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Effect of threonine level in a high-protein diet on the growth performance and carcass characteristics of 50- to 91-day-old slow-growing indigenous male Boschveld chickens

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Broiler chickens are economically, nutritionally, and culturally very important. An experiment was conducted to determine the effect of dietary threonine level on the growth performance of slow-growing 50- to 91-day-old male Boschveld chickens. A total of 75 chickens were used in a complete randomized design comprising five dietary treatments, with three replicates and five chickens per replicate. The dietary treatments had similar nutrient contents but different threonine levels: 4.0, 7.5, 8.0, 8.5, and 9.0 g/kg dry matter (DM). The collected data were subjected to one-way analysis of variance using SAS version 9.4. A quadratic equation was used to determine the dietary threonine levels for the optimal performance of the chickens. The threonine levels affected (p<0.05) the feed intake, body weight gain, feed conversion ratio, metabolizable energy intake, nitrogen retention, abdominal fat weight, and the meat crude protein and threonine levels of the chickens. The body weight gain, feed conversion ratio, metabolizable energy intake, nitrogen retention, abdominal fat weight, and the meat crude protein and threonine levels of the chickens were optimized at dietary threonine levels of 6.12, 6.82, 5.71, 6.0, 6.9, 5.9, and 5.7 g/kg DM, respectively. However, the feed intake of the chickens decreased (p<0.05) when the dietary threonine levels were increased. The dietary threonine levels did not affect (p<0.05) the pH values of chicken meat. However, the threonine level in the diet affected (p<0.05) the chicken meat color. The chicken meat lightness and the hue angle had a positive relationship (p<0.05) with the dietary threonine levels, while the meat redness, yellowness, and chroma had a negative relationship (p<0.05) with the dietary threonine levels. It was concluded that the production parameters of male Boschveld chickens were optimized at different dietary threonine levels.

KEYWORDS

threonine, chickens, growth, gut, carcass

Introduction

One of the major challenges in the poultry industry faced by developing countries, such as those in Africa, is the feed cost due to the high prices of protein and energy sources (Abbas, 2013). Indigenous chicken breeds are extremely important, particularly in developing countries, as they are a major source of protein for the people. However, there are limited data on the dietary nutrient levels for the optimal performance of slow-growing indigenous chicken breeds (Manyelo et al., 2020; Ng'ambi et al., 2017). NRC (1994) suggested lower dietary nutrient levels for the optimal performance of slow-growing chicken breeds compared with those of fast-growing broiler chickens. Slow-growing indigenous female Venda chickens had lower dietary threonine levels for optimal performance than fast-growing broiler chickens (Ng'ambi et al., 2017). The authors also observed that the threonine requirements for optimal performance were dependent on the chicken parameters, i.e., feed intake, feed conversion ratio (FCR), growth rate, and carcass traits, among others. Threonine is an essential amino acid in chickens as their body is not capable of synthesizing threonine *de novo* (Pokoo-Aikins et al., 2022; Lehninger et al., 2000; NRC, 1994). Thus, dietary threonine levels are important for the optimal performance of broiler chickens as deficiency of this amino acid may result in increased FCR, decreased weight gain and nitrogen retention, low carcass characteristics, poor intestinal morphology, weak immunity, and slow functional development (Qaisrani et al., 2018; Manyelo and Ng'ambi, 2024). Chickens fed a diet deficient in threonine perform poorly (Kidd et al., 2004; Dozier et al., 2000). Threonine is known to be the most important amino acid for the maintenance of the intestinal mucosa as it is used in the synthesis of mucin that covers the surface area of the mucosa (Ahmed et al., 2020). According to Debnath et al. (2020), threonine is also known to maintain the intestinal strength and growth in addition to providing energy for normal intestinal functions. An increase in supplemental threonine levels has been reported to provide sufficient concentrations for the high turnover of mucosal tissues (Rezaeipour et al., 2012). Increasing the dietary L-threonine improved the morphological aspects of the small intestine of chickens, resulting in a greater development rate compared with chickens fed threonine-deficient diets, which exhibited a decreased nutrient absorption in the digestive tract (Wasman, 2022). Increases in the number of heterophils are an essential measure of immune system function, and threonine increases the globulin levels in the blood and lymphocytes (Azzam et al., 2011). There are no data in the literature on the threonine levels in the diet for optimal performance of the slow-growing indigenous Boschveld chicken breed found in South Africa. The objective of this study was to determine the threonine levels in a high-protein diet for the optimal performance of slow-growing male Boschveld chickens aged between 50 and 91 days.

