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Original Article

Effects of Bee Pollen Inclusion on the Performance and Gut Morphology of Ross 308 Broiler Chickens

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■Keywords

Antibiotics, bee pollen, body weight, broiler chickens, gut morphology.

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ABSTRACT

This study was conducted to determine the effect of bee pollen inclusion on the performance and gut morphology of Ross 308 broiler chickens. A total of 240-day-old chicks (120 males and 120 females) were allocated to 4 treatments in a randomized complete block design (RCBD) with sex as a block. Each experimental group was replicated 3 times with 10 chicks per replicate, with an average weight of 40 ± 5g per bird. Body weight and feed intake were measured on a weekly basis to calculate the feed conversion ratio. Gut morphology was measured on days 21 and 42. Data were analysed using the General Linear Model procedures of the Statistical Analysis System.Bee pollen inclusion in starter diets had an effect (p<0.05) on body weight and live weight gain of male Ross 308 broiler chickens. The different inclusion levels of bee pollen had an effect (p<0.05) on the gut morphology of Ross 308 broiler chickens. The ileum lengths of female broiler chickens were significantly wider (p<0.05) in comparison with male chickens. This may suggest that bee pollen inclusion has a beneficial effect on broiler chickens gut morphology during the early stages of development. It can be concluded that natural substances such as bee pollen can be a possible feed additive to replace synthetic antibiotics, since such compounds are essential for the growth and development of poultry gut.

INTRODUCTION

Chicken production has a major impact on employment and income, being an important aspect of food security for the people of Africa (Ngongolo *et al*., 2018). Soon after chick hatching, chicks start feeding on solid feeds while they depend on the remaining yolk on their body (Sklan, 2003). This process results in weight loss after hatching (Willemsen *et al*., 2010). Chicks require diets that help meet their nutrient requirements to avoid weight loss after hatching, with the main goal of achieving their full growth potential (Gous, 2010). Antibiotics have been used to improve feed utilization in chickens (Rosen,1996). However, the use of antibiotics as growth promoters has been banned in many countries, thus there is a need to find safe additives that will have no adverse effects on the health of animals, humans, and the environment (Zhang *et al*., 2005). In some countries, bee pollen is considered medicinal (Brindza *et al*., 2010). Honeybees collect pollen from different plants and it is mixed with their digestive enzymes (Kalafova *et al*., 2014). Bee pollen is a rich source of protein, essential amino acids, oils, vitamins, minerals, enzymes, and carbohydrates (Xu *et al*., 2009). Some studies revealed that bee pollen can be used as a growth promoter and immune system stimulator in broiler chickens (Wang *et al*., 2005). Phenolic

constituents and antioxidants in bee pollen have been identified as possible growth promoters in chickens and rabbits (Saric *et al*., 2009). Amino acids, vitamins, and trace elements of bee pollen stimulate the early development, proliferation, and differentiation of intestinal cells. The environments for intestinal microbial ecosystems are also improved (Dias *et al*., 2013). Several studies have shown the possible potential bee pollen has on the growth performance in chicken production (Attia *et al*., 2014; Hosseini *et al*. 2016; Zafarnejad *et al*., 2016). This type of natural substance can promote gut health, and digestibility, while also decreasing pathogens in poultry (Duarte *et al*., 2014). A clear perspective on bee pollen as an alternative to synthetic antibiotics in poultry production is necessary. Therefore, this study was conducted to determine the effect of bee pollen inclusion on the performance and gut morphology of Ross 308 broiler chickens.

MATERIALS AND METHODS

Study site

The study was conducted at the University of Limpopo, Animal Unit, Limpopo Province, South Africa. The University of Limpopo lies at latitude 27.55ºS and longitude 24.77ºE. The mean ambient temperature around the study area is 28ºC during winter and 36ºC during summer (Shiringani, 2007).

The experimental procedures were conducted in accordance with the University of Limpopo (UL) Ethics committee, reference number: AREC/06/2020:PG.

