Research Article

Influences of Amaranth Leaf Meal on Performance, Blood Profiles, and Gut Morphology in Boschveld Chickens

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Abstract | Protein ingredients are the most expensive inputs in poultry production even though not included in large quantities compared to energy ingredients. The study aimed to determine whether amaranth leaf meal supplementation could improve the performance, blood parameters, and gut morphology of indigenous Boschveld chickens. A total of 200 one-day-old indigenous Boschveld chicks were randomly allocated to five treatments in a completely randomized design each with four replicates of ten chicks. Amaranth leaf meal (ALM) levels evaluated in this study were 0, 5, 10, 15, and 20% with recording weekly body weights, feed intakes, and feed conversion ratios. Gut organ weights, lengths, pH, and meat characteristics were determined, and the general linear model was used to analyze the collected data. ALM supplementation levels did not affect feed intakes, body weights, feed conversion ratios, and live weights of indigenous Boschveld chickens. At the age of 42 days chickens that were supplemented with 0, 10, and 20% ALM had higher ($P < 0.05$) white blood cells, heterophils, monocytes, eosinophils, and basophils compared to other treatments. At the age of 90 days, the chickens which were treated with 5, 10, and 15% ALM had higher ($P < 0.05$) heterophils, lymphocytes, eosinophils, and basophils than other treatments. However, 5% ALM had a higher ($P < 0.05$) nutrient digestibility in the Boschveld chickens. ALM inclusion did not affect the gastrointestinal tract, small intestine, caeca, and large intestine weights of chickens aged 21, 42, and 90 days, respectively.

Keywords | Amaranth, broiler chickens, hematology, nutrient digestibility, performance

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INTRODUCTION

Amongst all other farm animal products, poultry has seen significant growth at an alarming rate. This might be attributed to the fact that these products are cost-effective, nutritious, and preferred by the majority of households (Nkukwana, 2018). In most developing countries, the poultry sector is composed of both exotic and indigenous chicken breeds. In South Africa, one of the prolific indigenous chicken breeds is the Boschveld which according to McCullough (2017) is a crossbred between Venda, Matabele, and Ovambo native chickens This indigenous chicken breed is mostly liked by rural households because it is hardy, resilient, survive on scavenging and also can escape from predators (Manyelo et al., 2020). It is thus important to maximize the potential of the Boschveld chickens by supplying them with the nutritional requirement they need for optimal production (Okoro et al., 2017).

Due to high feed cost constraints, the formulation of diets that meet nutrient requirements for optimal productivity of indigenous chickens has lagged (Rezaei et al., 2004). Perhaps, this might be because energy and protein sources are the second-largest components of poultry diets and the most expensive (Iji et al., 2017). This then defeats the purpose of keeping indigenous chickens in a low-resource setting. Furthermore, because of erratic macroclimatic conditions and social changes, soybean production has decreased due to the rise in its prices (FAO, 2015). Hence the need for many developing countries to search for alternative protein sources.

According to Van der Poel et al. (2013), nutritional and technological aspects, such as the variability in nutrients levels and quality, particularly the essential amino acid balance and anti-nutritional factors of recommended protein sources, must be taken into consideration before being introduced into poultry diets (Rangel et al., 2004). Amaranth leaf meal has gained momentum as one of the most suitable protein sources which can be used in poultry diets (Manyelo et al., 2020). In many countries, amaranth vegetables have been reported to be used as feed for monogastric animals, such as chickens, pigs, and rabbits (Peiretti, 2018, Molina et al., 2018). Despite the high nutritional content present in amaranth leaves, there is little information regarding its utilization in poultry feeding as a protein source for growth performance, feed digestibility, blood profiles, and gut organ characteristics. Such information is needed in identifying feeding strategies, to improve indigenous chicken production considering that the use of protein sources such as fish and soybean meal are costly. Therefore, the current study was designed to explore the influences of amaranth leaf meal supplementation at different dietary levels on body weight, feed intake, and the feed conversion ratio, blood profiles, and gut morphology of indigenous Boschveld chickens.

MATERIALS AND METHODS

Study location and approval

The study was conducted at the University of Limpopo Livestock unit (latitude of 27.55°S and longitude of 24.77°E). The study area has ambient temperatures ranging from 20 and 36°C in the summer months (June – September) and between 5 and 25°C in the winter months (December - March). Mean annual rainfall ranges between 446.8 and 468.44 mm. This study was conducted during the winter period.

The experimental procedures were conducted following the University of South Africa (UNISA) and University of Limpopo's (UL) ethics code for the use of live animals in research, ethics reference number: 2019/CAES_AREC/154 and AREC/12/2020: IR, respectively.