Materials and methods

Diets, design, and procedures

This experiment was performed at the University of Limpopo (–27.55° S and 24.77° E). The study was approved by the University of Limpopo Animal Research Ethics Committee (AREC/20/2023: PG). This study determined the threonine levels for the optimal performance of 50- to 91-day-old male Boschveld chickens. A total of 75 male chickens weighing an average of 600 ± 10g were allocated into five treatment groups, with three replicates and five chickens per replicate (5 × 3 × 5 = 75 chickens), in a completely randomized design (Okpanachi et al., 2015; Matius et al., 2022). The diets were isocaloric (14 MJ/kg dry matter, DM) and isonitrogenous [210g crude protein (CP)/kg DM] but with threonine levels of 4.0 ($MT_{4.0}$), 7.5 ($MT_{7.5}$), 8.0 (MT_{8}), 8.5 ($MT_{8.5}$), or 9.0 g/kg DM ($MT_{9.0}$) (Tables 1, 2). The chickens had been raised on a diet with 220g CP/kg DM and 12 MJ/kg DM before the commencement of the experiment. The chickens were raised in a well-ventilated house with 15 m^2 × 2 m^2 pens of wire mesh. The house temperature was maintained between 23°C and 30°C. Light was provided for 20h to ensure adequate growth and development of the chickens (National Audit of Continence Care, 2010). Feed and water were available all the time.

Data collection

The live weights of the chickens were taken at the beginning of the experiment and weekly thereafter. The daily diet intakes were determined. The daily live weight gains and feed efficiencies were calculated. Apparent nutrient digestibility measurements were carried out when the chickens were between 84 and 91 days old. Two birds were randomly selected from each replicate, placed in metabolic cages with dimensions of 60cm × 50cm with a wire mesh bottom of 1cm × 1cm, and were used for digestibility determination. The fecal matter was collected from day 4 until day 7. The feces were weighed, dried at 70°C in an oven for 48h, and then weighed to determine nutrient digestibility. Thereafter, digestibility was calculated using the following formula:

$$\begin{aligned}\text{Digestibility}\ (\%) &= (\text{Nutrient consumed} \\ &\quad - \text{Nutrient excreted})/(\text{Nutrient consumed}) \times 100\end{aligned}$$

TABLE 1 Dietary treatment codes for the experiment.

Code	Diet description
$MT_{4.0}$	Slow-growing male chickens fed a diet containing 4.0g threonine/kg DM
$MT_{7.5}$	Slow-growing male chickens fed a diet containing 7.5g threonine/kg DM
$MT_{8.0}$	Slow-growing male chickens fed a diet containing 8.0g threonine/kg DM
$MT_{8.5}$	Slow-growing male chickens fed a diet containing 8.5g threonine/kg DM
$MT_{9.0}$	Slow-growing male chickens fed a diet containing 9.0g threonine/kg DM

DM, dry matter.

TABLE 2 Diet ingredients for the experiment.

Feed (%)	Treatment				
	$MT_{4.0}$	$MT_{7.5}$	$MT_{8.0}$	$MT_{8.5}$	$MT_{9.0}$
Corn	60.5	54.0	54.0	54.0	54.0
Corn gluten	1.14	0.37	2.50	2.35	2.18
Wheat bran	3.06	3.22	3.03	3.00	3.00
Soya bean	27.20	30.16	29.00	29.11	29.21
Lysine	0.10	0.23	0.10	0.10	0.10
DL-Methionine	0.10	0.64	0.50	0.50	0.50
Threonine	0.0	0.01	0.04	0.09	0.14
Vitamins + minerals	0.10	0.15	0.15	0.15	0.15
Limestone	1.76	1.76	1.77	1.77	1.77
Salt	0.30	0.44	0.44	0.44	0.44
Monocalcium phosphate	1.64	1.61	1.61	1.61	1.61
Sodium bicarbonate	0.10	0.10	0.10	0.10	0.10
Sunflower oil	4.00	7.30	6.75	6.77	6.79
Total	100	100	100	100	100

The ingredients contained in the vitamin–mineral premix were as follows (per kilogram of diet): vitamin A, 12,000 IU; vitamin D3, 3,500 IU; vitamin E, 30.0 mg; vitamin K, 32.0 mg; thiamine, 2 mg, riboflavin, 6 mg; pyridoxine, 5 mg; vitamin B12, 0.02 mg; niacin, 50 mg; pantothenate, 12 mg; biotin, 0.01 mg; folic acid, 2 mg; Fe, 60 mg; Zn, 60 mg; Mn, 80 mg; Cu, 8 mg; Se, 0.1 mg; Mo, 1 mg; Co, 0.3 mg; and I, 1 mg.

