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Propolis Supplementation on Broiler Chicken Performances, Nutrient Digestibility, and Carcass Characteristics: A Meta-Analysis

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ABSTRACT

This meta-analysis was conducted to investigate the effect of dietary propolis supplementation on broiler performance, carcass characteristics, and nutrient digestibility evaluated at the starter and finisher phases. An online literature search was conducted using scientific platforms of Science Direct, PubMed, and Google Scholar to identify the studies utilizing propolis as dietary supplementation in broiler chickens. A total of 39 publications comprising of 58 experiments met the inclusion criteria. A mixed model procedure for meta-analysis was performed, considering the studies as a random effect and levels of propolis as a fixed effect. Regression analysis suggested that average daily gain (ADG), body weight, and average daily feed intake (ADFI) at starter and finisher phases were quadratically affected by supplementing propolis in the diet ($p < 0.01$). Propolis also lowered the feed conversion ratio (FCR) throughout the periods ($p < 0.05$). In addition, a positive effect of propolis inclusion was also observed on breast meat percentage ($p < 0.05$). However, there was a negative linear relationship between propolis and the digestibility of dry matter (DMD) and organic matter (OMD) ($p < 0.01$) at the starter period. Conversely, there was a linear increase in DMD ($p < 0.05$) and a tendency to improve apparent metabolizable energy (AME) and phosphorus digestibility at the finisher period ($p < 0.10$). Overall, it can be concluded that dietary propolis supplementation promoted better growth performance and feed efficiency when given at ~1.66-2.13 g/kg diets for starter and finisher phases in the broiler chickens. The effect of propolis on nutrient digestibility was positive at the finisher phase while it was detrimental at the starter phase.

Keywords: bioactive compounds; broiler chicken; growth promoter; nutrient utilization; propolis

INTRODUCTION

Demand for natural-based additive in modern broiler production is increasing, in line with the increasing consumer awareness of healthy animal products. In the past, broiler producers received many benefits from the use of antibiotics at sub-therapeutic doses as growth-promoting (AGPs) additive, particularly related to the effect on cost-effective production (Allen *et al.*, 2013). However, since antibacterial resistance has become a global issue due to the extensive use of AGPs,

their applications in the poultry feed industry have been prohibited massively (Abudabos *et al.*, 2019). Since then, significant progress in investigating antibiotics alternatives for broiler chickens has been prompted by scientists worldwide, including the use of propolis, the resinous and balsamic substances produced by bees which have received a growing interest in the last decades.

Propolis is a complex resinous substance produced by honey bees for the purpose of building honeycombs in the hives and is known to contain more than 300 chemical compounds (Przybyłek & Karpiński, 2019).

Among the biological and pharmacological properties, polyphenols and terpenoids groups are considered to be the most potent antioxidant, antimicrobial, immunomodulatory, and anti-inflammatory compounds (Pimenta *et al.*, 2015; Silva *et al.*, 2017). There are two mechanisms of propolis in modulating broiler metabolism: the first mechanism is attributed to the direct action as antimicrobial activity. The second mechanism is related to the immune-stimulating effects, facilitating the activation of the natural defense system of the animal (Sforzin, 2016). In regard to its antibacterial effect, substances of propolis were reported to effectively disrupt the permeability of the cellular membrane and adenosine triphosphate (ATP) production of bacteria (Przybyłek & Karpiński, 2019). In broiler, propolis supplementation is beneficial for balancing the microbial composition of the digestive system, whether offered in the form of extract or crude propolis (Eyng *et al.*, 2017). The authors also suggest that propolis supplementation also has the potential to improve nutrient utilization by enhancement of enzyme secretion as well as absorptive functions. Another advantage is that propolis does not have any residue or toxic effect on the carcass, which is beneficial for human health (Denli *et al.*, 2005).