Experimental procedures and design

A total of 240-day-old chicks (120 males and 120 females) were allocated to 4 treatments in a randomized complete block design (RCBD) with sex as a block. Each experimental group was replicated 3 times with 10 chicks per replicate, with an average weight of 40 ± 5g per bird. Bee pollen inclusion levels were 0, 4, 8, or 12 g/kg DM feed (Table 1). Bee pollen used in the current study was purchased from a company in Tzaneen, Polokwane. Bee pollen was dried in a well-ventilated laboratory to obtain a constant weight and milled into powder through a 1 mm sieve by using a hammer mill, before being added to the formulated diets (Table 2 and Table 3). After 21 days, the chickens remained in their treatment groups. This experiment lasted for 42 days, and feed and water were provided *ad libitum* throughout the experimental period.

Table 1 – Dietary treatment for the experiment.

Treatment code	Treatment description
MBP_0	Male Ross 308 broiler chickens fed a 22% CP starter mash without bee pollen
MBP_4	Male Ross 308 broiler chickens fed a 22% CP starter mash with 4g of bee pollen per kg DM
MBP_8	Male Ross 308 broiler chickens fed a 22% CP starter mash with 8g of bee pollen per kg DM
MBP_{12}	Male Ross 308 broiler chickens fed a 22% CP starter mash with 12g of bee pollen per kg DM
FBP_0	Female Ross 308 broiler chickens fed a 22% CP starter mash without bee pollen
FBP_4	Female Ross 308 broiler chickens fed a 22% CP starter mash with 4g of bee pollen per kg DM
FBP_8	Female Ross 308 broiler chickens fed a 22% CP starter mash with 8g of bee pollen per kg DM
FBP_{12}	Female Ross 308 broiler chickens fed a 22% CP starter mash with 12g of bee pollen per kg DM

BP_*: Treatments were supplemented with 0, 4, 8, or 12g of bee pollen per kg DM feed

CP: Crude protein

Table 2 – Feed ingredients and nutrient composition of the starter diets.

Variable	Treatment#			
	BP_0	BP_4	BP_8	BP_{12}
Feed ingredient (%)				
Yellow maize	41.57	40.20	40.00	40.00
Soybean full fat	17.73	16.50	16.06	14.06
Wheat	14.75	14.75	12.75	12.75
Sunflower	12.39	11.85	11.00	10.00
Fishmeal	5.66	5.02	5.00	4.00
Vitamins-minerals premix	0.50	0.50	0.50	0.50
Oil sunflower	2.50	2.50	1.94	1.79
Na bicarbonate	1.50	1.50	1.50	1.50
Limestone	1.50	1.50	1.50	1.50
Salt	1.30	1.30	1.30	1.30
Monocalcium phosphate	0.20	0.20	0.20	0.20
DL methionine	0.15	0.15	0.15	0.15
L threonine	0.15	0.15	0.15	0.15
L lysine	0.10	0.10	0.10	0.10
Bee pollen (g/kg DM)*	0	4	8	12
Total	100	100	100	100
Nutrients				
Crude protein (%)	22	22	22	22
Energy (MJ/kg DM)	16.1	16.0	16.1	16.1
Lysine (%)	1.08	1.08	1.08	1.08
Methionine (%)	0.53	0.53	0.53	0.53
Threonine (%)	0.89	0.89	0.89	0.89
Fat (%)	2.27	2.27	2.27	2.27
Ca (%)	1.07	1.07	1.07	1.07
Available P (%)	0.50	0.50	0.50	0.50

†The active 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 K3 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.

*: Bee pollen inclusion at 0 (no bee pollen), 4, 8, or 12g/kg DM in starter diets.

#: The treatments were bee pollen inclusion at 0 (no bee pollen, BP_0), 4 (BP_4), 8 (BP_8) or 12. (BP_{12})g/kg DM in starter diets.

Table 3 – Feed ingredients and nutrient composition of the grower diets.

