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β-sitosterol as an alternative to oxytetracycline: Effect on growth performance, feed intake and utilization efficiency and viscera macromorphometry of Cobb 500 broiler chickens

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ABSTRACT

Antibiotics are used to fortify broiler chicken feeds as growth promoters. Chronic antibiotic use pollutes the environment and causes the development of antibiotic resistance. Natural alternatives that mimic the properties of antibiotics, without causing health and environmental challenges are required. β-sitosterol has antimicrobial, antioxidant, digestive and immune system modulating and growth stimulating activities. We evaluated its potential to replace oxytetracycline as a growth-promoter in broiler chicken feeds. Two hundred and forty, one-day-old Cobb 500 broiler chicks were randomly allocated to four diets where β-sitosterol replaced oxytetracycline at 0 mg/kg (control; fortified with 50 mg/kg oxytetracycline), 500 mg/kg, 1000 mg/kg and 1500 mg/kg (w/w) feed and fed for 6 weeks: 2 weeks for each growth phase. Each diet was replicated thrice with 20 chicks per replicate. Initial, weekly and terminal body mass (TBM) and daily feed intake (FI) were measured. Body mass gain (BMG), average daily gain (ADG) and feed conversion ratio were computed. Terminally, the chickens were fasted for 4 h then slaughtered and dressed. Gastrointestinal tract (GIT) and GIT accessory viscera masses and small and large intestine lengths were measured. Dietary fortification with β-sitosterol had similar effects ($P > 0.05$) to oxytetracycline on the chickens' TBM, BMG, ADG, FI and utilisation efficiency and GIT organ macromorphometry. In conclusion, β-sitosterol can replace oxytetracycline in Cobb 500 broiler chicken feeds without compromising growth performance, feed intake and utilisation efficiency and GIT organ growth and development.

1. Introduction

The global human population is predicted to be 10.9 billion by 2050 and the demand for food is expected to increase by 70% (Wrachien et al., 2021). According to Lynch et al. (2018) meat production should increase by 200 million tons to reach 525 million tons annually to meet the demand of the growing global population by 2050. In sub-Saharan Africa the increase in the demand of chicken meat and eggs (Nkukwana, 2019) is driven by an increase in population, expansion of urban settlements and improved economic status of the younger populace (Wickramasuriya et al., 2022). Chicken meat is one of the most common and widely consumed meats (Wahyono & Utami, 2018) and together with chicken eggs make a significant source of animal-derived protein for human consumption. Compared to beef, pork, lamb, mutton and chevon, broiler chicken meat and eggs are relatively more affordable (King et al., 2018). Importantly, chicken meat compared to red meats has lower fat, saturated fatty acid and cholesterol content which from a consumer health perspective, is nutritionally a better product (Zhao et al., 2019). Despite chicken meat being relatively more affordable and nutritionally superior compared to other meats, the South African poultry industry fails to meet local demand of broiler chicken meat such that the country resorts to imports to make up for the shortfall (Jörnling, 2017).

In order to enhance broiler chicken productivity and improve profitability, antibiotics are often used at sub-therapeutic doses as growth promoters in broiler chicken feeds (Mehdi et al., 2018). Antibiotics boost broiler chicken productivity by inhibiting the growth of harmful intestinal microbes and promoting growth of favourable intestinal gut microbiota (Lillehoj et al., 2018). Antibiotic-induced reduction in unfavourable gut microbiota results in improved gut health and reduced inflammation of the GIT mucosa translating into increased functional

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efficiency (Mehdi et al., 2018) characterised by increased digestion, absorption and assimilation of nutrients (Kallam & Sejian, 2021). However, use of antibiotics as growth promoters in the production of broiler chicken and other livestock is a key driver of the development of antibiotic resistant bacteria, in addition to polluting the environment with antibiotic residues in chicken waste (Selaledi et al., 2020). In addition to causing the development of antibiotic resistant bacteria, the accumulation of antibiotic residues in chicken meat and eggs negatively impacts human health (Mund et al., 2017). The threat of antibiotic-resistant pathogens and residual environmental pollution has led to a growing interest in the exploration and development of alternative growth promoters for use in poultry production (Mehdi et al., 2018).