At the age of 91 days, three chickens from each replicate were slaughtered using the cervical dislocation method and, within 10 s after dislocation, the birds were killed (AVMA, 2020). Breast meat samples were analyzed for nutrient contents (AOAC, 2012), cooking loss (McDonald et al., 2010), and sensory attributes (American Meat Science Association, 1995) (Table 3).

An electronic scale was used to measure the dressed carcass and carcass part weights of the chickens. The digestive organ weight and length were measured using an electric scale and a measuring tape, respectively. A digital pH meter (BASIC 20 pH Meter; Crison, Barcelona, Spain) was used to measure the digesta and meat pH values. Meat color was determined with the use of the Hunter-Lab test system, where L^* is the lightness, a^* is the redness, b^* is the yellowness, and h is the hue angle (angular position of a color on the color wheel). Shear force values of the meat were determined using the Warner–Bratzler shear force (Novakovi and Tomaševi, 2017).

Chemical analysis

Samples of feeds and feces were analyzed for nutrient contents (AOAC, 2012). The threonine in the feeds was determined using ion exchange chromatography (HPLC, University of Limpopo, South Africa). The dietary apparent metabolizable energy (ME) content was calculated as described by McDonald et al. (2010).

Data analysis

The collected data were analyzed using the general linear model (GLM) procedure of the statistical analysis of variance (SAS, 2020), version 9.4. Where significant treatment effects were detected, the means were separated using Duncan's test for multiple comparisons (p<0.05). The statistical model used was as follows:

$$Y_{ij} = \mu + T_i + e_{ij}$$

where Y_{ij} is the performance response of the chickens, μ is the overall mean, T_i is the effect of dietary threonine level, and e_{ij} is the random error.

The following quadratic equation was used to determine the threonine level in the diet for optimal performance (SAS, 2020):

$$Y = a + b_1 x + b_2 x^2 + e$$

where Y is the optimal performance, a is the intercept, b is the coefficient of the equation, x is the dietary threonine level, $-b_1/2b_2$ is the threonine level for optimal response, and e is the error.

A linear regression equation (SAS, 2020) was used to determine the relationship between dietary threonine level and chicken performance.

$$Y = a + bx$$

where Y is the chicken performance, a is the intercept, b is the coefficient of the linear equation, and x is the threonine level in the diet.

TABLE 3 Sensory attribute evaluation scores.

Score	Sensory attribute			
	Tenderness	Juiciness	Flavor	Overall acceptability
1	Too tough	Too dry	Very bad flavor	Highly rejected
2	Tough	Dry	Poor flavor	Rejected
3	Neither tough nor tender	Neither dry nor juicy	Neither bad nor good flavor	Neither accepted nor rejected
4	Tender	Juicy	Good flavor	Accepted
5	Too tender	Too juicy	Very good flavor	Highly accepted

Source: American Meat Science Association (1995).

TABLE 4 Nutrient composition of the diets.

Nutrient[a]	Treatment[b]				
	$MT_{4.0}$	$MT_{7.5}$	$MT_{8.0}$	$MT_{8.5}$	$MT_{9.0}$
Crude protein (%)	21 ± 1.2	21 ± 1.2	21 ± 1.2	21 ± 1.2	21 ± 1.2
Energy (MJ/kg DM)	14 ± 1.30	14 ± 1.30	14 ± 1.30	14 ± 1.30	14 ± 1.30
Threonine (g/kg DM)	4.0^{e} ± 0.13	7.5^{d} ± 0.12	8.0^{c} ± 0.13	8.5^{b} ± 0.12	9.0^{a} ± 0.10
Fat (%)	68.0 ± 9.10	60.1 ± 5.20	64.1 ± 4.30	63.1 ± 4.40	64.3 ± 4.30
Ash (g/kg DM)	77.3 ± 8.30	82.7 ± 4.20	84.2 ± 4.03	82.2 ± 3.20	68.7 ± 16.02
Ca (g/kg DM)	10.2 ± 1.10	10.2 ± 1.00	10.1 ± 1.20	10.1 ± 1.20	10.1 ± 1.10
Sodium (g/kg DM)	3.0 ± 0.12	3.1 ± 0.10	3.1 ± 0.20	3.0 ± 0.10	3.1 ± 0.10

[a, b, c, d, e] Means in the same row not sharing a common superscript are significantly different (p<0.05).
DM, dry matter.
[a]Values are presented as the mean ± standard error.
[b]Diet with 4.0 ($MT_{4.0}$), 7.5 ($MT_{7.5}$), 8.0 ($MT_{8.0}$), 8.5 ($MT_{8.5}$), or 9.0g threonine/kg DM ($MT_{9.0}$).