In general, previous studies reported an improvement in immunological indices, intestinal microbial composition, and broiler performance by using a diet supplemented with propolis (Attia *et al.*, 2014; Attia *et al.*, 2017; Zafarnejad *et al.*, 2017). Since promoting the growth of beneficial bacteria could enhance intestinal health, this mechanism is suggested to improve nutrient digestibility (Ao & Choct, 2013; Seven *et al.*, 2012). Moreover, it has been demonstrated that propolis supplementations reduce the negative effects of heat stress in broiler chickens due to their antioxidant properties as well as macrophages activation and antibody synthesis (Eyng *et al.*, 2015). Since there are large variations in propolis properties according to their origin, different pieces of evidence may exist. For instance, Eyng *et al.* (2014) reported contrary results that the inclusion of propolis extract impaired digestive enzyme secretion at the pre-starter phase, especially sucrose activity, thus lowered broiler performance at this stage. However, a recovery effect was observed at the finisher phase reflected by the improvement effect in the broiler receiving propolis. Also, Eyng *et al.* (2017) did not find any effect on intestinal microbiota and broiler performance by supplementing both raw propolis and ethanolic extract of propolis, although they found changes in the caecal bacterial composition. From this point of view, inconsistent results from available studies need to be elucidated.

A quantitative review can facilitate to summarize and explain the factors causing the different effects. A meta-analysis is a statistical tool that is being increasingly popular to be used in the area of animal nutrition. This statistically robust method facilitates researchers to integrate available studies and summarize the effect of size from different studies quantitatively, thus increase statistical power and reduce publication bias (Sauvant *et al.*, 2008). By integrating studies examining the effect of propolis inclusion in the broiler diets, it can contribute to explaining the gap of knowledge synthesized

from this meta-analysis. Therefore, this study aimed to quantify the effect of diet supplemented with propolis on broiler performance, nutrient digestibility, and carcass characteristics according to inclusion levels using meta-analysis.

MATERIALS AND METHODS

Literature Search

Studies containing information about the use of propolis in broiler diet were retrieved from the online scientific database of Science Direct, PubMed Central, and Google Scholar (Table 1). In searching the literature, we used a combination of several words and Boolean statement as follow: "propolis" [MeSH Terms] OR "propolis" [All Fields] AND broiler [All Fields] AND performance [All Fields] AND digestibility [All Fields]. Relevant articles were downloaded according to their title and further reviewed for database development. Several inclusion criteria were determined in order to select the suited articles as well as to reduce the publication bias. To be eligible, the article has to be: (1) published in English in a peer-reviewed academic journal to ensure the quality; (2) reported the level of propolis supplementation in the diet; (3) reported the mean value, variations (standard deviation or standard error of means), number of replications, and number of birds used in the experiment; and (4) provided a clear methodology particularly the environmental situation. At this stage, articles that did not meet the prerequisite criteria were excluded from the study. Data were compiled with LibreOffice version 6.3.6.2. After carefully reviewing the database, a total of 39 studies met our pre-determined criteria and therefore were used for analysis. These studies are composed of 58 experiments consisting of 196 data lines.

The parameters included in the database were growth performance at the starter and finisher phases [e.g. body weight (BW), average daily gain (ADG), daily feed intake (DFI), feed conversion ratio (FCR), and mortality], nutrients digestibility [e.g., apparent metabolizable energy (AME), calcium digestibility (CD), crude-protein digestibility (CPD), ether-extract digestibility (EED), nitrogen digestibility (ND), dry-matter digestibility (DMD), organic-matter digestibility (OMD), and phosphorus digestibility (PD)], and carcass characteristics (carcass, breast, legs, wings, abdominal fat, cecum, cloaca, esophagus and crop, gizzard, heart, liver, proventriculus, spleen, and thymus).

Statistical Analysis

Data with different units were transformed into the same measurement units prior to analysis. The summary of the data was processed using the linear mixed model method (St-Pierre, 2001), whereas the experimental studies were declared as a random effect, and the level of supplemental propolis was set as fixed effects. The mathematical model of the linear mixed model is as follows:

Table 1. Studies using propolis as an additive on broiler chickens included for meta-analysis