Housing and management of chickens

A total of 200, day-old, male indigenous Boschveld chicks were brought from a local hatchery. An open-sided structure was used to house the chickens. For proper ventilation long axis was situated along an east-west direction in one m^2 pen of wire mesh. Natural (V-shaped windows) and artificial means (supplying fans) were used as ventilation mechanisms to enhance the birds' microclimatic conditions and to maintain the natural convection act. Moreover, the house had temperatures maintained at 30 to 33°C during the starter and 23 to 25°C at the grower phase. Two weeks before the start of the experiment, paraformaldehyde was used to disinfect the poultry house. Lighting was provided 24 hours daily utilizing natural lighting and artificial lighting using 175watt infrared ruby lamps. The bedding for the chickens was prepared using wood shaved sawdust and it was changed weekly. Birds were given fresh amaranth-based diets (Table 2) and drinking water *ad libitum* throughout the experiment. Disinfectants were used to wash and cleaned feeders and drinkers daily in the morning before being used. The chicks were vaccinated against infectious bronchitis and Newcastle virus disease using live attenuated virus vaccine (Nobilis® IB 4-91, $\log_{10}$ 3, 6 EID_{50}/dose) on day 7, and on days 18 and 28 birds were given live lentogenic virus vaccine (PESTIKAL 1000 dose, Lasota ≥$10^{6.0}$ EID_{50}).

Experimental diets, design, and procedures

A total of 200 one-day-old indigenous Boschveld chicks with an initial live weight of 42±8 g/bird were randomly allocated to five dietary treatments in a complete randomized design. Each group consists of four replicates of ten birds. Amaranth leaf meal treatments were 0, 5, 10, 15, and 20%. *Amaranth cruentus* (*L)* leaves were grown in the North-West Province, South Africa under a controlled field trial. The mean temperatures around the area in summer are above 22°C and in winter below 20°C and lie at a latitude of 25.6200°S and a longitude of 27.9800°E. The *Amaranth cruentus* used in the current study was grown in September 2019, under dryland conditions, which receives a mean annual rainfall of less than 250 mm. Amaranth leaves were hand-harvested and dried in a well-ventilated laboratory to obtain constant weights, milled into powder using a hammer mill with a 1 mm sieve, and subjected to the analysis and formulated diets (Tables 1 and 2). The experimental period lasted for 42 days.

Data collection

Growth performance: The live weights of each chicken were determined at the start of the experiment, thereafter, weekly weights were taken using an electronic weighing scale (model: RADWAG AS 220/C/2). The daily feed intakes were determined by subtracting the weights of feed leftover from the total weights of the feed supplied daily

Table 1: Proximate composition (g/100g), gross energy (kcal/g), and amino acids composition (%) of *Amaranthus cruentus* leaf meal (ACLM)

Composition (g/100g)	ACLM
DM	92.65
CP	23.23
CF	17.14
NDF	15.40
ADF	7.14
ADL	1.95
GE	14.50
EE	1.12
Starch	0.38
Ash	21.18
Amino acids	
Histidine	0.29
Arginine	0.90
Threonine	0.85
Lysine	1.73
Tyrosine	0.52
Methionine	0.34
Valine	1.51
Leucine	1.55
Serine	0.90
Glycine	0.94
Aspartic acid	2.16
Glutamine	2.94
Alanine	1.27
Proline	0.87
Isoleucine	0.83
Phenylalanine	0.66

Values are means of duplicate analysed amaranth leaves samples

and the difference was divided by the total surviving chickens in each replicate for 13 weeks. The feed conversion ratios (FCR) were calculated using the following formulae:

Apparent Nutrient digestibility: Apparent nutrient digestibility measurements were carried out at 37 and 42 days. The faecal matter was collected in trays beneath each metabolic cage for 48 hours; dried at 70°C in an oven for 48 h, and weighed to determine nutrient digestibility. Standardized methods of the AOAC (2012) were used to determine the moisture content, ash, crude protein (N × 6.25), fat, and crude fiber of feeds, and faecal matter. The adiabatic bomb calorimetry (Gallenkamp, Autobomb, and London, UK) was used to determine gross energy content of the milled samples, whereas ether extracted lipid contents were estimated using TecatorSoxtec. Waters Acquity Ultra Performance Liquid Chromatography (UPLC), fitted with a photodiode array (PDA) detector was used to determine amino acid separation and detection. This required 1µl of sample/standard solutions injected into the mobile phase which conveyed derivatized amino acids onto a Waters UltraTax C 18 column (2.1 x 50mm x 1.7µm) maintained at 60°C. Elutions off the column were performed by running a gradient. Analytes eluting off the column were detected by the PDA detector, with individual amino acids coming off the column at unique retention times.

Blood sampling: On days 42 and 90, 60 blood samples were collected from 60 birds (three birds per pen) per feeding group and 2.5 ml were placed in EDTA tubes. A drop of blood, containing an anticoagulant was smeared on a glass slide for blood smear preparation. May-Grunwald and Giemsa stains were used to smear stained (Campbell, 1995). An improved Neubauer hemocytometer was used to determine the total blood cell count (Merck Sigma Aldrich).

Gut characteristics

At the age of 21, 42, and 90 days, following the recommendations of the University of South Africa and the University of Limpopo's ethical guidelines three chickens per pen were slaughtered using the cervical dislocation method. Thus, a total number of 135 birds were sacrificed by the end of the experiment. To harvest the internal organs, birds were immersed in hot water to be de-feathered, cleaned, and dissected. Measuring tape, electronic weighing scale, and digital pH meter (Crison, Basic 20 pH Meter) were used to measure gut organ lengths, weights, gut organ pH. The pH meter was first calibrated using the following buffer solutions: pH 4, 7, and 10. Thereafter, an electrode from the pH meter was inserted in the gut organs digesta to measure the pH values.

