



Effects of dietary supplementation with garlic powder (*Allium sativum* L.) on broiler performance, carcass traits, lymphoid organ development and intestinal biometrics

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Abstract

Aim of the study: To assess the effects of dietary supplementation with garlic (*Allium sativum* L.) powder as a growth-promoting additive on the productive performance, carcass traits, intestinal biometrics, and lymphoid organ development of broilers.

Area of study: Machado, Minas Gerais, southeast region of Brazil.

Material and methods: A total of 660 one-day-old chicks were used in a completely randomized design with 5 treatments (0, 2.5, 5.0, 7.5, and 10.0 g/kg garlic powder), 6 replications, and 22 animals per experimental unit during 42 days of production, divided into phases: initial (1 to 7 days), growth (8 to 22 days), and finishing (23 to 42 days).

Main results: In the total production period, the birds that were fed rations containing 5, 7.5 and 10 g/kg garlic powder showed increased weight gain and feed conversion ratio ($p < 0.05$). Among the carcass yield variables, live weight and carcass yield showed a positive linear effect ($p < 0.05$). Birds supplemented with garlic powder showed increased weight ($p < 0.05$) and relative length of the small intestine ($p < 0.05$). The absolute weight of the lymphoid organs, thymus and bursa of Fabricius, and the relative weight of the thymus, bursa of Fabricius and spleen also increased linearly with the level of dietary supplementation with garlic powder ($p < 0.05$).

Research highlights: Dietary supplementation with 5 g/kg and higher concentrations of garlic powder is recommended as a growth promoter for broilers because this feed additive enhances lymphoid organ development, improves intestinal biometric variables and consequently maximises the productive performance and carcass yield of broilers from 1 to 42 days of age.

Additional key words: average daily gain; Bursa of Fabricius; immunonutrition; immunity; phytobiotics; natural growth promoter.

Abbreviations used: BW (body weight); BWG (body weight gain); CUY (cut yield); CY (carcass yield); FCR (feed conversion ratio); FI (feed intake).

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Introduction

The poultry industry has been in constant evolution, aimed at increasingly producing high-quality, low-cost chicken, with high competitiveness. In the search for improvements and new technologies for maintaining this production growth, management techniques, health control, facilities, genetics, and feed have been optimised. In terms of diet, one of the main nutritional factors that currently favour high poultry productivity is the use of feed additives.

Subtherapeutic dosages of antibiotics are commonly used in broiler diets as growth promoters, but prolonged and excessive misuse is linked to the increased presence of their residues in bird tissues, inducing the selection of new strains of antibiotic-resistant microorganisms (Saleha et al., 2009), harming human health (Min et al., 2016).

Poultry meat production still suffers significant losses due to contamination and to the presence of harmful agents and pathogens, mainly in the gastrointestinal tract, directly impacting feed efficiency, productive performance, and bird survival (Khoobani et al., 2020). The use of natural growth-promoting feed additives from different sources in broiler feed is an alternative to the use of antibiotics, improving feed efficiency and reducing antibiotic residues.

Among the wide variety of additives and supplements used in poultry diets, phytobiotics have been studied in different diets, supplementation levels and processing methods, because they are “Safe Natural Additives” (Boka et al., 2013). Garlic (*Allium sativum* L.) has been identified as a key microbiota intestinal modulator for its ability to efficiently reduce the number of pathogenic bacteria living in the intestines of birds (Peinado et al., 2013). Additionally, garlic can stimulate the digestive system by controlling digestive pH and digestive enzyme activity (Herawati & Marjuki, 2011). Similarly, the activity of pancreatic enzymes is elevated with the properties present in garlic, providing a more appropriate environment for nutrient absorption and activating the digestive process (Peinado et al., 2013).

The use of garlic in poultry diets has been considered an important performance enhancer, possibly due to its hepatoprotective, antioxidant, antimicrobial (Sangilimadan et al., 2019), antiviral, antifungal, hypercholesterolemic, and immunostimulant properties (Corzo-Martínez et al., 2007).

Some recent studies (Elbaz et al., 2021; Ismail et al., 2021; Kumar et al., 2022) have investigated the effects of garlic in broiler chicken diets. However, these studies often examine the combination of multiple ingredients, resulting in limited exploration of the isolated effects of garlic. Moreover, there is a lack of homogeneity in the literature regarding recommended doses, and the effects of estimated doses for each studied parameter were not quantified.

The aim of this study was to assess the effect of different supplementation doses of garlic powder as a growth-promoting feed additive on the productive performance, carcass traits, intestinal biometrics, and lymphoid organ

development of broilers. And secondly, to quantify these effects by regression equations.