Variable	Treatment#			
	BP_0	BP_4	BP_8	BP_{12}
Feed ingredient (%)				
Yellow maize	44.91	43.00	43.30	42.00
Soybean full fat	15.30	12.39	11.00	11.00
Wheat	15.00	15.00	15.00	15.00
Sunflower	12.39	11.39	10.07	9.35
Fishmeal	4.00	4.00	3.60	3.00
Vitamins-minerals premix	0.50	0.50	0.50	0.50
Oil sunflower	2.50	2.50	2.50	1.75
Na bicarbonate	1.50	1.50	1.50	1.50
Limestone	2.00	2.00	2.00	2.00
Salt	1.30	1.30	1.30	1.30
Monocalcium phosphate	0.20	0.20	0.20	0.20
DL methionine	0.15	0.15	0.15	0.15
L threonine	0.15	0.15	0.15	0.15
L lysine	0.10	0.10	0.10	0.10
Bee pollen (g/kg DM)*	0	4.00	8.00	12.00
Total	100	100	100	100
Nutrients				
CP (%)	20.00	20.00	20.00	20.00
Energy (MJ/kg DM)	16.80	16.80	16.80	16.80
Lysine (%)	1.24	1.24	1.24	1.24
Methionine + Cysteine (%)	0.95	0.95	0.95	0.95
Threonine (%)	0.83	0.83	0.83	0.83
Fat (%)	5.5	5.5	5.5	5.5
Ca (%)	0.90	0.90	0.90	0.90
Available P (%)	0.45	0.45	0.45	0.45

Table 4 – Nutrient contents of bee pollen.

Component	Bee pollen
Dry matter (g/kg)	88.5
Ash (g/kg DM)	2.9
Crude protein (g/kg DM)	21.8
Crude fat (g/kg DM)	5.2
Gross energy, MJ/kg	404.3 KJ/100g
Aminoacids (mg/g DM)	
Methionine	0.47
Lysine	7.64
Threonine	4.63
Histidine	4.60
Leucine	11.45
Isoleucine	6.04
Valine	9.11
Phenylalanine	2.55
Tryptophan	1.02
Arginine	3.60
Minerals (%)	
K	42.5
Mg	7.0
N	2.1
Ca	15.7
P	31.2

Data collection

Live weights were determined at the start of the experiment and then weekly. Feed intake per chicken was determined by calculating the difference between the weight of feed offered and the weight of feed leftover, and the difference was then divided by the total number of chickens in the pen. Feed intake and weight gain were used to calculate the feed conversion ratio (McDonald *et al*., 2010).

At the ages of 21 and 42 days, 3 chickens per replicate were slaughtered using the cervical dislocation method to determine gut organ weights and lengths, and gut organ digesta pH. Before the slaughter, each chicken was weighed using an electronic weighing balance. Afterwards, carcasses were put inside a bucket containing hot water for a few seconds, subsequently being taken out. Carcasses were then put on a table for hand defeathering. They were cut open at the abdominal site and the digestive tracts were removed from the abdominal cavities. The carcass weight of each chicken was measured only at the age of 42 days, after slaughter. The gastrointestinal tract, small intestine, caeca, and large intestine lengths were determined using a tape measure (Kokoszyński *et al*., 2017). The pH of gut contents (crop, proventriculus, gizzard, ileum, caecum, and colon) was measured using a digital pH meter (Crison, Basic 20 pH meter). Breast, drumstick, thigh, crop, proventriculus, gizzard, small intestine, caeca, and large intestine weights were measured using an electronic weighing balance.

Dry matter of feeds, bee pollen, feed refusals, faeces, and meat were determined by drying the samples in the oven for 24 hours at a temperature of 105ºC AOAC (2012). Neutral and acid detergent fibre contents of feed and faeces were determined according to Van Soest *et al*. (1991). Ash contents of feeds, bee pollen, faeces, and meat samples were determined by ashing the sample at 600ºC in a muffle furnace overnight. Ash was analysed for calcium, magnesium, phosphorus, potassium, sodium, zinc, iron, copper, and manganese AOAC (2012). Nitrogen contents of feed and meat samples were determined by the Kjeldahl method AOAC (2012). The gross energy values of feeds and faeces were determined using a bomb calorimeter AOAC (2012). A full analysis for faeces and feeds was performed at the Pietermaritzburg Laboratory, Kwa-Zulu Natal, South Africa according to AOAC (2012).

