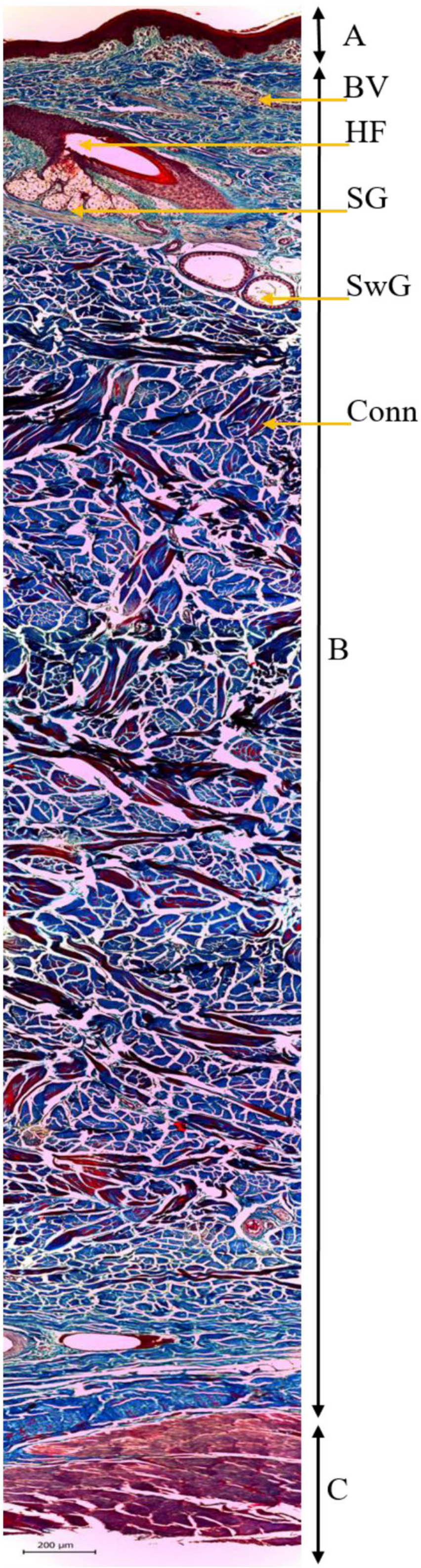

FIGURE 3 | Histological image of the skin of buffaloes (*B. bubalis*) stained by Mason's trichrome, assessed in light-field optical microscopy (100×): epidermis **(A)**, dermis and hypodermis **(B)**, and smooth musculature **(C)**, with emphasis on the identification of the hair follicle (HF), sebaceous glands (SG), sweat glands (SwG), blood vessels (BV), and conjunctive tissue (Conn).

test. For the biometeorological variables, one-way ANOVA (DBT and RH) and Kruskal–Wallis test (BGHI) were applied. For the parametric analyses of the physiological variables, fixed-effects (treatment-periods) and random-effects (animals and date) ANOVA were applied. The means between periods were compared by Tukey's test and, if needed, the data were transformed by BoxCox to meet the assumptions of the parametric analyses. All those analyses considered significance at 5%.

In addition, the factorial analysis by principal components (PCs) was performed to cluster and identify the behaviors of the correlations between the variables TS, ASG_surface, RR, CHS, and HTI. Bartlett's test of sphericity and Kaiser–Meyer–Olkin test were performed to assess whether the factorial analysis fitted the data. Finally, bivariate Pearson linear correlation analysis was carried out for the variables RR, BT, EST, CST, TST, RST, FST, CHS, BGHI, HF, HD, TS, HSwG, ASwG, ASwG_surface, ASG, ASG_surface, and HTI. The data were analyzed in the softwares SAS version 9.1.3 (Statistical Analysis System, RRID:SCR_008567) (59) and Rstudio version 1.4.1717 (R Project for Statistical Computing, RRID:SCR_001905) (60).

RESULTS

Based on the averages of the biometeorological variables, it was found that DBT ($p = 0.13251$) and RH ($p = 0.12783$) did not change during the observation times over the HTT. The mean maximum values of DBT were 33.15 ± 0.57°C with coefficient of variation of 1.72% recorded in Period 2. The mean maximum values of RH were 54.53 ± 2.16% with coefficient of variation of 3.96% recorded in Period 0. However, the non-parametric analysis by Kruskal–Wallis test indicated a significant difference for BGHI between the experimental periods ($p = 0.02731$), with the highest BGHI value of 98.46 ± 2.88 with coefficient of variation of 2.93% recorded in Period 1.

The ANOVA for the physiological and biophysical variance exhibited a significant effect ($p < 0.0001$) for all sources of variation and the means adjusted by least squares are presented in **Table 1**.

The animals staying for 1 h under full sunlight, from 12 p.m. to 1 p.m., resulted in an increase in physiological variables related to thermolysis, with an increase by 0.91°C in BT, 59 mov·min^{-1} in RR, and 5.41°C in CompST, with the highest increase in ST recorded in the rump region at 6.48°C.

In period 2, a decrease at the same proportion was found, by 59 mov·min^{-1} in RR, which returned to baseline values, with no difference among Periods 0, 2, and 3. The same behavior of reduction in body STs was observed regardless of the anatomic region assessed. Two hours after the animals returned to the shade (Period 3), CST and FST returned to baseline values, reflecting in the reduction in internal body temperature, demonstrated by HS and CHS, with a decrease by up to 0.6°C in BT, with no difference between Periods 2 and 3.

The descriptive analysis of the histomorphometry variables and the HTI of the animals is presented in **Table 2**.

The mean HTI recorded during the HTT was 9.66 ± 0.21, ranging from 9.20 to 10.00. For the histomorphometric variables,

TABLE 1 | Mean and standard deviation of the physiological and biophysical variables assessed in adult female buffaloes in the Eastern Amazon during the HTT.

Effects	Period 0	Period 1	Period 2	Period 3
Variable	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
BT	38.05 ± 0.11[c]	38.96 ± 0.16[a]	38.38 ± 0.12[b]	38.36 ± 0.12[b]
RR	22 ± 2.18[b]	81 ± 11.52[a]	22 ± 0.83[b]	20 ± 1.05[b]
EST	36.17 ± 0.68[c]	39.99 ± 1.07[a]	37.68 ± 0.61[b]	37.53 ± 0.45[b]
CST	35.37 ± 0.88[c]	39.89 ± 1.13[a]	36.62 ± 0.73[b]	36.33 ± 0.65[bc]
TST	34.68 ± 0.74[c]	40.59 ± 1.58[a]	36.04 ± 0.74[b]	35.92 ± 0.74[b]
RST	34.18 ± 0.71[c]	40.66 ± 0.89[a]	35.61 ± 0.98[b]	35.44 ± 1.38[b]
FST	33.66 ± 1.03[c]	39.97 ± 1.45[a]	35.66 ± 1.07[b]	35.08 ± 1.25[c]
CompST	34.82 ± 0.76[c]	40.23 ± 0.55[a]	36.33 ± 0.68[b]	36.06 ± 0.79[b]
HS	0[b]	0.0216 ± 0.0030[a]	−0.0138 ± 0.0032[c]	−0.0003 ± 0.0021[b]
CHS	0[c]	0.0649 ± 0.0091[a]	0.0233 ± 0.0123[b]	0.0222 ± 0.0117[b]

Period 0 (shade): From 10 a.m. to 12 p.m.; Period 1 (sun): From 12 p.m. to 1 p.m.; Period 2 (shade): From 1 p.m. to 2 p.m.; Period 3 (shade): From 2 p.m. to 3 p.m.
BT, Internal body temperature (°C); RR, Respiratory rate (mov·min^{-1}); EST, Eyeball surface temperature (°C); CST, Cheek surface temperature (°C); TST, Trunk surface temperature (°C); RST, Rump surface temperature (°C), FST, Forelimb surface temperature (°C); CompST, Compound surface temperature (°C); HS, Heat storage (W·m^{-2}); CHS, Cumulative heat storage (W·m^{-2} h^{-1}).
[a–c]Means followed by equal lowercase letters within the row do not differ statistically by Tukey's test ($p < 0.05$).

TABLE 2 | Number of observations (*n*), averages ($\bar{X}$), standard deviation (SD) coefficient of variation (CV), minimum (Min.) and maximum (Max.) values for the heat tolerance index, and histomorphometric characteristics of the skin of adult female buffaloes reared in the Eastern Amazon.

Variable	Unit	*n*	$\bar{X}$	SD	CV	Min.	Max.
HTI	-	40	9.66	0.21	2.15	9.20	10.00
HF	-	10	12.40	2.12	17.09	10.00	16.00
HD	hair·mm^{-2}	10	2.00	0.26	13.11	1.59	2.41
TS	mm	10	6.03	1.16	19.21	4.77	8.28
HSwG	μm	10	16.20	1.99	12.28	12.00	19.00
AswG	μm^2	10	17,283.9	4,449.8	25.75	13,102.1	26,450.9
ASwG_surface	%	10	1.57	0.38	24.16	1.14	2.30
ASG	μm^2	10	11,821.9	4,301.9	36.39	4,834.0	19,175.8
ASG_surface	%	10	1.08	0.39	35.98	0.44	1.73

HTI, Heat tolerance index; HF, Number of hair follicles; HD, Hair density; TS, Thickness of skin; HSwG, Height of sweat gland; ASwG, Mean value of the area of each sweat gland; ASwG_surface, Percentage of active sweat gland tissue in the sample; ASG, Mean value of the area of each sebaceous gland, ASG_surface, Percentage of active sebaceous gland tissue in the sample.

the averages of HD of 2.0 ± 0.26 mm^2, TS of 6.03 ± 1.16 mm, HSwG of 16.2 ± 1.99 μm, ASwG of 17,283.92 ± 4,449.85, and ASG of 11,821.95 ± 4,301.90 μm^2 were observed per animal. The percentage of active sweat gland tissue was 1.57 ± 0.38% and the percentage of active sebaceous gland tissue was 1.08 ± 0.39%.

The results obtained by the PCs technique, with the respective eigenvalues and percentages of variance explained by each one, show that the first two PCs accounted for 86.2% of the total variation on the thermolytic capacity of buffaloes, with principal component 1 (PC1) accounting for 45.2% and principal component 2 (PC2), for 41.0% of the data variance.

An analysis of the contribution of the variables in the dimensions and their respective weights shows that the variables that most contributed in dimension 1 were RR (0.766), TS (−0.914), and ASG_surface (−0.865), while the variables that most contributed to dimension 2 were HTI (−0.920), CHS (0.944), and HF (0.686) (**Figure 4**).

The correlations among internal body temperature, respiratory rate, body STs, stored heat, and the BGHI are presented in **Figure 5**. Analysis of the coefficients of Pearson's correlations showed significant positive correlations ($p < 0.0001$) for all pairs of these variables.

