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Original Research Article

A multi-strain probiotic administered via drinking water enhances feed conversion efficiency and meat quality traits in indigenous chickens

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ABSTRACT

Whereas the use of probiotics is commonplace in commercial production of improved chicken strains, little is known about the impact of these live microbial feed additives in indigenous chickens in South Africa. This study investigated the effect of a multi-strain probiotic (containing *Bacillus safensis*, *Bacillus subtilis*, *Bacillus megaterium* and *Cupriavidus metallidurans*, total bacteria number was 1.4×10^8 cfu/mL), administered via drinking water, on growth performance, blood parameters, and carcass and meat quality characteristics of Potchefstroom koekoek cockerels for a period of 12 weeks. A total of 140 five-week-old cockerels were randomly allocated to 4 experimental diets formulated to have similar energy and protein levels as follows: 1) negative control diet (CON; commercial chicken grower diet without both antibiotics and probiotics), 2) positive control diet (ANTIB; commercial chicken grower diet with antibiotics [0.05% Coxistac and 0.04% olaquindox] but no probiotics), 3) negative control diet plus 2.5 mL of probiotics per litre of water (PROB25) and 4) negative control with 5.0 mL of probiotics per litre of water (PROB50). There was a significant ($P < 0.05$) week and diet interaction effect on average weekly feed conversion efficiency. At 9 weeks of age, cockerels in PROB50 group had higher ($P < 0.05$) feed conversion efficiency than those in CON and ANTIB groups. However, 14-week-old cockerels in PROB50 group had lower ($P < 0.05$) feed conversion efficiency than those in ANTIB group. Treatments had no significant ($P > 0.05$) effect on overall feed intake, overall weight gain and haemato–biochemical parameters of cockerels. Gizzard and spleen weights were similar ($P > 0.05$) in PROB50, CON and PROB25 groups. Cockerels in PROB50 group had shorter ($P < 0.05$) small intestine than those in CON and PROB25 groups. Cockerels in PROB50 group had larger ($P < 0.05$) breast weight than those in PROB25 group. Cockerels in ANTIB and PROB50 groups had greater ($P < 0.05$) wing and thigh weights than those in CON and PROB25 groups. Shank weight was similar ($P > 0.05$) in PROB50, CON and ANTIB groups. Meat pH measured after 24 h of slaughter was the highest ($P < 0.05$) in CON and ANTIB groups followed by PROBO25 and PROB50 groups. Cockerels in CON group had lower ($P < 0.05$) cooking losses than those in ANTIB, PROB25 and PROB50 groups. It was concluded that probiotics can be used in place of prophylactic antibiotics in Potchefstroom koekoek cockerels.

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1. Introduction

Indigenous chickens (*Gallus gallus domesticus*) play important nutritional and socio-economic roles for people residing in rural communities because they provide high quality dietary protein and serve as a ready source of income (Kingori et al., 2010; Khobondo et al., 2015). These chickens have the potential to supply food to rural communities by converting accessible, non-conventional feed resources found around households into usable protein in the form of eggs and meat. However, their productivity is low mainly due to suboptimal nutrition causing low growth rates, poor egg production and high mortality rate (Atela et al., 2015; Khobondo et al., 2015). Commercial production of these naturally slow growing chickens would require an inexpensive ration as a strategy to reduce feed costs and the incorporation of probiotics to improve their performance without resorting to the use of antibiotic feed additives. Furthermore, selecting a breed such as the Potchefstroom koekoek for large-scale intensive production would be economically-friendly as the breed is known for its high ability to convert feed, high growth rates, excellent mothering abilities, and survivability under extreme environmental conditions compared to most of the indigenous breeds in South Africa (Matshogo et al., 2018).

To maintain poultry health in large-scale production units, the use of probiotics is inevitable. The rapid adoption of probiotics in poultry production gained attention after the social pressures to ban the use of in-feed antibiotics as growth promoters. This was due to the concerns regarding development of antibiotic-resistant microorganisms and the existence of antibiotic residues in animal products (Da Costa et al., 2011; Mnisi et al., 2017). As a consequence, poor performance and a rise in poultry diseases such as necrotic enteritis were reported (Cho et al., 2011). This prompted the need to identify and introduce probiotics as a viable alternative to antibiotics to enhance poultry performance. Probiotics have been reported to improve the performance of chickens by maintaining a healthy microbial balance within the intestine to promote gut integrity and prevent enteric diseases (Khan et al., 2011; Cox and Dalloul, 2015). The positive effects of probiotics are thought to be accomplished through three main mechanisms: competitive exclusion, bacterial antagonism, and stimulation of the immune system (Ohimain and Ofongo, 2012).