Statistical analysis

Collected data were analysed using the general linear model (GLM) procedure of SAS (2012). The Duncan test at a 5% level of probability was used for means separation where there were significant differences ($P < 0.05$).

The quadratic models were fitted to the experimental data by using the procedure of SPSS (2017). The response in optimum feed intake, body weight, internal organ weights, and blood profiles of the Boschveld indigenous chickens, were modeled using the following quadratic equation:

$$Y= a+ b_1x+ b_2x^2$$

Where y=optimum, a=intercept; b=coefficients of the quadratic equation; x= amaranth meal inclusion level and $-b_1/2b_1$ =x value for optimum response. The quadratic equation was the preferred model as it gives the optimum fit.

Table 2: Ingredients and calculated analysis of experimental diets

Amaranth leaf meal (ALM) inclusion levels (g/kg)					
Ingredients	0	5	10	15	20
Maize	40.00	40.00	40.00	40.00	40.00
Groundnut oil cake	20.00	18.00	15.00	12.00	10.00
Soya bean meal	8.00	8.00	8.00	8.00	8.00
Fish meal	2.00	2.00	2.00	2.00	2.00
Wheat offals	25.70	22.70	20.70	18.70	15.70
Bone meal	2.50	2.50	2.50	2.50	2.50
Limestone	0.50	0.50	0.50	0.50	0.50
Salt (NaCl)	0.50	0.50	0.50	0.50	0.50
Dl-Methionine	0.15	0.15	0.15	0.15	0.15
L-Lysine	0.15	0.15	0.15	0.15	0.15
Vit/Min Premix†	0.50	0.50	0.50	0.50	0.50
ALM	0.00	5.00	10.00	15.00	20.00
Total	100.00	100.00	100.00	100.00	100.00
Calculated analysis					
Crude protein (%)	20.00	20.00	20.00	20.00	20.00
Crude fibre (%)	4.52	4.57	4.71	4.70	4.73
Ether extract (%)	7.21	6.41	6.40	6.71	6.51
GE (kcal/100g)	462.40	462.30	462.10	461.50	461.70
Analysed composition					
Crude protein (%)	19.44	19.50	19.51	19.48	20.01
Crude fibre (%)	5.03	6.22	6.35	6.43	6.37
Ether extract (%)	7.61	7.65	7.80	7.81	7.79
GE (kcal/100g)	453.7	452.80	452.70	451.60	451.00

† The ingredients contained in the vitamin–mineral premix were as follows (per kg of diet): vitamin A 12000 IU, vitamin D3 3500 IU, vitamin E 30.0 mg, vitamin K 3 2.0 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, I 1 mg. inclusion. SEM: standard error of the mean.

RESULTS

Productive performance

Amaranth leaf meal inclusion did not affect the indigenous Boschveld chickens (Table 3) aged 21, 42, and 90 days. There were no linear or quadratic influences observed in any of the performance parameters at 21, 42, and 90 days with increased levels of ALM in their diets.

ALM levels did not affect lymphocytes of indigenous Boschveld chickens aged 42 days (Table 4). ALM inclusion levels significantly affect ($P < 0.05$) WBC, heterophils, monocytes, eosinophils, and basophils of indigenous Boschveld chickens at 42 days. Indigenous Boschveld chickens treated with 0% ALM had a higher ($P < 0.05$) white blood cell count (WBC), heterophils, and monocytes. However, indigenous Boschveld chickens treated with 10% ALM revealed higher ($P < 0.05$) eosinophils. There was a linear (p = 0.029, 0.047, and 0.052) effect on WBC, lymphocytes, and monocytes with increasing levels of ALM in their diets.

At 90 days, amaranth leaf meal did not affect monocytes of indigenous Boschveld chickens. Indigenous Boschveld chickens which were treated with 5, 10, and 15% ALM, had higher ($P < 0.05$) heterophils and basophils. Similarly, chickens supplemented with 5, 10, and 15% ALM had the same heterophils. Indigenous Boschveld chickens treated with 15% ALM revealed higher ($P < 0.05$) lymphocytes and eosinophils. However, chickens offered with diets containing 10 and 15% ALM had similar lymphocytes. ALM treatments in the diets showed a linear (p= 0.029, 0.003, 0.002, and 0.004) effect on heterophils, lymphocytes, eosinophils, and basophils, as well as a quadratic (p= 0.028) influence on monocytes.