Material and methods

The experiment was performed at the Experimental Poultry Farm of the Instituto Federal de Educação, Ciência e Tecnologia do Sul de Minas Gerais – Campus Machado. The field research of this study had been previously analysed and approved by the Comitê de Ética no Uso de Animais do Instituto Federal de Educação, Ciência e Tecnologia do Sul de Minas Gerais – CEUA/IFSULDEMINAS) under protocol number 057/2018-R4.

Diets, animals, and experimental design

In total, 660 one-day-old Cobb 500 male chicks with a mean weight of 43.53 ± 0.6 g were used. The experimental design was completely randomised with 5 treatments, namely 4 levels of supplementation with garlic powder and the control treatment, and 6 replicates, with 22 animals per experimental unit. The supplementation levels were 0, 2.5, 5.0, 7.5, 10.0 g/kg garlic powder from 1 to 42 days of age.

The experimental diets (Table S1 [suppl]) were formulated based on corn and soybean meal and were isoenergetic and isoproteic, as recommended by Rostagno et al. (2017) for male broilers of normal-average performance in the starter (1 to 7 days), grower (8 to 22 days) and finisher (23 to 42 days) feeding phases. The feeds were formulated with different levels of supplementation with garlic powder replacing the inert ingredient (kaolin).

The birds were housed at a population density of 8 birds/m², in 3-m² nesting boxes with a bell drinker, a tube feeder and wood shavings bedding. The heating system consisted of heat lamps, individually installed in each experimental box. The poultry shed was equipped with a curtain winching system to control the temperature and internal humidity.

Feed was provided twice a day (at 08:00 am and at 4:00 pm) and water ‘ad libitum’. Temperature and relative humidity were monitored twice a day, at 8:00 am and 3:00 pm, using a digital thermo-hygrometer, placed in the centre of the nesting box at rump height. The lighting program was set as recommended in the COBB Strain Manual (2012). The light supply was controlled by a timer, which turned the lights on and off during the night and dawn, according to the procedure adopted in commercial farms.

Productive performance

Performance was analysed by weekly weighing the birds and feed leftovers in each experimental unit. Feed intake (FI, g) was calculated as the difference between the amount of feed supplied and the leftovers adjusted to the

number of dead birds during each period. Body weight gain (BWG, g) was determined as the difference between the final mean weight and the initial mean weight of the birds at all phases of life. Feed conversion ratio (FCR) was calculated as: $FCR = FI / BWG$.

Carcass traits

At 42 days of age, two birds per experimental unit were randomly selected according to the mean weight of the experimental unit to evaluate carcass yield (CY), cuts (breast, thigh + drumstick, wing, back), absolute and relative weight of organs and intestinal biometrics.

In total, 12 birds were selected per treatment, subjected to a 6-hour pre-slaughter fasting, and subsequently weighed to determine the live weight at slaughter. The animals were tagged, stunned by cerebral concussion, and slaughtered by cervical dislocation at the slaughterhouse of the Instituto Federal de Educação, Ciência e Tecnologia do Sul de Minas Gerais – Campus Machado. Subsequently, they were bled and scalded at a controlled temperature ranging from 60 °C to 65 °C, for 20 to 15 seconds, respectively, according to the temperature.

After performing the normal slaughter procedures, stunning, bleeding, scalding, plucking and evisceration, the birds were weighed again to assess CY. During the evisceration, the heart, liver, proventriculus, gizzard and pancreas were collected. The liver was weighed immediately after being removed. The proventriculus and gizzard were opened and weighed after removing the contents.

The carcass yield was calculated using the following formula:

$$CY(\%) = \left(\frac{\text{Carcass weight (g)}}{\text{Live weight (g)}} \right) \times 100$$

where carcass weight without feathers, giblets, feet, and head. The cut yield (CUY) was determined using the following formula:

$$CUY(\%) = \left(\frac{\text{Cut weight (g)}}{\text{Live weight (g)}} \right) \times 100$$

thus calculating the breast, thigh+ drumstick, wing, and back yield. After the organs (liver, heart, proventriculus, gizzard and pancreas) were collected and weighed, the absolute weight of each organ was expressed in relation to the live weight at slaughter, thus calculating the relative weight.

Biometrics of the intestinal and lymphoid organs

The small and large intestines were collected during evisceration, separated by sections at the point where the duodenum emerges from the gizzard and at the beginning

of the ceca, and then cleaned and dried on paper towels. After weighing the small intestine, the duodenum, jejunum, ileum, and cecum were weighed separately on a 0.01g precision scale. Then, the lengths of the intestines (cm) were measured using a tape measure.