Albazaz and Buyukunal-Bal (2014) reported that probiotics improve resistance to pathogenic bacterial colonization and enhance mucosal immunity, and consequently reduce pathogenic load, which ultimately improve the performance and health status of the birds. Several studies have been conducted to determine the effects of probiotics on growth indices and their general effect on the microbiota and carcass characteristics of broiler chickens (Ashayerizadeh et al., 2011). However, there is paucity of information on the comparative effectiveness of probiotics in indigenous chickens, particularly Potchefstroom koekoek. Therefore, this study was designed to investigate the effect of 2 levels of probiotics as feed additives on growth performance, haematological parameters, serum biochemical indices, carcass characteristics and meat quality traits in Potchefstroom koekoek cockerels. We hypothesised that using probiotics in place of prophylactic antibiotics improves growth performance, health and meat quality in Potchefstroom koekoek cockerels.

2. Materials and methods

2.1. Ethics statement

All experimental procedures used to rear and slaughter chickens were reviewed and approved by the Animal Research Ethics Committee, North West University (AREC-MC) (approval No. NWU-00562-17-59).

2.2. Study site and sources of treatments

The study was conducted at North-West University Molelwane experimental farm (25°86'00"S; 25°64'32"E) located in the semi-arid region of the North West province, South Africa. The study was conducted during the summer season with temperatures ranging from 25 to 36 °C, and an average annual rainfall of 450 mm. All feed ingredients were purchased from Optifeeds (PTY) Ltd. (North West province, South Africa), whereas the probiotics were acquired from Molaplus Ltd. (Nakuru, Kenya). The probiotic (1.4×10^8 cfu/mL) contained the following beneficial bacteria, which were identified via 16S rRNA gene sequencing: *Bacillus safensis*, *Bacillus subtilis*, *Bacillus megaterium* and *Cupriavidus metallidurans*.

2.3. Dietary formulation and experimental design

Four dietary treatments were formulated to be isonitrogenous and isoenergetic using Format nutritional software from Optifeeds (PTY) Ltd. The treatments were formulated as follows: negative control diet (CON; commercial chicken growers' diet without both antibiotics and probiotics in the water), positive control diet (ANTIB; commercial chicken growers' diet with antibiotics [Coxistac and olaquindox] but no probiotics), negative control diet plus 2.5 mL of probiotics per litre of water (PROB25), and negative control with 5.0 mL of probiotics per litre of water (PROB50), as shown in Table 1. A total of 140 four-week-old male Potchefstroom koekoek indigenous chickens were purchased from a local farm (Zeerust, North West province, South Africa). The cockerels were initially fed a commercial grower diet until they reached five weeks of age. At 5 weeks of age, the birds were randomly and evenly allocated to 20 replicate pens (experimental units) measuring 3.5 m length × 1.0 m breadth × 1.85 m height, with each pen carrying

Table 1
Ingredient and chemical composition of diets on an as-fed basis (%, unless otherwise stated).

Item	CON	ANTIB
Ingredients		
Yellow maize-fine	69.9	69.9
Prime gluten 60	1.8	1.8
Full fat soya meal	5.1	5.1
Soybean meal	19.7	19.7
Limestone powder-fine	1.45	1.45
Mono calcium phosphate	0.72	0.72
NaCl (salt-fine)	0.32	0.32
Sodium carbonate	0.17	0.17
Choline powder	0.075	0.075
Lysine	0.279	0.279
L-threonine	0.041	0.041
Methionine	0.187	0.187
Growers-phytase	0.167	0.167
Coxistac	–	0.05
Olaquindox	–	0.04
Chemical composition		
Dry matter	92.35	92.35
Crude protein	18.94	18.94
Metabolisable energy, MJ/kg	12.10	12.10
Crude fat	6.24	6.24
Crude fibre	4.18	4.18
Calcium	0.85	0.85
Phosphorus	0.56	0.56
Sodium	0.18	0.18
Chloride	0.30	0.30
Potassium	0.73	0.73

CON = negative control diet (commercial chicken grower diet without both antibiotics and probiotics in the water); ANTIB = positive control diet (commercial chicken grower diet with antibiotics [Coxistac & olaquindox] but no probiotics).

seven birds replicated 5 times. The birds were allowed to adapt to the four treatments (CON, ANTIB, PROB25 and PROB50) for one week before the experiment commenced at 6 weeks of age. Feed and water were offered *ad libitum* for the entire duration of the experiment (12 weeks) under natural lighting.