Phytochemicals are secondary plant metabolites produced to mitigate herbivory and infection by microorganisms (Sethiya, 2015). They are potential targets to replace antibiotics as feed-based growth promoters in chicken production due to their health beneficial biological activities that mirror those of antibiotics (Valenzuela-grijalva et al., 2017). In addition to mirroring the biological activities of antibiotics, phytochemicals have been shown to boost feed flavour thereby increasing feed intake (Valenzuela-grijalva et al., 2017). Phytochemicals also stimulate secretion of digestive enzymes; resulting in enhanced broiler chicken production efficiency (Alagawany et al., 2021). However, supplementing broiler chicken diets with some phytochemical feed additives at dietary inclusion levels greater than 1500 mg/kg feed (w/w) has been reported to compromise feed intake due to the phytochemical-induced strong odour and flavour (Valenzuela-grijalva et al., 2017).

One of the well characterised phytochemicals is β-sitosterol, known as (3S,8S,9S,10R,13R,14S,17R)-17-[(2R,5R)-5-ethyl-6-methylheptan-2-yl]-10,13-dimethyl-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3-ol), a phytosterol which possesses antioxidant, antimicrobial, antibacterial, antifungal and immunomodulatory activities (Saeidnia et al., 2014). β-sitosterol has been shown to modulate and reduce adhesion of pathogenic gut bacteria and to increase nutrient availability to the host (Dhama et al., 2014). We hypothesised that β-sitosterol can potentially replace oxytetracycline as a growth-promoting supplement in broiler chicken feeds hence we evaluated its effects on broiler chicken growth performance, feed intake and feed utilisation efficiency. Previous studies have shown that dietary β-sitosterol has been successfully utilized as a growth promoter in Arbor Acres (Xie et al., 2022), White feather broiler (Ding et al., 2021) Yellow-feather (Feng et al., 2020), Partridge Shank (Zhao et al., 2019), Arbor Acres (Cheng et al., 2019), Ross 308 broiler chicken strains (Naji et al., 2014). In this study, the Cobb500 broiler chicken breed was selected as it has a good growth rate and good performance on low cost feed rations (Awad et al., 2020). Furthermore, the breed is internationally recognised as one of the world's most production efficient breed for meat production (Singh et al., 2021). It is also widely used in Africa (Cobb, 2022). Brazil is one of the main contributors of broiler chicken meat in the world, they produce about 60% of the Cobb 500 broiler chicken with a production of 14.3 million tonnes per year (Miller et al., 2022). Despite the reported successful utilization of the β-sitosterol as a growth promoter in different broiler chicken breeds, its potential has not been evaluated on the productive performance of Cobb 500 broiler chicken breed. Therefore, the present study evaluated the effect of dietary β-sitosterol on growth performance, feed intake and utilization efficiency, feed conversion ratio and viscera macromorphometry of Cobb 500 broiler chicken.

2. Materials and methods

2.1. Ethical clearance and study site

Ethical clearance for the study was granted (AREC number: 2018/50/10/B) by University of the Witwatersrand's Animal Research Ethics Committee. The feeding trial was conducted at the Wits Research Animal Facility in Johannesburg, South Africa.

2.2. β-sitosterol, oxytetracycline and feed ingredients

β-sitosterol (purity: ≥70%) was obtained from Sigma-Aldrich Private Limited, Germany. Groenvoer product cc (Olifantfontein, Pretoria, South Africa) supplied the oxytetracycline. Yellow maize grain, soyabean meal, canola oil, wheat bran, feed grade limestone, dicalcium phosphate, choline chloride, and sodium chloride were purchased from Obaro Animal Feed Manufacturers (Pty Ltd), Pretoria, South Africa. Corn gluten meal (60%) was bought from Tongaat Hullet Starch (Germiston, South Africa). The vitamin-mineral premix, synthetic lysine and methionine were purchased from Trouw Nutrition (Isando, Johannesburg, South Africa).

2.3. Diet formulation

The diets were formulated to meet the nutritional requirements of broiler chickens at starter, grower, and finisher growth stages as per National Research Council (NRC, 1994) recommendations. Table A.1 shows the ingredient and chemical composition of the starter, grower, and finisher diets.