No.	Levels	Strain	Sex	Period (day)			References
				Starter	Finisher	Total	
1	0-4000	ROSS 308	Male	1-28	29-42	1-42	Açıkğözü <i>et al.</i> (2005)
2	0-1000	ROSS 308	As hatched	1-21	22-47	1-47	Ziaran <i>et al.</i> (2005)
3	0-250	ROSS 308	As hatched	1-21	22-42	1-42	Shalmany & Shivazad (2006)
4	0-1500	ROSS 308	Male	1-21	22-42	1-42	Tekeli <i>et al.</i> (2010)
5	0-5000	ROSS 308	As hatched	-	-	1-42	Seven & Seven (2008)
5	0-3000	ROSS 308	Mixed	3-21	22-41	3-41	Seven <i>et al.</i> (2008)
6	0-3000	ROSS 308	As hatched	-	-	8-42	Khodanazary <i>et al.</i> (2011)
7	0-200	ROSS 308	Male	1-21	22-42	1-42	Daneshmand <i>et al.</i> (2012)
8	0-1000	ROSS 308	As hatched	3-21	22-41	3-41	Seven <i>et al.</i> (2012)
9	0-500	Cobb 500	Male	1-21	-	-	Eyng <i>et al.</i> (2014)
10	0-750	ROSS 308	Male	1-21	22-42	1-42	Mahmoud <i>et al.</i> (2013)
11	0-2500	ROSS 308	Male	-	-	1-28	Abbas (2014)
12	0-300	Arbor Acres	Mixed	1-21	22-35	1-35	Attia <i>et al.</i> (2014)
13	0-500	Cobb 500	Male	1-21	-	1-42	Duarte <i>et al.</i> (2014)
14	0-5000	Cobb 500	Male	1-21	-	1-42	Eyng <i>et al.</i> (2014)
15	0-500	Cobb 500	Mixed	-	-	1-42	Abou-Zeid <i>et al.</i> (2015)
16	0-200	ROSS 308	Male	1-21	22-42	1-42	Daneshmand <i>et al.</i> (2015)
17	0-4000	Cobb 500	Male	-	-	1-21	Eyng <i>et al.</i> (2015)
18	0-200	ROSS 308	As hatched	1-21	22-42	1-42	Torki <i>et al.</i> (2015)
19	0-5000	Cobb 500	Male	1-21	-	1-21	Eyng <i>et al.</i> (2017)
20	0-400	ROSS 308	Mixed	1-21	22-42	1-42	Haščík <i>et al.</i> (2016)
21	0-3000	ROSS 308	Male	1-21	22-42	1-42	Hosseini <i>et al.</i> (2016)
22	0-300	ROSS 308	Male	1-21	22-42	1-42	Gheisari <i>et al.</i> (2017)
23	0-570	ROSS 308	Male	1-21	22-28	1-28	Biavatti <i>et al.</i> (2003)
24	0-1000	ROSS 308	Mixed	1-21	22-42	1-42	Al-Jabri <i>et al.</i> (2005)
25	0-1000	ROSS 308	As hatched	1-21	22-42	1-42	Seven <i>et al.</i> (2010)
26	0-3000	ROSS 708	Male	-	-	1-42	Mahmoud <i>et al.</i> (2017)
27	0-1200	ROSS 308	Female	-	-	16-20	Sahin & Ozturk (2017)
28	0-2000	ROSS 308	Male	1-24	25-42	1-42	Shaddel-Tili <i>et al.</i> (2017)
29	0-4000	ROSS 308	Male	1-21	22-42	1-42	Chegini <i>et al.</i> (2018)
30	0-3000	ROSS 308	Male	-	-	1-35	Kinasi <i>et al.</i> (2018)
31	0-1000	ROSS 308	Mixed	1-21	22-42	1-42	Klarić <i>et al.</i> (2018)
32	0-1000	Cobb 500	As hatched	1-21	22-42	1-42	Al-Sultan <i>et al.</i> (2019)
33	0-500	Iraqi local rooster	Male	-	-	-	Khafaji <i>et al.</i> (2019)
34	0-400	ROSS 308	Mixed	1-21	22-42	1-42	Haščík <i>et al.</i> (2019)
35	0-3000	ROSS 308	As hatched	1-21	22-42	1-42	Hassan <i>et al.</i> (2018)
36	0-400	Cobb 500	Mixed	1-21	22-49	1-49	Rabie <i>et al.</i> (2018)
37	0-800	Cobb 500	Mixed	-	-	-	Abdelsalam <i>et al.</i> (2019)
38	0-1000	ROSS 308	Mixed	1-21	22-42	1-42	Alani <i>et al.</i> (2019)
39	0-1000	ROSS 308	Mixed	1-21	22-42	1-42	Prakatur <i>et al.</i> (2019)