The correlations among histomorphometric variables, the heat tolerance index, internal body temperature, and RR are presented in **Figure 6**. An analysis of the values of the linear coefficients showed a significant negative correlation ($p = 0.0157$) between RR and skin thickness and no significant correlation with internal body temperature.

DISCUSSION

The water buffaloes exhibited very high heat tolerance and used thermal polypnea as the main heat dissipation pathway. Their mean skin thickness observed was 6.03 ± 1.16 mm and, for the

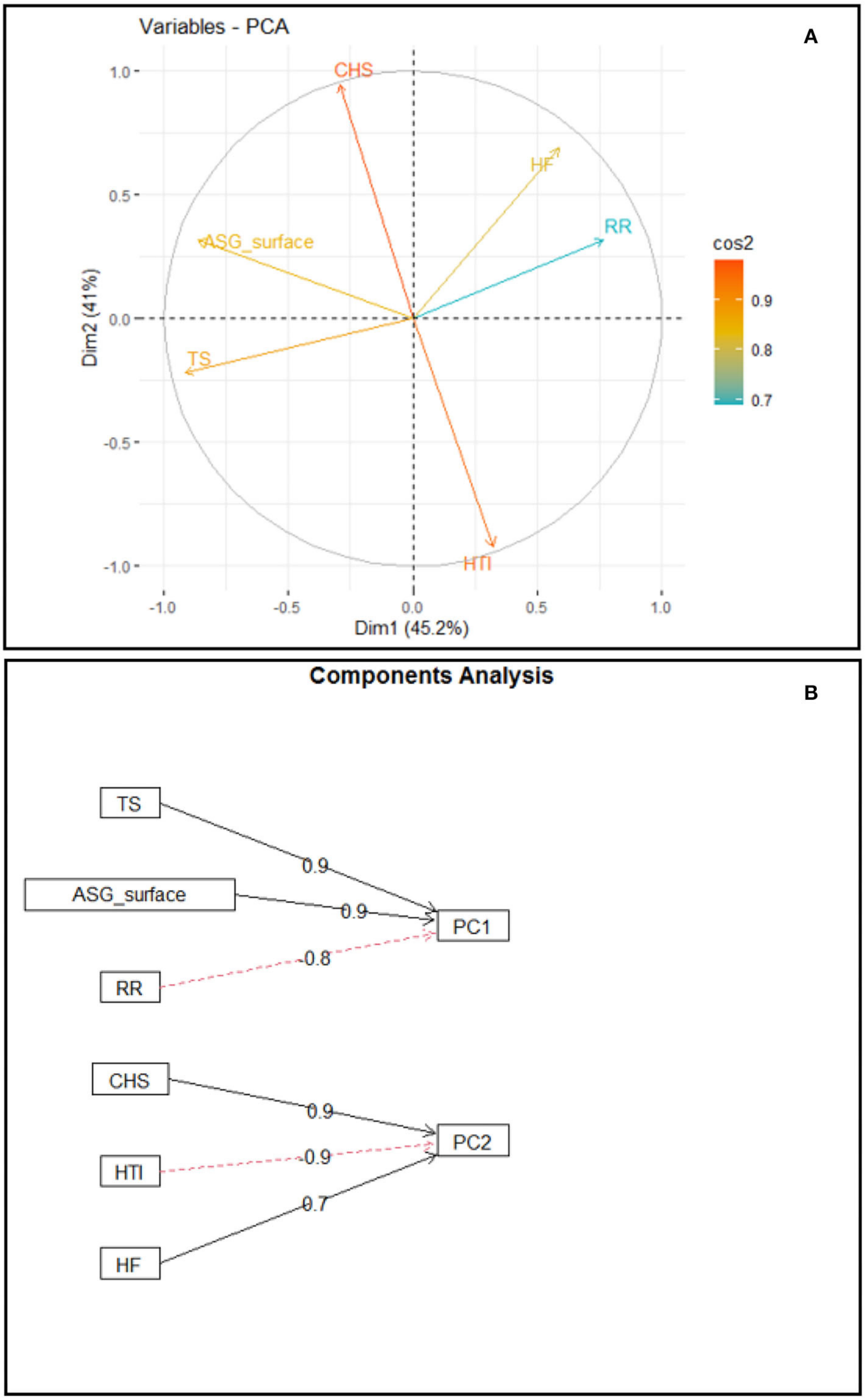

FIGURE 4 | Biplot of PC1 × PC2, indicating the contribution of the variables in the dimensions **(A)**; Weight of the contribution of variables TS, ASG_surface, and RR to PC1 and weight of the contribution of variables CHS, HTI, and number of HF to PC2 **(B)**.

first time, the morphometry of sweat glands and sebaceous glands of buffaloes was performed, with a histological approach that allowed indicating its secreting activity. The PCs analysis enabled identifying morphological and functional associations relevant for thermoregulation of buffaloes, such as the interrelation of TS with the proportion of ASG and with respiratory rate. The study also showed that the buffaloes exhibited a high positive correlation between eyeball temperature and internal

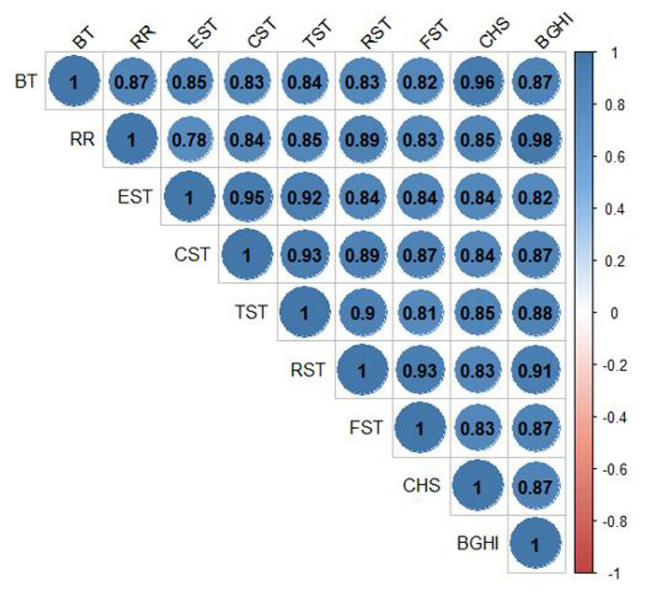

FIGURE 5 | Linear correlations between the BGHI and internal BT, RR, EST, CST, TST, RST, FST, and CHS during the HTT performed in adult female buffaloes reared in humid tropical climate.

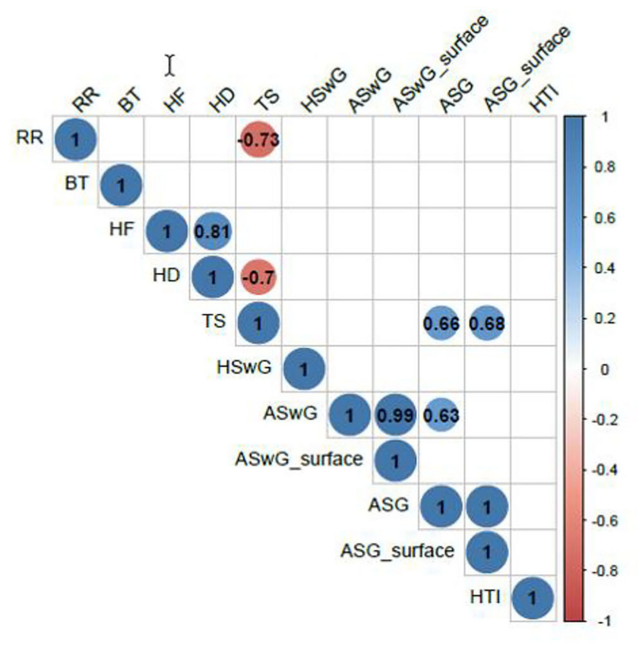

FIGURE 6 | Linear correlations among the HTI, internal BT, and RR and the histomorphometric variables number of HF, hair density (HD), TS, HSwG, ASwG, ASwG_surface, ASG, and ASG_surface of adult female buffaloes reared in humid tropical climate.

body temperature, a characteristic that may be explored in the future for the development of non-invasive diagnosis resources and remote monitoring of animal health and well-being.

The data in this study were collected during November and December, during the less rainy season of the Amazon biome, which goes from July to December (46). Although there was no significant difference in the dry-bulb temperature and relative air humidity values between the experimental periods, it was seen that the animals were in an environmental condition susceptible to heat stress since the mean air temperature values were above 32°C in all periods over the HTT.

The animals were exposed to environmental temperatures above the thermal comfort zone, characterized as an air temperature range in which the animal may exhibit its minimum metabolic rate and where homeothermy regulation is performed *via* exchange of sensible heat with the environment (61). According to Goswami and Narain (62), the thermoneutral zone for the water buffaloes ranges from 15.5°C to 21.2°C, while Shafie (63) places such zone between 13.0°C and 24.0°C. These authors suggest that the critical high temperature for buffaloes is above 29°C and 23.6°C, respectively, indicating that the animals in this study had to activate their heat dissipation pathways to maintain homeostasis as the lowest air temperature to which they were exposed was 32.14°C in the beginning of the HTT (Period 0).

Despite the high air temperatures during the tolerance test, relative air humidity remained with minimum values of 48% in Period 1 and maximum values of 56% in Period 0, staying within the relative humidity range considered optimal for most domestic species, which ranges from 40 to 70% (64). That can be explained by the time of day recommended for the application of the heat thermotolerance test, which coincided with moments of lower relative humidity in the circadian cycles occurring in the humid tropical climate subtype (46).

In this study, the classification of the BGHI indicates a danger situation (54), even when the animals were kept in the shade, with BGHI above 81. When the animals were exposed to sunlight, to identify their heat acquisition under a condition of direct solar radiation, the BGHI indicated an emergency situation (54), with maximum value of 101, since during that period the maximum black globe temperature value recorded was 52.1°C. The environmental thermal challenge situation for the animals is therefore evident, compelling them to activate their thermolysis pathways, an effect demonstrated by the alterations in physiological and biophysical variables monitored.

The relative air humidity recorded during the HTT favored heat exchange through respiratory evaporation, demonstrated by the pronounced increase in RR when the animals were under direct solar radiation, reaching maximum values of 116 mov·min^{-1}. The increase in RR is an essential thermoregulatory response to maintain homeothermy and is used by buffaloes as a proactive thermolytic response to prevent hyperthermia in heat challenge situations (65).