2.4. Chicken management: housing and feeding

One-day-old Cobb 500 broiler chicks vaccinated against Marek's, Newcastle and infectious bursal disease were purchased from Alfa Kuikensplaas chicks (Onderstepoort, Pretoria, South Africa). During the two-day habituation period, before the feeding trial, the chicks were fed a starter diet and dewormed with piperazine (Kyron Laboratories Pvt Ltd, Johannesburg, South Africa) in drinking water at 90 mg/L. The chicks were housed in a deep litter system with each group of 20 chicks housed in a pen measuring 1.7 m × 1.1 m × 1.3 m length, width, and height, respectively. Clean dry wood shavings served as bedding. Feed and cleaning drinking water were provided *ad libitum*. The controlled housing temperature was set to meet the requirements of broiler chicken at starter, grower and finisher growth stages as according to Zhao et al. (2019). Infra-red lights provided supplemental heat throughout the experiment. A 12-hlighting programme was followed: with lights on from 6:00 h to 18:00 h as recommended by the South African Poultry Association (SAPA, 2012).

2.5. Experimental design and measurements

Two hundred and forty-one-day-old Cobb 500 broiler chicks (n=240) were, in a completely randomised design, allocated to four diets where β-sitosterol replaced oxytetracycline at 0 mg/kg (control; fortified with 50 mg/kg oxytetracycline), 500 mg/kg, 1000 mg/kg and 1500 mg/kg (w/w) for diets 1 to 4, respectively. The chicks were fed for 6 weeks: 2 for each of the starter, grower, and finisher phase with similar β-sitosterol inclusion in the starter, grower, and finisher diets. Each diet was replicated thrice with 20 chicks per replicate. Initial, weekly and terminal body mass and daily feed intake were measured.

2.6. Computations

The body mass gain, average daily gain and feed conversion ratio were computed from data on body mass and feed intake. The body mass gain (BMG) and average daily gain (ADG) were computed using the equations:

i BMG = final body mass – initial body mass and
ii ADG = body mass gain/duration (days) of the feeding trial, respectively.

Daily feed intake (FI) by growth phase and trial were computed using the equation:

FI = offered feed – residual feed.

The FI and BMG were used to compute the feed conversion ratio (FCR) by growth phase and for the trial period using the equation:

FCR = feed intake (g) /body mass gain (g).

2.7. Terminal procedures, measurements and sample collection

Three days after the end of the 6-weeks feeding trial, thirty broiler chickens from each dietary treatment group were randomly selected and then subjected to a 4-h fast with access to clean drinking water. Due to constraints with availability of the slaughter facilities all the birds were killed three days after ending of the performance measurements of the feeding trial. However, the birds were kept on their respective diets, groups and pens until slaughter. Each fasted chicken was weighed and then humanely slaughtered by decapitation using a guillotine (Harvard Apparatus, Massachusetts, United States). Feathers were hand-plucked and GIT (proventriculus, ventriculus, caecum, small and large intestine) and accessory GIT (liver and pancreas) viscera were dissected out. Viscera masses were measured using an electronic balance (SnowrexEQ-1200, Snowrex International Company, Taipei, Taiwan). Prior to weighing each GIT segment, digesta was gently squeezed out. The small and large intestines were gently stretched on a dissection board and their lengths measured using a ruler.

2.8. Statistical analyses

Data are presented as mean ± standard deviation. GraphPad Prism version 8.0.2 (GraphPad Software, San Diego, California, USA) was used to analyse the data. All multiple-group parametric data were analysed using one-way ANOVA. The differences between the treatment means were determined using Tukey *post hoc* test. Significance was set at $P < 0.05$.

3. Results

3.1. Mortality

During the trial, an overall 3% mortality spread as 0.00%, 8.33%, 3.33% and 1.67% for diets 1 through to 4, respectively was recorded.

3.2. Growth performance

The effect of dietary β-sitosterol on the growth performance, feed intake and utilization efficiency by growth phase and trial are shown in Table A.2. β-sitosterol had similar ($P > 0.05$) effects as oxytetracycline on the chickens' terminal body mass (TBM) and total body mass gain (TBMG) as well as their BMG, ADG, FI and FCR by growth phase and overall.

