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Propolis supplementation affects performance, intestinal morphology, and bacterial population of broiler chickens

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Abstract

A meta-analysis was conducted to examine the effect of supplementing the diet of broiler chickens with propolis on growth, bacterial population of the intestine, antiviral serum concentration, intestinal morphology, and digestive enzyme activities in broiler chickens. Forty peer-reviewed articles that had been published between 2003 and 2019 were identified using the PRISMA protocol and included in the study. Data were analysed with mixed model methodology, in which the studies with mixed model methodology, whereas the level of supplemental propolis was considered a fixed effect. Responses to propolis supplementation in bodyweight (BW) and average daily gain (ADG) were quadratic, but average daily feed intake (ADFI) was not affected. Propolis supplementation improved feed conversion ratio (FCR) significantly as a linear function of the level of supplement. The optimum level of supplementation was between 256 and 262 mg/kg feed and produced maximum ADG and final BW. There was a tendency for mortality to decrease because of propolis supplementation. Propolis had no detectable effect on serum antiviral concentration, intestinal bacterial population or intestinal morphology. Among digestive enzymes, only sucrase increased linearly as propolis was increased. Thus, supplementation with propolis increased the growth performance of broiler chickens positively and the effect was dose dependent. This may have been partly because of an improvement in sucrase activity and other factors related to the nutritional content of propolis. Future study to evaluate specific bioactive compounds of propolis is therefore warranted.

Keywords: bee glue, digestive enzymes, growth, meta-analysis

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Introduction

Successful broiler production is determined largely by rearing management, including disease prevention and proper use of medication. Antibiotics are commonly used as therapeutic agents to control diseases, and used to be a popular growth-promoting feed additive (AGP). Antibiotic metabolites, which can inhibit the growth of microorganisms in low doses, are produced by fungi and algae and are manufactured chemically (Nir & Ve-Senkoylu, 2000). Their use as AGPs was aimed at improving feed efficiency, but raised problems because bacteria acquired resistance to antibiotics from uncontrolled use (Bronzwaer *et al.*, 2002; FAO & IFIF, 2010; Attia *et al.*, 2019a, b). Thus, the use of antibiotics as AGPs is no longer allowed worldwide. Consequently, there has been growing interest in replacing AGPs with natural products that are readily available and safe for poultry, including spices, herbs, plant extracts, antioxidants, enzymes, probiotics, and prebiotics (Khattak *et al.*, 2006; Toghyani *et al.*, 2011; Abdel-Kareem & El-Sheikh, 2015;

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Omar *et al.*, 2016; Cimrin *et al.*, 2020; Attia *et al.*, 2016). Among these natural resources, propolis has shown potent ¹¹ as an AGP (Attia *et al.*, 2014; Abou-Zeid *et al.*, 2015; Klarić *et al.*, 2018).

Propolis or 'bee glue' is a resinous substance that is collected by honeybees from flowers and shoots of trees such as willow, poplar and wild chestnut. Its bioactive components consist of polyphenols, phenol aldehydes, aromatic compounds, steroids, fatty acids, enzymes, essential minerals and vitamins, levels of which vary according to plant species, location and time of collection (Lotfy, 2006; Krocko *et al.*, 2012; Klarić *et al.*, 2018). In practice, propolis has been used among others as an antioxidant, antimicrobial, anti-inflammatory, antiradiation, and hepatoprotective substance in animals and humans (Bankova, 2005; Yamaguchi *et al.*, 2006; Pascoal *et al.*, 2014). Studies reported effective use of propolis as a therapeutic agent against many human diseases, including heart disease, cancer, diabetes mellitus and inflammation (Mishima *et al.*, 2005). In several animal species, propolis has been reported to promote animal growth, improve the quality and safety of animal products, increase immune response, and regulate the intestinal tract (Liu *et al.*, 2010). In broiler chickens, propolis reportedly enhanced performance and health status (Attia *et al.*, 2014; Abou-Zeid *et al.*, 2015; Rabie *et al.*, 2018). In rabbits, propolis was effective in replacing zinc bacitracin (antibiotic) with positive improvements in growth performance, economic benefit, immune status, and reproduction (Attia *et al.*, 2015, 2019a, 2019b).