The increase or decrease in RR depends on the intensity and duration of the heat stress to which the animals are submitted (66). This study showed the importance of heat loss through the respiratory pathway for buffaloes to maintain homeothermy. The pronounced reduction in RR occurred as soon as the animals returned to the shade after the heat challenge period (81–22 mov·min^{-1}) confirms the rapid tolerance recovery of buffaloes after a heat stress situation, which reflected in a reduction by 0.58°C in internal body temperature. Several studies have described this rapid recovery of buffaloes in comparison with cattle (14, 67, 68).

When the animals were kept in a thermal comfort environmental condition provided by shade, their RR decreased and returned to values considered normal, with no significant difference among Periods 0, 2, and 3. According to Shafie (63), the RR of buffaloes ranged from 18 to 30 mov–min^{-1} for animals in thermal comfort. Thus, the importance of shade to maintain or favor homeothermy in buffaloes is evident. Indeed, recent reports indicate that the natural shade provided by silvopastoral systems contributes to the buffalo's well-being, to the expression of their normal behavior, and to increased performance (39). In tropical regions, providing shade becomes a requirement as it helps decrease excess direct irradiation received by the epidermis of buffaloes through direct solar radiation, particularly during the hottest hours of the day (61). Other studies carried out in the Amazon region have also indicated beneficial effects of shade on the physiological responses of buffaloes (69–75). In addition to seeking shade to rest and facilitate heat dissipation, buffaloes have the habit of wallowing (76). With this behavior, the animals lay on mud to favor body cooling *via* conduction, combined with the effects of convection caused by the wind and evaporation of the water in the mud (19). Since in this study, the animals were kept standing during the thermotolerance test to avoid interferences in the determination of HS, the effect of wallowing on body thermoregulation was not assessed.

This study recorded temperatures of eyeball surface, cheek, and forelimb, due to the greater microvascular blood flow of those anatomical regions (77), in addition to the trunk and rump, as those regions are more susceptible to oscillations in environmental conditions (78), particularly of solar radiation incidence. An assessment of the body STs when the animals were exposed to direct solar radiation showed a more significant increase in rump temperature (34.18–40.66°C) followed by increases in forelimb, trunk, cheek, and eyeball temperatures in relation to the baseline period. It is known that the epidermis temperature of the animal is influenced by the thermal condition of the environment, changes in blood flow, fur characteristics, and intensity of the skin latent thermolysis pathway (79). The increases in rump and trunk temperatures are directly related to environmental conditions in Period 1, when the daily irradiation level is historically maximum in the region (46), associated with morphofunctional characteristics of the epidermis of buffaloes, which favor heat acquisition. The epidermis of buffaloes has a high melanin concentration, which provides them heightened protection against ultraviolet radiation, however, with high absorbance (11). Moreover, the number of HF in the body surface makes them more susceptible to visible and infrared radiations, which are absorbed at higher intensity due to the color of the epidermis (10).

The increase in forelimb and cheek temperatures likely occurred due to the peripheral vasodilation in those regions. When an animal is exposed to heat stress, the initial thermoregulatory response that is set off is peripheral vasodilation (61, 79). In addition, considering the body area/mass ratio, greater blood flow is observed in the skin of the limbs and head than in the other regions of the body, which can be considered a thermoregulatory strategy for exchange of sensitive heat through radiation, conduction, and convection in those regions (77). It was found that only those two anatomic regions did not exhibit significant differences for STs recorded in Periods 0 and 3, which confirms vasodilation enables increased loss of sensitive heat, resulting in a reduction in forelimb and cheek temperatures when the animals were in environmental thermal comfort condition.

The orbital region has sympathetic fibers of the face nerve that innerve the capillary vessels of the facial and infraorbital artery, which respond to stressful stimuli (19). It was found that the lower alteration in STs between Periods 0 and 1 occurred in the eyeball, with an increase by only 3.82 °C. Such lower alteration may be related to the orbital anatomy, since, according to Casas–Alvarado et al. (80), adrenergic sympathetic fibers are sensitive to the neurosecretion of epinephrine and norepinephrine, which promotes vasoconstriction of capillaries, thus reducing the heat exchange rate and serving as a local thermoregulating mechanism.

An analysis of linear correlations showed that, among the body STs assessed, eyeball temperature exhibited the highest correlation with internal BT ($r = 0.84523$, $p < 0.0001$). The orbital region more closely corresponds to the internal BT since it suffers less alteration relative to the effects of environment temperature (81). Such results corroborate those found by Brcko et al. (50) and Barros et al. (43), who assessed the domestic buffaloes reared in the Amazon region. Bertoni et al. (82) also concluded that temperatures of the orbital region, of the nasal septum, and of the vulva have proved efficient in evaluating the thermal comfort of female buffaloes.

Pearson correlation between the variables studied and RR showed a greater positive correlation with the STs of the rump ($r = 0.88598$, $p < 0.0001$) and trunk ($r = 0.84612$, $p < 0.0001$) as those anatomic regions are more prone to temperature alterations when the animal is exposed to direct solar radiation (82). The negative correlation between RR and skin thickness ($r = -0.73371$, $p = 0.0157$) makes the animal spend less energy to dissipate heat since its RR is not as intense. Such characteristic observed is favorable to the animal and its productivity since altering the RR and depth results in a deviation of energy to respiration, which could be used in metabolic processes (79) such as growth, maintenance of gestation, or milk production.

The greater thermal gradient between the composite ST and air temperature measured in a dry-bulb thermometer was recorded in Period 1, with $\Delta T = 7.29$°C. That greater gradient facilitates the dissipation of metabolic heat accumulated through the sensitive pathway and, due to the relative air humidity recorded, it also favored heat losses through the latent pathway, demonstrated by the alteration in respiratory rate. When the animals returned to a thermal comfort environment (Periods 2 and 3), a gradual reduction was observed in the calculated values of HS. Shade enabled a reduction by 0.0427 W·m^{-1} h^{-1} in stored heat, which reflected in a difference of 0.31°C in internal BT when compared with the internal temperatures measured between Periods 0 and 3. According to Pereira et al. (65), when in a thermal comfort condition, buffaloes exhibit a notable loss of body heat, provided by the reduction in stored heat.

Given the results of the tolerance index, the animals were classified as individuals of very high heat tolerance, with HTI values above 9.51 (45). Such thermotolerance capacity is directly related to the rapid physiological recovery of the animals after the heat stress situation due to the activation of their latent and sensitive thermolysis pathways. That result corroborates the findings by Pantoja et al. (75), who evaluated buffaloes reared in the Amazon region, in a humid tropical climate, indicating high heat tolerance in individuals from herds of that region.

A search of the scientific literature on the tegumental structures of buffaloes shows a lack of information and, at times, some controversies. Therefore, this study brings a valuable contribution regarding the skin histomorphometry of domestic buffaloes. The most striking characteristic of buffalo skin is the thickness of the epidermis, since the stratum corneum is more eminent and can be twice as thick than in cattle (14). The results from this study indicate that the mean skin thickness was 6.03 mm, corroborating the values reported by Ermetin (83), who determined a variation in skin thickness of adult domestic buffaloes of 6.0–7.6 mm. The findings also agree with the results found by Taneja and Bhatnagar (84), who indicate skin thickness of female Murrah buffaloes between 6.0 and 6.4 mm.

In addition, several recesses characterized by the extension of countless papillae in the dermis are found in the interface between the epidermis and dermis (14). According to Hafez and Anwar (16), those papillae have an important thermoregulatory role as the epidermis, when expanding into the dermis, changes the distribution of blood vessels. Thus, the arteries branch out more frequently, originating countless arterioles and capillaries, which favor heat dissipation *via* vasodilation. That effect can be observed in this study through the increase in ST of the animals when exposed to direct solar radiation, which was made evident by the results obtained with the use of infrared thermography.

Domestic buffaloes are animals that stand out for their low hair density (18). The number of hairs per unit of area decreases with age, making the adult animals nearly glabrous (85). This study found mean density of 2.00 HF per mm^2, matching the results by Debbarma et al. (20) and Raheem, Elias and Ahmed (18), who indicate hair density in buffaloes of 1.91 and 2.78 HF per mm^2, respectively. However, the number of HF in the literature varies, with values ranging from 91.84 cm^{-2} for Murrah animals (86), 135–145 cm^{-2} for Mediterranean buffaloes (17), and 394 cm^{-2} for Egyptian buffaloes (15). In fact, the lower hair density of buffaloes may have a double effect. It may facilitate heat dissipation by convection and evaporation, but it also reduces thermal insulation as the skin is more exposed to direct solar radiation (61).

In this study, the mean epithelium height of the sweat gland was 16.20 μm, with variation between 12.00 and 19.00 μm. The mean areas of the sweat and sebaceous glands were 17,283.92 μm^2 and 11,821.95 μm^2, respectively. The percentage of active sebaceous gland tissue was 1.08 $\pm$ 0.39%. In turn, the percentage of active sweat gland tissue in the sample analyzed was 1.57 $\pm$ 0.38%. That variable represents the ratio between the sum of the areas of the lumen of sweat glands identified in an image and the total area of tissue evaluated in the histological cut. Since the lumen of a sweat gland depends on it being filled by the sweat produced (79), the higher the percentage, the greater gland activity will be, representing the functionality of the sweat gland. Thus, the greater the gland activity at a given moment, the higher the possibility of the sweat produced being secreted, favoring the reduction of the skin ST. To the best of our understanding, such information is a first since no reference values were found in the literature to compare those variables. The recent studies have assessed the type and diameter of sweat (20) and sebaceous (21) glands in different regions of the body of buffaloes, in addition to their distribution (86); however, with no mention of the percentage of gland area, which is an indicative of activity of the respective glands.

Given the determination of the number of PCs, it was found that the first two PCs generated based on this analysis have eigenvalues >1 ($\lambda i > 1$) (87). The eigenvalues observed, of 2.709 for Dimension 1 and 2.458 for Dimension 2, accounted for 86.2% of the total variance in the dataset. Thus, the first two PCs effectively summarize the total sampling variance and may be used to study the set of those data. The weights of the variables that most contribute to Dimension 1 and the relationship between the variables and their placements along PC1, which models 45.2% of the variance of the data matrix, show that RR has an opposite sign to skin thickness and to the activity of sebaceous gland tissue. Such relationship is in accordance with the thermolytic response of buffaloes since skin thickness may condition the amount of energy that will be transferred to the core region of the body of the animal. The thicker the skin, the greater the protection against body overheating *via* reduction of thermal conductivity (88).

Moreover, the association of greater epidermis thickness with larger percentage of active sebaceous gland tissue favors the reduction in radiant thermal load absorbed by the skin. That occurs due to the greater reflectance of solar radiation in consequence of lipid secretion of the sebaceous glands, which spreads over the epidermis (10, 89), thus leading to a lower need of latent thermolysis and activation of thermal polypnea. Therefore, it is supposed that the first PC1 models the behavior of the preference of thermolytic pathway in buffaloes.