Indigenous Boschveld chickens which were treated with 5% ALM (Table 5) had higher ($P < 0.05$) CP and CF digestibility. Indigenous Boschveld chickens supplemented

Table 3: Effect of amaranth leaf meal inclusion on feed intake (FI, g/bird), weight gain (gain, g/bird) and feed conversion ratio (FCR, g: g, FI: BWG) of Indigenous Boschveld chickens

	1-21 days			22-42 days			43-90 days		
ALMg/kg	**FI**	**BW**	**FCR**	**FI**	**BW**	**FCR**	**FI**	**BW**	**FCR**
0	249.23	164.66	1.54	525.68	427.06	1.23	1506.42	1358.75	1.12
5	259.44	184.43	1.46	557.22	456.14	1.22	1779.47	1531.71	1.16
10	223.12	154.35	1.53	560.25	434.28	1.29	1849.53	1498.42	1.23
15	234.42	161.30	1.52	576.77	455.96	1.27	1853.36	1559.06	1.19
20	227.93	165.12	1.41	540.09	458.97	1.18	1893.12	1554.30	1.29
SEM	12.706	19.234	0.165	15.122	25.839	0.062	63.031	67.555	0.029
P-value									
Treatment	0.275	0.848	0.976	0.465	0.683	0.733	0.251	0.564	0.094
Linear	0.183	0.607	0.315	0.203	0.517	0.769	0.064	0.104	0.610
Quadratic	0.486	0.898	0.560	0.526	0.149	0.379	0.059	0.183	0.198

Diets: ALM0g/kg= a diet having no amaranth leaf meal inclusion, ALM5g/kg= a diet having 5 g/kg of amaranth leaf meal inclusion, ALM10g/kg=a diet having 10 g/kg of amaranth leaf meal inclusion. ALM15g/kg= a diet having 15 g/kg of amaranth leaf meal inclusion. ALM20g/kg = a diet having 20 g/kg of amaranth leaf meal inclusion. SEM: standard error of the mean

Table 4: Effect of amaranth inclusion leaf meal on blood profiles of Indigenous Boschveld chickens

	WBC	**Heterophils**	**Lymphocytes**	**Monocytes**	**Eosinophils**	**Basophils**
ALM%						
42 days						
0	27.38^{a}	54.63^{a}	43.00	6.88^{a}	0.00^{b}	7.25^{b}
5	23.80^{ab}	26.05^{c}	53.43	5.08^{b}	0.00^{b}	4.33^{c}
10	25.05^{ab}	31.00^{b}	48.00	6.25^{ab}	1.00^{a}	5.25^{c}
15	19.00^{b}	34.25^{b}	56.00	3.00^{c}	0.00^{b}	1.75^{d}
20	18.85^{b}	16.00^{d}	65.25	2.75^{c}	0.00^{b}	9.25^{a}
SEM	2.134	1.351	9.735	0.454	0.000	0.452
P-value						
Treatment	0.047	<0.000	0.573	<0.000	<0.000	<0.000
Linear	0.029	0.129	0.047	0.052	0.419	0.900
Quadratic	0.163	0.379	0.189	0.225	0.643	0.403
90 days						
0	7.24	21.50^{ab}	51.00^{c}	4.00	2.00^{b}	0.75^{b}
5	9.71	28.50^{a}	59.25^{bc}	6.00	4.00^{b}	2.25^{a}
10	8.90	32.75^{a}	71.50^{ab}	6.75	9.00^{a}	0.00^{b}
15	8.90	32.75^{a}	74.25^{a}	6.75	9.00^{a}	0.00^{b}
20	9.12	16.00^{b}	51.00^{c}	6.75	2.50^{b}	0.75^{b}
SEM	1.929	3.859	4.383	1.666	1.245	0.371
P-value						
Treatment	0.919	0.029	0.003	0.747	0.001	0.004
Linear	0.383	0.816	0.729	0.082	0.655	0.519
Quadratic	0.455	0.098	0.185	0.028	0.201	0.838

Diets: ALM0%= a diet having no amaranth leaf meal inclusion, ALM5%= a diet having 5 g/kg of amaranth leaf meal inclusion, ALM10% =a diet having 10 g/kg of amaranth leaf meal inclusion. ALM15%= a diet having 15 g/kg of amaranth leaf meal inclusion. ALM20% = a diet having 20 g/kg of amaranth leaf meal inclusion. SEM: standard error of the mean.

Table 5: Effect of amaranth leaf meal inclusion on nutrient digestibility of Indigenous Boschveld chickens

Nutrient digestibility						
ALM%	**DM (%)**	**CP (%)**	**GE (MJ/kg)**	**CF (%)**	**EE (%)**	**Ash (%)**
0	95.01	70.81^{c}	15.11	60.20^{e}	8.32^{e}	62.36^{a}
5	95.19	79.15^{a}	15.97	64.71^{a}	15.60^{b}	59.40^{b}
10	95.20	77.07^{b}	15.73	63.17^{b}	14.69^{c}	58.79^{c}
15	95.26	69.86^{d}	15.08	61.04^{d}	15.90^{a}	57.73^{d}
20	95.43	68.13^{e}	15.05	62.25^{c}	10.51^{d}	56.82^{e}
SEM	0.012	0.012	0.012	0.012	0.012	0.012
P-value						
Treatment	0.624	0.021	0.679	0.035	0.018	0.027
Linear	0.012	0.412	0.539	0.951	0.725	0.012
Quadratic	0.090	0.286	0.401	0.705	0.118	0.036

a, b, c, d, e Means in the same row not sharing a common superscript are different ($p<0.05$). Diets: ALM0%= a diet having no amaranth leaf meal inclusion, ALM5%= a diet having 5 g/kg of amaranth leaf meal inclusion, ALM10% =a diet having 10 g/kg of amaranth leaf meal inclusion. ALM15%= a diet having 15 g/kg of amaranth leaf meal inclusion. ALM20% = a diet having 20 g/kg of amaranth leaf meal inclusion. SEM: standard error of the mean.