2.4. Chemical analysis

The basal diets were analysed for laboratory dry matter (DM, AOAC, 2005; method No. 930.15) and total nitrogen using the standard macro-Kjeldahl method (AOAC, 2005, method No. 984.13). Total nitrogen was converted to crude protein (CP) using a factor of 6.25. Crude fibre was determined using the ANKOM^{2000} Fibre analyser (ANKOM Technology, New York, USA) by refluxing with 0.255 mol/L crude fibre acid solution followed by 0.313 mol/L crude fibre base solution. Crude fat and metabolisable energy were predicted using the models from the near infrared reflectance spectroscopy SpectraStar XL (Unity Scientific, Australia). Minerals (calcium, phosphorus, sodium, chloride and potassium) were analysed following guidelines from Agri Laboratory Association of Southern Africa (AgriLASA, 1998).

2.5. Feed intake and growth performance

Average weekly feed intake (AWFI) per cockerel was measured from 7 to 18 weeks of age by subtracting the weight of the feed refused from that of the feed offered and dividing the difference by the total number of cockerels in the pen. The initial live-weights of the cockerels were measured at the beginning of the experiment. Thereafter, average live-weights were measured weekly by weighing all the birds in each pen. These live-weights were used to calculate the average weekly weight gain (AWG) per bird as follows:

$$AWG = W(T) - W(t_0),$$

where t_0 = initial time (d); T = final time (d); $W(T)$ = final body weight/bird (g), and $W(t_0)$ = initial body weight/bird (g). Feed conversion efficiency was calculated as AWG divided by AWFI per bird.

2.6. Haematology and serum biochemistry parameters

At 17 weeks of age, 2 birds from each pen were randomly selected prior to feeding and about 4 mL of blood was drawn from the brachial vein for blood analyses. The blood was collected into two sets of sterilised tubes. Tubes containing ethylenediaminetetraacetic acid as an anticoagulant were used to collect blood for haematological analyses, whereas tubes without anticoagulant were used to collect blood for serum biochemical analyses. The blood samples were analysed using automated IDEXX LaserCyte Haematology Analyser (IDEXX Laboratories, Inc.). The haematological indices analysed were erythrocyte count, haemoglobin, leucocyte count, neutrophils, lymphocytes and monocytes. While, the serum biochemical indices measured were total protein, albumin, glucose, cholesterol, chloride, sodium, potassium, creatinine, bilirubin, calcium, alanine transaminase, aspartate transaminase, phosphate inorganic and urea using an automated IDEXX Vet Test Chemistry Analyser (IDEXX Laboratories, Inc.).

2.7. Slaughter procedure, carcass traits

At the end of the feeding trial, all the birds were starved for 13 h to ensure the complete emptying of the crop (Ari et al., 2013). All cockerels were transported to a local abattoir for slaughter (Rooigrond, South Africa). At the abattoir, the birds were electrically stunned before being slaughtered by cutting the jugular vein with a sharp knife and left hanging until bleeding ended. After defeathering, the cockerels were taken to the Animal Science Laboratory of the North-West University for determination of internal organs and carcass characteristics. Carcasses were weighed immediately after slaughter to obtain the hot carcass weight (HCW) and after 24 h of chilling to obtain the cold carcass weight (CCW). The dressing out percentage was calculated as the proportion of HCW to slaughter weight. Weights of internal organs and carcass parts were expressed as a proportion (%) of HCW, except for intestines whose size (cm) was measured with a measuring tape.

2.8. Meat quality traits

2.8.1. Meat pH and colour

Meat pH was measured on the breast muscle of each bird 24 h after slaughter using a portable digital pH meter (CRISON pH24, CRISON Instruments SA, Spain) with a piercing electrode. After every 20 measurements, the pH meter was calibrated with pH 4, pH 7 and pH 10 standard solutions (Ingold Messtechnik AG, Udorf, Switzerland). The colour of meat (lightness [L*] and yellowness [b*]) was determined on the breast meat 24 h after slaughter using Minolta colour-guide (Spectrophotometer CM 2500c, Konika Minolta, Osaka, Japan) with a 20 mm diameter measurement area with innovative 45° a:0 geometry optics. The colour measurements were taken on the dorsal surface of the left breast fillet (bone side) in triplicate.