1) $Y_{ij} = \beta_0 + \beta_1 Level_{ij} + Experiment_i + Experiment_i Level_{ij} + \epsilon_{ij}$

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

where 1) is mathematical model of the linear mixed model (LMM) order 1; 2) is a mathematical model of LMM order 2; fixed effect is $\beta_0 + \beta_1 Level_{ij}$ (ordo 1) and $\beta_0 + \beta_1 Level_{ij} + \beta_2 Level_{ij}^2$ (ordo 2); random effect is $Experiment_i + Experiment_i Level_{ij} + \epsilon_{ij}$ (ordo 1 and 2); Y_{ij} is dependent variable, β_0 is value when level intersects the Y axis for

all random effect combinations; β_1 is coefficient of the 1st order level; β_2 is coefficient of the 2nd order level; $Level_{ij}$ is increased level of random effect; $Experiment_i$ is experiment at i, and ϵ_{ij} is model error.

The statistical models used are p-values, root mean square errors (RMSE), and Akaike information criterion (AIC). The significance was denoted when $p < 0.05$ and when $0.05 < p < 0.10$, it was declared as tendency. The data were analyzed using a script built using R version 3.6.3 software with the "nlme" package (Pinheiro *et al.*, 2020; R Core Team 2020).

RESULTS

Broiler Performance and Carcass Characteristics

48 In the present meta-analysis, the effect of propolis supplementation on broiler performance was evaluated during starter and finisher periods. As shown in Table 2, propolis supplementation linearly increased the ADG, BW, and DFI of broiler chickens both in starter and finisher phases, giving a significant improvement in final broiler performance (p<0.01). This study also suggested that there was a dependent relationship between daily gain with the levels of propolis supplementation, 39 shown with a significant quadratic effect (Table 2, p<0.05). In addition, feed conversion ratio (FCR) also decreased linearly as propolis inclusion increased at starter and finisher periods (p<0.01). The positive effect of propolis was also noticed on mortality throughout the period of broiler chickens as the mortality tends to decrease at starter and finisher periods and consequently at the final period in a quadratic manner (p<0.1). When the carcass characteristics were evaluated, no effect

was observed on carcass yield, abdominal fat, digestive organs, and most of the visceral organs such as liver, thymus, and hearth (p>0.05). However, breast meat and spleen weight linearly increased with increasing propolis supplementation (p<0.05).

Nutrient Digestibility

The effect of propolis supplementation on nutrient digestibility was investigated during the starter and finisher phases, where the regression model 45 is presented in Table 3. At the starter phase (day 1 up to 21 d of age), 49 levels of propolis linearly reduced digestibility of dry matter (DMD) and organic matter (OMD) 11 (p<0.01). At the finisher phase, the DMD is quadratically affected by the levels of propolis (p<0.01). OMD, on the other hand, tends to increase at a linear pattern (p<0.1) as propolis level increased. In addition, there was also a tendency to decrease crude protein digestibility (CPD) as a result of propolis supplementation (p<0.1). In this study, we confirmed a marginal increase in apparent metabolizable energy (AME) as affected by propolis

3 Table 2. Regression equations on the effect of dietary propolis supplementation (mg/kg diet) on broiler chicken performances