Table 6: Effect of amaranth leaf meal inclusion on essential amino acid digestibility (%) of Indigenous Boschveld chickens

Essential amino acid digestibility %									
ALM%	**Arg**	**His**	**Ile**	**Leu**	**Lys**	**Met**	**Phe**	**Thr**	**Val**
0	0.76^{b}	0.78^{b}	0.50^{c}	0.92^{b}	0.62^{b}	0.74^{b}	0.71^{b}	0.44^{b}	0.67^{c}
5	0.93^{a}	0.84^{a}	0.59^{a}	0.96^{a}	0.78^{a}	0.78^{a}	0.94^{a}	0.56^{a}	0.76^{a}
10	0.96^{a}	0.79^{b}	0.49^{c}	0.92^{b}	0.76^{a}	0.76^{ab}	0.93^{a}	0.55^{a}	0.72^{b}
15	0.69^{c}	0.77^{b}	0.55^{b}	0.90^{bc}	0.65^{b}	0.74^{b}	0.66^{c}	0.44^{b}	0.67^{c}
20	0.60^{d}	0.76^{b}	0.47^{c}	0.87^{c}	0.61^{b}	0.73^{b}	0.67^{c}	0.43^{b}	0.64^{c}
SEM	0.012	0.013	0.012	0.012	0.012	0.011	0.011	0.012	0.012
P-value									
Treatment	<0.000	0.010	0.0002	0.004	<.0001	0.052	<.0001	<.0001	0.0003
Linear	0.312	0.404	0.596	0.128	0.629	0.420	0.500	0.575	0.393
Quadratic	0.169	0.592	0.655	0.169	0.246	0.329	0.397	0.337	0.261

a, b, c Means in the same row not sharing a common superscript are different ($p<0.05$). Diets: ALM0%= a diet having no amaranth leaf meal inclusion, ALM5%= a diet having 5 g/kg of amaranth leaf meal inclusion, ALM10% =a diet having 10 g/kg of amaranth leaf meal inclusion. ALM15%= a diet having 15 g/kg of amaranth leaf meal inclusion. ALM20% = a diet having 20 g/kg of amaranth leaf meal inclusion. SEM: standard error of the mean.

Table 7: Effect of amaranth leaf meal inclusion on non-essential amino acid digestibility (%) of Indigenous Boschveld chickens

Non-essential amino acid digestibility (%)							
ALM%	**Ala**	**Asp**	**Glu**	**Gly**	**Pro**	**Ser**	**Tyr**
0	0.78^{c}	0.80^{b}	0.62^{b}	0.65^{c}	0.69^{cd}	0.84^{ab}	0.42^{c}
5	0.91^{a}	0.86^{a}	0.66^{a}	0.76^{a}	0.85^{a}	0.86^{a}	0.62^{a}
10	0.85^{b}	0.84^{a}	0.66^{a}	0.69^{b}	0.72^{c}	0.83^{ab}	0.54^{b}
15	0.85^{b}	0.79^{b}	0.66^{a}	0.65^{c}	0.79^{b}	0.82^{b}	0.44^{c}
20	0.78^{c}	0.75^{c}	0.54^{c}	0.64^{c}	0.66^{d}	0.82^{b}	0.38^{d}
SEM	0.012	0.012	0.016	0.012	0.012	0.011	0.011
P-value							
Treatment	<.0001	0.0005	<.0001	0.0001	<.0001	0.136	<.0001

Linear	0.782	0.263	0.408	0.489	0.691	0.139	0.482
Quadratic	0.289	0.108	0.092	0.510	0.508	0.403	0.282

a, b, c, d Means in the same row not sharing a common superscript are different ($p<0.05$). Diets: ALM0%= a diet having no amaranth leaf meal inclusion, ALM5%= a diet having 5 g/kg of amaranth leaf meal inclusion, ALM10% =a diet having 10 g/kg of amaranth leaf meal inclusion. ALM15%= a diet having 15 g/kg of amaranth leaf meal inclusion. ALM20% = a diet having 20 g/kg of amaranth leaf meal inclusion. SEM: standard error of the mean.

Table 8: Effect of amaranth leaf meal inclusion on gut organ weights of Indigenous Boschveld chickens