2.8.2. Cooking losses and meat tenderness

For the determination of cooking losses, raw breast muscle samples were individually weighed to obtain initial weight of the breast muscle. The samples were then placed in foil plate and oven broiled (dry heating) at 140 °C for 20 min. The broiled samples were then removed from the oven and left to cool for 20 min. The samples were then re-weighed to obtain the cooked weight. The cooking losses were calculated as the difference between the weight of raw meat and cooked meat. Meat tenderness was determined on the breast muscle samples used to determine cooking losses. The subsamples measuring 2 cm high × 2 cm wide × 15 cm long were sheared perpendicular to the fibre direction using a Meullenet-Owens Razor Shear Blade (A/MORS) mounted on a Texture Analyser (TA XT plus, Stable Micro Systems, Surrey, UK). The reported value represented the average of the peak force measurements on each sample in Newtons (N).

2.9. Statistical analyses

All reported parameters were tested for normality using the NORMAL option in the Proc Univariate statement before being subjected to analysis of variance. Average weekly feed intake, weight gain and feed conversion efficiency data were analysed using repeated measures analysis (SAS, 2010). The following statistical linear model was employed:

$$Y_{ijk} = \mu + T_i + W_j + (T \times W)_{ij} + E_{ijk},$$

where Y_{ijk} = dependant variable, μ = population mean, T_i = effect of treatments, W_j = effect of week, $(T \times W)_{ij}$ = effect of interaction between treatments and week, E_{ijk} = random error associated with observation *ijk*, assumed to be normally and independently distributed.

Overall feed intake, overall weight gain, haematology, serum biochemistry, carcass characteristics and meat quality parameters

were analysed using the general linear models procedure of SAS (2010). The following linear statistical model was employed:

$$Y_{ij} = \mu + T_i + E_{ij},$$

where Y_{ij} = observation of the dependent variable, μ = population mean, T_i = effect of treatments, E_{ij} = random error associated with observation *ij*, assumed to be normally and independently distributed. For all statistical tests, significance was declared at $P < 0.05$. Least squares means (LSMEANS) were compared using the probability of difference option in the LSMEANS statement of SAS.

3. Results

Repeated measures analysis showed a significant ($P < 0.05$) week and diet interaction effect on feed conversion efficiency but not on AWFI and AWG. Table 2 shows that at the age of 9 weeks, cockerels in PROB50 group had higher ($P < 0.05$) feed conversion efficiency than those in CON and ANTIB groups. There was no difference ($P > 0.05$) between 9-week old cockerels in PROB50 and PROB25 groups in terms of feed conversion efficiency. At 14 weeks of age, cockerels in PROB50 group had lower ($P < 0.05$) feed conversion efficiency than those in ANTIB group. There were no differences ($P > 0.05$) in terms of feed conversion efficiency among 14-week old cockerels in PROB25, CON and ANTIB groups. Dietary treatments had no significant ($P > 0.05$) effects on overall feed intake (5.81 to 6.32 kg/bird) and overall weight gain (1.12 to1.37 kg/bird). Table 3 shows that probiotics had no effect ($P > 0.05$) on haematological and serum biochemical parameters of Potchefstroom koekoek cockerels.

Table 4 shows that probiotics significantly ($P < 0.05$) influenced the size of gizzards, spleens and small intestines. Cockerels in PROB50 and ANTIB groups had similar ($P < 0.05$) gizzard and spleen weights. Cockerels in PROB50 group had shorter ($P < 0.05$) small intestines compared to those in CON and PROB25 groups. Cockerels in PROB50 and ANTIB groups had similar ($P > 0.05$) small intestines lengths.

Table 5 shows that dietary treatments had significant ($P < 0.05$) influence on breast, wing, thigh and shank weights. Cockerels in PROB50 group did not differ ($P > 0.05$) with those in CON and ANTIB groups in terms of breast weights. However, treatment PROB50 promoted larger ($P < 0.05$) breast weights than treatment PROB25. Treatments ANTIB and PROB50 promoted greater ($P < 0.05$) wing and thigh weights compared to treatments CON and PROB25, which did not differ ($P > 0.05$). Cockerels in PROB25 and PROB50 groups did not differ ($P > 0.05$) in terms of shank weights. There were no differences ($P > 0.05$) among PROB50, CON and ANTIB cockerels in terms of shank weights.