TABLE 5 Effect of the dietary threonine level on the performance of slow-growing male Boschveld chickens aged 50–91 days.

Variable[a]	Treatment[b]				
	$MT_{4.0}$	$MT_{7.5}$	$MT_{8.0}$	$MT_{8.5}$	$MT_{9.0}$
Diet intake (g DM per bird per day)	141.0^{a} ± 1.00	111.7^{b} ± 0.104	135.6^{a} ± 0.03	136.3^{a} ± 0.25	105.9^{b} ± 3.33
Body weight gain (g per bird per day)	22.8^{a} ± 0.65	23.4^{a} ± 0.56	23.5^{a} ± 0.20	23.7^{a} ± 0.35	19.8^{b} ± 0.22
Feed conversion ratio (g intake/g body weight gain)	6.2^{a} ± 0.10	4.8^{c} ± 0.10	5.8^{b} ± 0.18	5.8^{b} ± 0.18	5.4^{b} ± 0.23
Metabolizable energy intake (MJ/kg DM)	10.0^{b} ± 0.10	10.2^{a} ± 0.02	10.2^{a} ± 0.02	10.0^{b} ± 0.10	10.0^{b} ± 0.10
N retention (g per bird per day)	1.3^{c} ± 0.05	1.4^{b} ± 0.01	1.7^{a} ± 0.13	1.3^{c} ± 0.10	1.4^{b} ± 0.01

[a, b, c] Means in the same row not sharing a common superscript are significantly different (p<0.05).
DM, dry matter.
[a]Values are presented as the mean ± standard error.
[b]Diet with 4.0 ($MT_{4.0}$), 7.5 ($MT_{7.5}$), 8.0 ($MT_{8.0}$), 8.5 ($MT_{8.5}$), or 9.0g threonine/kg DM ($MT_{9.0}$).

Results

The treatments had similar nutrients, except for threonine (Table 4). The dietary threonine level affected the feed intake, the live weight gain, the feed efficiency, the ME intake, and the N retention of 50- to 90-day-old slow-growing male Boschveld chickens (p<0.05) (Table 5). The quadratic equations indicated that the live weight gain, the FCR, the ME intake, and the N retention of the chickens were optimized at dietary threonine levels of 6.12 (r^2 = 0.634), 6.82 (r^2 = 0.521), 5.71 (r^2 = 0.619), and 6.0 g/kg DM (r^2 = 0.41), respectively. However, the feed intake of the chickens decreased as the dietary threonine level increased (Y=4.30 + 157.90x, r^2 = 0.358, p<0.05).

The dietary threonine level did not affect the gut organ digesta pH and the length of slow-growing chickens aged 91 days (p > 0.05) (Table 6). Similarly, the weights of the gizzard, ileum, duodenum, cecum, and large intestine of the chickens were not affected by the threonine level in the diet (p > 0.05). However, the weights of the crop and jejunum of the chickens were affected by the threonine level in the diet (p<0.05). The weights of the crop ($Y = -12.40 + 10.00x + -0.84x^2$, r^2 = 0.831) and jejunum (Y=14.88 + 2.92x + $-0.26x^2$, r^2 = 0.238) of Boschveld chickens were optimized at 6.0g threonine/kg DM diet.

The weights of the carcass, thigh, drumstick, and breast of the slow-growing 91-day-old male Boschveld chickens were affected (p<0.05) by the threonine level in the diet (Table 7). Positive relationships were observed between the threonine level in the diet and the carcass (r^2 = 0.947), thigh (r^2 = 0.870), drumstick (r^2 = 0.801), and breast weights (r^2 = 0.078) of the chickens (Table 8). The abdominal fat weight of Boschveld chickens was optimized at a threonine level of 6.9 g/kg DM diet ($Y = -12.21 + 8.66x + -0.63x^2$, r^2 = 0.396). Different dietary threonine levels of 5.9 (Y=47.982 + 7.040x – 0.590x^2, r^2 = 0.827) and 5.7 g/kg DM (Y=1.988 + 0.340x – 0.030x^2, r^2 = 0.745) optimized the meat CP and threonine contents, respectively.