An analysis of the relationship between variables and their placements along PC2, which models 41.0% of the variance in the data matrix, showed that the HTI has an opposite sign to stored heat and number of HF. The relationship of those variables in the PC corresponds to the thermolytic responses of buffaloes since the heat stored along the day results in an increase in internal body temperature, leading to a lower heat tolerance index. The opposite relationship between the HTI and the number of HF demonstrates the preference of buffaloes in using latent thermolysis through the respiratory pathway in contrast with the cutaneous one. According to Marai and Haeeb (10), buffaloes have lower efficiency in losing heat through cutaneous pathways and the respiratory pathway is very relevant in dissipating endogenous heat to maintain homeothermy. A shortcoming of this study is that the local microclimate conditions, particularly the high relative air humidity, did not favor the direct quantification of sweat production by the standard technique, which uses paper disks impregnated with cobalt chloride (90). Therefore, it would be interesting to replicate the experimental protocol under climate conditions other than humid tropical climate. Furthermore, the thermoregulatory associations measured in this study were limited to young females and could be assessed in animals in other age groups and physiological conditions, such as calves and lactating or pregnant females.

CONCLUSIONS

The quantification of the dynamic responses to the HTT and of the morphofunctional characteristics of buffalo tegument, such as the activities of sweat and sebaceous glands, hair density, and skin thickness, are the resources that allow measuring the thermotolerance capacity of buffaloes in a humid tropical environment. Buffalo skin thickness influences the conformation of dermal papillae and the distribution of peripheral blood vessels, which are identifiable *via* infrared thermography and whose participation in thermal exchanges can be successfully monitored in regions such as eyeball, cheek, trunk, rump, and forelimb. In humid tropical climate conditions, water buffaloes are able to quickly reduce their internal BT when protected from direct solar radiation, with the activation of thermal polypnea. In addition, eyeball ST can be used as a non-invasive indicator of thermal comfort of buffaloes as it exhibits a high correlation with internal body temperature.

Nomenclature: Resource Identification Initiative

FLIR: FLIR tools, RRID:SCR_016330; HOBO: HOBOware Pro, RRID:SCR_021915; Leica Microsystems: Leica Microsystems, RRID:SCR_008960; Software Image J: ImageJ, RRID:SCR_003070; SAS: Statistical Analysis System, RRID:SCR_008567; R Studio Team: R Project for Statistical Computing, RRID:SCR_001905.

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 study was carried out in accordance with the Brazilian Guideline for Care and Use of Animals in Teaching or Scientific Research Activities. The procedures were approved by the Animal Bioethics Commission of Embrapa Eastern Amazon (Protocol 00715) and the results were reported according to the ARRIVE Guidelines: Animal Research Reporting in vivo Experiments.

AUTHOR CONTRIBUTIONS

RV, JL, MJ, and AG: conceptualization. RV, JL, MJ, AB, and AG: methodology. RV, AB, and AG: data curation. MP, MJ, JL, RV, and CO: investigation. AB and AG: formal analysis. RV and AG: writing the original draft. JL and AG: funding acquisition. MJ, JL, and AG: reviewing, and editing. AG: project administration, supervision. All authors have read and agreed to the published version of the manuscript.

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Grass composition and distribution patterns as determinants of behavioral activities and weight accumulation of Nguni and Boran cattle post-relocation

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Grass biomass composition and distribution patterns within the paddock as determinants of behavioral activities and animal performance of Nguni (NG) and Boran (BR) cattle post-relocation to a novel environment were examined. Ten steers of each breed aged 9 months were bought from two different farms and sent to Honeydale research facilities, where they were reared on rangelands for 12 weeks. Identification and classification of grass species were done every sampling week before introducing cattle to each paddock. Direct visual observations and durations of behavior and paddock occupancy patterns were recorded every fortnight between 0500 and 1900 h every week. Individual animal weights and body condition scores (BCS) were recorded two times per week. Location within paddocks hugely affected ($P < 0.0001$) the composition of the vegetation as most grass species were found everywhere on pastures, near the watering points and along fencelines. However, the distribution patterns of the grass species significantly differed at different locations. *Aristida congesta* was dominant ($P = 0.0014$) everywhere in the pasture and along fenceline than in areas with a high density of trees. Except in shaded areas, *Cynodon dactylon* ($P = 0.0003$) and *Eragrostis chloromelas* ($P = 0.0008$) were highly abundant near the watering points, pastures, and along the fenceline. *Themeda triandra* ($P < 0.0001$) was only prevalent everywhere on pastures except in shade areas, near the water sites, and along fenceline. In terms of palatability and ecological groups, highly palatable species ($P < 0.0001$) and decreasers ($P = 0.0010$) were more frequent everywhere in the paddocks. From Weeks 1 to 3, NG spent more time walking ($P < 0.0001$), while the BR showed a significant decline in grazing activities ($P < 0.0001$) in spite of several differences in vegetation composition. Both breeds showed a significant decline in weight gain ($P < 0.0001$) and body condition score ($P < 0.0001$) in the first 3 weeks. However, the two cattle breeds quickly compensated for their behavioral activities and weight gain, and this shows a good ability to cope with stress caused by heterogeneous environmental conditions.

KEYWORDS

novel environment, beef cattle, climate change, foraging behavior, rangelands

Introduction

A cattle ranching holds a significant contribution as a tool to alleviate the poverty crisis among the disadvantaged rural people in South Africa and beyond (1), where three-quarters of the population primarily relies on livestock farming as a source of income (2). Seventy-five percent of cattle production in the country is undertaken in areas, where land and the surrounding environments are harsh and not suitable for other viable use (3). Cattle production in rural areas basically relies on natural pastures as a source of feed (4). Herbaceous forages, browse species, and crop residues are the main feed resources offering a range of goods and services, such as cattle grazing, an important component of extensive livestock production (5). In South Africa, normally cattle production has two distinct seasons: rainy and dry seasons (6). During the rainy season, from October to March, cattle typically have a reasonable amount of forage available (7). Conversely, during the dry season, from April to September, pastures often present restricted forage quantity and quality (8). Thus, the nutritional and productivity of natural pastures throughout the year are not enough for grazing animals to reach their full productive potential (9). Under such challenging situations, cattle farmers are forced to establish supplementary plans depending on the production goals and socio-economic status of the farmer (10). Supplementation involves additional costs to the farmer (8). In most instances, only 20%, if not less, of the farmers could afford the additional feed costs and other management inputs due to the consistent lack of a sustainable income source (11).

Animal performance is dependent on forage intake, which, in turn, is influenced by consumption patterns (6). In heterogeneous pastures such as native grassland, animals change the mechanisms of forage harvest and ingestion, keeping nutrient supply constant (12), and similar behavior may occur when animals have access to better quality pasture. In turn, pastures with higher fiber content cause the animal to reduce forage consumption (13). When it is possible for the animal to select a better diet quality, changes occur in patterns of ingestion and rumination of grazing animals (9). For this reason, foraging behavior is one of the prominent activities in extensively reared cattle as it affords them a better opportunity to learn about their environment by searching for better quality forage, locating water sources (13, 14), and engaging with their herd mates to build social relationships (15). For ruminants, grazing and ruminating activities are essential in nutrient capture and, ultimately, animal performance (16). Up to date, little is known about how the fobs and grass biomass composition at different locations within a paddock may impact different behavioral activities and weight accumulation where animals' food base is natural pastures. A better understanding on how grass biomass composition and distribution patterns determine grazing activities and weight accumulation is important for managing both animals, and their rangelands as climate change through recurrent droughts continue to be an area of concern to many countries (17).

At the same time, high-producing cattle breeds like Simmental, Limousine, and others are experiencing difficulties to adapt to harsh environmental conditions with less available feed resources than their area of origin (13). Consequently, livestock ownership changes involving the relocation of cattle became a norm as farmers were forced to sell a portion of the stock to reduce drought-related financial and production losses (15). Tropical cattle breeds like Nguni and Boran are highly reputed for their ability to strive under nutritive restrictive environments where hot and humid conditions impede productivity (18). For this reason, tropical breeds like Nguni and Boran were allowed to enter the growing commercial sector and extensive recording facilitated breed improvement (19). Thus, while the breeds were improved in the commercial sector, they were being eroded in the rural areas (20). Fortunately, the inherent hardiness of the breeds allowed them to survive, and purebred animals are still found in limited numbers in rural communities. In an attempt to address this problem, there has been a growing interest calling for the mainstreaming of indigenous cattle breeds as a climate-resilient model to improve the tolerance and herd productivity in communal farming setups (21). With climate change impacts occurring at a faster rate than predicted, the current study sought to deposit a portion of the information by examining the distribution and vegetation composition as determinants of foraging behavior and weight accumulation of Nguni and Boran cattle post-relocation to a novel environment.

Materials and methods

Ethical approval

Accommodation and care of animals were in accordance with the recommendations of the University of Fort Hare's Research Ethics Policy. The project guidelines were reviewed and permitted under the ethical clearance certificate number MUC551SSLA01 from the Institutional Animal Research Ethics Committee.

Source of animals and description of experimental site

The animals were sourced from two different farms in the Eastern Cape (Nguni bought in Morgan Bay while the Boran was bought in Bathurst) and sent to Honeydale Research farm at the University of Fort Hare in Alice, South Africa (Figure 1). The Nguni cattle are widely acknowledged to be the outstanding beef breed for optimal production under harsh African conditions. Nguni cattle are heat and light tolerant and can handle extreme heat and cold alike (3). They are adaptable and hardy and possess excellent resistance to internal and external parasites with natural immunity to tick-borne diseases (10). It is slightly smaller in size compared to the large beef breeds of other countries but this just enables it to live in the Highveld regions of Africa (21). Bulls are medium sized and weigh between 500 and 600 kg. They are muscular and display typical male characteristics with well-developed, muscular cervicothoracic humps, which mean that the hump is in front of the foreleg. The cows are small and weigh between 300 and 400 kg. They are feminine with sleek, delicate lines around the neck and forequarter, and a prominent wedge shape with the weight in the stomach and hindquarter area. The sloping rump is a distinctive characteristic of the Nguni cow and ensures ease of calving. The udder is small to medium, well-attached with small, functional teats. On the other hand, the Boran cattle are a medium-sized beef animal. They can be gray, fawn, or red in color. They are recognized for their high fertility, good mothering ability, excellent temperament, and great survivability under harsh conditions (22). Their early maturity and good meat quality will ensure their value in crossbreeding projects aimed at improving the productivity of beef herds. The experimental area is located at 32.8° latitude and 26.9° longitude at approximately 520 m above the sea level. The local climate is classified as semi-arid, with a mean annual temperature of 28.7°C and a mean annual rainfall of 453 mm. The experimental site is 210 ha, which was divided into 36 paddocks of 5.84 ha each. These paddocks were characterized by high heterogeneity in cattle grazing patterns, with some areas showing some signs of degradation and encroachment. The soils in the area are comprised of deep alluvial-derived types in arable lands, which are mostly shallow with $<$450-mm depth (23). The area lies in the lowland characterized by steep, isolated mountains, and hills with several dams and water streams. The ecological area and veld type predominantly belong to the Bhisho Thornveld (24). The vegetation is composed of several trees, shrubs, and grass species, with *Vachelia karroo, Themeda triandra, Digitaria eriantha, Eragrostis spp*., and *Pennisetum clandestinum* being the dominant plant species (25).