The beneficial effects of propolis on growth performance can be explained by several mechanisms. Klarić *et al.* (2018) revealed a positive effect on the health status of chickens as shown by haematological parameters when they consumed a diet supplemented with propolis. Its efficacy as an immunomodulator was demonstrated by improvement in blood globulin levels ²³ and a positive response in humoral immunity (Hassan *et al.*, 2018; Attia *et al.*, 2016). In addition, propolis and bee pollen had beneficial effects on intestinal morphology, increasing the surface area for nutrient absorption in broilers (Chegini *et al.*, 2018; Prakatur *et al.*, 2019; Attia *et al.*, 2019a, b). Therefore propolis was suggested as an effective alternative additive for use in intensive animal production (Attia *et al.*, 2019a).

Although a number of studies have discussed the effects of propolis on broiler production, none has attempted to summarize the findings and to provide robust conclusions for propolis use. The present study therefore aimed to evaluate the effects of dietary propolis supplementation on growth performance, bacterial count in the intestinal tract, immune response, morphology of the small intestine, and activity ¹⁰ of digestive enzymes in broilers. The study was performed using data from the literature and employing a meta-analysis approach based on a mixed-model methodology to analyse them quantitatively.

Materials and Methods

A systematic literature search following the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analysis) protocol (Page *et al.*, 2021) was conducted to identify studies reporting immune and enzymatic responses and intestinal morphology of broiler chickens fed a diet containing propolis. A combination of search terms that included 'propolis', 'broiler', 'bacterial population', and 'intestinal morphology', was applied to databases of Science Direct (2021), PubMed (2021), and Scopus (2021). Only articles published in international peer-reviewed journals were retained in the screening process. A total of 75 articles were identified from title and abstract evaluation. These were further screened on a full-text basis according to pre-determined criteria, namely i) the article must report the use of propolis in the diet; ii) the form of propolis should be explained, that is, whether crude or extracted; iii) inclusion level must be reported; and iv) the effects on growth performance, number of bacteria, immune response, and antioxidant activity must be recorded. A total of 39 articles met these criteria (Table 1).

The database contained the authors and year of publication, strain of broiler chicken, sex, form of propolis, rearing phase (starter or finisher), levels of propolis inclusion, and response variables reported in the articles, which included BW, ADG, daily feed intake, FCR ratio, mortality, intestinal bacteria population (Bacteroidaceae, *Bifidobacterium* spp., Clostridiaceae), anti-viral response (anti-Newcastle disease serum titer), intestinal morphology (height and width of villi in the duodenum, jejunum and ileum), and activity of the digestive enzymes sucrase, maltase, amylase, chymotrypsin, lipase and trypsin.

Table 1 Literature that provided data for a meta-analysis on the effects of propolis (mg/kg of feed) on broilers