The dietary threonine level did not affect Boschveld chicken meat pH values (p > 0.05) (Table 9). However, the chicken meat color was affected (p<0.05) by the dietary threonine level. Negative relationships were observed between the dietary threonine levels and the meat redness, yellowness, and chroma (p<0.05), while positive relationships were observed between the dietary threonine levels and the meat lightness and hue angle (p<0.05)

TABLE 6 Effect of threonine level in the diet on the gut organ pH, length, and weight of 91-day-old slow-growing indigenous male Boschveld chickens.

Variable[a]	Diet[b]				
	$MT_{4.0}$	$MT_{7.5}$	$MT_{8.0}$	$MT_{8.5}$	$MT_{9.0}$
Gut organ digesta pH					
Crop	7.0 ± 0.106	7.2 ± 0.125	7.1 ± 0.037	7.1 ± 0.081	7.0 ± 0.170
Gizzard	7.2 ± 0.018	7.1 ± 0.080	7.2 ± 0.088	7.2 ± 0.018	7.2 ± 0.051
Ileum	7.1 ± 0.101	7.0 ± 0.222	7.0 ± 0.152	7.0 ± 0.202	7.1 ± 0.122
Jejunum	6.8 ± 0.010	6.9 ± 0.059	6.9 ± 0.160	6.8 ± 0.099	6.8 ± 0.058
Duodenum	7.0 ± 0.098	7.0 ± 0.206	7.0 ± 0.230	7.0 ± 0.296	7.1 ± 0.049
Cecum	7.3 ± 0.191	7.2 ± 0.245	7.4 ± 0.173	7.2 ± 0.023	7.3 ± 0.110
Large intestine	7.2 ± 0.102	7.4 ± 0.119	7.1 ± 0.162	7.1 ± 0.201	7.1 ± 0.182
Gut organ length (cm)					
Gastrointestinal tract	144 ± 2.720	147 ± 6.724	139 ± 7.277	144 ± 2.617	150 ± 4.024
Ileum	53 ± 1.297	53 ± 1.297	53 ± 1.934	52 ± 2.397	55 ± 0.404
Jejunum	53 ± 1.982	54 ± 5.980	52 ± 5.184	53 ± 5.636	56 ± 1.049
Duodenum	22 ± 0.795	24 ± 0.436	22 ± 1.494	23 ± 0.231	23 ± 0.884
Cecum	32 ± 1.471	33 ± 1.650	33 ± 1.934	34 ± 0.698	35 ± 1.300
Large intestine	6 ± 0.318	7 ± 0.533	6 ± 0.673	5 ± 0.858	5 ± 0.973
Gut organ weight (g)					
Crop	14^{b} ± 0.866	17^{a} ± 1.443	12^{c} ± 1.375	12^{d} ± 0.764	10^{e} ± 0.088
Gizzard	54 ± 2.107	49 ± 4.140	49 ± 3.910	55 ± 1.761	46 ± 6.540
Ileum	17 ± 0.644	18 ± 0.717	17 ± 0.921	16 ± 1.644	16 ± 1.229
Jejunum	22^{ab} ± 1.212	24^{a} ± 1.415	21^{ab} ± 0.426	20^{b} ± 0.231	22^{ab} ± 0.841
Duodenum	12 ± 0.293	11 ± 0.774	11 ± 0.864	11 ± 0.824	13 ± 0.677
Cecum	9 ± 0.565	9 ± 0.306	9 ± 0.968	8 ± 1.195	9 ± 0.289
Large intestine	2 ± 0.248	2 ± 0.233	2 ± 0.338	2 ± 0.265	2 ± 0.238

[a, b, c, d, e] Means in the same row not sharing a common superscript are significantly different (p<0.05).
DM, dry matter.
[a]Values are presented as the mean ± standard error.
[b]Diet with 4.0 ($MT_{4.0}$), 7.5 ($MT_{7.5}$), 8.0 ($MT_{8.0}$), 8.5 ($MT_{8.5}$), or 9.0g threonine/kg DM ($MT_{9.0}$).