Experimental design and animal management

Upon arrival at the experimental farm, the steers were supplemented with Vitamin B complex and inoculated with Blanthrax® against Anthrax and other related diseases are known to be prevalent in the area. The steers were allocated into one paddock (500 × 500 m per paddock, length × width) irrespective of their source of origin to allow the acclimatization and buildup of social relationships with their new herd mates. A 6-month resting period was applied to each paddock to ensure there is sufficient forage before introducing the animals. A 24-h accessible watering system was located at the center of each paddock. Different numbered and colored ear tags (green for Boran and white for Nguni) were fitted on each animal for identification. An individual code was marked on the back of each steer using a washable dye to differentiate between steers with the same body color during behavioral observations. The steers were dipped with DRASTIC DEADLINE® fortnightly, as is the new requirement to control tick infestation in the area. The steers had free access to water and were allowed to free-range together throughout the trial period. Rotational grazing was adopted for feeding the animals, and they were moved to a new paddock after every 14 days. A total number of six paddocks were used to carry the animals in the current study.

Vegetation assessments and species identification

The species composition of the vegetation was estimated from different locations within a paddock using a step-point method (26). The nearest plant and basal strikes were recorded from 250 point observations per location. The point observations were placed at 2-m intervals, and records were made over the length of the plot in five straight parallel lines with a distance of 4 m between them. Grasses were classified based on the succession and ecological information for the arid and semi-arid regions of South Africa (27) as follows: (i) highly palatable species; those that develop on rangeland in good condition and decrease with high grazing pressure (decreaser), (ii) palatable species; those that appear in rangeland in good condition and increase with moderate grazing pressure (increaser IIa), and (iii) less palatable species; those that occur in rangeland in good condition and increase with severe utilization (increaser IIb and IIc). Those grasses that could not be identified were collected with full inflorescences and sent to the South African National Biodiversity Institute (SANBI) Herbarium in Pretoria for identification. In each location, four 0.25 m^2 quadrants were randomly placed for above-ground grass biomass sampling. Forages within each quadrant were harvested to a stubble height, bulked, and oven dried for 48 h at 60°C. Dried samples were weighed to measure the dry matter (DM) yield.

Inter-observer reliability and recording of foraging and drinking behavior

Five observers with more than 3 years of participation in data collection involving behavior monitoring were selected in the current experiment. At the beginning of the trial,

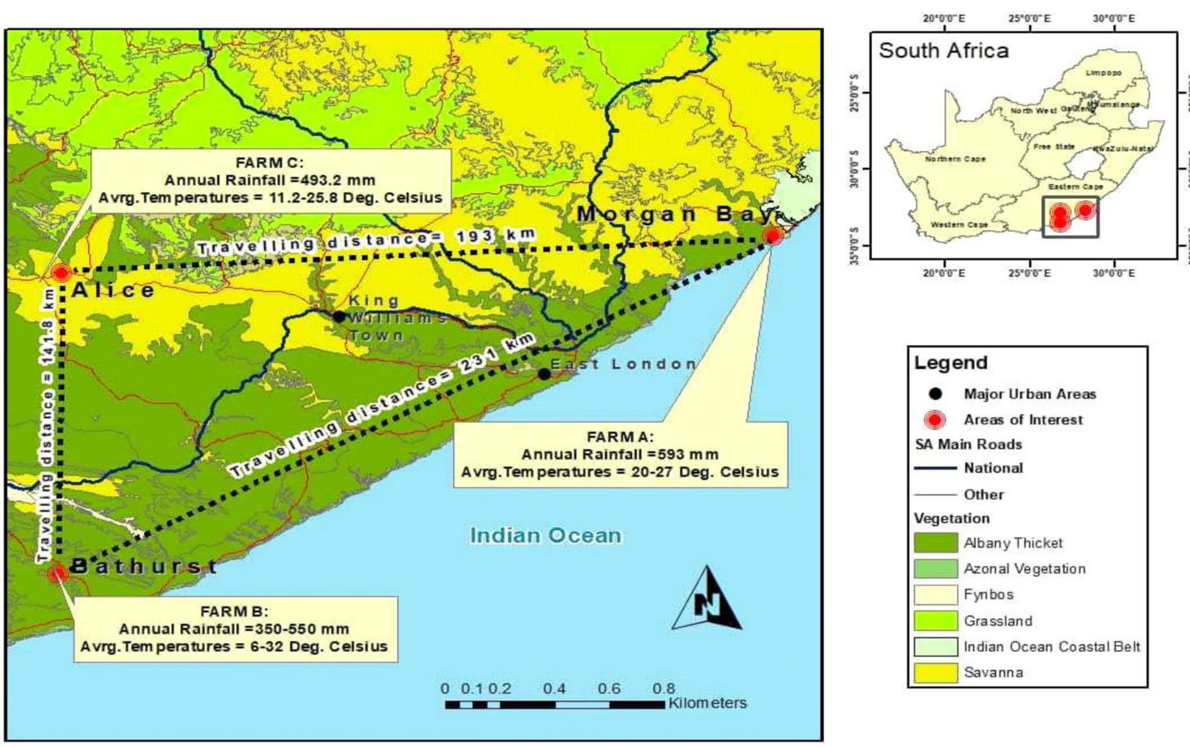

FIGURE 1
Geographic location of the three farms that participated in the current project.

definitions of behaviors were set, and the observers were trained to gather data and were subjected to a preliminary trial as a protocol to test their understanding and to ensure inter-observer reliability. Using data collection sheets, the five trained personnel recorded the animals' behavioral patterns from 0500 h in the morning to 1,900 h the same day. The procedure was done on three consecutive days each observation week. The same data collectors were used throughout the trial period. Time spent grazing (mouth in contact with grass species), drinking water (head pointing down and mouth in contact with water at the watering point), standing idle or either ruminating, lying down (body in contact with the ground using either lateral or sternal recumbency), browsing (mouth in contact with forage browse tree or shrub) and walking (moving from one place to the next with head raised up) was recorded for every first 20 min of every hour through the scan sampling procedure by Martin and Bateson (28). The location of the animals in the paddock (shade, pasture, fenceline, or water source) was recorded every 20 min per hour. The animal was considered in the shade when the head and most of its body were covered by the shade, at the water source and fenceline, and when standing or lying at <5 m from the water source and fence. Amount of time recorded for each activity and location was expressed as a percentage of the total time spent for all the activities by the animals in the veld. Using the same five trained observers, drinking frequencies were recorded continuously and simultaneously for each animal. The number of instances for each drinking action (drinking bouts) and time spent by each animal drinking water was recorded using a stopwatch. "Drinking bouts" were defined as when at least 4 min was spent without any drinking.

Live weight and body condition scores

The steers were driven into the handling facility to collect individual animal weights and BCS two times per week. Animals were individually weighed using a digital weighing band (individual scale CAUDURO 40100–1,500 kg, Cachoeira do Sul; Brazil), and live weights were recorded. The steers were weighed at 8 o'clock, after 3 h of fasting. The animals were palpated, and body condition scoring was assessed by experienced personnel using a 1–5 point scale (3). These measurements were taken every fortnight. The average daily gain (ADG) was determined by the difference between weights on Weeks 1, 3, 5, 7, 9, and 12 divided by the number of days between each measurement.

Statistical analysis

The data obtained were checked for homogeneity of variance and the presence of outliers using the extreme observation table, while normality was tested using PROC UNIVARIATE. Data on foraging activities were arc sin transformed, while the BCS were square-root transformed before the generalized linear model analysis was run. This adjustment was made to ensure the normality of the data. Outliers were set to missing, and the analysis was re-run to determine if any new outliers appeared. This process was repeated until all outliers were removed from the data set. A comparison of means was made using the PDIFF option. The model used was as follows:

$$Yijl = u + \alpha i + \beta j + hk + \alpha\beta ij + \alpha hij + \beta hjl + Eijk,$$

where, Y_{ijl} = proportion of time spent on different behavioral activities (grazing, browsing, resting, walking, pasture, shade, fenceline, and watering points); water intake (number and duration of drinking bouts); and animal performance (ADG and BCS. Whereas, μ = overall mean; α_i = effect of breed (i = Boran, Nguni); βj = effect of observation week (1, 3, 5, 7, 9, and 12); hk = effect of observer (1, 2, 3, 4, and 5); $\alpha\beta_{ij}$ = interaction of genotype and observation week; αh_{ik} = interaction of breed and observer; βh_{jk} = interaction of observation week and observer; E_{ijk} = random errors. Pearson correlation coefficients for the two breeds were used to determine the relationship among the tested variables.

Results

Abundance of common grass species, ecological, and palatability groups

The current study identified six common grass species at different locations within the paddocks during the trial period (Table 1). All the common grass species were more abundant except the *Sporobolus fimbriatus*. However, the distribution patterns of the grass species significantly differed at different locations. *Aristida congesta* was dominant ($P = 0.0014$) everywhere in the pasture and along fenceline than in areas with a high density of trees. Except in shaded areas, *Cynodon dactylon* ($P = 0.0003$) and *Eragrostis chloromelas* ($P = 0.0008$) were highly abundant near the watering points, pastures, and along the fenceline. *Themeda triandra* ($P \leq 0.0001$) was only prevalent everywhere on pastures except in shade areas, near the water sites, and along the fenceline. In terms of palatability and ecological groups, highly palatable species ($P \leq 0.0001$) and decreasers ($P = 0.0010$) were more frequent everywhere in the paddocks. Biomass production significantly varied ($P < 0.0001$) between location sites, with more DM yield found everywhere on pasture than in other areas.

Biomass production according to different locations within a paddock is presented in Figure 2. Anywhere within the paddock and fenceline had higher biomass production ($P < 0.0001$) except along the water points and fencelines.