Reference	Level, mg/kg diet	Broiler strain	Sex	Rearing period, d		
				Starter	Finisher	Total
Biavatti <i>et al.</i> , 2003	0 - 570	Ross 308	Male	1 - 21	22 - 28	1 - 28
Açıkgoz <i>et al.</i> , 2005	0 - 2000	Ross 308	Male	1 - 28	29 - 42	1 - 42
Taheri <i>et al.</i> , 2005	0 - 1000	Ross 308	Mixed	1 - 21	22 - 42	1 - 42
Ziaran <i>et al.</i> , 2005	0 - 1000	Ross 308	-	1 - 21	22 - 47	1 - 47
Shalmany & Shivazad, 2006	0 - 250	Ross 308	-	1 - 21	22 - 42	1 - 42
Seven & Seven, 2008	0 - 1500	Ross 308	Male	1 - 21	22 - 42	1 - 42
Seven <i>et al.</i> , 2008	0 - 5000	Ross 308	-	-	-	1 - 42
Seven <i>et al.</i> , 2008	0 - 3000	Ross 308	Mixed	3 - 21	22 - 41	3 - 41
Khodanazary <i>et al.</i> , 2011	0 - 1000	Ross 308	-	1 - 21	22 - 42	1 - 42
Tekeli <i>et al.</i> , 2011	0 - 3000	Ross 308	-	-	-	8 - 42
Daneshmand <i>et al.</i> , 2012	0 - 200	Ross 308	Male	1 - 21	22 - 42	1 - 42
Seven <i>et al.</i> , 2012	0 - 1000	Ross 308	-	3 - 21	22 - 41	3 - 41
Eyng <i>et al.</i> , 2013	0 - 500	Cobb 500	Male	1 - 21	-	-
Mahmoud <i>et al.</i> , 2013	0 - 750	Ross 308	Male	1 - 21	22 - 42	1 - 42
Abbas, 2014	0 - 2500	Ross 308	Male	-	-	1 - 28
Attia <i>et al.</i> , 2014	0 - 300	Arbor Acres	Mixed	1 - 21	22 - 35	1 - 35
Duarte <i>et al.</i> , 2014	0 - 500	Cobb 500	Male	1 - 21	-	1 - 42
Eyng <i>et al.</i> , 2014	0 - 5000	Cobb 500	Male	1 - 21	-	1 - 42
Abou-Zeid <i>et al.</i> , 2015	0 - 500	Cobb 500	Mixed	-	-	1 - 42
Daneshmand <i>et al.</i> , 2015	0 - 200	Ross 308	Male	1 - 21	22 - 42	1 - 42
Eyng <i>et al.</i> , 2015	0 - 4000	Cobb 500	Male	-	-	1 - 21
Torki <i>et al.</i> , 2015	0 - 200	Ross 308	-	1 - 21	22 - 42	1 - 42
Haščík <i>et al.</i> , 2016	0 - 400	Ross 308	Mixed	1 - 21	22 - 42	1 - 42
Hosseini <i>et al.</i> , 2016	0 - 3000	Ross 308	Male	1 - 21	22 - 42	1 - 42
Eyng <i>et al.</i> , 2017	0 - 5000	Cobb 500	Male	1 - 21	-	1 - 21
Gheisari <i>et al.</i> , 2017	0 - 300	Ross 308	Male	1 - 21	22 - 42	1 - 42
Mahmoud <i>et al.</i> , 2017	0 - 3000	Ross 708	Male	-	-	1 - 42
Sahin & Ozturk, 2017	0 - 400	Ross 308	Female	-	-	16 - 20
Shaddel-Tili <i>et al.</i> , 2017	0 - 2000	Ross 308	Male	1 - 24	25 - 42	1 - 42
Chegini <i>et al.</i> , 2018	0 - 5000	Ross 308	Male	1 - 21	22 - 42	1 - 42
Kinasih <i>et al.</i> , 2018	0 - 1000	Ross 308	Mixed	1 - 21	22 - 42	1 - 42
Klarić <i>et al.</i> , 2018	0 - 1000	Cobb 500	-	1 - 21	22 - 42	1 - 42
Rabie <i>et al.</i> , 2018	0 - 500	Iraqi rooster	Male	-	-	-
Hassan <i>et al.</i> , 2018	0 - 400	Ross 308	Mixed	1 - 21	22 - 42	1 - 42
Al-Sultan <i>et al.</i> , 2019	0 - 3000	Ross 308	-	1 - 21	22 - 42	1 - 42
Abdelsalam <i>et al.</i> , 2019	0 - 400	Cobb 500	Mixed	1 - 21	22 - 49	1 - 49
Alani <i>et al.</i> , 2019	0 - 800	Cobb 500	Mixed	-	-	-
Haščík <i>et al.</i> , 2019	0 - 1000	Ross 308	Mixed	1 - 21	22 - 42	1 - 42
Khafaji <i>et al.</i> , 2019	0 - 000	Ross 308	Mixed	1 - 21	22 - 42	1 - 42