Outcome variables	Unit	Model	N	Parameter estimates				Model estimates			Interpretation		
				Intercept	SE _{Intercept}	Slope	SE _{Slope}	p-value	RMSE	AIC ¹	Trend	X ²	Y ³
Broiler performance at starter period													
Bodyweight	g	Q	103	723.7	32.9	439.6	132.11	0.001	2.42	1,205.2	Max.	2,135	770.6
						-1,029.3	320.55	0.002					
ADG	g/h/d	Q	105	29.6	1.2	22.9	6.02	<0.001	2.49	571.6	Max.	2,245	32.14
						-50.9	14.64	0.001					
DFI	g/h/d	Q	101	47.1	2.1	20.8	6.25	0.001	2.15	585.8	Max.	2,173	49.40
						-47.9	15.15	0.002					
FCR		L	101	1.59	0.03	-0.40	0.22	0.071	2.39	-140.8	Min.	2,405	1.55
						0.83	0.53	0.121					
Mortality	%	Q	8	4.25	3.29	-319.5	149.00	0.099	0.95	47.9	Min.	519.9	-4.05
						3,072.2	1,527.57	0.115					
Broiler performance at finisher period													
Bodyweight	g	Q	86	2,064.2	61.43	2,203.8	463.09	<0.001	1.87	1,151.2	Max.	1,762	2,258
						-6,254.0	1,401.6	<0.001					
ADG	g/h/d	Q	81	73.4	3.84	62.4	15.39	<0.001	1.57	564.9	Max.	1,656	78.6
						-188.5	46.86	<0.001					
DFI	g/h/d	Q	77	152.4	7.92	45.9	32.82	0.168	1.75	649.81	Max.	1,451	155.8
						-158.3	99.45	0.118					
FCR		Q	77	2.08	0.04	-1.05	0.51	0.045	2.00	-51.38	Min.	2,041	1.97
						2.5870	1.53	0.097					
Mortality	%	Q	8	7.89	4.84	-127.8	36.5	0.025	0.95	32.5	Min.	426.4	5.16
						1,498.6	379.3	0.017					
Broiler performance at overall period													
Bodyweight	g	Q	151	2,143.7	85.1	835.9	242.02	0.001	2.08	2,034.9	Max.	2,322.4	2,240.8
						-1,799.7	585.73	0.003					
ADG	g/h/d	Q	151	61.2	4.4	24.1	7.03	0.001	1.90	1,019.3	Max.	2,273.2	63.9
						-52.9	16.71	0.002					
DFI	g/h/d	Q	147	140.7	20.8	20.1	9.91	0.046	2.22	1,195.4	Max.	2,350.1	143.0
						-42.7	23.54	0.073					
FCR		Q	147	2.1	0.1	-0.4	0.23	0.065	2.13	-45.5	Min.	2,422.2	2.01
						0.9	0.54	0.108					
Mortality	%	Q	27	5.9	1.7	-9.2	4.68	0.065	1.28	144.9	Neg.		

Note: ADG= Average daily gain; AIC= Akaike information criterion; DFI=Daily feed intake; FCR= feed conversion ratio; Int= Intercept; L= Linear; Max.= maximum; Min.= minimum; N= number of data; Neg.= Negative; Q= quadratic; RMSE= Root mean square errors; SE= standard error; AIC¹= an estimator of the relative quality of statistical models for a given set of data; X²= predicted optimal level of dietary propolis supplementation (mg/kg); Y³= predicted optimal outcome of the response parameter as influenced by dietary propolis supplementation (mg/kg).

Table 3. Regression equations on the effect of dietary propolis supplementation (mg/kg diet) on carcass characteristics (% BW) of broiler chickens

No.	Variables	Model	N	Parameter estimates				Model estimates			Interpretation			
				Intercept	SE _{Intercept}	Slope	SE _{Slope}	p-value	RMSE	AIC	Trend	X ²	Y ³	
1.	Carcass	L	83	74.12	1.68	2.33	1.45	0.114	2.00	360.9		Pos.		
2.	Breast	Q	34	29.26	2.39	9.44	3.03	0.005	1.36	129.4		Max.	3,284.5	30.8
3.	Legs	L	20	27.60	4.13	-2.74	1.29	0.053	1.49	76.9		Neg.		
4.	Wings	L	24	16.35	3.46	-14.36	6.98	0.212	1.73	113.4		Pos.		
5.	Abdominal fat	L	59	1.50	0.12	-0.10	0.31	0.752	1.40	29.8		Neg.		
6.	Cecum	L	15	0.34	0.09	-0.12	0.13	0.356	1.12	-40.5		Neg.		
7.	Cloaca	L	11	0.24	0.01	0.07	0.04	0.151	1.22	-51.3		Pos.		
8.	Esophagus and crop	L	20	0.42	0.07	0.004	0.03	0.906	1.19	-59.9		Pos.		
9.	Gizzard	L	69	1.64	0.09	0.06	0.09	0.556	1.45	-48.8		Pos.		
10.	Heart	L	52	0.53	0.09	0.10	0.08	0.222	1.44	-135.8		Pos.		
11.	Liver	L	82	2.17	0.11	-0.04	0.14	0.776	1.44	-1.6		Neg.		
12.	Proventriculus	L	28	0.44	0.06	-0.03	0.06	0.599	1.04	-75.9		Neg.		
13.	Spleen	L	44	0.13	0.01	0.07	0.02	0.008	1.30	-211.6		Pos.		
14.	Thymus	L	24	0.39	0.09	0.01	0.13	0.956	1.46	-47.8		Pos.		