(Table 10). The meat juiciness, flavor, overall acceptability, and cooking loss values of Boschveld chickens were not affected by the threonine level in the diet (p > 0.05) (Table 11). However, the meat tenderness increased with an increase in dietary threonine level (Y=0.29x+1.02, r^2 = 0.799, p<0.05).

Discussion

The diets used in this study had similar nutrient levels except for dietary threonine, which ranged from 4.0 to 9.0 g/kg DM. The body weight gain, the FCR, the ME intake, the nitrogen retention, the abdominal fat weight, and the meat CP and threonine levels of the chickens were optimized at dietary threonine levels of 6.12, 6.82, 5.71, 6.0, 6.9, 5.9, and 5.7 g/kg DM, respectively, possibly indicating different threonine requirements for the different chicken production parameters (Ciftci and Ceylan, 2024; Ng'ambi et al., 2017; NRC, 1994). Ng'ambi et al. (2017) reported that 6.1, 6.4, 5.7, and 6.1g threonine/kg DM diet optimized the body weight gain, the feed efficiency, the ME intake, and the nitrogen retention of slow-growing 50- to 91-day-old Venda chickens, respectively. The present results indicate that the voluntary feed intake of male Boschveld chickens decreased with an increase in the dietary threonine level, possibly indicating that the dietary threonine level for optimal voluntary feed intake was lower than the dietary threonine levels used. However, Ng'ambi et al. (2017) reported that the optimal feed intake for Venda chickens was achieved at a dietary threonine level of 6.1 g/kg DM. On the other hand, Najafi et al. (2017) reported increased feed intake by 5.1%, increased body weight gain by 6.4%, and better FCR when chickens were fed diets

TABLE 7 Effect of threonine level in the diet on the meat part weights of 91-day-old slow-growing male Boschveld chickens.

Variable[a]	Treatment[b]				
	$MT_{4.0}$	$MT_{7.5}$	$MT_{8.0}$	$MT_{8.5}$	$MT_{9.0}$
Carcass (g)	937^{c} ± 15.50	1057^{b} ± 23.53	1118^{ab} ± 60.46	1139^{a} ± 35.94	1189^{a} ± 21.94
Thigh (g)	62.9^{c} ± 0.06	75.7^{b} ± 1.55	75.7^{b} ± 2.40	82.7^{a} ± 0.09	77.3^{b} ± 0.58
Drumstick (g)	70.3^{c} ± 1.16	76.5^{b} ± 1.80	75.1^{b} ± 3.32	77.3^{b} ± 0.87	82.0^{a} ± 0.37
Breast (g)	166.6^{c} ± 0.15	164.2^{d} ± 2.22	148.1^{e} ± 4.47	172.2^{b} ± 1.24	191.6^{a} ± 1.59
Abdominal fat (g)	12.2^{c} ± 0.38	19.0^{a} ± 1.44	16.0^{b} ± 0.58	12.0^{c} ± 0.75	16.7^{ab} ± 1.82
Crude protein (g/100g DM)	67.0^{d} ± 0.021	67.6^{c} ± 0.013	69.5^{a} ± 0.012	68.5^{b} ± 0.023	66.4^{e} ± 0.014
Threonine content (g/100g DM)	2.86^{c} ± 0.001	2.94^{b} ± 0.021	2.99^{a} ± 0.023	2.84^{d} ± 0.001	2.81^{e} ± 0.002

[a, b, c, d, e] Means in the same row not sharing a common superscript are significantly different (p<0.05).
DM, dry matter.
[a]Values are presented as the mean ± standard error.
[b]Diet with 4.0 ($MT_{4.0}$), 7.5 ($MT_{7.5}$), 8.0 ($MT_{8.0}$), 8.5 ($MT_{8.5}$), or 9.0g threonine/kg DM ($MT_{9.0}$).

TABLE 8 Relationship between the threonine level in the diet and the meat part weights of 91-day-old slow-growing male Boschveld chickens.

Factor	Formula	Coefficient of determination (r^2)	Probability
Carcass (g)	Y=47.50x+736.14	0.947	0.005
Thigh (g)	Y=3.42x+49.52	0.870	0.021
Drumstick (g)	Y=1.91x+62.13	0.801	0.040
Breast (g)	Y=2.22x+152.10	0.078	0.648

supplemented with threonine levels up to 0.93%. Kheiri and Alibeyghi (2017) reported that broilers fed diets containing 0.9% threonine had 1.1% increased feed intake, 3.2% higher body weight gain, and 1.7% better FCR. Min et al. (2017) reported that broiler chickens fed a diet containing 0.75% threonine had 1.6% higher feed intake, 6% higher average daily gain, and 4.2% better FCR. The differences in the results between the studies may be due to the different diets and chicken genotypes used (NRC, 1994).