Table 6 shows that probiotic supplementation affected ($P < 0.05$) meat pH and cooking losses. Cockerels in CON and ANTIB groups had the highest ($P < 0.05$) meat pH measured after 24 h of slaughter, followed by those in PROB25 group and lastly those in PROB50 group. Cockerels in CON group had lower ($P < 0.05$) cooking losses (19.84%) compared to those in ANTIB, PROB25 and PROB50 groups, which did not differ ($P > 0.05$).

Table 2
Effect of probiotic supplementation on feed conversion efficiency of Potchefstroom koekoek cockerels.

Item	CON	ANTIB	PROB25	PROB50	SEM	Significance
Week 7	0.370	0.393	0.332	0.451	0.0424	NS
Week 8	0.304	0.282	0.259	0.284	0.0233	NS
Week 9	0.191[a]	0.202[a]	0.306[ab]	0.370[b]	0.0398	*
Week 10	0.219	0.194	0.231	0.239	0.0165	NS
Week 11	0.282	0.232	0.233	0.237	0.0152	NS
Week 12	0.244	0.241	0.272	0.319	0.0217	NS
Week 13	0.249	0.188	0.208	0.211	0.0261	NS
Week 14	0.205[ab]	0.275[b]	0.252[ab]	0.188[a]	0.0216	*
Week 15	0.223	0.199	0.254	0.192	0.0196	NS
Week 16	0.159	0.151	0.150	0.198	0.0160	NS
Week 17	0.141	0.130	0.147	0.133	0.0098	NS
Week 18	0.131	0.123	0.223	0.137	0.0396	NS

CON = negative control diet (commercial chicken grower diet without both antibiotics and probiotics in the water); ANTIB = positive control diet (commercial chicken grower diet with antibiotics [Coxistac & olaquindox] but no probiotics); PROB25 = negative control diet plus 2.5 mL of probiotics per litre of water; PROB50 = negative control with 5.0 mL of probiotics per litre of water.
NS indicates the difference was not significant ($P > 0.05$), and * indicates a significant difference ($P < 0.05$).
[a, b] In a row, means with different superscripts significantly differ at $P < 0.05$.

Table 3
Effect of probiotic supplementation on haematological and serum biochemical parameters of Potchefstroom koekoek cockerels.

Item	CON	ANTIB	PROB25	PROB50	SEM
Haematological parameters					
Erythrocyte count, $\times 10^{12}$/L	2.96	2.80	2.80	2.86	0.079
Haemoglobin, g/dL	10.70	9.94	10.24	10.32	0.320
Leucocyte count, $\times 10^{9}$/L	21.50	21.22	16.26	21.46	2.475
Lymphocytes, $\times 10^{9}$/L	18.98	19.34	11.68	19.50	2.424
Monocytes, $\times 10^{9}$/L	0.38	0.36	0.74	0.74	0.277
Neutrophils, $\times 10^{9}$/L	1.52	1.14	3.16	0.82	0.695
Serum biochemical parameters					
Albumin, g/L	15.70	15.20	16.30	15.70	0.477
ALT, U/L	0.70	0.90	2.40	0.60	0.473
AST, U/L	190.5	181.3	186.0	190.5	5.141
Bilirubin, μmol/L	0.58	0.46	1.00	0.36	0.205
Calcium, mmol/L	3.30	2.98	3.22	3.28	0.114
Chloride, mmol/L	112.5	113.4	111.9	112.4	1.025
Cholesterol, mmol/L	3.18	3.04	3.18	3.24	0.134
Creatinine, μmol/L	18.70	18.40	18.70	18.10	0.411
Glucose, mmol/L	12.50	13.22	13.84	14.02	0.410
Phosphate inorganic, mmol/L	2.26	2.12	2.16	2.00	0.094
Potassium, mmol/L	4.28	3.90	4.06	3.88	0.168
Sodium, mmol/L	146.9	146.4	146.2	146.9	0.360
Total protein, g/L	39.50	41.70	43.30	42.30	1.329
Urea, mmol/L	0.24	0.32	0.30	0.26	0.030

CON = negative control diet (commercial chicken grower diet without both antibiotics and probiotics in the water); ANTIB = positive control diet (commercial chicken grower diet with antibiotics [Coxistac & olaquindox] but no probiotics); PROB25 = negative control diet plus 2.5 mL of probiotics per litre of water; PROB50 = negative control with 5.0 mL of probiotics per litre of water; ALT = alanine transaminase; AST = aspartate transaminase.