3.3. Viscera macromorphometry

Table A.3 shows the effect of β-sitosterol on the macromorphometry of GIT and GIT accessory organs. β-sitosterol similarly ($P > 0.05$) affected the proventriculi, ventriculi, small and large intestines and caeca, pancreata and liver masses and small and large intestine lengths of the chickens as oxytetracycline.

4. Discussion

4.1. Growth performance, feed intake and utilisation efficiency

We evaluated the potential of β-sitosterol, supplemented at 500 mg/kg, 1000 mg/kg and 1500 mg/kg of feed, respectively, to replace oxytetracycline as a growth promoter in broiler chicken starter, grower, and finisher diets, respectively. Our findings show that β-sitosterol-supplemented chickens had similar growth performance, feed intake and utilisation efficiency as their oxytetracycline-supplemented (control) counterparts. These findings show that supplemental β-sitosterol at the three dietary inclusion levels used in the current study did not compromise the growth performance, feed intake, and feed utilization efficiency of the broiler chickens. The three dietary inclusion levels of β-sitosterol were equally effective in promoting the growth performance of the broiler chickens, suggesting that one can save on production costs by making use of the lowest dietary β-sitosterol inclusion level without compromising performance. It must be noted that in our study we used general poultry guidelines for diet formulation according to the National Research Council (NRC, 1994). The diet was formulated to mimic common practice in general broiler chicken rearing where farmers typically purchase feeds that are commercially available without targeting specific breeds. Additionally, the study did not target breeder Cobb 500 stock where there is a greater emphasis on precision diet formulation.

Ding et al. (2021) reported that β-sitosterol, at 40 and 80 (mg/kg) dietary inclusion as a growth promoting dietary supplement in broiler chicken diets resulted in improved average daily gain (ADG) and average daily feed intake (ADFI). Although findings from the current study did not show β-sitosterol-induced improvement in FCR, it is important to note that the β-sitosterol did not compromise feed utilization efficiency. Thus, the broiler chicken grew significantly. Our results are consistent with the findings of other authors who observed similar effects of β-sitosterol on DFI, ADG and FCR of broiler chickens (Feng et al., 2020; Zhao et al., 2019) using lower dietary concentrations (20, 40, 80 mg/kg) of β-sitosterol as well as the findings of Naji et al. (2014) who made use of higher (25 000, 50 000 and 75 000 mg/kg) dietary concentrations of the phytosterol compared to ours. In the current study, we supplemented β-sitosterol at 500, 1000 and 1500 mg/kg dietary inclusion levels. However, we observed similar growth performance, feed intake and feed utilisation with oxytetracycline supplement control counterparts. The 42-day terminal body mass of the broiler chicken fed β-sitosterol fortified diets in the current study ranged from 1938 – 2016 g which was similar to that of the oxytetracycline control counterparts.

Our findings differ from those of Naji et al. (2014) who reported an increased final body mass (3104 – 3286 g) in broiler chicken whose diets were supplemented with different doses of β- sitosterol (25 000, 50 000 and 75 000 mg/kg of feed) compared to the control birds final body mass after 6 weeks (2881 g). However, there was no difference reported in FI and FCR in their study across the dietary treatments. It is important to note that Naji et al. (2014) made use of substantially higher dietary β-sitosterol inclusion levels and Ross308 broiler chicken in their study. Ross strains 308 and 708 have been reported to be rapidly growing broiler chicken reaching 2.2-2.9 kg by 35-40 days (Bedford et al., 2017). Ding et al. (2021) reported an increased final body mass (2670 – 2731 g) in White Feathered broiler chicken with lower doses of β-sitosterol (10 – 80 mg/kg of feed) than in the current study. Possible reasons for the differences in the terminal body mass of broiler chickens in the current study compared to other studies include breed differences, diet formulations, β-sitosterol doses, feeding environment and management. Importantly, our findings show similarities in terminal 42-day mass, BMG, ADG, DFI and FCR of chicken fed β-sitosterol supplemented diets and those fed the oxytetracycline supplemented diet which suggests that β-sitosterol was equally effective as oxytetracycline in sustaining the growth performance and efficient feed utilization by the Cobb 500

Table A1
The ingredient and chemical nutrient composition of the starter, grower and finisher diets.