Statistical analysis was conducted using a mixed-model methodology following the examples of St-Pierre (2001) and Sauvant *et al.* (2008), in which the studies were considered random effects and the level of propolis inclusion was a fixed effect. The mathematical models used in this study were as follows:

$$Y_{ij} = \beta_0 + \beta_1 Level_{ij} + Experiment_i + Experiment_i Level_{ij} + e_{ij}$$

$$Y_{ij} = \beta_0 + \beta_1 Level_{ij} + \beta_2 Level_{ij}^2 + Experiment_i + Experiment_i Level_{ij} + e_{ij}$$

These models differ only by the inclusion of a quadratic effect of level in the second model. In these models, Y_{ij} is the dependent variable, β_0 is the intercept, β_1 and β_2 are linear and quadratic regression coefficients, $Level_{ij}$ is level of propolis used to produce the observed response in $Experiment_i$, and e_{ij} is random residual error. Statistics that were used to establish the importance of effects included in the models were the P -value, root mean square error (RMSE), and Akaike information criterion (AIC). Effects were considered significant when $P \leq 0.05$, and tendency was thought to exist when $0.05 < P \leq 0.10$. Initially, the quadratic model was applied. When $P > 0.10$ was observed for the quadratic effect of level, then the corresponding linear model was applied. Data were analysed using R software version 3.6.3 within the 'nlme' library (Pinheiro *et al.*, 2020; R Core Team, 2020).

Results and Discussion

Propolis contains more than 300 compounds, which make its composition complex (Sahin & Ozturk, 2018). Geographical differences, climatic characteristics, bee genetics, and seasons also affect its composition, making it difficult to determine which compounds affect responses to its use as a dietary supplement. The average composition of crude propolis is approximately 50% resin and balsam, 30% wax, 10% essential and aromatic oils, 5% pollen, and 5% impurities (Marcucci, 1995). To the authors' knowledge, no studies on the effects of specific bioactive compounds of propolis have been reported for broiler chickens.

Bodyweight and ADG exhibited quadratic responses to the level of propolis supplementation ($P < 0.01$). Bodyweight had a predicted maximum value of 2186 g when propolis was supplemented at 262 mg/kg. Likewise, a maximum ADG of 60.8 g/d was obtained when propolis was included at 256 mg/kg. Inclusion of propolis in the diet did not affect daily feed intake significantly. However, it decreased FCR linearly. In addition, there was a tendency for linear decrease in mortality owing to an increase in the level of propolis supplementation. The effects of propolis supplementation on the growth performance of broiler chickens are presented in Table 2.

Table 2 Regression equations describing effects of dietary propolis (mg/kg feed) on the performance of broilers

Response variable	N	Parameter estimates			P-value		RMSE	AIC
		Intercept	Linear	Quadratic	Linear	Quadratic		
BW, g	130	2072 ± 101	870 ± 253	1.658 ± 0.5930	0.001	0.006	2.13	1730
ADG, g/d	130	57.6 ± 4.46	25.0 ± 7.20	-0.0489 ± 0.0167	0.001	0.004	1.97	836
DFI, g/d	126	122 ± 17.70	4.35 ± 3.93		0.271		2.24	958
FCR	126	1.99 ± 0.09	-0.538 ± 0.259		0.040		1.98	677
Mortality, %	27	5.96 ± 1.70	-9.20 ± 4.68		0.065		1.28	145

BW: bodyweight, ADG: average daily gain, AIC: Akaike information criterion, DFI: daily feed intake, FCR: feed conversion ratio, N: number of observations, RMSE: root mean square error