Lymphoid organs, namely the thymus, spleen, and the bursa of Fabricius, were collected and separated, expressing their weight in grams. With the absolute weights of the lymphoid organs, their relative weight was determined in relation to the live weight. The relative weights of the small intestine segments and lymphoid organs were determined using the formula:

$$\text{Relative organ weight}(\%) = \left(\frac{\text{Organ weight (g)}}{\text{Live weight (g)}} \right) \times 100$$

The relative length of each small intestine segment (SIS) was calculated according to the following formula:

$$\text{SIS relative length}(\%) = \left(\frac{\text{Length (cm)}}{\text{Live weight (kg)}} \right)$$

Statistical analysis

The data were analysed using the statistical software R (R Core Team, 2019), assessing the normality of the residuals using the Shapiro-Wilk test and the homogeneity of variances using Bartlett's test. The model described below was adopted to assess significant treatment effects, whenever the p value was lower than 0.05.

$$Y_{ik} = \beta_0 + \beta_i + \epsilon_{ik}$$

wherein: Y_{ik} = variable measured in the experimental unit k , fed a diet containing an amount i of garlic powder; β_0 = general constant; β_i = effect of the level of supplementation with garlic powder; ϵ_{ik} = random error associated with each observation.

Subsequently, the effects of garlic powder levels were estimated by analysis using linear and quadratic regression models, according to the best fit for each variable. The estimate of the optimal dietary level of garlic powder was calculated by splitting the sum of squares of the level of garlic powder using orthogonal polynomials. Then, differences were tested using the Dunnett's test at 5% probability, comparing the control treatment (0 g/kg garlic powder) with the others (2.5, 5, 7.5 and 10 g/kg garlic powder).

Results

During the study period, average min. and max. temperatures of $20.66 \pm 3.01^\circ\text{C}$ and $29.21 \pm 1.36^\circ\text{C}$ and min. and max. average relative air humidity of $32.22 \pm 6.98\%$ and $84.03 \pm 5.05\%$ were recorded in the morning, respectively. In the afternoon, average min. and max.

Table 1. Effect of dietary garlic powder supplementation on the productive performance of broilers from 1 to 42 days.

Items ^[1]	Garlic powder levels in the diet (g/kg)					SEM ^[2]	p-value ^[3]		
	0 (control)	2.5	5.0	7.5	10		D	L	Q
Starter phase (1-7 days)									
BWG (g/bird)	144.6	148.4	145.3	145.6	147.0	0.0200	0.983	0.9022	0.9367
FI (g/bird)	177.5	180	172.5	176.6	178.3	0.0260	0.938	0.9342	0.6760
FCR	1.226	1.212	1.209	1.213	1.211	0.0400	0.731	0.3502	0.3772
Grower phase (8-22 days)									
BWG (g/bird)	759.0	728.1	723.2	763.2	745.6	0.0078	0.403	0.8840	0.2851
FI (g/bird)	1035.1	1030.7	1010.8	1008.2	988.8	0.0178	0.939	0.3983	0.9361
FCR	1.385	1.373	1.341	1.312	1.460	0.0194	0.282	0.3980	0.0736
Finisher phase (23-42 days)									
BWG (g/bird)	1751.0	1815.3	1807.3	1728.1	1851.2	0.0228	0.451	0.4926	0.8110
FI (g/bird)	3236.9	3384.7	3213.8	3266.4	3342.7	0.0232	0.088	0.5430	0.6273
FCR	1.857	1.868	1.727	1.885	1.815	0.2116	0.120	0.6470	0.4154
Overall period (1-42 days)									
BWG (g/bird)	2585.7	2726.3	2763.5*	2766.9*	2778.8*	0.0233	0.038	0.0073	0.1044
FI (g/bird)	4459.5	4554.8	4466.0	4443.4	4579.8	0.0267	0.385	0.4988	0.5114
FCR	1.796	1.664	1.630*	1.625*	1.625*	0.2118	0.034	0.0080	0.0747

^[1] BWG = body weight gain; FI = feed intake; FCR = feed conversion ratio. ^[2] SEM = standard error of the mean. ^[3] D = p value of Dunnett's test; L = p value of linear analysis; Q = p value of quadratic analysis. Means with an asterisk differ from the control treatment by Dunnett's test at 5% probability (n = 132 birds/treatment); FCR (1 - 42d) = $-0.1521x + 1.7445$ ($R^2 = 0.67$); BWG (1 - 42d) = $0.1708x + 2.6389$ ($R^2 = 0.71$).

temperatures of $24.62 \pm 0.90^\circ\text{C}$ and $31.33 \pm 1.62^\circ\text{C}$ and min. and max. average relative air humidity of $33.80 \pm 13.55\%$ and $58.5 \pm 13.05\%$ were recorded, respectively.