Ingredients	Starter				Grower				Finisher			
	Diet 1	**Diet 2**	**Diet 3**	**Diet 4**	**Diet 1**	**Diet 2**	**Diet 3**	**Diet 4**	**Diet 1**	**Diet 2**	**Diet 3**	**Diet 4**
Yellow maize meal (g/kg)	447.94	447.94	447.94	447.94	494.09	494.09	494.09	494.09	520.65	520.65	520.65	520.65
Soyabean meal (g/kg)	383.95	383.95	383.95	383.95	264.69	264.69	264.69	264.69	225.62	225.62	225.62	225.62
Wheat bran (g/kg)	18.28	18.28	18.28	18.28	105.88	105.88	105.88	105.88	121.49	121.49	121.49	121.49
Corn gluten meal (g/kg)	118.84	118.84	118.84	118.84	88.23	88.23	88.23	88.23	82.44	82.44	82.44	82.44
Canola oil (g/kg)	-	-	-	-	22.94	22.94	22.94	22.94	26.03	26.03	26.03	26.03
Limestone (g/kg)	20.11	20.11	20.11	20.11	14.12	14.12	14.12	14.12	13.88	13.88	13.88	13.88
DL-Methionine, 99% (g/kg)	1.28	1.28	1.28	1.28	1.24	1.24	1.24	1.24	1.21	1.21	1.21	1.21
Dicalcium phosphate (g/kg)	2.74	2.74	2.74	2.74	2.21	2.21	2.21	2.21	2.17	2.17	2.17	2.17
Salt (g/kg)	2.29	2.29	2.29	2.29	2.21	2.21	2.21	2.21	2.17	2.17	2.17	2.17
*Vitamin and min premix (g/kg)	4.57	4.57	4.57	4.57	4.41	4.41	4.41	4.41	4.34	4.34	4.34	4.34
Oxytetracycline (mg/kg)	50	-	-	-	50	-	-	-	50	-	-	-
β-sitosterol (mg/kg)	-	500	1000	1500	-	500	1000	1500	-	500	1000	1500
Analysed chemical composition												
Dry matter (%)	91.94	92.00	91.98	91.89	92.00	92.16	92.08	91.89	92.00	92.10	92.04	92.07
Crude protein (% DM)	31.62	30.80	30.25	30.92	24.51	24.26	24.44	24.38	21.11	21.24	21.61	21.30
Crude fibre (% DM)	27.96	28.00	28.15	24.68	19.11	19.23	20.12	19.48	19.51	19.62	18.04	18.38
Ash (%DM)	28.13	28.49	28.29	28.64	28.04	26.91	28.20	27.07	28.34	28.27	28.50	28.43
Ether extract (% DM)	2.35	2.35	2.35	2.34	6.10	6.15	6.15	6.12	5.75	5.75	5.80	5.76
Neutral detergent fibre (%DM)	10.40	10.43	10.42	10.44	13.49	13.44	13.45	13.44	15.90	15.88	15.89	15.90
Acid detergent fibre (%DM)	4.66	4.56	4.45	4.55	5.11	5.07	5.09	5.08	5.97	6.19	6.06	6.04
Calcium (%)	0.90	0.91	0.90	0.90	0.81	0.81	0.80	0.81	0.90	0.89	0.90	0.90
Phosphorus (%)	0.50	0.51	0.50	0.50	0.46	0.45	0.46	0.46	0.46	0.44	0.47	0.47
Magnesium (%)	0.16	0.15	0.16	0.15	0.12	0.11	0.12	0.12	0.12	0.13	0.12	0.10
Potassium (%)	1.26	1.25	1.26	1.26	1.20	0.19	1.20	1.20	1.15	1.16	0.15	1.13
Gross energy (MJ/kg DM)	17.66	17.62	17.80	17.66	18.51	18.72	18.14	18.48	18.07	17.93	17.81	18.12