In this study, the threonine level in the diet did not affect the gut organ digesta pH and length of the 91-day-old male Boschveld chickens, possibly indicating that the dietary threonine levels for optimal gut organ digesta pH and length were lower than or equal to the levels used in the experiment. Ng'ambi et al. (2017) reported no dietary threonine level effect on the digesta pH values of Venda chickens. A dietary threonine level of 8.0 g/kg DM was recommended for the optimal gut morphology of 22- to 42-day-old fast-growing broiler chickens (NRC, 1994). Jamroz (2005) observed that longer intestines improved the feed digestibility in broiler chickens. The weights of the gizzard, ileum, duodenum, cecum, and large intestine of male Boschveld chickens were not affected by the threonine levels in the diet; however, the weights of the crop and jejunum were affected by the dietary threonine levels.

TABLE 9 Effect of the dietary threonine level on the breast color and pH values of 91-day-old slow-growing male Boschveld chickens.

Variable[a]	Treatment[b]				
	$MT_{4.0}$	$MT_{7.5}$	$MT_{8.0}$	$MT_{8.5}$	$MT_{9.0}$
L^* (lightness)	52.24^{d} ± 0.557	56.39^{c} ± 0.159	65.91^{a} ± 0.465	65.96^{a} ± 0.537	61.68^{b} ± 0.958
a^* (redness)	6.83^{a} ± 0.156	4.81^{c} ± 0.081	3.98^{d} ± 0.087	3.80^{e} ± 0.023	6.24^{b} ± 0.062
b^* (yellowness)	12.21^{a} ± 0.167	11.43^{b} ± 0.240	8.50^{c} ± 0.256	5.53^{e} ± 0.095	7.95^{d} ± 0.225
C^* (chroma)	14.04^{b} ± 0.228	12.51^{c} ± 0.167	9.98^{e} ± 0.274	14.82^{a} ± 0.032	11.50^{d} ± 0.427
h (hue angle)	60.87^{d} ± 0.566	64.78^{b} ± 0.502	58.49^{e} ± 0.286	72.58^{a} ± 1.316	62.57^{c} ± 0.828
Breast pH	6.87 ± 0.067	6.80 ± 0.058	6.87 ± 0.067	6.77 ± 0.033	6.80 ± 0.058

[a, b, c, d, e] Means in the same row not sharing a common superscript are significantly different (p<0.05).
[a]Values are presented as the mean ± standard error.
[b]Diet with 4.0 ($MT_{4.0}$), 7.5 ($MT_{7.5}$), 8.0 ($MT_{8.0}$), 8.5 ($MT_{8.5}$), or 9.0g threonine/kg dry matter (DM) ($MT_{9.0}$).

TABLE 10 Relationship between the dietary threonine level and breast color of 91-day-old slow-growing male Boschveld chickens.

Factor	Formula	r^2 (coefficient of determination)	Probability
L^* (lightness)	Y=2.47x+42.14	0.659	0.095
b^* (yellowness)	Y = −1.05 + 16.89	0.585	0.132
a^* (redness)	Y = −0.39x+8.05	0.333	0.308
C^* (chroma)	Y = −0.37x+15.30	0.142	0.532
h (hue angle)	Y = −0.93x+56.99	0.116	0.575

A threonine level of 6 g/kg DM diet optimized both the crop and jejunum weights of male Boschveld chickens. However, Rezaeipour et al. (2012) reported that dietary threonine levels did not affect the gut organ weights of broiler chickens.