Parameters									
ALM%	**GIT**	**Crop**	**ProGizz**	**Gizz**	**Liver**	**Spleen**	**SI**	**Caeca**	**LI**
21 days									
0	30.50	1.51	1.58	10.00	6.48	0.35	11.81	1.50	1.26
5	35.67	1.67	1.55	9.61	6.05	0.42	12.82	1.79	1.66
10	34.33	1.27	1.51	9.80	6.05	0.28	12.46	2.29	1.43
15	32.85	1.37	1.53	10.52	5.80	0.28	11.73	1.46	1.78
25	31.30	1.55	1.50	9.15	5.43	0.32	11.97	1.78	1.40
SEM	2.614	0.110	0.123	0.685	0.352	0.040	0.838	0.255	0.240
P-value									
Treatment	0.628	0.147	0.998	0.706	0.353	0.152	0.868	0.209	0.560
Linear	0.884	0.719	0.065	0.688	0.088	0.345	0.671	0.861	0.622
Quadratic	0.261	0.738	0.151	0.795	0.067	0.672	0.632	0.726	0.520
42 days									
0	76.70	3.72	3.52	23.65	15.85	1.50^{a}	38.35	5.38	1.63
5	79.28	4.20	3.59	22.58	16.46	1.48^{a}	55.82	5.65	1.82
10	77.96	4.26	3.86	22.71	15.76	1.32^{ab}	38.02	6.01	1.61
15	72.65	4.57	3.69	26.18	16.76	1.53^{b}	54.02	5.76	1.62
20	67.26	2.85	3.26	21.95	15.76	1.02^{b}	42.10	5.31	1.87
SEM	4.261	0.576	0.238	1.178	0.941	0.120	7.476	0.428	0.325
P-value									
Treatment	0.311	0.296	0.517	0.149	0.909	0.048	0.304	0.779	0.058
Linear	0.084	0.596	0.624	0.976	0.948	0.207	0.867	0.979	0.558
Quadratic	0.018	0.213	0.157	0.942	0.806	0.391	0.839	0.080	0.741
90 days									
0	159.50^{c}	7.95^{a}	6.20	36.45	29.12	3.30	43.82^{b}	8.30	3.02
5	163.50^{c}	5.62^{b}	5.62	35.22	27.12	2.45	39.87^{b}	7.62	2.85
10	187.25^{ab}	7.05^{ab}	6.37	40.95	34.82	3.17	47.55^{a}	8.12	3.22
15	184.75^{b}	5.47^{b}	5.45	39.55	26.07	3.02	39.32^{ab}	7.10	2.80
20	193.00^{a}	5.45^{b}	5.50	39.00	28.35	3.87	35.65^{b}	8.45	3.95
SEM	2.031	0.574	0.437	2.301	2.330	0.776	2.685	0.693	0.635
P-value									
Treatment	0.025	0.017	0.651	0.341	0.543	0.278	0.035	0.623	0.546
Linear	0.024	0.173	0.302	0.248	0.847	0.360	0.300	0.920	0.272
Quadratic	0.117	0.450	0.644	0.487	0.890	0.284	0.455	0.621	0.344

Diets: ALM0%= a diet having no amaranth leaf meal inclusion, ALM5%= a diet having 5 g/kg of amaranth leaf meal inclusion, ALM10% =a diet having 10 g/kg of amaranth leaf meal inclusion. ALM15%= a diet having 15 g/kg of amaranth leaf meal inclusion. ALM20% = a diet having 20 g/kg of amaranth leaf meal inclusion. SEM: standard error of the mean.

Table 9: Effect of amaranth leaf meal inclusion on gut organ lengths of Indigenous Boschveld chickens

Parameters				
ALM%	**GIT**	**SI**	**Caeca**	**LI**
21 days				
0	95.50	74.93	8.50	4.50
5	92.00	73.12	8.50	4.37
10	93.62	75.35	8.93	4.12
15	92.62	80.60	8.93	4.62
20	84.75	71.81	8.50	4.20
SEM	4.418	4.512	4.266	0.349
P-value				
Treatment	0.508	0.700	0.459	0.860
Linear	0.101	0.926	0.638	0.663
Quadratic	0.216	0.807	0.381	0.916
42 days				
0	138.75	124.25	13.56	8.06
5	135.18	113.00	13.93	6.81
10	138.50	127.75	13.75	7.12
15	140.00	113.62	12.87	7.18
20	133.25	114.50	12.18	7.43
SEM	4.822	6.273	0.718	0.447
P-value				
Treatment	0.847	0.352	0.432	0.393
Linear	0.566	0.464	0.079	0.624
Quadratic	0.728	0.803	0.033	0.926
90 days				
0	153.50	132.75	18.00	10.25
5	153.50	123.50	14.75	8.75
10	176.75	137.00	17.37	9.75
15	164.62	126.50	16.25	9.75
20	158.00	130.25	17.00	10.00
SEM	9.840	5.833	1.066	0.500
P-value				
Treatment	0.671	0.534	0.230	0.341
Linear	0.593	0.924	0.919	0.824
Quadratic	0.452	0.994	0.788	0.637

Diets: ALM0%= a diet having no amaranth leaf meal inclusion, ALM5%= a diet having 5 g/kg of amaranth leaf meal inclusion, ALM10% =a diet having 10 g/kg of amaranth leaf meal inclusion. ALM15%= a diet having 15 g/kg of amaranth leaf meal inclusion. ALM20% = a diet having 20 g/kg of amaranth leaf meal inclusion. SEM: standard error of the mean.