4. Discussion

The use of antibiotics in animal feed is widely frowned-upon due to the proliferation of drug-resistant pathogenic bacteria (Gong et al., 2014) as well as the negative, direct effects of antibiotic residues in animal products on human health. This has resulted in rising demand for antibiotic-free animal products produced using green and pollution-free additives (Phillips et al., 2004). Probiotic supplementation is said to modify gut microbiota and improve physiological responses of poultry birds (Alloui et al., 2013; Wang et al., 2017), and thus could be a viable alternative to improve the performance of indigenous chickens. This study represents the first attempt to investigate the effect of probiotics, administered through drinking water, on physiological response and meat quality characteristics of a South African indigenous chicken strain, Potchefstroom koekoek.

Repeated measures analyses showed a significant diet and week interaction effect on feed conversion efficiency, suggesting that the efficiency of the cockerels in converting experimental diets into body mass depended on the age of the cockerels. Feeding 5.0 mL/L probiotics to 9-week old cockerels resulted in higher feed conversion efficiency, signifying that this higher dosage of probiotics improved the efficiency of feed conversion. However, as the

Table 4
Effect of probiotic supplementation on size of internal organs (% HCW, unless otherwise stated) of Potchefstroom koekoek cockerels.

Item	CON	ANTIB	PROB25	PROB50	SEM	Significance
Gizzards	3.02[a]	3.84[b]	2.89[a]	3.47[ab]	0.173	***
Proventriculi	0.56	0.57	0.52	0.54	0.037	NS
Hearts	0.82	0.92	0.77	0.80	0.05	NS
Livers	2.33	2.49	2.18	2.20	0.093	NS
Spleens	0.28[a]	0.36[b]	0.27[a]	0.32[ab]	0.019	*
Small intestines, cm	125.5[b]	114.8[ab]	124.8[b]	109.0[a]	3.783	*
Large intestines, cm	4.25	4.73	4.71	4.36	0.379	NS

HCW = hot carcass weight; CON = negative control diet (commercial chicken grower diet without both antibiotics and probiotics in the water); ANTIB = positive control diet (commercial chicken grower diet with antibiotics [Coxistac & olaquindox] but no probiotics); PROB25 = negative control diet plus 2.5 mL of probiotics per litre of water; PROB50 = negative control with 5.0 mL of probiotics per litre of water.
NS indicate the difference was not significant ($P > 0.05$), and * or *** indicate a significant difference ($P < 0.05$ or $P < 0.01$).
[a, b] In a row, means with different superscripts significantly differ at $P < 0.05$.

Table 5
Effect of probiotic supplementation on carcass traits (% HCW, unless otherwise stated) of Potchefstroom koekoek cockerels.

Item	CON	ANTIB	PROB25	PROB50	SEM	Significance
Heads	5.05	4.77	4.64	4.90	0.297	NS
Necks	5.20	5.93	5.19	5.50	0.309	NS
Breasts	8.79[ab]	11.45[b]	6.80[a]	10.68[b]	0.872	***
Wings	4.29[a]	6.41[b]	4.02[a]	6.56[b]	0.461	***
Thighs	5.02[a]	7.39[b]	4.63[a]	7.71[b]	0.573	***
Drumsticks	5.43	7.43	5.17	7.34	0.669	NS
Shanks	3.30[b]	3.48[b]	2.85[a]	3.15[ab]	0.153	*
HCW, kg	1.13	0.97	1.14	1.12	0.045	NS
CCW, kg	1.10	0.95	1.12	1.09	0.047	NS
Dressing out, %	62.09	56.82	56.17	58.42	11.48	NS

HCW = hot carcass weight; CON = negative control diet (commercial chicken grower diet without both antibiotics and probiotics in the water); ANTIB = positive control diet (commercial chicken grower diet with antibiotics [Coxistac & olaquindox] but no probiotics); PROB25 = negative control diet plus 2.5 mL of probiotics per litre of water; PROB50 = negative control with 5.0 mL of probiotics per litre of water; CCW = cold carcass weight.
NS indicate the difference was not significant ($P > 0.05$), and * or *** indicate a significant difference ($P < 0.05$ or $P < 0.01$).
[a, b] In a row, means with different superscripts significantly differ at $P < 0.05$.