Data analysis

Data was analysed using the General Linear Model (GLM) procedures of the statistical analysis of variance

SAS (2012) to detect dietary treatment effects. The statistical model $Y_{ijk} = \mu + T_i + B_j + (TB)_{ij} + e_{ijk}$ was applied, where Y_{ijk} = the observation on feed intake, digestibility, live weight, gut morphology, carcass characteristics, feed conversion ratio, and mortality due to dietary treatment effects; μ = the overall mean; T_i = the i^{th} effect of bee pollen inclusion in starter diets; B_j =sex as a block factor; $(TB)_{ij}$ = interaction between bee pollen inclusion and sex; and e_{ijk} = the residual effect (error). Where significant differences were observed, mean separation was conducted using Tukey test at a 5% level of significance (SAS, 2012). The responses to bee pollen inclusion levels observed in optimal feed intake, live weight, growth rate, digestibility, feed conversion ratio, metabolisable energy, gut morphology, and carcass characteristics were modelled using the quadratic equation (SAS 20012).

RESULTS

The growth performance of broiler chickens aged one to 21 and 22 to 42 days (Table 4) was analysed. Between the age of one and 21 days, bee pollen inclusion level had no effect (p>0.05) on diet DM intake, growth rate, and FCR of male and female Ross 308 broiler chickens. Similarly, bee pollen inclusion levels had no effect (p>0.05) on the live weight of male Ross 308 broiler chickens aged 21 days. However, the bee pollen inclusion level affected (p<0.05) the live weight of female Ross broiler chickens aged 21 days. Female Ross 308 broiler chickens on a diet with 8g/kg bee pollen per kg DM feed had higher (p<0.05) live weights than those with 0, 4, or 12 g of bee pollen per kg DM. Bee pollen inclusion level had no effect (p>0.05) on the diet DM intake and FCR values of male Ross 308 broiler chickens aged 42 days.

Table 5 – Effect of bee pollen inclusion level on diet DM intake, growth rate, feed conversion ratio, and live weight of Ross 308 broiler chickens aged 1-21 and 22-42 days.

	Parameter							
Day	1-21				22-42			
Treatment	FI	FCR	GR	LW	FI	FCR	GR	LW
Male Ross 308 broiler chickens								
BP_0	101.8±5.13	37.3±4.87	3.1±0.15	760.2±10.46	193.1±14.02	52.6^{c}±1.40	2.2±0.68	2249.9^{b}±58.89
BP_4	92.1±10.98	36.8±4.98	3.2±0.11	778.8±37.59	188.5±4.47	54.9^{c}±1.13	2.1±0.75	2342.1^{b}±46.22
BP_8	97.9±2.88	39.4±2.47	3.2±0.09	726.1±30.26	192.2±6.10	59.3^{b}±1.69	3.1±0.81	2580.3^{a}±75.12
BP_{12}	104.3±2.78	43.1±1.41	3.3±0.08	745.3±28.73	206.5±15.10	63.0^{a}±0.77	3.3±0.97	2686.3^{a}±31.72
Female Ross 308 broiler chickens								
BP_0	78.1±2.29	46.8±1.17	2.1±0.54	742.4^{c}±7.22	161.9±17.99	44.6^{b}±1.36	1.2±0.88	2142.6^{c}±38.71
BP_4	81.3±8.28	45.6±1.79	2.4±0.56	762.9^{bc}±36.31	171.4±16.64	46.5^{b}±1.38	2.2±1.05	2182.2^{c}±69.61
BP_8	91.3±12.5	45.3±1.97	2.5±0.23	899.6^{a}±22.06	179.6±8.19	50.6^{a}±1.19	3.2±1.09	2446.2^{b}±27.47
BP_{12}	94.6±9.76	47.5±1.14	2.6±0.07	793.6^{b}±25.04	170.57±17.05	48.3^{ab}±1.29	3.2±1.07	2541.9^{a}±48.26
Chicken Sex								
Male	102.4^{a}±2.89	41.4±1.28	3.2±0.56	811.8^{a}±18.58	194.6±15.68	55.1±5.52	2.5±0.34	2360.3^{a}±79.74
Female	93.8^{b}±2.78	42.5±1.52	2.1±0.63	775.2^{b}±12.09	165.6±13.55	43.9±6.87	2.1±0.22	2179.0^{b}±98.67
Significance								
Sex	0.001	0.003	0.143	0.005	0.063	0.356	0.471	0.038
Treatment	0.578	0.001	0.074	0.001	0.050	0.007	0.097	0.009
Sex×Treatment	0.011	0.130	0.044	0.130	0.061	0.841	0.786	0.678

a, b, c, d: Means with different superscripts in the same column indicate significant differences between treatments (p<0.05).