*Vit A: 2000000.000, Vit B_1 (Thiamine): 003.000g, Vit D_3 (500 000): 3000000.000 IU, Vit E (500 IU): 40000.000 IU, Vit K_3 (43%): 003.000g, Vit B_2 (80%): 010.000g, Vit B6 98% (pyrod): 005.000g, Vit B_{12} 1g/kg (m): 100.000mg, Niacine 99.5%: 060.000g, Choline (Chloride 60): 606.060g, Biotine 2%: 200.000 mg, Manganese ($MnSO_4$-31%): 160.000g, Copper ($CuSO_4$-25.2%): 005.000g, Cobalt ($CoSO_4$- 20%): 100.000mg, Selenium (Na_2SeO_3 4.5%): 400:000mg, Calcium pantothenate: 020.000g, Folic acid (96 pure): 001.000g, Anty-ox Vit Dry: 100.000g, Zinc ($ZnSO_4$-35%): 090.000g, Iodide (KI 76.45%): 001.000g, Ferrous (FeSO4-30%): 035.000g, Limestone: 2647.133g; DL-Methionine with purity of 99%.

broiler chicken. At 30-45-days of age, Cobb 500 broiler chickens are expected to have a body mass range of approximately 2000 to 2500 g (Singh et al., 2021) which was observed in our study. Some phytochemicals when used as natural growth promoters enhance the flavour (palatability) and aroma of feeds following stimulation of the olfactory nerve and taste buds which increases appetite and hence feed intake (Valenzuela-grijalva et al., 2017; Jiang et al., 2007). Our findings show similar DFI across dietary treatments which (similarities in DFI) seem to suggest that β-sitosterol, though a phytochemical, may not have had any stimulatory effect on appetite and hence feed intake.

4.2. *Viscera macromorphometry*

The gastrointestinal tract is the first point of contact for ingested substances, thus it interfaces directly with the exogenous environment (Mukonowenzou et al., 2021). Constituents of this external environment, for example, feed ingredient type and form, feed additives and diet nutrient composition impact the development and functional efficiency of the GIT (Kogut, 2019). Phytochemicals through their effects on gut microflora can mediate precocious maturation of the GIT that enhances nutrient digestion and absorption (Mukonowenzou et al., 2021). Therefore, we evaluate β-sitosterol's effects on broiler chicken growth performance and feed intake and utilisation efficiency we also determined its effects on GIT organ and GIT accessory organ macromorphometry. We observed similarities in the masses of the proventriculi, ventriculi, small and large intestine, pancreata and livers and the lengths of the small and large intestines of broiler chickens supplement with β-sitosterol and control counterparts whose diets were fortified with oxytetracycline. The similarities in the GIT and GIT accessory organ macromorphometry suggest that dietary β-sitosterol neither stimulated nor compromised the growth and development of the GIT. However, our findings show significantly lower absolute and relative liver masses compared to the results observed by Feng et al. (2020) despite them having supplemented the broiler chicken diets with β-sitosterol at 25 mg/kg of feed which lower than the including level used in our study.

On the contrary, Naji et al. (2014) observed that supplemental β-sitosterol caused significant increases in the liver and proventriculi masses of the chicken. We speculate that the high dietary inclusion level of β-sitosterol by Naji et al. (2014) might have mediated "hypertrophy" of the chickens' proventriculi and livers. Apart from its cholesterol-lowering effect, β-sitosterol has been reported to have liver-protective properties (Feng et al., 2020). Contradicting results have been reported on the effect of phytosterols on the liver mass and index in broiler chickens with higher or lower dietary phytosterol doses. In a study with layer chicken Shi et al. (2014) observed that supplementing the diets with β-sitosterol at 400 and 800 mg/kg of feed resulted in similar liver masses with those fed the control diet; a finding that is similar to observations from the current study albeit differences in dietary inclusion levels of the phytosterol. The liver, which plays an important role in detoxification, is an important organ for nutrient metabolism and a sensitive indicator of toxicity, (Dong et al., 2020). Higher dietary inclusion levels of phytosterols have been shown to affect liver lipid metabolism largely mediating increased hepatic fat accumulation a sign of fatty liver disease in broiler chicken liver (Sissener et al., 2017). This did not seem to be the case in our study.