Productive performance

The levels of dietary supplementation with garlic powder of broilers did not significantly affect productive performance ($p > 0.05$) in the starter (1 to 7 days), grower (8 to 22) and finisher (23 to 42 days) phases, as outlined in Table 1. However, weight gain and feed conversion increased linearly with supplementation with garlic powder ($p < 0.05$) in the total production period (1 to 42 days). The analysis of comparisons showed that, during the total period of production, the birds fed rations containing 5, 7.5 and 10 g/kg had a higher weight gain ($p < 0.05$) and better FCR ($p < 0.05$) than the birds fed diets without supplementation with the natural feed additive, with no significant effect on FI ($p > 0.05$) from 1 to 42 days.

Carcass traits

The effects of dietary supplementation with garlic powder on broiler carcass traits at 42 days are presented in Table 2. Live weight and CY increased linearly with the levels

of dietary supplementation with garlic powder ($p < 0.05$). Supplementation with 7.5 and 10 g/kg garlic powder provided a higher weight gain than the control treatment (0 g/kg garlic powder). Broilers fed natural additive had higher CY than control broilers ($p < 0.05$), regardless of the level of supplementation. The yields of retail cuts, breast, back, wing and thigh + drumstick, were not significantly affected by the treatments ($p > 0.05$).

The levels of dietary supplementation with garlic powder did not significantly affect the relative weight of the heart ($p > 0.05$) and digestive organs, namely liver, proventriculus, gizzard and pancreas ($p > 0.05$), of broilers at 42 days of age.

Intestinal biometrics

Supplementation with garlic powder had an increasing linear effect on the relative weight ($p < 0.05$) and a quadratic effect on the relative length ($p < 0.05$) of the small intestine (Table 3). When compared with broilers fed control ration, all levels of supplementation with garlic powder tested in this study resulted in increased weight ($p < 0.05$) and relative length of the small intestine ($p < 0.05$).

The relative weight of the duodenum and ileum significantly and linearly increased with the garlic powder levels ($p < 0.05$), upon dietary supplementation with 7.5 and 10 g/kg garlic powder when compared with the control treat-

Table 2. Effect of dietary garlic powder supplementation on carcass traits of 42-day-old broiler.

Items	Garlic powder levels in the diet (g/kg)					SEM ^[1]	p-value ^[2]		
	0 (control)	2.5	5.0	7.5	10		D	L	Q
Yield of carcass and cuts (% of live weight)									
Live weight (g)	2752.6	2799.9	2878.1	2916.4*	2925.3*	0.0191	0.010	0.0005	0.4314
Carcass	71.74	73.53*	73.63*	73.86*	74.10*	0.2046	0.001	0.0002	0.0524
Breast	25.47	26.23	26.81	26.32	26.56	0.2206	0.387	0.1502	0.2539
Drumstick + thighs	21.91	22.10	22.12	22.12	22.05	0.1057	0.971	0.7145	0.5543
Back	17.32	16.07	16.22	16.53	16.84	0.2256	0.424	0.7432	0.0891
Wing	8.17	8.63	8.61	8.43	8.46	0.0635	0.145	0.3724	0.0552
Relative weights of heart and digestible organs (% of live weight)									
Heart	0.502	0.504	0.499	0.500	0.524	0.0225	0.997	0.8103	0.7971
Liver	1.93	2.16	2.08	2.05	2.10	0.0383	0.436	0.4022	0.3315
Proventriculus	0.369	0.373	0.341	0.360	0.363	0.1490	0.971	0.8208	0.7161
Gizzard	1.505	1.519	1.519	1.504	1.518	0.0363	0.999	0.9690	0.9599
Pancreas	0.219	0.221	0.200	0.207	0.212	0.0040	0.473	0.3120	0.3132

^[1] SEM = standard error of the mean. ^[2] D = p value of Dunnett's test; L = p value of linear analysis; Q = p value of quadratic analysis. Means with an asterisk differ from the control treatment by Dunnett's test at 5% probability (n = 12 birds/treatment); Live weight = $0.0185x + 2.7621$ ($R^2 = 0.9371$); Carcass yield = $0.2017x + 72.372$; ($R^2 = 0.7234$).

ment ($p < 0.05$). The levels of garlic powder had a quadratic effect on the relative length of the relative length of the jejunum and cecum ($p < 0.05$) with maximum relative length of 38.18 and 16.90 cm/kg, with estimated supplementation of 6.35 and 5.17 g/kg of garlic powder in the diet, respectively. The relative length of the ileum increased linearly with the levels of supplementation with garlic powder ($p < 0.05$). When compared with the control treatment, broilers fed diets containing 2.5, 5 and 7.5 g/kg garlic powder and 2.5, 5 and 10 g/kg showed increased relative length of the jejunum and ileum ($p < 0.05$), respectively. The relative length of the duodenum was not affected by the levels of dietary supplementation with garlic powder ($p > 0.05$).