*** :** Treatments were bee pollen inclusion at 0 (BP_0), 4 (BP_4), 8 (BP_8) or 12 (BP_{12}) g/kg DM.

#: Values presented as mean ± standard error (SE).

FI : Feed intake (g/bird/day).

FCR : Feed conversion ratio (g feed/g live weight gain.

GR : Growth rate (g/bird/day).

LW : Live weight (g).

The results in Table 6 show that there was no effect (p>0.05) on the gastrointestinal tract (GIT), duodenum, jejunum, caeca, and ileum lengths of male Ross 308 broiler chickens aged 21 days. Moreover, at the age of 22-42, bee pollen inclusion had no effect (p>0.05) on the gastrointestinal tract (GIT), duodenum, jejunum, caeca, and ileum lengths of male Ross 308 broiler chickens. However, bee pollen inclusion had an effect (p<0.05) on the ileum lengths of female broiler chickens at 21 days, and an effect (p<0.05) on the GIT and duodenum lengths of female broilers at the age of 42 days.

Table 6 – Effect of bee pollen inclusion levels on the gut organ lengths (cm) of Ross 308 broiler chickens aged 21 and 42 days.

	Parameter											
Day	1-21						22-42					
Treatment	GIT	Duodenum	Jejunum	Ileum	Caecum	LI	GIT	Duodenum	Jejunum	Ileum	Caecum	LI
Male Ross 308 broiler chicken												
BP0	157.5±8.72	12.0±0.62	57.0±3.06	56.4±2.39	13.0±0.58	6.0^{c}±0.58	195.0±18.16	15.7±0.96	91.3±7.45	83.0±5.86	20.0±1.00	13.3±0.88
BP4	156.3±2.33	11.7±0.88	57.2±3.31	58.0±0.89	13.2±0.73	5.7^{c}±0.33	195.0±12.65	13.7±2.83	87.3±6.49	85.0±2.52	21.3±2.03	12.7±0.33
BP8	158.3±2.98	12.0±0.58	57.3±0.74	58.3±1.20	13.0±0.68	7.3^{b}±0.33	220.3±3.28	15.3±0.63	85.3±5.53	79.7±4.93	21.0±0.58	12.0±1.48
BP12	159.2±0.58	12.5±0.03	56.8±1.48	59.3±0.88	13.0±0.88	8.7^{a}±0.33	221.7±13.78	17.3±0.77	87.7±2.33	87.0±3.52	19.3±0.88	12.0±1.16
Female Ross 308 broiler chicken												
BP0	136.5±32.51	10.2±1.36	44.2^{b}±3.40	46.8^{c}±2.80	9.5±3.50	7.7±0.33	201.0^{b}±4.73	12.3^{c}±0.97	72.3±9.33	78.0±10.08	20.3±1.20	11.3±2.77
BP4	137.3±22.88	11.0±0.58	47.6^{b}±3.51	53.0^{bc}±3.46	12.5±0.68	5.3±1.20	239.0^{a}±4.58	12.7^{c}±0.77	83.7±3.84	77.0±13.77	21.3±0.88	12.7±0.77
BP8	163.3±3.18	11.0±1.00	57.7^{a}±2.03	56.3^{ab}±2.73	13.5±0.50	7.3±1.20	236.0^{a}±5.51	14.3^{b}±0.33	85.0±4.73	84.7±7.54	21.3±0.33	14.3±0.47
BP12	160.3±0.88	11.3±0.88	60.3^{a}±0.88	61.0^{a}±2.52	12.3±0.83	8.7±0.33	229.7^{a}±18.81	16.0^{a}±0.57	80.7±3.51	91.0±2.31	18.7±2.40	14.0±0.57
Chicken sex												
Male	150.8±3.08	11.5±0.31	54.3±2.21	54.4±1.35	12.9±0.30	7.3±0.39	216.3^{b}±8.29	14.9^{a}±0.31	83.7±1.94	84.7±2.57	20.4±0.58	12.4^{b}±0.04
Female	152.0±3.12	10.8±0.44	56.4±2.35	51.8±1.37	11.9±0.71	7.2±0.51	233.9^{a}±7.73	13.8^{b}±0.51	81.9±3.36	82.0±2.34	20.9±0.69	13.1^{a}±0.45
Significance												
Sex	0.328	0.325	0.334	0.888	0.150	0.866	0.048	0.050	0.261	0.060	0.304	0.051
Treatment	0.062	0.457	0.009	0.002	0.261	0.002	0.755	0.736	0.889	0.924	0.099	0.784
Sex × Treatment	0.534	0.834	0.357	0.480	0.276	0.904	0.755	0.295	0.582	0.462	0.241	0.547