Table 10: Effect of amaranth leaf meal inclusion on gut organ pH of Indigenous Boschveld chickens

Parameters						
ALM%	**Crop**	**Pro**	**Gizz**	**SI**	**Caeca**	**LI**
21 days						
0	3.09^{b}	3.45^{b}	3.23	6.27	6.86	6.48
5	3.81^{ab}	3.20^{b}	2.72	5.91	6.88	6.58

10	4.20[ab]	4.31[b]	3.67	6.12	6.36	6.21
15	4.52[a]	4.58[ab]	3.65	5.95	6.30	6.03
20	4.84[a]	4.97[a]	2.95	5.99	6.65	6.25
SEM	0.378	0.438	0.295	0.199	0.348	0.321
P-value						
Treatment	0.044	0.051	0.143	0.699	0.663	0.750
Linear	0.003	0.022	0.823	0.327	0.304	0.165
Quadratic	0.006	0.131	0.797	0.542	0.359	0.405
42 days						
0	5.08	4.66	3.34	18.53	6.61	6.47
5	5.57	4.87	4.04	18.73	6.13	6.27
10	5.51	4.35	3.27	17.79	6.38	5.99
15	5.90	4.48	3.88	17.98	6.43	6.63
20	5.77	4.47	3.60	18.42	6.29	6.14
SEM	0.287	0.211	0.262	0.510	0.101	0.217
P-value						
Treatment	0.352	0.482	0.217	0.672	0.507	0.294
Linear	0.060	0.284	0.784	0.515	0.619	0.765
Quadratic	0.141	0.620	0.917	0.545	0.776	0.934
90 days						
0	5.33	4.61	4.20	6.29	6.57	6.76
5	5.24	4.74	3.37	6.39	6.62	6.47
10	5.18	5.09	3.73	6.16	6.58	6.66
15	5.45	4.92	4.15	6.27	6.85	6.58
20	4.98	5.05	3.78	6.34	6.59	6.79
SEM	0.165	0.269	0.330	0.161	0.098	0.095
P-value						
Treatment	0.346	0.547	0.213	0.289	0.317	0.412
Linear	0.457	0.089	0.964	0.953	0.549	0.741
Quadratic	0.698	0.212	0.851	0.811	0.745	0.407

Diets: ALM0%= a diet having no amaranth leaf meal inclusion, ALM5%= a diet having 5 g/kg of amaranth leaf meal inclusion, ALM10% =a diet having 10 g/kg of amaranth leaf meal inclusion. ALM15%= a diet having 15 g/kg of amaranth leaf meal inclusion. ALM20% = a diet having 20 g/kg of amaranth leaf meal

with a 15% ALM had higher (P < 0.05) EE digestibility. Indigenous Boschveld chickens treated with 0% ALM revealed higher (P < 0.05) ash digestibility. There was a linear (p=0.012) increase in DM and ash digestibility with increasing levels of ALM in the diets.

ALM inclusion significantly affects (P < 0.05) essential amino acids' digestibility of indigenous Boschveld chickens as revealed in Table 6. Indigenous Boschveld chickens fed with 5% ALM revealed overall higher (P < 0.05) amino acid digestibility. There was no linear or quadratic effect observed in any amino acid digestibility of indigenous Boschveld chickens when increasing levels of ALM in the diets, even though there was a treatment effect observed in all amino acids' digestibility.

Indigenous Boschveld chickens supplemented with 5% ALM in Table 7 revealed higher (P < 0.05) non-essential amino acids' digestibility. Similarly, chickens supplemented with 5 and 15% ALM revealed the same Asp and Glu digestibility. Interestingly, all non-essential amino acids' digestibility showed no linear or quadratic influence.

ALM inclusion did not affect gut organ weights of indigenous Boschveld chickens 21 days old as shown in Table 8. ALM inclusion did not affect GIT, crop, proventriculus, gizzard, liver, small intestine, caeca, or large intestine weights of indigenous Boschveld chickens aged 42 days. At the age of 42 days, indigenous Boschveld chickens supplemented with 0 and 5% ALM revealed higher (P < 0.05) spleen weights. At the age of 42 days, there was a linear (p = 0.048) effect on spleen weight and a quadratic (p = 0.018) effect on GIT weights with increasing levels of

ALM in their diets.

At the age of 90 days, ALM significantly affects ($P < 0.05$) GIT, crop, and small intestine weights of indigenous Boschveld chickens. Indigenous Boschveld chickens supplemented with 20% ALM revealed higher ($P < 0.05$) GIT weights. Indigenous Boschveld chickens which were fed diets with 0% ALM revealed higher ($P < 0.05$) crop weights than those on diets containing 5, 10, 15, and 20% ALM. Indigenous chickens fed 10% ALM revealed higher ($P < 0.05$) small intestine weights. ALM inclusion in the diets showed a linear ($p = 0.024$) effect on the GIT weight. ALM did not affect GIT, small intestine, caeca, or large intestine weights of indigenous Boschveld chickens aged 21, 42, and 90 days, respectively as shown in Table 9. There were neither linear nor quadratic effects observed in any gut organ lengths with increasing levels of ALM in their diets.

ALM did not affect the gizzard, small intestine, caeca, or large intestine pH of indigenous Boschveld chickens aged 21 days. ALM significantly (Table 10, $P < 0.05$) affected crop and proventriculus pH of indigenous Boschveld chickens 21 days old. Indigenous Boschveld chickens treated with 15 and 20% ALM revealed higher ($P < 0.05$) crop pH values. Linear ($p = 0.004$ and 0.0.022) and quadratic ($p = 0.006$) effects were observed on crop and proventriculus pH with increasing levels of ALM in their diets.