Table 6
Effect of probiotic supplementation on meat quality parameters of Potchefstroom koekoek cockerels 24 h after slaughter.

Item	CON	ANTIB	PROB25	PROB50	SEM	Significance
pH	5.94[c]	5.93[c]	5.80[b]	5.49[a]	0.033	***
L*	65.01	60.63	64.94	65.44	2.082	NS
b*	17.30	16.77	17.60	19.58	0.974	NS
Cooking losses, %	19.84[a]	26.52[b]	25.00[b]	25.67[b]	1.642	*
Peak force, N	7.72	8.39	11.09	8.89	1.126	NS

CON = negative control diet (commercial chicken grower diet without both antibiotics and probiotics in the water); ANTIB = positive control diet (commercial chicken grower diet with antibiotics [Coxistac & olaquindox] but no probiotics); PROB25 = negative control diet plus 2.5 mL of probiotics per litre of water; PROB50 = negative control with 5.0 mL of probiotics per litre of water; L* = lightness; b* = yellowness.
NS indicate the difference was not significant ($P > 0.05$), and * or *** indicate a significant difference ($P < 0.05$ or $P < 0.01$).
[a, b, c] In a row, means with different superscripts significantly differ at $P < 0.05$.

cockerels in PROB50 group grew older, their efficiency in converting feed declined, suggesting that feed utilization declined with age. Several studies have reported that probiotic supplementation improves growth performance but the efficacy of the probiotics depends on the application method, administration level, basal diet, the type of strains and the concentration of the probiotic (Patterson and Burkholder, 2003). However, in this study, supplementation with probiotics had no significant effect on overall feed intake and overall weight gain. This is in line with studies conducted by Lee et al. (2010) and Zhang et al. (2011), who reported no positive results on growth performance of chickens fed with various probiotic supplements.

Haematological and serum biochemical parameters are useful indicators of physiological responses of animals to the diet they are consuming (Madubuike and Ekenyem, 2006). In this study, probiotic supplementation had no effect on blood parameters, with all reported values falling within the normal ranges for healthy indigenous chickens (Ibrahim, 2012). The lack of differences in blood parameters of chickens offered probiotics and those on the positive control (antibiotics), suggests that probiotics and antibiotics equally promoted optimal health status in Potchefstroom koekoek cockerels.

Cockerels in PROB50 group had shorter small intestines compared to those fed on the negative control diet. This was expected as probiotics are known to adhere to the intestinal walls and enhance nutrient utilisation and increase the rate of digestion, thereby reducing the time of the digesta remains in the gut. However, without probiotic supplementation, the digesta will remain in the gut for prolonged periods, resulting in the elongation of small intestines as an adaptive mechanism to accommodate higher quantities of digesta. Treatments ANTIB and PROB50 increased gizzard and spleen weights when compared with treatments CON and PROB25, confirming the potential of probiotic supplementation to replace antibiotics in diets of Potchestroom koekoek cockerels, however, results indicated that a higher dosage of probiotics is more effective.

Although, there was no significant effect on overall weight gain, while supplementation with probiotics influenced some carcass characteristics such as wing, breast, thigh and shank weights. This was in agreement with the findings of Wang et al. (2017) that probiotics have a growth promoting effect on body weights of chickens. Furthermore, treatments were shown to influence meat pH measured 24 h after slaughter, indicating that pH of meat changes with storage time. However, the addition of probiotics reduced meat pH, which was in contrast with the findings of Hossain et al. (2012) and Saleh (2014), who reported that probiotic supplements improve meat pH. It is not clear why probiotics negatively affected the pH of meat which is a parameter that can be influenced by several factors as explained by Muchenje et al. (2009). Supplementation of probiotics had no effect on meat colour and tenderness; however, it negatively affected cooking losses, which is in line with the findings by Castellini et al. (2002) who reported that a drop in pH ultimately affects the ability of meat to retain its water, resulting in high cooking losses.

5. Conclusion

This study reveals that the multi-strain probiotic has potential for use as an alternative to prophylactic antibiotics in diets of

Potchefstroom koekoek cockerels. The probiotic has a positive effect on feed conversion efficiency and some carcass and meat quality parameters of Potchefstroom koekoek cockerels.

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