Note: AIC= Akaike information criterion; N= number of data; Neg.= Negative; Pos.= Positive; Q= quadratic; RMSE= Root mean square errors; SE= standard error; AIC= an estimator of the relative quality of statistical models for a given set of data; X²= predicted optimal level of dietary propolis supplementation (mg/kg); Y³= predicted optimal outcome of the response parameter as influenced by dietary propolis supplementation (mg/kg); % BW= % body weight.

Table 4. Regression equations on the effect of dietary propolis supplementation (mg/kg diet) on nutrient digestibility (%)

Variables	Unit	Model	N	Parameter estimates				Model estimates			Interpretation			
				Intercept	SE _{Intercept}	Slope	SE _{Slope}	p-value	RMSE	AIC	Trend	X ²	Y ³	
Nutrient digestibility in the starter period														
DMD	%	L	6	74.51	3.74E-15	-1.25	8.01E-17	<0.001	297.45	-398.0		Neg.		
OMD	%	L	6	77.00	1.45E-15	-0.95	8.01E-17	<0.001	297.45	-403.7		Neg.		
Nutrient digestibility in the finisher period														
DMD	%	Q	10	75.78	3.35	-25.66	3.57	0.006	0.74	46.56		Min.	2,381.1	72.7
						53.88	8.24	0.007						
OMD	%	Q	12	77.52	2.75	-28.04	9.92	0.047	0.94	65.81		Min.	2,401.4	74.2
						58.39	22.84	0.063						
CPD	%	Q	12	74.29	4.90	-37.73	17.12	0.092	0.88	79.29		Min.	2,453.5	69.7
						76.89	39.44	0.123						
EED	%	Q	10	81.99	4.02	-31.07	6.11	0.015	0.75	53.72		Min.	2,127.4	78.7
						73.02	14.08	0.014						
ND	%	L	10	57.27	2.59	12.53	5.28	0.055	0.97	61.34		Pos.		
CAD	%	Q	10	43.61	0.36	-6.33	4.10	0.183	1.14	25.85		Min.	3,661.1	42.4
						8.64	8.82	0.372						
PD	%	L	10	48.84	0.55	3.50	1.31	0.037	0.97	32.23		Pos.		
AME	%	L	10	11.62	0.19	1.43	0.65	0.071	1.12	15.17		Pos.		

Note: AIC= Akaike information criterion; AME= apparent metabolizable energy; CAD= calcium digestibility; CPD= crude protein digestibility; DMD= dry matter digestibility; EED= ether extract digestibility; ND= Nitrogen digestibility; OMD= Organic matter digestibility; PD= Phosphor digestibility; L= Linear; Max.= maximum; Min.= minimum; N= number of data; Neg.= Negative; Q= quadratic; RMSE= Root mean square errors; SE= standard error; AIC= an estimator of the relative quality of statistical models for a given set of data; X²= predicted optimal level of dietary propolis supplementation (mg/kg); Y³= predicted optimal outcome of the response parameter as influenced by dietary propolis supplementation (mg/kg).

inclusion (p<0.07), while a more positive effect was observed on phosphorus digestibility (PD) and ether extract digestibility (EED) which showed a significantly increased at the linear pattern (p<0.05).