Threonine is important for protein synthesis in chickens (Lehninger et al., 2000; NRC, 1994). Increasing the dietary threonine levels improved the carcass, thigh, drumstick, and breast weights of 50- to 91-day-old male Boschveld chickens, possibly indicating that the threonine levels used were lower than the optimal requirements for these production variables. However, Ng'ambi et al. (2017) observed that 6.2g threonine/kg DM diet optimized the carcass and breast weights of indigenous Venda chickens, which is lower than the dietary threonine levels of 7.5, 8.0, 8.5, and 9.0 g/kg DM used in the present study. Al-Hayani (2017); Kidd et al. (2004), and NRC (1994) reported 6.8, 6.5, and 8.6g threonine/kg DM diet, respectively, for optimal breast weights of 22- to 42-day-old broiler chickens. Dietary threonine levels of 5.9, 5.7, and 6.9 g/kg DM optimized the meat CP content, threonine content, and abdominal fats in male Boschveld chicken, respectively, possibly indicating different threonine requirements for the different chicken production parameters (Ng'ambi et al., 2017; NRC, 1994). The improved meat CP and threonine contents due to the increased threonine levels in the diet did not affect the meat juiciness, flavor, overall acceptability, or the cooking loss of Boschveld chicken. Ng'ambi et al. (2017) also observed that dietary threonine levels of 4–8 g/kg DM did not have any effect on the meat sensory attributes of slow-growing female Venda chickens. However, dietary amino acids affected the meat sensory attributes in chickens (Lawrie, 2006). The meat pH values of Boschveld chickens were not affected by the threonine levels in the diet, possibly indicating that the dietary threonine levels for optimal meat pH values were equal to or lower than the levels used in the present study. Schiavone et al. (2017) reported that the dietary threonine level had no effect on the meat pH values of broiler chickens. The meat color of Boschveld chickens was affected by the dietary threonine level. Negative relationships were observed between the dietary threonine levels and the meat redness, yellowness, and chroma, while positive relationships were observed between the threonine level in the diet and the lightness and hue angle of the meat. Zadeh et al. (2019) reported that 6.7 and 8.1g threonine/kg DM in the diet resulted in similar quail meat colors. Alaa El- Din et al. (2019) reported that the color of fresh meat and its stability can vary widely among species and cuts from the same animal due to differences in the anatomical, physiological function, and biochemical processes of the muscles where the meat is obtained from.

Conclusion and recommendations

The dietary threonine levels used in the present study affected the growth performance of 50- to 91-day-old male Boschveld chickens. The live weight gain, the feed efficiency, the ME intake, the nitrogen retention, and the abdominal fat weight and threonine levels of the chickens were optimized at dietary threonine levels of 6.14, 6.82, 5.71, 6.0, 6.9, and 5.9 g/kg DM, respectively, possibly

TABLE 11 Effect of the dietary threonine level on the meat sensory attributes and cooking loss of 91-day-old slow-growing male Boschveld chickens.

Variable[a]	Treatment[b]				
	$MT_{4.0}$	$MT_{7.5}$	$MT_{8.0}$	$MT_{8.5}$	$MT_{9.0}$
Tenderness	2.33^c ± 0.333	3.00^b ± 0.0001	3.00^b ± 0.0001	3.67^a ± 0.333	4.00^a ± 0.0001
Juiciness	3.67 ± 0.333	3.33 ± 0.333	3.33 ± 0.333	3.00 ± 0.664	4.00 ± 0.338
Flavor	3.67 ± 0.333	3.33 ± 0.333	3.33 ± o.333	3.67 ± 0.333	3.67 ± 0.333
Overall acceptability	3.33 ± 0.333	3.33 ± 0.333	3.00 ± 0.338	3.67 ± 0.333	3.67 ± 0.333
Cooking loss	35.82 ± 2.518	32.45 ± 5.921	30.54 ± 8.600	24.89 ± 10.413	32.00 ± 3.753

[a, b, c] Means in the same row not sharing a common superscript are significantly different (p<0.05).
[a]Values are presented as the mean ± standard error.
[b]Diet with 4.0 ($MT_{4.0}$), 7.5 ($MT_{7.5}$), 8.0 ($MT_{8.0}$), 8.5 ($MT_{8.5}$), or 9.0g threonine/kg dry matter (DM) ($MT_{9.0}$).

indicating that the threonine requirements for optimal performance of the chickens are dependent on the particular production variables of interest, i.e., feed intake, FCR, body weight gain, and carcass characteristics, among others. It is recommended that when formulating diets for indigenous Boschveld chickens, the threonine levels should be based on the parameters of interest and breed as broiler chicken lines are constantly being improved through efficient breeding. In conclusion, more research is required to explore the biological and physiological reasons behind this.

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 approved by University of Limpopo Animal Research and Ethics Committee. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

TS: Data curation, Project administration, Visualization, Writing – original draft, Writing – review & editing, Formal analysis. JN: Data curation, Formal analysis, Software, Writing – original draft, Writing – review & editing. TM: Conceptualization, Data curation, Investigation, Methodology, Project administration, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

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Conflict of interest

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Supplementary material

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