$^{a, b, c}$: Means with different superscripts in the same column indicate significant differences between treatments (p<0.05). * : Treatments were bee pollen inclusion at 0 (BP_0), 4 (BP_4), 8 (BP_8) or 12 (BP_{12}) g/kg DM. # : Values presented as mean ± standard error (SE). GIT : Gastrointestinal tract. LI : Large intestine.

Table 7 – Effect of bee pollen inclusion levels on the gut organ weights (g) of Ross 308 broiler chickens aged 21 and 42 days.

	Parameter													
Day	1-21							22-42						
Treatment	Crop	Provent.	Gizzard	Liver	SI	Caecum	LI	Crop	Provent.	Gizzard	Liver	SI	Caecum	LI
Male Ross 308 broiler chicken														
BP_0	18.2^{b} ± 1.20	4.8^{d}± 0.35	38.8 ±10.95	22.3 ± 1.89	43.4^{c} ± 2.48	7.0 ± 0.27	1.4^{c} ± 0.17	18^{c} ± 1.53	10.5^{c} ± 0.69	61^{a} ± 1.48	49^{c} ± 4.73	99^{d} ± 2.22	17 ± 3.30	12.3± 2.78
BP_4	25.3^{a} ± 0.97	5.4^{c}± 0.07	53.8 ± 7.41	21.6 ± 1.99	45.8^{bc}± 2.90	6.4 ± 1.25	1.8^{bc} ± 0.25	15^{c}± 2.27	10.2^{c} ± 0.17	54^{ab}± 7.41	56^{c} ± 1.82	131^{c} ± 1.48	16 ± 2.16	13.8± 2.39
BP_8	15.0^{b} ± 5.12	5.9^{b} ±0.15	39.5 ±13.23	26.3 ± 2.89	54.9^{a} ± 1.71	7.1 ± 0.23	2.1^{b} ± 0.12	20^{b} ± 0.67	12.3^{b} ±0.96	40^{b}±13.23	66^{b} ± 3.89	152^{b} ± 3.89	19 ± 0.70	18.2± 5.89
BP_{12}	21.3^{b}± 2.84	7.0^{a}± 0.41	48.1 ± 7.59	22.9 ± 0.75	48.1^{b} ± 1.71	7.2 ± 0.27	2.4^{a} ± 0.16	30^{a} ± 5.03	16.3^{a}± 0.87	48^{b}± 7.59	7^{a} ± 4.94	179^{a} ± 4.94	22 ± 2.89	14.9± 0.69
Female Ross 308 broiler chicken														
BP_0	7.7± 1.34	5.4± 0.15	23.7^{c} ± 0.58	20.7 ± 1.47	45.3^{b} ± 2.01	4.9± 0.18	1.8± 0.27	12^{bc}±1.62	13 ± 4.22	50^{c} ± 6.83	63 ± 1.52	65^{d} ± 6.01	10± 11.7	12± 2.78
BP_4	7.5± 1.66	4.0 ± 3.16	24.5^{c} ± 0.67	23.9 ± 1.73	51.3^{a} ± 2.04	7.8^{a}± 0.53	2.0± 0.62	11^{c} ± 1.04	14 ± 2.74	62^{b} ± 3.83	60 ± 3.98	79^{c} ± 2.34	12.± 5.0	14± 2.33
BP_8	11.5± 3.09	3.6 ± 5.59	37.4^{a} ± 0.94	21.9 ± 2.14	51.9^{a} ± 2.01	4.7^{bc}±0.87	1.7± 0.27	15^{b} ± 1.77	16 ± 0.18	74^{a} ± 1.18	53 ± 9.85	86^{b} ± 0.78	13± 6.7	18± 5.80
BP_{12}	10.1± 3.99	4.9 ± 3.34	32.8^{b} ± 0.29	20.9 ± 1.86	55.4^{a} ± 3.13	5.6^{b}±0.51	1.8± 0.15	29^{a} ± 0.38	14 ± 2.24	74^{a} ± 3.64	53 ± 10.32	133^{a} ± 2.13	20± 3.3	14± 0.72
Chicken sex														
Male	14.6 ± 2.53	5.8^{a}±0.28	28.2±1.08	22.8±1.10	49.7^{a}±1.87	5.8^{a}±0.44	2.1^{a}±0.15	19.5±6.64	12.6±0.71	71.3±3.26	67.5^{a}±5.47	79.6±15.66	12.5±1.29	8.5±3.79
Female	11.5±1.77	5.2^{b}±0.30	26.4±1.04	21.9±0.95	44.6^{b}±2.60	4.9^{b}±0.43	1.8^{b}±0.16	17.3±2.19	13.7±0.94	65.0±3.78	46.8^{b}±1.59	97.9±9.06	9.9±1.37	15.2±1.57
Significance														
Sex	0.267	0.053	0.063	0.538	0.046	0.050	0.051	0.063	0.070	0.974	0.009	0.061	0.071	0.067
Treatment	0.002	0.001	0.001	0.081	0.046	0.024	0.052	0.001	0.001	0.001	0.001	0.006	0.080	0.075
Sex × Treatment	0.915	0.042	0.096	0.116	0.020	0.036	0.167	0.844	0.063	0.090	0.055	0.541	0.098	0.695