Behavioral activities displayed by the Nguni and Boran cattle during the observation weeks

The proportion (expressed in percentages) of time spent by the Nguni (NG) and Boran (BR) steers on each behavioral activity during the observation weeks is shown in Table 2. BR had a higher proportion ($P < 0.0001$) of grazing time than NG. However, the proportion of time spent grazing between the two breeds significantly declined ($P < 0.0001$) from Week 1 to Week 5. There was a significant interaction ($P = 0.0002$) between breed and observation period with regard to the proportion time spent on grazing. A higher proportion of time spent on walking events was noted in NG than in BR. The two genotypes showed an increase ($P < 0.0001$) in time spent walking on Week 1 than in the successive weeks. Significant interactions ($P < 0.0001$) existed between breed and observation period with regard to the proportion of time spent walking. BR had higher proportion ($P < 0.0001$) of time spent browsing than NG. Time spent walking by the two breeds showed a significant difference ($P < 0.0001$) with regard to the observation period. A notable decline in browsing activities was observed in Weeks 1–5 than in the subsequent weeks. There was a significant interaction ($P = 0.0006$) between the breed and observation period. The proportion of time spent resting remained insignificantly low ($P > 0.05$) irrespective of the breed and observation period.

Spatial distribution of the Nguni and Boran steers according to observation weeks

The effect of breed and period on animal distribution in the paddock is presented in Figure 3. The proportion of time spent on pasture remained the same ($P = 0.0559$) irrespective of the breed. However, a significant variation with regard to the time spent on pasture ($P = 0.0005$) was noted within the observation period. From Week 5 to Week 9, the steers spent more time on pasture than in the preceding weeks. NG had a higher ($P < 0.0001$) proportion of time spent along the fenceline than BR. Week 1 and Week 3 showed a higher ($P < 0.0001$) proportion of time spent along fenceline than in the successive weeks. Significant interactions ($P < 0.0001$) were also noted between breed and observation period with regard to the

TABLE 1 Relative abundance (%) of common grass species, ecological, and desirability groups at different locations within a paddock.

Grass species	Water points	Pastures	Shade	Fenceline	M.S.E	Probability
Aristida congesta	2.94[b]	5.88[a]	1.47[c]	2.94[b]	1.42	0.0014
Cynodon dactylon	5.88[b]	7.35[a]	2.94[c]	4.41[c]	1.65	0.0003
Digitaria eriantha	2.94[c]	8.82[a]	2.94[c]	4.41[b]	1.44	<0.0001
Eragrostis chloromelas	5.88[b]	7.35[a]	1.47[d]	2.94[c]	2.37	0.0008
Sporobolus fimbriatus	1.47[b]	2.94[a]	1.47[b]	1.47[b]	1.63	0.0322
Themeda triandra	1.47[c]	10.29[a]	2.94[b]	1.47[c]	3.05	<0.0001
Ecological groups						
Decreasers	9.38[c]	18.75[a]	7.29[c]	12.50[b]	1.51	0.0010
Increaser I	-	2.08	-	-	0.10	<0.0001
Increaser II	7.29[c]	15.63[a]	6.25[c]	10.42[b]	1.42	0.0002
Increaser III	1.04[c]	3.13[a]	-	2.08[b]	0.71	0.0426
Invaders	1.04[b]	2.08[a]	-	1.04[b]	0.33	0.0500
Palatability groups						
Highly palatable	14.14[b]	22.22[a]	8.08[c]	15.15[b]	1.62	<0.0001
Moderately palatable	8.08[b]	19.19[a]	4.04[c]	7.07[b]	1.21	0.0025
Less palatable	3.03[a]	1.01[b]	1.01[b]	1.01[b]	1.24	0.0375
Virtually unpalatable (forbs)	2.02	2.02	-	-	0.81	0.5074

Different letters "a, b, c, and d" represent significant differences ($P \leq 0.05$) between sampling locations within a paddock.

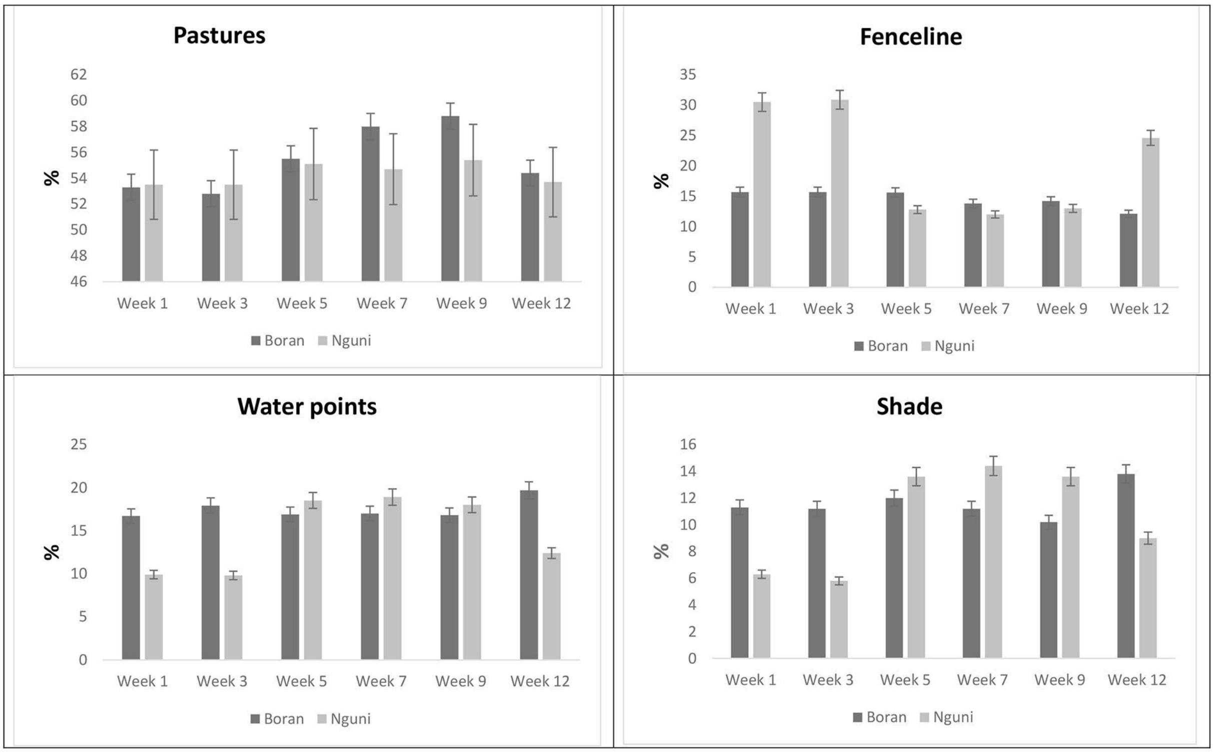

FIGURE 2
LSMeans for the number and duration of drinking bouts according to breed during the observation period.

time spent along fenceline. BR spent more ($P < 0.0001$) time along water points than NG. A variation in time spent along water sources was more dominant ($P < 0.0001$) in Weeks 1–3 than in the successive weeks. Genotype and observation period showed a significant interaction ($P < 0.0001$) in response to time spent along water points. The two breeds spent similar

TABLE 2 LSMeans for the proportion of time spent on different behavioral activities according to breed and observation period.

		Period							Statistics		
	Breed	Week 1	Week 3	Week 5	Week 7	Week 9	Week 12	±SEM	B	P	B x P
Grazing	Boran	44.20^{b,x}	39.00^{c,y}	42.80^{b,y}	50.10^{a,x}	51.80^{a,y}	51.80^{a,x}	1.412	<0.0001	<0.0001	0.0002
	Nguni	35.80^{c,y}	40.00^{c,y}	44.20^{b,y}	42.80^{b,y}	49.80^{b,y}	43.60^{b,y}				
Walking	Boran	33.70^{b,x}	38.30^{b}	33.60^{b}	23.10^{c,y}	18.80^{d,y}	20.60^{c,x}	1.295	<0.0001	<0.0001	<0.0001
	Nguni	42.00^{a,y}	38.80^{b}	32.60^{b}	33.20^{b,x}	25.20^{c,x}	32.50^{b,y}				
Browsing	Boran	12.70^{b,x}	12.50^{b,x}	13.10^{b,x}	18.40^{a,x}	16.70^{a,x}	18.70^{a,x}	0.739	<0.0001	<0.0001	0.0006
	Nguni	11.60^{b,x}	11.40^{b,x}	13.00^{b,x}	13.10^{b,y}	14.40^{b,y}	13.30^{b,y}				
Resting	Boran	9.40^{b,y}	10.20^{a,x}	10.50^{a,x}	8.40^{b,y}	12.70^{a,x}	8.90^{b,y}	0.979	0.4450	0.3499	0.2048
	Nguni	10.60^{a,x}	9.80^{b,x}	10.20^{a,x}	10.90^{a,x}	10.60^{a,x}	10.60^{a,x}				

S.E.M, standard error of means. Different letters "a, b, c" represent significant differences ($P \leq 0.05$) between periods of observation. "x and y" represent significant differences ($P \leq 0.05$) between genotypes. B, Breed; P, Period; B x P, Breed x Period interaction.

time in shade ($P = 0.1014$) during the observation periods. However, the observation period showed a significant variation ($P = 0.0004$) with regard to time spent by the steers near or under the shade. The proportion of time spent under the shade showed significant interaction ($P < 0.0001$) between breed and observation period.

Water consumption patterns of the Nguni and Boran steers during the observation weeks

No significant interactions were noted between breed and observation period on the number of drinking bouts ($P = 0.4007$) and duration of drinking bouts ($P = 0.1042$) as shown in Figure 4. BR had a higher number ($P < 0.0001$) of drinking bouts than NG throughout the study. Contrastingly, NG had a longer duration ($P < 0.0001$) per drinking bout than BR during the period of behavior observation.

Average daily weight gain and body condition scores of Nguni and Boran cattle

The average daily gain and BCS of the Nguni and Boran steers are presented in Table 3. BR had higher ADG ($P < 0.0001$) and BCS ($P < 0.0001$) than NG, even though they were of similar age. Both breeds showed a significant decline in ADG ($P < 0.0001$) on Weeks 1 and 3 than Weeks 5–12. On the other hand, the two breeds showed a significant decline ($P = 0.0028$) on BCS during Week 1 only. No significant interactions ($P = 0.3129$) were noted with regard to ADG.