DISCUSSION

Broiler Performance and Carcass Characteristics

Among naturally occurring feed additives, propolis has been acknowledged for its beneficial effect on broiler

growth and health. As confirmed in this meta-analysis, our findings supported previous experiments in which propolis improved broiler performance both in normal conditions and when the animals were situated under heat stress challenge (Tatli Seven et al., 2008; Seven et al., 2012). The explanation regarding the positive impact of propolis incorporated in the diet on the broiler chicken performances is attributed to the multiple mechanisms associated with active compounds presented in the propolis. Propolis contains a very complex chemical composition (Abdelsameea et al., 2013), including vita-

mins, minerals, and amino acids, and a number of bioactive compounds (Attia *et al.*, 2014; Nasution *et al.*, 2015; Attia *et al.*, 2017). The beneficial effect of phenolic compounds presented in the propolis is known to have multiple pharmacological functions, such as antibacterial, immunomodulator, and antioxidants (Wang *et al.*, 2004; Dziedzic *et al.*, 2013; Eyng *et al.*, 2015). The presence of vitamins, amino acids, and trace elements such as Zn also contributed to the improved growth performance of poultry (Seven *et al.*, 2012; Idayat *et al.*, 2020).

As an antioxidant, several authors have reported that propolis could alleviate the negative effect of heat stress and improved antioxidant parameters such as the activities of superoxide dismutase (SOD), malondialdehyde (MDA), and catalase (CAT) (Seven *et al.*, 2012). Improvement of antioxidant status can further promote body protein synthesis, decrease oxidative stress, and it can also enhance the digestive enzyme activity (Seven *et al.*, 2012; Attia *et al.*, 2017). In addition, the immunomodulatory effect of propolis allows broiler chickens to activate the macrophages and stimulate antibody secretion as well as improve lymphoid organ weight (Eyng *et al.*, 2015), thereby improving the gut health of broiler chickens (Kleczek *et al.*, 2014). Intestinal macrophages are the first phagocytic cells responsible for initiating and developing the innate immune system by inhibiting pathogens as well as clearing the bacterial metabolite products that is essential to protect against prolonged inflammation and to maintain immune homeostasis (Wang *et al.*, 2019).

Furthermore, these mechanisms play an important role in improving the intestinal health of chickens, where the process of nutrients absorption from the digested feed is carried out there. Temizer *et al.* (2017) reported that propolis could perform an anti-bacterial function, thereby reducing the number of pathogenic bacteria in the gut, as reported by Kačaniová *et al.* (2012). The function of propolis as an anti-bacterial has an effect on improving intestinal health, which in turn increases the absorption of nutrients by the intestine (Tayeb & Sulaiman, 2014) so that the effect is to increase the efficiency of using feed as shown in this study.

Nutrient Digestibility

It has been suggested that propolis could indirectly improve nutrient digestibility (Eyng *et al.*, 2014). This effect is attributable to the role of propolis to modulate intestinal bacteria by enriching beneficial bacteria and suppressing pathogenic bacteria (Guo *et al.*, 2003). A good bacterial composition can promote the secretion of digestive enzymes and the immune system, thus improve nutrient digestion and absorption in the intestine (Romier *et al.*, 2009). There is a well-explained mechanism regarding the effect of microbial stimulation on enzyme secretion. Modulation of intestinal microbiota to promote a higher beneficial bacterial population provides several advantages. First, good bacteria can produce antimicrobial substances such as bacteriocins and short-chain fatty acids (SCFA) that can inhibit the growth of pathogens by disrupting the environment for the growth of pathogenic microbes (Jha *et al.*, 2020).

Second, the bacteria are able to secrete extracellular enzymes such as amylase, xylanase, protease, and lipase. In addition, balancing microbial population also contribute to improving intestinal integrity and immunity (Oakley *et al.*, 2016). Moreover, active compounds of propolis such as flavonoids were also suggested to have a growth hormone activity because they have an aglycone hydroxyl group (Przybyłek & Karpiński, 2019).