$^{a, b, c, d}$: Means with different superscripts in the same column indicate significant differences between treatments (p<0.05). * : Treatments were bee pollen inclusion at 0 (BP_0), 4 (BP_4), 8 (BP_8) or 12 (BP_{12}) g/kg DM. # : Values presented as mean ± standard error (SE). Provent : Proventriculus. SI : Small intestine. LI : Large intestine.

Table 7 represents the effect of bee pollen inclusion level on the gut organ weights (g) of Ross 308 broiler chickens aged 21 and 42 days. Female and male Ross 308 broiler chickens aged 21 days with 0, 4, 8, or 12 g of bee pollen per kg DM had similar (p>0.05) crop, gizzard, and liver weights. However, male Ross 308 broiler chickens had heavier (p<0.05) proventriculus, small intestines, and large intestines. Bee pollen inclusion had no effect (p>0.05) on the caecum and large intestine weights of male Ross 308 broiler chickens aged 42 days. However, bee pollen inclusion affected (p<0.05) the gizzard, crop, proventriculus, liver, and small intestine weights of male Ross 308 broiler chickens aged 42 days.Male Ross 308 broiler chickens with 12 g of bee pollen per kg DM had heavier (p<0.05) crop, proventriculus, and liver weights than those on diets with 0, 4, or 8 g of bee pollen per kg DM.There were positive and significant relationships (p<0.05) between bee pollen inclusion levels and the liver and small intestine weights of male Ross 308 broiler chickens aged 42 days.

DISCUSSION

In the present study, bee pollen inclusion had no effect on diet DM intake, growth rate, and FCR of male and female broiler chickens aged one to 21 days. Similarly, bee pollen inclusion levels in starter diets had no effect on the live weight of male broilers aged 21 days. However, the current study is inconsistent with that of (Liu *et al*., 2010; Attia *et al*., 2011; Hascik *et al*., 2012; Eyng *et al*., 2014), who observed improved diet intake and live weights after including bee pollen in broiler diets. The improved performance may be due to the high nutrition found in bee pollen, consequently making it a suitable feed supplement for chickens (Hascik *et al*., 2017). Thus, bee pollen could be included in broiler diets without any effect on chicken performance.