Correlation coefficients of Nguni and Boran steers among the tested variables

Table 4 shows relationships between all the tested variables for the Boran (top) and Nguni (bottom) diagonal. For BR steers, the proportion of time spent on grazing positively correlated with browsing ($r = 0.017$ at $P < 0.0001$). On the other hand, time spent on grazing activities negatively correlated with resting ($r = -0.963$ at $P = 0.0025$). Browsing negatively correlated with the proportion of time spent on resting ($r = -0.767$ at $P = 0.0098$) and pastures ($r = -0.699$ at $P = 0.0246$). Positive correlations ($r = 0.634$ at $P = 0.0492$) were noted between the proportion of time spent on browsing and fenceline. Resting positively correlated with walking (r = 0.836 at $P = 0.0026$), while negatively correlated ($r = -0.661$ at $P = 0.0379$) with ADG. Walking positively correlated with time spent along the fenceline ($r = 0.741$ at $P = 0.0142$), while negatively correlated (r = -0.701 at $P = 0.0240$) with ADG. The proportion of time spent under the shade negatively correlated with time spent on pasture ($r = -0.733$ at $P = 0.0158$) and fenceline ($r = -0.780$ at $P = 0.0078$). Time spent under the shade positively correlated with time spent around water points ($r = -0.947$ at $P < 0.0001$) and the number of drinking bouts ($r = 0.782$ at $P = 0.0264$).

The amount of time spent on pasture positively correlated with time spent along the fenceline ($r = 0.931$ at $P < 0.0001$) and the number of drinking bouts ($r = 0.778$ at $P = 0.0081$). Time spent near the water points negatively correlated with the amount of time spent along the fenceline ($r = -0.679$ at $P = 0.0308$) and ADG ($r = -0.794$ at $P = 0.0061$). Positive correlation ($r = 0.701$ at $P = 0.0239$) existed between the amount of time spent around water points and drinking bouts. The proportion of time spent along the fenceline positively ($r = 0.683$ at $P = 0.0295$) correlated with the number of drinking bouts. For NG steers, the proportion of time spent on grazing negatively correlated with time spent on browsing ($r = -0.666$ at $P = 0.0357$), resting ($r = -0.659$ at $P = 0.0384$), walking

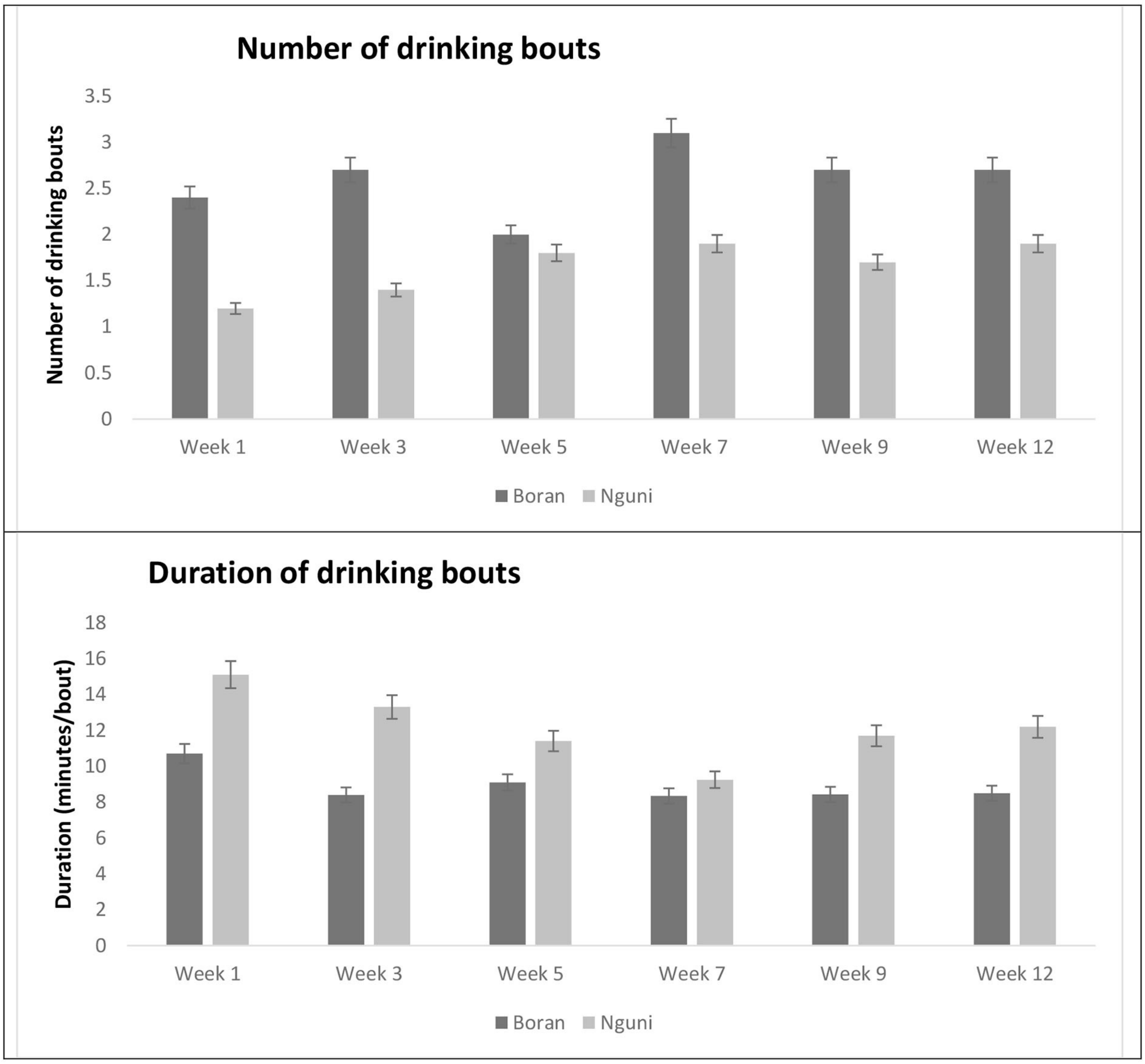

FIGURE 3
LSMeans for the proportion of time spent on pasture water points, fenceline, and shade according to breed and observation period.

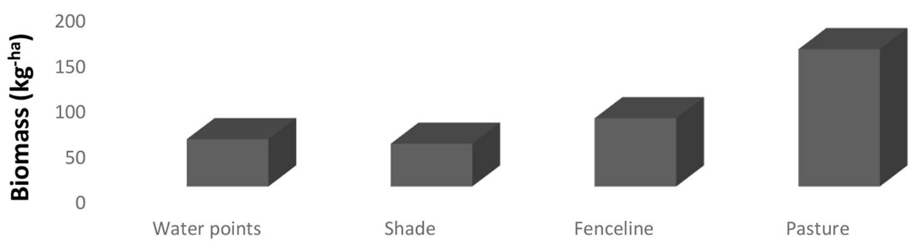

FIGURE 4
Mean forage biomass production (kg $^{ha-1}$) in different locations within a paddock.

TABLE 3 LSMeans for the average daily gain (ADG) and body condition scores (BCS) according to breed and observation period.

		Period						Statistics			
	Breed	Week 1	Week 3	Week 5	Week 7	Week 9	Week 12	±SEM	B	P	B × P
ADG* (kg/day)	Boran	$0.36^{c,x}$	$0.38^{c,x}$	$0.47^{a,x}$	$0.50^{a,x}$	$0.46^{a,x}$	$0.43^{a,x}$	0.012	<0.0001	<0.0001	0.3129
	Nguni	$0.30^{d,y}$	$0.31^{b,y}$	$0.41^{b,y}$	$0.41^{b,y}$	$0.42^{b,y}$	$0.35^{b,y}$				
BCS	Boran	$3.00^{c,x}$	$4.00^{a,x}$	$3.70^{b,x}$	$3.70^{b,x}$	$3.60^{b,x}$	$3.50^{b,x}$	0.181	<0.0001	0.0028	0.1564
	Nguni	$2.40^{e,y}$	$2.60^{d,y}$	$2.70^{d,y}$	$3.00^{c,y}$	$3.10^{c,y}$	$2.60^{c,y}$				

*ADG, average daily gain; BCS, body condition score; s.e.m, standard error of means. Different letters "a, b, c, d, and e" represent significant differences ($P \leq 0.05$) between periods of observation. "x and y" represent significant differences ($P \leq 0.05$) between breeds. B, Breed; P, Period; B x P, Breed x Period interaction.

TABLE 4 Correlation values among the tested variables of interest for the Boran (top) and Nguni (bottom) diagonal.

	Grazing	Browsing	Resting	Walking	Shade	Pasture	WP	FL	DB	ADG
Grazing	–	0.017***	−0.963***	−0.838**	−0.146	0.296	0.202	−0.463	−0.289	0.451
Browsing	−0.666*	–	−0.767**	−0.122	0.127	−0.699*	−0.031	0.634*	−0.288	0.370
Resting	−0.659*	0.479	–	0.836*	0.372	−0.183	0.048	−0.241	−0.102	−0.661*
Walking	−0.707*	−0.750*	−0.749*	–	−0.291	−0.043	−0.295	0.741*	0.588	−0.701*
Shade	0.104	0.247	0.439	−0.696*	–	−0.733*	0.947***	−0.780**	0.782**	−0.250
Pasture	0.191	−0.554	−0.297	0.423	−0.637**	–	−0.422	0.931***	0.778**	0.419
WP	−0.606	0.551	0.161	0.217	0.974***	0.080	–	−0.679*	0.701*	−0.794**
FL	0.042	−0.147	−0.388	0.396	−0.652*	0.684*	−0.980***	–	0.683*	0.219
DB	−0.711*	0.396	0.045	0.563	−0.848**	0.144	0.701*	0.895***	–	−0.010
ADG	0.260	−0.696*	−0.704*	−0.701	−0.308	0.650*	−0.173	0.195	0.195	–

Significance at *($P < 0.05$), **($P < 0.01$), ***($P < 0.001$), WP, water point; FL, fenceline; DB, drinking bouts; ADG, average daily gain and BCS, body condition score.

($r = -0.826$ at $P = 0.0033$), number of drinking bouts ($r = -0.711$ at $P = 0.0210$), and BCS ($r = -0.739$ at $P = 0.0145$). Browsing negatively correlated with the amount of time spent on walking ($r = -0.750$ at $P = 0.0168$) and ADG ($r = -0.696$ at $P = 0.0255$). Resting positively correlated with fecal DM ($r = 0.746$ at $P = 0.0133$), while negatively correlated with time spent on walking ($r = -0.749$ at $P = 0.0125$). Positive correlations ($r = 0.813$ at $P = 0.0042$) were noted between the amount of time spent on pastures and a number of drinking bouts. Negative correlations ($r = -0.696$ at $P = 0.0253$) existed between the amount of time spent on walking and under the shade. Time spent under the shade negatively correlated with the amount of time spent on pasture ($r = -0.637$ at $P = 0.0015$), fenceline ($r = -0.652$ at $P = 0.0411$), and drinking bouts ($r = -0.848$ at $P = 0.0020$). The proportion of time spent under the shade positively correlated with the amount of time spent along water points ($r = 974$ at $P < 0.0001$) and BCS ($r = 0.661$ at $P = 0.0376$). A positive correlation existed between the amount of time spent on pasture and fenceline. The amount of time along water points positively correlated with the BCS ($r = 0.646$ at $P = 0.0437$), while negatively correlated with time spent along the fenceline. Time spent along the fenceline positively correlated with the number of drinking bouts ($r = 0.895$ at 0.0005), while at the same time negatively correlated ($r = -0.739$ at $P = 0.0417$) with the BCS. ADG positively correlated with the amount of time spent on pasture ($r = 0.650$ at $P = 0.0419$).