Lymphoid organ development

The absolute weight of the lymphoid organs thymus and bursa of Fabricius increased linearly with the dietary levels of garlic powder ($p < 0.05$). Thus, the relative weight of the thymus, bursa of Fabricius and spleen increased linearly with the dietary levels of garlic powder ($p < 0.05$). Dietary supplementation with garlic powder had a quadratic effect on the absolute weight of the bursa of Fabricius ($p < 0.05$) with an estimated maximum weight of 8.11 g (Fig. 1).

Discussion

The exploration of safe and effective substitutes has become increasingly frequent after the disuse of antibiotics.

Natural compounds such as probiotics (Alagawany et al., 2021a), prebiotics (Yaqoob et al., 2021), essential oils (Alagawany et al., 2021b), and green synthesised nanoparticles (Abd El-Ghany et al., 2021) have been increasingly researched to find compounds and properties applicable as growth-promoting feed additives for their beneficial effects on intestinal health, antioxidant capacity, immune system, feed efficiency and, consequently, growth and performance.

Numerous medicinal plants and herbs have been highlighted as important phytobiotics with active biological elements that can be used in broiler nutrition, such as sweet pepper (*Capsicum annuum* L.) (Abd El-Hack et al., 2022), ginger (*Zingiber officinale* Roscoe) (Samadi et al., 2020), drumstick tree (*Moringa oleifera* Lam.) (Mickdam et al., 2022), soursop (*Annona muricata* L.) (Alkassar & Alaboudy, 2021), turmeric (*Curcuma longa* L.) (Xie et al., 2019), Indian frankincense (*Boswellia serrata*) (Mohamed et al., 2021), liquorice (*Glycyrrhiza glabra* L.) (Geravand et al., 2021) and garlic (*Allium sativum* L.) (Elbaz et al., 2021; Ismail et al., 2021; Kumar et al., 2022). However, most studies include mixtures or assess the synergistic effect of phytobiotics, which reduces the reliability of the isolated effects under study.

Some studies published in the literature demonstrate the isolated effect of garlic powder as a growth promoter, but recommendations are still inconsistent. While some studies show better results upon dietary supplementation with 3% garlic extract (Elagib et al., 2013), other demonstrate efficient results between 0.25% (Jimoh et al., 2012) and 0.5% (Saleem et al., 2021) garlic, and most studies does