The meta-analysis confirmed the results of numerous individual studies that reported promising effects of propolis supplementation on the performance of animals (Attia *et al.*, 2014; 2016; 2019a,b). For instance, Rabie *et al.* (2018) reported that broilers fed diets containing propolis (400 mg/kg diet) had higher BW as a result of higher ADG. Similarly, Attia *et al.* (2014) reported that supplementation of 300 mg/kg propolis to broiler diets increased BW significantly by 12%. Results from other studies in which propolis at various levels of inclusion and in either crude or extracted forms showed increased BW gain of broilers (Seven, 2008; Seven *et al.*, 2008; Klarić *et al.*, 2018; Hassan *et al.*, 2018). The current study showed little effect of propolis

on bacterial population in the digestive tract of broiler chickens, although other studies revealed positive modulation effects of propolis on gut microbiota (Klarić, 2014; Eyng *et al.*, 2017). The results of the current study are supported by the finding that the addition of ethanolic extract of propolis at various levels in broiler chicken feed did not affect intestinal microbiota (Eyng *et al.*, 2017). Propolis possibly promoted growth performance in broilers because of the increase of certain enzyme activities, primarily sucrase, as shown in this study. Other plausible reasons are related to the nutritional and bioactive contents of propolis, such as vitamins, flavonoids, minerals, and essential oils (Figure 1) (Awadalla & Kamel, 2000; Gardana *et al.*, 2007; Easton-Calabria *et al.*, 2019).

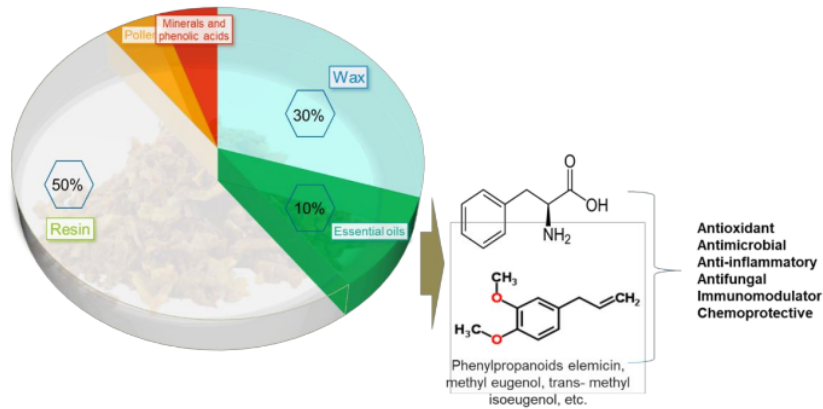


Figure 1 Raw propolis composition and its main functions (adapted from Easton-Calabria *et al.*, 2019) trans-methyl

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Propolis had no effect on some species of intestinal bacteria population, although this has to be interpreted cautiously because of limited data (Table 3). There was no effect ($P > 0.05$) on Newcastle disease antibody titer.

Table 3 Linear regression analysis of the size of intestinal bacteria populations and Newcastle disease antibody titers on the level of propolis supplementation in the diet (mg/kg feed)

Response variable	N	Intercept	Slope	P-value	RMSE	AIC
Bacterial composition						
Bacteroidaceae, log ₁₀ cfu/g	12	4.36 ± 0.33	0.195 ± 1.51	0.900	1.22	36.4
Clostridiaceae, log ₁₀ cfu/g	12	4.51 ± 0.67	-3.63 ± 3.12	0.274	1.25	53.8
Enterobacteriaceae, log ₁₀ cfu/g	18	3.64 ± 1.15	-0.178 ± 1.34	0.896	1.30	54.1
Newcastle disease antibody titers						
Starter phase	20	1547 ± 426	-2816 ± 2194	0.218	1.13	303
Finisher phase	33	1258 ± 293	-925 ± 935	0.331	1.33	513

AIC: Akaike information criterion, N: number of observations, RMSE: root mean square error

The addition of propolis did not affect ($P > 0.05$) villus height, crypt depth of the duodenum or the ratio of villus height to crypt depth in the duodenum, jejunum, and ileum. There were significant linear increases ($P < 0.05$) of the sucrase enzyme in the duodenum and jejunum. However, amylase, chymotrypsin, maltase

and trypsin were not influenced ($P > 0.05$). Table 4 presents the intestinal morphology and enzyme activity of broiler chickens as influenced by levels of propolis.