In the present study, we found contrary results with the available literature, especially at the starter phase, whereas propolis increased the digestibility of DM and OM. However, these results were in agreement with the study of Eyng *et al.* (2014), who reported a detrimental effect of propolis on broiler chickens during the first week of life. According to the literature, however, we did not find any effect on enzyme secretion and activity since there was no available information. Thus, we suggested that the mechanism underlying how propolis disrupts DM, OM, and CP digestibility and broiler growth can be connected with enzyme secretion and the negative effect of propolis on palatability. In the first week of life, broilers secrete very low digestive enzymes (Noy & Sklan, 1999). Thus they need a diet with easily degradable ingredients such as broken rice, and soy-protein isolate to stimulate indigenous enzyme secretion and activity (Ebling *et al.*, 2015). Introducing a diet containing various bioactive compounds was less favorable to pre-starter broilers that may disrupt enzyme secretion to degrade the feed to release nutrients. The second reason is regarding the negative effect of propolis on palatability. In these regards, studies reported that propolis inclusion reduced feed intake on broilers (Seven *et al.*, 2008; Eyng *et al.*, 2014). Another study demonstrated that supplementing a diet with a high level of propolis suppressed protein digestion and growth (Açıkgöz *et al.*, 2005). The reason to elucidate the adverse effect of propolis on DM, OM, and CP digestibility is that antibiotics or most alternatives do not directly benefit nutrient digestibility (Mountzouris *et al.*, 2010). In the case of increasing nutrient utilization caused by AGP, it was promoted by reducing metabolic activities of the digestive bacteria (Miles *et al.*, 2006). Instead, the protective effect in the intestinal barrier resulted from alternative AGP had different modes of action whereas consequently required a higher energy cost by microbes for their growth (Attia *et al.*, 2017).

For the finisher phase, most of the previous findings where dietary propolis improved nutrient digestibility (Seven *et al.*, 2012; Chegini *et al.*, 2019) were confirmed in the present results, especially for EE and phosphorus digestibility. There were some possible reasons explaining how propolis or its properties improved nutrient utilization in the finisher phase. First, it can be related to the enzyme stimulating effect. Seven *et al.* (2012) found a remarkable increase in digestive enzyme activities of saccharase, amylase, and phosphatase on broiler chickens treated with a propolis-supplemented diet. This effect was not found in the starter phase, probably because enzyme secretion in the starter period is very low (Noy & Sklan, 1999), thus adding a less palatable additive such as propolis decreased nutrient digestion as was shown in the current result (Table 3).

Second, antioxidant effects could also partially explain the improvement of protein digestibility by interfering with oxidative protein denaturation. Moreover, propolis also exhibited a synergistic effect for protective actions on the intestinal barrier as a result of the ability of their substances such as phenolic compounds and flavonoids in modulating the gut ecosystem, enhancing absorptive capacity thus increase nutrient absorption (Prakatur *et al.*, 2019). To support this theoretical reason, there were a number of pieces of evidence showing a significant improvement in length, high, and wide of the villi in the duodenum, jejunum, and ileum of broiler chickens receiving propolis treatment in their diet (Wang *et al.*, 2007; Tekeli *et al.*, 2010; Eyng *et al.*, 2014; Prakatur *et al.*, 2019). These parameters indicate a higher proliferation in the intestinal mucosa, whereas it is a clear indicator of better nutrient utilization. Furthermore, this result was also highly related to the major findings that propolis inclusion improved broiler performance at the finisher phase (Eyng *et al.*, 2014).

CONCLUSION

Dietary propolis supplementation was effective to improve broiler chicken performances during the starter and finisher periods. The most optimum supplementary levels for to obtain optimum body weight and feed conversion ratio were between 1.66-2.13 g/kg diets. Dietary propolis supplementation was also effective to improve nutrient digestibility at the finisher phase rather than the starter phase as reflected by the substantial increase in phosphorus digestibility. However, as the effect of increasing propolis supplementation in the starter phase was negative, it can be taken into consideration for future studies.

CONFLICT OF INTEREST

The authors (i.e., Nahrowi and Anuraga Jayanegara) serve as editorial boards of the Tropical Animal Science Journal, but they have no role in the decision to publish this article. The authors also declare that there is no conflict of interest with any financial, personal, or other relationships with other people or organization related to the material discussed in the manuscript.

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