ALM did not affect the crop, proventriculus, gizzard, small intestine, or large intestine pH of indigenous Boschveld chickens for 42 days. ALM inclusion significantly affected ($P < 0.05$) caeca pH at 42 days. Indigenous chickens fed 0% ALM revealed higher ($P < 0.05$) caeca pH values. However, chickens fed 0, 10, 15, and 20% ALM had similar caeca pH values. ALM did not affect the crop, proventriculus, gizzard, small intestine, or large intestine pH of indigenous Boschveld chickens aged 90 days. There was no linear, nor quadratic, influence observed in any nutrient digestibility of indigenous Boschveld chickens with increasing levels of ALM in the diets.

DISCUSSION

In the present study, amaranth leaf meal (ALM) did not affect feed intakes, body weights, or feed conversion ratios of indigenous Boschveld chickens of 21, 42, and 90 days. To the best of our knowledge, there are no studies available in the literature on the use of ALM in indigenous Boschveld chickens' feed, nor any other indigenous chicken. Furthermore, the non-effect on performance parameters is an indication that ALM can be included in chicken diets, at any level, without negatively influencing productivity. However, ALM inclusion did not affect DM and GE (gross energy) digestibility of indigenous Boschveld chickens but affected crude protein (CP), CF (crude fiber), EE (ether extracts), and ash digestibility of indigenous Boschveld chickens at a 5% ALM inclusion level in the diets. The high protein digestibility values found in the present study, when ALM levels were at their highest level of 5%, reflect protein of good quality raw materials for chicken feed formulation, with low levels of secondary metabolites which might have increased with an increase of ALM levels (Dias et al., 2005). Because of the limited data on the use of ALM inclusion in indigenous Boschveld chickens, we had to compare our findings with the results of the studies which used poultry species. Indigenous Boschveld chickens fed with 5% ALM revealed higher essential and non-essential amino acids than those on diets having 0, 5, 10, and 15% ALM inclusion levels. However, there is no basis for comparison found in the literature. The reason why a 5% ALM inclusion had the best amino acids' digestibility, might be because the chickens were able to tolerate low levels of secondary metabolites which are contained in the diets of a 5% ALM inclusion. In the poultry industry, the study of blood profiles is important to examine the cellular and fluid of blood to assess the health status of the animal (Aikpitanyi and Egweh, 2020). This is normally overseen when most of the conventional feeds are tested in livestock production. At the age of 42 days, ALM inclusion affected WBC, Heterophils, monocytes, eosinophils, and basophils. Indigenous Boschveld chickens which were fed with diets containing 0, 10, and 20% ALM inclusion levels, had higher WBC, monocytes, heterophils, eosinophils, and basophils than those fed with diets having 5, 10, 15, and 20% ALM inclusion levels. According to Imaseun and Ijeh (2017), monocytes are the largest member of the white blood cells and are capable of traveling to various parts of the body to eliminate the harmful matter. This may imply that affected monocytes and white blood cells count, in the current study, were not a result of inflammation or diseased chickens, but were due to the antioxidative properties of ALM, which are responsible for maintaining total monocytes as to white blood cells count. At the age of 90 days, indigenous Boschveld chickens which were fed with diets containing 5, 10, and 15% ALM inclusion levels, had higher lymphocytes, heterophils, eosinophils, and basophils. However, blood profiles were affected at various ALM inclusion levels, unfortunately, no literature is available to back up our observations. Blood profiles in the current study, were, however, across all the various levels in acceptable ranges for normal chickens.

ALM inclusion levels did not affect gut organ weights of indigenous Boschveld chickens aged 21, 42, and 90 days, respectively. However, ALM inclusion levels affected crop and proventriculus pH of indigenous Boschveld chickens aged 21 days. ALM inclusion levels affected the caeca pH of indigenous Boschveld chickens aged 42 days. However, there is no basis for comparison found in the literature. According to Rodrigues and Choct (2018), the principal functions of the gastrointestinal tract (GIT) organs are to digest and absorb ingested nutrients and excrete waste products. Most nutrients, which come from conventional feed sources, are ingested in a form that is either too complex for absorption, or insoluble, and therefore, indigestible or incapable of being digested. However, in the current study, ALM had shown no adverse effects on the GIT organs of indigenous Boschveld chickens. This might be a good indication that nutrients that are available in ALM, do favor the productivity of the chickens.

CONCLUSION

Amaranth leaf meal can be included in indigenous Boschveld chickens' diets without having any adverse effects on the chickens' performance. Moreover, the nutrient digestibility of amaranth leaf meal as a potential protein feed source has been indicated. Nutrient utilization and affected parameters showed in favor of an inclusion level of 5, 10, and 15% in broiler diets, without any adverse effects. From the results obtained in this study, it is concluded that various amaranth leaf meal (ALM) inclusion levels can be incorporated into the diets of indigenous Boschveld chickens. However, 5, 10, and 15% ALM inclusion levels in the diet are recommended, as they showed the most favor in nutrient digestibility and blood profile of indigenous Boschveld chickens.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTIONS

Conceptualization, M.M.; writing—original draft preparation, T.G.M., and Z.M.H.; review and editing, N.A.S., J.W.N. and M.M.; visualization, N.A.S., and M.M. All authors have read and agreed to the current version of the manuscript.

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