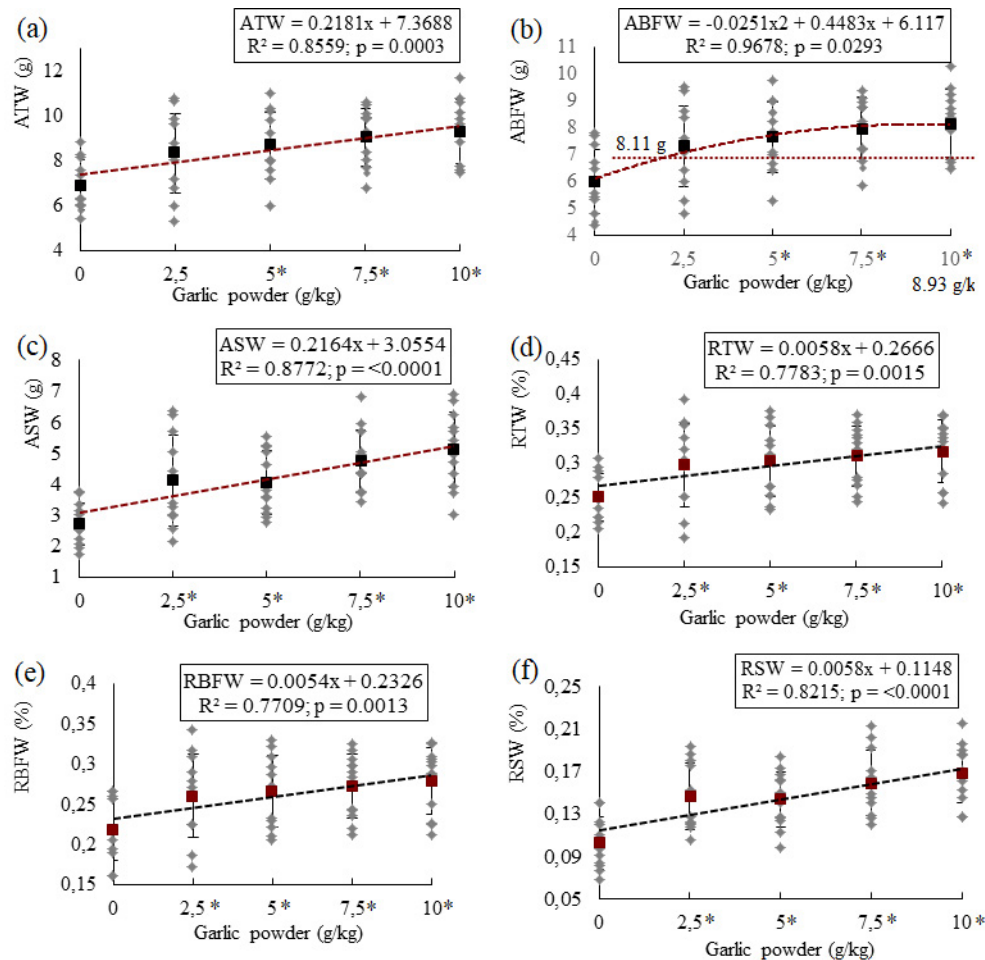


Figure 1. Effect of dietary garlic powder supplementation on absolute weight (a-c) and relative weight (d-f) of lymphoid organs of 42-day-old broilers. ATW = absolute thymus weight; ABFW = absolute bursa of Fabricius weight; ASW = absolute spleen weight; RTW = relative thymus weight; RBFW = relative bursa of Fabricius weight; RSW = relative spleen weight; $p < 0.05$ = Linear or quadratic effect in relation to the inclusion of garlic in the diet. Means with an asterisk differ from the control treatment by Dunnett's test at 5% probability ($n = 12$ birds/treatment).

not recommend a level of dietary supplementation with garlic powder for broilers.

The productive performance findings of this study indicate that supplementation with garlic powder did not affect the weight gain, FI or feed conversion of broilers in the starter (1 to 7 days), grower (8 to 22 days) and finisher (23 to 42 days) phases separately. Although weight gain was higher in absolute values in all phases, a significant difference was only found when analysing the total production period (1 to 42 days), with weight gain increasing with the dietary levels of garlic powder ($p < 0.005$). The same variation was observed in feed conversion, demonstrating that birds fed garlic powder perform better than those fed the control treatment.

Feed intake remained similar between treatments during the total production period, explaining the increased feed conversion because these birds had higher weight gain with the same nutrient intake. In addition, FI was similar, as

expected because the diets were properly formulated with the same nutritional quality (isoenergetic and isoproteic). These results also demonstrate that the change in the smell and taste of the rations as a result of the addition of garlic in the diets was not enough to impact FI. The FI results were similar to those found by Khan et al. (2017) with broiler dietary supplementation with garlic extract in the total production period. Adebiyi et al. (2017), when evaluating broilers supplementation with 1%, 2% and 3% garlic powder, also failed to observe any phytobiotic effect on FI. The difference between the studies is basically attributed to the source of allicin, used in pure form, and the content of the supplemented source (garlic) in the other studies.

The weight gain results corroborate the findings of Hayat et al. (2022). The authors evaluated dietary supplementation with 0, 0.3, 0.6 and 0.9% garlic powder and observed that the phytobiotic had no effect on weight gain and feed conversion in the first three weeks, but

Table 3. Effect of dietary garlic powder supplementation on intestinal biometrics of 42-day-old broilers.

Items	Garlic powder levels in the diet (g/kg)					SEM ^[2]	p-value ^[3]		
	0 (control)	2.5	5.0	7.5	10		D	L	Q
Relative weights (%)									
Small intestine	2.106	2.663*	2.656*	2.6621*	2.658*	0.7291	0.049	0.0285	0.0611
Duodenum	0.397	0.427	0.428	0.433*	0.438*	0.0440	0.028	0.0045	0.2014
Jejunum	1.045	0.991	1.070	1.011	1.056	0.0104	0.091	0.5540	0.4707
Ileum	0.640	0.663	0.685	0.692*	0.701*	0.0068	0.025	0.0014	0.4270
Cecum	0.503	0.495	0.559	0.537	0.507	0.0082	0.070	0.3599	0.0545
Relative lengths (cm/kg of BW) ^[1]									
Small intestine	69.82	76.23*	76.10*	76.28*	75.78*	0.7672	0.023	0.0220	0.0284
Duodenum	12.11	12.73	12.40	12.55	11.94	0.1563	0.508	0.6304	0.1397
Jejunum	28.07	31.32*	31.68*	32.31*	30.86	0.1095	0.010	0.0166	0.0092
Ileum	28.54	31.48*	31.41*	28.54	32.17*	0.3603	0.010	0.0121	0.2566
Cecum	15.72	17.00	16.67	16.63	16.08	0.1699	0.119	0.7690	0.0179