Bee pollen inclusion did not affect the GIT, duodenum, caeca, and large intestine lengths of female Ross 308 broiler chickens aged 21 days. However, it positively affected the large intestine lengths of males as well as the jejunum and ileum lengths of female broilers. Similar results were obtained by Hascik *et al*. (2017) and Hashmi *et al*. (2012). This could have been due to amino acids, vitamins, minerals, and coenzymes, which are important for digestibility and cell growth (Wang *et al*., 2007).

The inclusion of bee pollen had no effect on the gizzard, liver, and caecum weights of male broilers as well as the crop, proventriculus, liver, and large intestine weights of females aged 21 days. Similar observations were made by Zeedan *et al*. (2017). However, bee pollen inclusion positively affected the crop, proventriculus, small intestine, and large intestine weights of male chickens aged 21 days. Moreover, it also improved the gizzard, small intestine, and caecum weights of female broilers. This is in agreement with Fazayeli-Rad *et al*. (2015) and Hascik *et al*. (2013). Nonetheless, there is limited information on the gut organ weights of broiler chickens.

The inclusion of bee pollen tended to improve the growth rate and live weight of female Ross 308 broiler chickens aged 22 to 42 days. Similar results were reported by Hascik *et al*. (2017) and Hascik *et al*. (2013), who observed that bee pollen inclusion in the diets improved the diet intake, digestibility, and live weights of broiler chickens.

The present study showed that there was an effect of bee pollen supplementation on the FCR of male Ross 308 broiler chickens. This is similar to the observations made by Fazayeli-Rad *et al*. (2015), who observed that the addition of BP in diets significantly improved the FCR values of Ross 308 male broiler chickens.

In the present study, bee pollen increased the GIT and duodenum lengths of Ross 308 female broiler chickens. These results are similar to those reported by Fazayeli-Rad *et al*. (2015), who observed increased sizes of the GIT and intestine of broiler chickens, which could have been due to the increased digestive enzyme caused by the adaptation effect in broiler chickens.

The inclusion of bee pollen in the present study increased the crop, proventriculus, gizzard, liver, and small intestine weights. Amerah *et al*. (2009) reported that gizzard volume increases in weight when diets contain structural components. This may have been the reason why the gizzard was affected. Bee pollen contains several components that are important for biological activities, such as phenols and flavonoids (Rzepecka-Stojko *et al*., 2015). Fazayeli-Rad *et al*. (2015) and Hashmi *et al*. (2012) observed similar results in liver weights of broiler chickens, reporting that treated livers were heavier than those in the control group. These findings may suggest that the antioxidant properties of flavonoids positively impact the alimentary canal of broilers. Sarikaya *et al*. (2018) observed no statistical differences amongst groups in terms of weights of the liver, gizzard, intestines, as well as in the lengths of intestines, when including bee pollen in quail diets at the 0.025% and 0.50% levels.

CONCLUSIONS

During each phase, dietary treatments had similar nutrient content levels that met the nutrient requirements of the broiler chickens. Thus, any differences in responses must have been due to the bee pollen that was supplemented in broiler diets. The effect of bee pollen in starter diets of Ross 308 broiler chickens at 12g/kg resulted in better live weights. There was a positive and significant relationship between bee pollen inclusion in the starter diet and live weights of male Ross broiler chickens aged 42 days. Therefore, it can be concluded that bee pollen has a positive effect on the gut of chickens, which was shown through the increased lengths and weights of the gut organs. The results of this study show that the inclusion level of 12g/kg of bee pollen had a significant effect on the gut of the chickens. It is suggested that further studies are conducted to support this finding.

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AUTHORS' CONTRIBUTIONS

MFD, SD, E:collected the data for this study, analysed the data, and wrote the initial draft of this manuscript; JWN: collaborated in the interpretation of the results and by drafting the manuscript; and TG: interpreted the results and finalized the manuscript. All the authors approved the final version of the manuscript.

CONFLICT OF INTEREST DECLARATION

No authors have any conflicts of interest to declare.

DATA AVAILABILITY STATEMENT

Data of the current study is available from the authors on request.

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