Discussion

Transferring cattle to a new environment results in a heterogeneous use in different parts of the paddock. However, this might be influenced by the vegetation composition and its distribution patterns since most of the animals rely on natural pastures as a source of nutrition. Findings obtained from this study noted differences in distribution patterns of common grass species, with most of them found along water points and everywhere on pastures. Differences in the abundance of grass species within a specified area is highly influenced by a number of factors, such as weather or climate and management practices (17, 29). Such factors are known to influence the grazing patterns and weight accumulation of cattle that solely depend on native grasslands as a source of feed (13). However, behavioral changes and weight accumulation differences between the two cattle breeds could be attributed to a combination of the new environment and vegetation composition. Exposing cattle to a new environment result in temporal disruptions in their foraging routine, water footprint, and weight accumulation as a response mechanism to the imposed stimuli (10). The response of the animals and time needed to adapt differs with each animal species, sex, breed, age, physiological status, and the production system (15, 30). It is well-accepted and documented in the literature that taking animals to a new environment compel them to make substantial changes in their time budgets, and this negatively impacts their welfare and productivity (31, 32). Previous studies done in cattle production used activity time budgets to measure the response as well as to get some insights into the adaptation potential of different breeds to several climate change shocks (10). Nonetheless, there is limited information about how different cattle genotypes learn to adapt when sent to a novel environment, as this has become a norm in the era of climate change. In the current study, the proportion of time spent on walking became the dominant activity shown by the Nguni and Boran steers during the first 3 weeks post-relocation. The difference in time spent on walking could be attributed to the fact that the Nguni cattle are known for their ability to walk long distances in search of grazing sites and water points (18). At the same time, the Nguni cattle are selective grazers and browsers; hence, the need to walk a longer distance to search for better quality forage, while the Boran are effective grazers (21). Some studies argue that an increase in locomotion may reflect social instability and curiosity of animals to cope with environmental changes (33). With this information, a combination of the season through insufficient forage and novelty of the environment might have played a crucial role in elevated time spent walking with intents to search for better quality food as the study was conducted during dry months. Other studies documented the restriction on time spent grazing as another response made by animals in adjusting to their unknown environment (34). Similar results were achieved in the current study, where the amount of time spent on grazing and browsing significantly dropped in the first 3 weeks of introduction to the new area. Breed differences in time spent grazing and browsing could be attributed to different feeding mechanisms shown by the steers. The remarkable ability shown by the Nguni and Boran cattle to obtain the nutritional value from the available natural vegetation proves to be very beneficial to their excellent adaptation under challenging conditions which could be counterproductive to bulk grazers such as exotic cattle breeds (12).

The proportion of time spent resting remains unchanged in both breeds throughout the study, which could be due to the fact that Nguni and Boran cattle are energetic cattle breeds capable to of adapting harsh climatic conditions and extreme temperatures (3). The two cattle breeds spent equal time on pastures, and this could be due to the fact that cattle are social animals and prefer to graze in groups. Without the social support of familiar group members, individuals may feel vulnerable to predation and spend more time moving up and down as they do not feel safe to rest (20). Similar findings were reported by Barbieri et al. (35), who noted that during the day, cattle spent most of their time grazing in groups. However, the time spent by the steers on pasture appears to be determined by various factors like weather conditions, social relationships of the animals, and forage availability (36). Introducing cattle to an unfamiliar environment results in an unstable social and foraging routine and cattle display a high proportion of heterogeneous patterns with regard to the occupancy distribution (5). For instance, in the first 3 weeks, the steers spent longer time around fenceline, with Nguni steers showing high proportion than the Boran steers. This implies that individual animals differ in their levels of inquisitiveness and motivation to explore novel situations, which is essential for learning and familiarization with a new environment. Grazing around the fenceline could also be a territorial marking and learning mechanism used by animals to familiarize themselves with their new environment (30). On the other hand, other studies argue that time spent along the fenceline could not be the sole indicator of adaption as it can be influenced by a number of factors, including forage availability within the paddock (14). Since the time spent along the fenceline was only dominant during the first 3 weeks, it is agreed that the steers were in the process of familiarizing themselves with their new environment. However, future studies should bring up the issue of seasonality in relation to forage availability and how this influences the spatial distribution of cattle after being exposed to a novel environment. Moreover, the social structure of the herd during grazing movements should be put into consideration as it also had an influence on the spatial distribution of the steers during the period of behavior observations.

There is a general lack of adapted genetic material suited to the prevailing harsh climatic conditions. For instance, exotic breeds tend to lack the adaptive traits necessary for survival and production in the rigorous environment accompanied by extreme temperatures and low forage availability than their area of origin (18). Even though it was minimal, the steers appeared to share equal chances of seeking shade irrespective of the breed. Tropical cattle breeds like Nguni and Boran are heat tolerant, and their medium body frame appears to be very instrumental to harsh and heterogeneous conditions (1). The availability of shade through trees, in this case, is essential for cattle reared on natural pastures as their absence can reduce animal well-being and subsequently alter their daily routine (37). Other studies claim that cattle prefer to graze or hide in dense areas as a protective mechanism to escape from predators and other mechanical intruders (38). Hiding under the trees or dense areas is an adaptation phenomenon used by cattle to protect and defend themselves against potential threats that are perceived as predation risks (6). Under such conditions, time spent by cattle under the shade primarily depends on the degree of disturbance stimuli (15). Predation risks play a prominent role in shaping the activity patterns of many foraging animals; hence, they resort to shifting some activities over others to avoid or reduce risks or extent of interference in competition for resources (7). In the absence of insufficient shade, cattle spend more of their time around water points (39). Similar observations were made in the current study, where Boran cattle were observed to spend more time around water points than the Nguni steers during different observation weeks. Frequent access and distance of cattle from the water sources depend on daily weather conditions like extreme temperatures, humidity, and wind speed (3, 19). Temperature and humidity have a direct relationship with cattle water consumption (4). Cattle tend lose more water from their bodies through consistent perspiration and defecation resulting from increasing physiological demand due to dehydration hence the need for regular access to water sources. Rearing cattle under natural pastures is accompanied by lots of water availability constraints due to erratic rainfalls, as many cattle farmers rely on natural resources like dams, streams, and ponds (2). As a result, water stress is an area of concern nowadays as most cattle breeds, particularly high-producing or precious genotypes, fail to adapt when sent to low-rainfall areas.

Boran cattle had a higher number of drinking bouts than the Nguni, even though no significant interactions were noted between the breed type and the period of behavior observation. A similar observation was made by Simelane et al. (10) who noted that drinking frequency in cattle could be affected by several factors, including weather conditions. In a study conducted by Williams et al. (39), an elevated degree of THI (temperature-humidity index) resulted in cows drinking more water, spending more time at the drinker, making more visits to the drinker, and competing more at the drinker. A similar observation was made in the current study, where cattle aggressively interacted with each other around the water points. It was noted that the Nguni had longer drinking bouts than the Boran steers. These results are unlikely to be explained by the weather conditions alone but might be related to physiological demand and unstable social relationships between the two cattle breeds. The two cattle breeds showed a significant decline in ADG and BCS even though it was just for the first 3 weeks. The difference in ADG and BCS between the two breeds could be that Boran had higher grazing time while the Nguni had walked longer hours during the first 3 weeks. A similar observation was made by Kabasingiza et al. (40) who recorded an increase in body weight loss in the 1st week's post-relocation. Both breeds quickly compensated for their weight loss in the successive weeks. Scientific evidence showed that the Nguni and Boran cattle performed well-under optimal conditions while the exotics performed poorly under the prevailing management practices of the communal system. Small framed cattle breeds like Nguni and Boran have a lower maintenance requirement which is more easily met by the available forage even during dry months (21). This may be due to the maintenance of a high blood urea when the nitrogen content of the pasture drops. As seen in previous studies by Katiyatiya et al. (3) and Simelane et al. (10), the Nguni maintained a level of 13% in winter while the blood urea levels of the Simmental fell to 7 mgs%, approaching the minimum for proper N balance. However, the authors note that the ability to maintain body condition may be due to adaptation to one or more stress factors.

Negative correlations between time spent grazing and the proportion of time spent on resting and walking. At any given time, the grazing will always be the preferred activity shown by the Boran instead of browsed (3, 19). Nguni steers showed negative correlations between the amount of spent grazing and other variables such as browsing, resting, walking, and a number of drinking bouts. It is widely accepted that under optimal conditions, browsing will always be the first preference activity shown by the Nguni cattle (10, 18). A negative correlation between browsing and ADG could be due to the high proportion of time spent by the Nguni while walking in search of browsing. Depending on the area and nature of vegetation, cattle breeds like Nguni and other browsers like goats tend to walk longer distances in search of browse species, and this is accompanied by lots of energy expenditure hence the weight loss (31, 34).

Conclusion

The study demonstrated adaptation differences with regard to behavioral activities and occupancy patterns of the Nguni and Boran steers post-relocation could be influenced by a combination of the unfamiliar environment and vegetation composition. This had a negative effect on the weight gain and BCS as the two breeds showed a consistent decline in grazing activities and spent more time walking in the first 3 weeks of exposure. The ability of the Nguni and Boran cattle to respond differently to the stimuli and quickly compensate for the weights implies that the two breeds coped very well in spite of several constraints. Reintroducing the indigenous cattle breeds should be a welcomed idea or possible mitigation approach to improve the tolerance of struggling cattle breeds subjected to harsh and heterogeneous environmental conditions. Nguni and Boran cattle should be prioritized in livestock development and breeding programs to better the genetic capacity of the struggling cattle breeds.

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 animal study was reviewed and approved by accommodation and care of animals was in accordance with the recommendations of the University of Fort Hare's Research Ethics Policy. The project guidelines were reviewed and permitted under the ethical clearance certificate number MUC551SSLA01 from the institutional Animal Research

Ethics Committee. Written informed consent was obtained from the owners for the participation of their animals in this study.

Author contributions

Conceptualization: MS and YN. Methodology: LZ and MS. Data curation and writing—original draft preparation: MS. Writing—review and editing: MS, YN, and LZ. All authors have read and agreed to the published version of the manuscript.

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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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