^[1] BW = body weight. ^[2] SEM = standard error of the mean. ^[3] D = p value of Dunnett's test; L = p value of linear analysis; Q = p value of quadratic analysis. Means with an asterisk differ from the control treatment by Dunnett's test at 5% probability (n = 12 birds/treatment); relative weight small intestine = $0.0441x + 2.3291$ ($R^2 = 0.4953$); relative weight duodenum = $0.0035x + 0.4075$ ($R^2 = 0.7508$); relative weight ileum = $0.0061x + 0.646$ ($R^2 = 0.9412$); relative length small intestine = $-0.1546x^2 + 2.025x + 70.52$ ($R^2 = 0.8643$) (77.15 cm/kg – 6.54 g/kg); relative length jejunum = $-0.0974x^2 + 1.2377x + 28.258$ ($R^2 = 0.9205$); (maximum response = 38.18 cm/kg; maximum response level of garlic powder = 6.35 g/kg); relative length ileum = $0.2433x + 29.572$ ($R^2 = 0.4583$); relative length cecum = $-0.0385x^2 + 0.3986x + 15.875$ ($R^2 = 0.7827$); (maximum response = 16.90 cm/kg; maximum response level of garlic powder = 5.17 g/kg).

in the total production period, the broilers fed a ration supplemented with garlic powder showed a higher weight gain than those in the control treatment, with the highest level of supplementation providing the highest weight gain, as in the present study. The same trend was observed by El-Katcha et al. (2016), where supplementation with garlic (in the form of allicin extract) did not affect weight gain in the first three weeks, but this variable was significantly increased at the end of the study.

The higher productive performance indices of broilers supplemented with garlic powder in the total production period may be associated with the increase in the enzymatic activity of the pancreas, increasing nutrient digestion and absorption efficiency (Ismail et al., 2021). Performance improvements can be attributed to the active allicin compounds that promote beneficial gut microflora, thereby increasing digestion efficiency, nutrient retention, and energy utilization (Sheoran et al., 2017; Al-Massad et al., 2018). In addition, the performance of broilers supplemented with garlic powder may have improved as a result of different biological activities of garlic, including antioxidant, antimicrobial, hepatoprotective, antitumor properties and its content of sulphur compounds, various enzymes, allicin, alliin, ajoene, adenosine and glycoside (Sangilimadan et al., 2019).

The digestive system of the newborn chick is sterile, immature and highly susceptible to infection. Any enteric disease at this stage can impair the immune response,

feed efficiency, growth, and health status. The early establishment of the intestinal microbiota of chicks is directly related to the health of the birds by maintaining intestinal homeostasis and fostering the maturation of the mucosa and the development of the immune system by inhibiting colonisation by intestinal pathogens (Maynard et al., 2012). Diet can directly interact with the intestinal microbiota and its composition can modulate the intestinal bacterial population, as is the case of the effect of growth promoters on the maintenance of immune and microbiota homeostasis (Yegani & Korver, 2008).

The first weeks of broiler life are crucial for performance because, at this stage, birds are susceptible to several pathogenic microorganisms that increase their vulnerability to diseases that can negatively impact performance (Dehghani-Tafti & Jahanian, 2016). Although dietary supplementation of garlic powder did not influence performance in the first weeks of life, probably because the birds were not challenged in this study, chickens that received supplemental garlic showed higher absolute values of weight gain and better feed conversion. This fact may have contributed to improvements in performance when evaluated in the total period of experimentation.

The linear increase in live weight and CY ($p < 0.05$) as a function of dietary garlic levels was already expected based on weight gain results in the total production period. Saleem et al. (2021) also identified increases in carcass weight and yield after supplementation with garlic.

The authors explained such increases by the presence of an alkaline antibiotic substance, which decreases the number of pathogenic bacteria and aflatoxin-producing fungi in the intestine of birds, thereby improving nutrient absorption and increasing carcass weight (Kharde & Soujanya, 2014). Similarly, Lukanov et al. (2015) found better CY results in broilers under dietary supplementation with garlic.

Carcass and CUY may vary in the same line with age, sex, and weight at slaughter (Moreira et al., 2004). In the present study, the same effect of supplementation with garlic powder on live weight at slaughter was observed in CY. Notwithstanding the higher CY in general, the yields of retail cuts, breast, thigh + drumstick, back and wing, were not affected by the dietary levels of garlic. Therefore, the yields of specific cuts presumably often vary with factors such age, stage of life at slaughter, sex, line (Gotardo et al., 2016) and quality of the protein fraction of the rations.

The relative weights of digestible organs remained constant despite the increasing dietary levels of garlic ($p>0.05$). The relative weight of liver should have been higher in broilers supplemented with garlic powder given the greater weight gain because liver is the main metabolic organ of the body and the greater metabolic demand generated in birds with higher performance could alter liver size.

The results of the relative weight of the pancreas and proventriculus were similar to those found by Saleem et al. (2021). However, the same authors reported higher liver and heart weight in birds supplemented with garlic powder and in line with the findings of Eltazi (2014). In another study, Aydogan et al. (2020) found no differences in the relative weight of the heart, liver, and pancreas of broilers under dietary supplementation with 5 g/kg garlic powder. Similarly, Samanthi et al. (2015) reported that the relative weights of the organs (heart and liver) of broilers under dietary supplementation with garlic were similar to those of broilers in the control treatment.