5. Conclusion

β-sitosterol supplemented diets had similar effects on the broiler chickens' terminal body mass, body mass gain, feed intake, feed conversion ratio and GIT and accessory GIT viscera macromorphometry as with oxytetracycline-fortified control diet. We therefore conclude that β-sitosterol can replace oxytetracycline as growth promoter in broiler chicken diets with no risk of compromising growth performance, feed intake and utilisation efficiency and the growth and development of GIT and accessory GIT organs.

Table A.2
Effect of dietary β-sitosterol on the growth performance, feed intake and feed utilisation efficiency of broiler chicken.

Parameter	Growth phase	Dietary treatments				Significance level
		Diet 1	**Diet 2**	**Diet 3**	**Diet 4**	
Initial body mass (g)		63.00 ± 1.23	63.00 ± 2.10	63.43 ± 0.65	62.30 ± 0.35	0.7513
Terminal body mass (g)		2204.00 ± 181.60	1938.00 ± 105.2	1956.00 ± 161.00	2016.00 ± 119.4	0.6167
BMG (g)	Starter	257.10 ± 52.71	259.00 ± 24.06	271.60 ± 52.91	272.40 ± 36.26	0.9549
	Grower	641.20 ± 91.74	676.60 ± 50.49	668.50 ± 48.57	695.70 ± 119.00	0.8793
	Finisher	968.70 ± 77.72	939.20 ± 81.55	914.90 ± 84.20	985.70 ± 85.88	0.8333
Total BMG (g)		1867.00 ± 124.00	1875.00 ± 104.10	1893.00 ± 161.50	1953.00 ± 119.40	0.8416
ADG (g)	Starter	18.33 ± 3.76	18.53 ± 1.71	19.43 ± 3.79	19.47 ± 2.63[a]	0.9518
	Grower	45.83 ± 6.57	48.33 ± 3.59	46.93 ± 4.17	49.67 ± 8.52[a]	0.8739
	Finisher	69.20 ± 1.25	67.10 ± 5.81	65.33 ± 3.15	70.40 ± 1.13[a]	0.8326
ADG trial (g)		44.47 ± 2.91	44.63 ± 2.52	44.13 ± 5.29	45.80 ± 4.04	0.9538
FI (g)	Starter	891.60 ± 80.30	947.10 ± 28.45	905.60 ± 74.44	908.50 ± 43.40	0.8913
	Grower	1162.00 ± 103.30	1203.00 ± 34.51	1243.00 ± 68.04	1225.00 ± 52.02	0.5464
	Finisher	2654.00 ± 113.10	2899.00 ± 208.00	2826.00 ± 174.80	2791.00 ± 83.06	0.3266
FI trial (g)		4708.00 ± 135.71	5065.00 ± 226.30	5009.00 ± 274.80	4925.00 ± 163.90	0.2411
FCR	Starter	3.60 ± 0.96	3.68 ± 0.34	3.45 ± 0.85	2.83 ± 0.08	0.9629
	Grower	1.83 ± 0.14	1.78 ± 0.11	1.80 ± 0.21	1.80 ± 0.33	0.9954
	Finisher	2.74 ± 0.17	3.09 ± 0.23	3.20 ± 0.85	2.83 ± 0.08	0.5794
FCR trial		2.52 ± 0.10	2.70 ± 0.05	2.67 ± 0.37	2.53 ± 0.24	0.6906

ns = not significant $P > 0.05$. BMG – body mass gain, ADG – average daily gain, FI – feed intake, FCR – feed conversion ratio. Diet 1 – oxytetracycline (50 mg/kg of feed); diet 2 – 500 mg/kg of feed of β-sitosterol; diet 3 – 1000 mg/kg of feed of β-sitosterol; diet 4 – 1500 mg/kg of feed of β-sitosterol. Data is presented as mean ± standard deviation; for growth performance n = 60 broiler chickens per dietary treatment group.

Table A.3
Effect of dietary β-sitosterol on viscera gastrointestinal organ and accessory organ masses and lengths of broiler chicken.