Table 4 Linear regression analysis of intestinal morphology and enzyme activity of broilers on the level of propolis supplementation in the diet (mg/kg feed)

Response variable	N	Intercept	Slope	P-value	RMSE	AIC
Duodenum						
Villus height, μm	25	1,609 \pm 251	-45.6 \pm 148.5	0.762	1.3	324
Crypt depth, μm	25	206 \pm 54	21.8 \pm 26.0	0.412	1.43	239
VH/CD	25	8.89 \pm 1.03	-0.494 \pm 2.12	0.818	1.5	100
Sucrase, U/mg	12	4.07 \pm 0.48	8.64 \pm 2.24	0.004	1.33	45.9
Maltase, U/mg	12	24.4 \pm 1.81	13.4 \pm 8.41	0.146	1.14	77.6
Jejunum						
Villus height, μm	21	901 \pm 86	-53.8 \pm 85.1	0.536	1.2	249
Crypt depth, μm	21	159 \pm 19	-10.1 \pm 9.7	0.313	1.13	163
VH/CD	21	5.9 \pm 0.65	-0.106 \pm 0.45	0.813	1.41	31.0
Sucrase, U/mg	12	5.26 \pm 0.85	10.4 \pm 3.80	0.023	1.04	57.9
Maltase, U/mg	12	27.1 \pm 1.92	10.5 \pm 8.93	0.269	1.08	79.0
Ileum						
Villus height, μm	15	638 \pm 89	52.9 \pm 60.8	0.403	1	165
Crypt depth, μm	15	126 \pm 16	10.2 \pm 14.6	0.498	1.06	120
VH/CD	15	5.09 \pm 0.20	0.04 \pm 0.35	0.918	1.22	4.47
Sucrase, U/mg	12	6.3 \pm 0.66	2.77 \pm 1.85	0.168	1.19	39.1
Maltase, U/mg	12	31.1 \pm 2.75	1.98 \pm 9.79	0.844	1.16	79
Amylase, nmol/mg	12	4.55 \pm 0.44	-0.146 \pm 2.02	0.944	1.19	43.4
Chymotrypsin, nmol/mg	12	4.86 \pm 0.27	-0.703 \pm 1.25	0.587	1.34	31.9
Lipase, U/mg	12	16.5 \pm 3.15	0.71 \pm 7.69	0.928	1.08	73.5
Trypsin, nmol/mg	12	26.3 \pm 6.35	-1.28 \pm 10.5	0.906	1.12	82.3

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AIC: Akaike information criterion, N: number of observations, RMSE: Root mean square error; VH/CD: ratio of villus height to crypt depth

The present meta-analysis failed to show an effect of propolis supplementation on the immune function of broilers, possibly because studies that evaluate the immune-modulatory effects of propolis on broiler chickens are few, with large variation among them making it difficult to generalize. A number of studies reported a positive effect in increasing immunoglobulins IgA, IgM, and IgY (Seven *et al.*, 2010), and on the formation of the viral antibody (Seven *et al.*, 2012; Eyng *et al.*, 2013a; Eyng *et al.*, 2013b), but they should be interpreted cautiously. The present study also failed to provide evidence on the modulating effect of propolis on intestinal bacterial population. Eyng *et al.* (2015) reported increases in macrophage phagocytes and in red blood cells in broilers that received a diet containing 500 mg/kg propolis, which indicated that propolis increased cellular response through macrophage cell activation pathways. This was effective in increasing the immune response of broilers and could increase the number of monocytes, but had no effect on the number of basophil cells or nitric oxide enzymes (Khan, 2017). In addition, propolis inclusion could reduce significantly the number of heterophile cells and lymphocytes in broilers that are

induced by phytohemagglutinin, which indicated that propolis inhibited tissue damage from pathogens and viruses by increasing white blood cells (Abdelsalam *et al.*, 2019).