Concerning the effect of garlic on lymphoid organs, it has been reported that it has immunomodulatory compounds that benefit the immune response of supplemented animals and reduce the decline in immune capacity with age (Rehman & Munir, 2015). Broiler dietary supplementation with garlic as a growth-promoting feed additive was related to increased leukocyte counts at 0.3% garlic (Fadlalla et al., 2010) and antibody titer against Infectious Bursal Disease Virus (IBDV) at 0.9% garlic. Ismail et al. (2021) observed a significant increase in immunoglobulins G (IgG) and M (IgM) in broilers under dietary supplementation with garlic, especially at 7.5 g/kg garlic. The same authors identified an increase in the relative weight of the bursa of Fabricius and thymus upon dietary supplementation with 5 to 7.5 g/kg garlic and in spleen at 7.5 g/kg garlic. In the present study, the relative weights of the lymphoid organs had a positive linear effect as a function of the increase in garlic in the diets,

indicating the beneficial effect of garlic as an immune system modulator. Lymphoid tissues are organised structures of the immune system that support responses to tissue damage or other stimuli in the form of hyperplasia, hypertrophy, or atrophy (Haley, 2017). Accordingly, the greater lymphoid organ development found in this study may be attributed to the improvement in immune capacity caused by dietary supplementation with the phytobiotic. According to Perozo et al. (2004), the body of the chicken is correlated with the relative weight of lymphoid organs. In other words, as a primary lymphoid organ, the bursa of Fabricius grows directly proportional to broiler BW and immune activity. This relationship would also explain the greater absolute and relative weights of lymphoid organs in birds with greater weight gain. Likewise, the greater development of lymphoid organs, in the sense of improving the immune system, would also explain the greater weight gain observed in the present study.

In addition, Rahimi et al. (2011) assessed the effects of dietary supplementation with garlic on broilers and reported an increase in the relative weight of the bursa of Fabricius in comparison with the control treatment. Conversely, Aydogan et al. (2020) found no differences in the relative weight of lymphoid organs or in the productive performance of broilers fed rations supplemented with 5g/kg garlic powder.

The evidence found in this study indicated a possible decrease in the immune capacity of control birds due to a decline in the development of lymphoid organs, which probably also impacted the productive performance of non-supplemented birds. Lymphoid organ development (thymus, spleen, and the bursa of Fabricius) is correlated with the small intestine because greater intestinal activity and health ensures greater T and B lymphocyte production and increases the relative weight of these hematopoietic organs in birds (Rohollahzadeh et al., 2018).

The results of intestinal biometrics demonstrate increased intestinal tract development, especially of the small intestine, in birds supplemented with garlic powder. However, the weight and relative length of the cecum were not affected by the treatments. The increased requirement and consequently growth of the cecum is associated with intrinsically dietary factors, such as the proportion of fermentable material and the length of the period during which chyme remains in this intestinal section (Herrera et al., 2017). In this study, these factors did not vary between diets, thus explaining the lack of effect on this segment of the intestine.

On the contrary, birds supplemented with 2.5 g/kg garlic powder and higher concentrations showed increased weight and relative length of the small intestine, as well as increased relative length of its jejunum and ileum segments ($p<0.05$); however, the relative weight of the duodenum and ileum only increased from 7.5 g/kg garlic powder. Khaligh et al. (2011) evaluated broiler dietary supplementation with different mixtures of medicinal

plants and observed no effect of treatments on the weight and relative length of different segments of the digestive system and lymphoid organs. However, there is no more information in the literature about the influence of dietary garlic on the intestinal biometry of broiler chickens.

Despite the strong relationship between broiler small intestine weight and BW (Awad et al., 2009), the nutrient absorption capacity of the small intestine depends more on the overall villous surface area than on intestinal length and mass (Wijten et al., 2012). The increase in surface area, combined with the improvement in villi characteristics, maximizes the digestive and absorptive function of the intestine. Thus, the increase in intestinal length found in the present research could produce an increase in the digestive and absorptive function of the intestine.

Therefore, the improved feed digestibility and absorption capacity and increased surface area for nutrient absorption, together with the immunity achieved by supplemented birds, could explain the increase feed efficiency and the improved productive performance in the total production period, with effects even on carcass yield.

In conclusion, dietary supplementation with garlic (*Allium sativum* L.) powder is recommended from 5 g/kg as a growth promoter for broilers because this feed additive enhances lymphoid organ development, improves intestinal biometric variables and therefore maximises the productive performance and carcass yield of broilers from 1 to 42 days of age.

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