Parameter	**Dietary treatments**				**Significance Level**
	Diet 1	**Diet 2**	**Diet 3**	**Diet 4**	
Heart (g)	12.01 ± 2.17	11.82 ± 2.69	11.87 ± 3.05	12.24 ± 2.80	0.9320
Relative to body mass (%)	0.51 ± 0.09	0.51 ± 0.10	0.49 ± 0.07	0.51 ± 0.08	0.9078
Liver (g)	43.13 ± 9.72	41.12 ± 10.04	41.88 ± 10.52	40.90 ± 9.98	0.8262
Relative to body mass (%)	1.83 ± 0.35	1.76 ± 0.32	1.75 ± 0.22	1.70 ± 0.28	0.3988
Pancreas (g)	4.18 ± 0.84	4.00 ± 0.75	4.20 ± 1.09	4.07 ± 0.78	0.7830
Relative to body mass (%)	0.18 ± 0.04	0.17 ± 0.03	0.18 ± 0.03	0.17 ± 0.03	0.7548
Spleen (g)	2.05 ± 0.61	1.94 ± 0.49	1.97 ± 0.58[a]	2.02 ± 0.64	0.8905
Relative to body mass (%)	0.09 ± 0.02	0.08 ± 0.02	0.08 ± 0.02[a]	0.08 ± 0.02	0.9007
Proventriculus (g)	8.24 ± 1.44	7.90 ± 1.95	7.61 ± 1.67[a]	7.76 ± 1.55	0.5089
Relative to body mass (%)	0.35 ± 0.06	0.34 ± 0.07	0.32 ± 0.04[a]	0.33 ± 0.06	0.1757
Ventriculus (g)	40.00 ± 5.29	39.37 ± 7.45	40.33 ± 9.68	40.87 ± 8.06	0.8991
Relative to body mass (%)	1.71 ± 0.19	1.70 ± 0.25	1.69 ± 0.23	1.73 ± 0.29	0.9642
Small intestines (g)	42.99 ± 6.10	40.78 ± 8.52	42.74 ± 9.28	43.04 ± 8.20	0.6618
Relative to body mass (%)	1.84 ± 0.24	1.76 ± 0.30	1.80 ± 0.21	1.82 ± 0.30	0.6798
Small intestines length (mm)	1684.00 ± 148.90	1690.00 ± 247.50	1681.00 ± 206.00	1698.00 ± 216.30	0.9886
Relative to body mass (mm/g)	0.73 ± 0.10	0.74 ± 0.12	0.73 ± 0.13	0.73 ± 0.14	0.9761
Large intestine (g)	3.21 ± 0.51	3.44 ± 0.96	3.20 ± 1.02	3.05 ± 0.99	0.4082
Relative to body mass (%)	0.14 ± 0.03	0.15 ± 0.05	0.14 ± 0.03	0.13 ± 0.03	0.1025
Large intestines length (mm)	101.80 ± 18.84	101.20 ± 19.10	99.63 ± 21.09	100.30 ± 24.49	0.9801
Relative to body mass (mm/g)	0.05 ± 0.01	0.06 ± 0.01	0.05 ± 0.01	0.05 ± 0.02	0.7228
Caecum (g)	7.15 ± 1.44	7.06 ± 1.36	6.70 ± 1.50	6.56 ± 1.27	0.3041
Relative to body mass (%)	0.31 ± 0.06	0.31 ± 0.07	0.28 ± 0.05	0.28 ± 0.05	0.0777
Visceral fat (g)	39.12 ± 16.51	41.77 ± 14.81	42.73 ± 13.65	45.15 ± 14.88	0.4752
Relative to body mass (%)	1.64 ± 0.58	1.92 ± 0.62	1.82 ± 0.62	1.98 ± 0.67	0.2189

ns = not significant $P > 0.05$. Diet 1 – oxytetracycline (50 mg/kg of feed); diet 2 – 500 mg/kg of feed of β-sitosterol; diet 3 – 1000 mg/kg of feed of β-sitosterol; diet 4 – 1500 mg/kg of feed of β-sitosterol, data is presented as mean ± standard deviation; n = 28-32 birds per dietary treatment group.

Ethical statement

Ethical clearance for the study was granted (AREC number: 2018/50/10/B) by University of the Witwatersrand's Animal Research Ethics Committee. The feeding trial was conducted at the Wits Research Animal Facility in Johannesburg, South Africa.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Malebogo Bopape reports financial support was provided by National Research Foundation. Malebogo Bopape reports a relationship with South Africa Department of Higher Education and Training that includes: funding grants.

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Appendices

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