Propolis could play a role in counteracting free radicals by increasing the activity of superoxide dismutase, catalase and glutathione peroxidase and significantly reducing the activity of malondialdehyde. This mechanism indicated that propolis exhibited immunomodulation and inhibition of tissue damage caused by free radicals by activating antioxidant enzymes in broiler chickens (Abou-Zeid *et al.*, 2015). An interconnected factor could explain the beneficial effects of propolis on immune response and intestinal characteristics and ecology (Figure 2). Propolis improved productive and reproductive performance in rabbits, as shown by higher litter size, survival, and growth rates of kits (Attia *et al.*, 2015, 2019a). A subsequent study indicated that supplementation with propolis produced increased white blood cell and lymphocyte counts, greater phagocytic activity, and increased levels of serum β -globulin, indicative of higher antibody response (Attia *et al.*, 2019b). Many authors reported positive effects on intestinal morphology (Eyng *et al.*, 2016; Klarić *et al.*, 2018; Prakatur *et al.*, 2019) and on immune response and enzyme activity (Wang *et al.*, 2007; Abdel-Mohsein *et al.*, 2014; Attia *et al.*, 2019b). However, no positive effects on the morphology of broiler small intestines were observed in the present study, which provided further evidence of inconsistent responses to propolis supplementation. This variability in response to propolis supplementation might be influenced by its complex composition

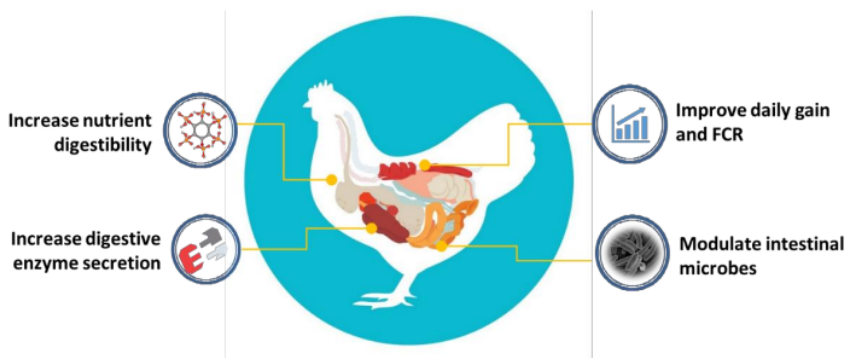


Figure 2 Mode of action of propolis as growth-promoting additive in broiler chickens

There is a correlation between intestinal microbiota and morphology (Hanhineva *et al.*, 2010; Abdel-Mohsein *et al.*, 2014). Active compounds of propolis can produce aromatic metabolites when metabolized by intestinal bacteria, which may interact with bacterial cells and inhibit their growth (Biavatti *et al.*, 2003; Açıkgöz *et al.*, 2005). Consequently, intestinal morphology may be improved, and this may promote enzyme production and nutrient absorption (Abdel-Mohsein *et al.*, 2014; Prakatur *et al.*, 2019). Interestingly, in the present study an increase was observed in sucrase activity, particularly in the duodenum and jejunum. Phenolic compounds of propolis could contribute to glucose metabolism because they were reported to stimulate insulin secretion (Taheri *et al.*, 2005; Shalmany & Shivazad, 2006).

Conclusion

Propolis supplementation has an apparent dose-dependent growth-promoting effect on broiler chickens. Its addition to the diet at 256 - 262 mg/kg was predicted to produce maximum ADG and final BW. However, it affected only the digestive enzyme sucrase, which increased linearly with the amount provided. Further study would be indicated to investigate how the specific components of propolis affect the performance and health of broiler chickens

Authors' Contributions

S, EW, AI, and RS conceptualized the research design and conducted the literature selection. MMS, RPS, and ACI performed database development and formal analysis. S and AI wrote the original draft of manuscript, AS, N, and AJ checked the database, supervised the research, and revised the manuscript.

Conflict of Interest Declaration

All authors declare that there are no conflicts of interest

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