



# Castor and Propolis Extracts as Antibiotic Alternatives to Enhance Broiler Performance, Intestinal Microbiota and Humoral Immunity

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**Abstract** | This study to explore the impact of the addition of castor extract (CE), propolis extract (PE) and the castor and propolis extracts mix (CE+PE) as antibiotic alternatives in broiler diets from 1-35 day of age. A total 250 one day old male Arbor acres broiler chicks were partitioned into five gatherings, five replicates of ten chicks each. Five experimental diets were formulated, diet one was a basal eating regimen with no addition (control group), diets 2-5 were the control diet supplemented with antibiotic 1 g/Kg diet, CE 1.5 g/Kg diet, PE 1.5 g/Kg diet and mixture of CE 0.75 and PE 0.75 g/Kg diet, respectively. Growth performance, carcass characteristics, intestinal parameters, intestinal microbiota and the immune response have been measured. The results showed that at grower, finisher and overall periods, chicks fed diets containing CE and CE+PE were significantly ( $P < 0.05$ ) recorded the best body weight gain (BWG) and feed conversion ratio (FCR) compared to the control group. In addition to the similarity of significantly ( $P < 0.05$ ) improvement of BWG and FCR recorded between the chicks fed diets containing either PE or antibiotic compared to the control. Birds fed diets containing CE, CE+PE recorded the best intestinal length, diameter and zero lesion score compared to the other treatments. In addition the birds fed CE and CE+PE measured the highest Enumeration of Lactic acid bacteria (beneficial bacteria) and nil for intestinal colonization of *Clostridium perfringens* (pathogenic bacteria) compared to all other treatments. Furthermore, the best improvement in the immune response against both of Newcastle (ND) and avian flu (H5) vaccine in the birds fed diets containing CE and CE+PE. Finally, it could be concluded that, the addition of CE or CE+PE to the broiler's diet gave superior in all measurements than the addition of antibiotic. Also, using of PE gave the comparable results to the antibiotic treatment. Therefore, these more effective and safety materials have to use instead of using antibiotic as growth promoter in broiler diets.

**Keywords** | Broiler, Castor extract, Propolis extract, Antibiotic, Growth performance, Intestinal microbiota and Immune response

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## INTRODUCTION

The antibiotic used as growth promoter from the 1940s, when researchers found that animals eaten dried mycelia, which was containing chlortetracycline residues, enhanced their performance. This enhanced of the animal performance related to the effect of antibiotic on the intes-

tinal microbiota. (Castanon 2007, Niewold, 2007). Nevertheless, it is dangerous to use antibiotic because it induce bacterial resistance in both animals and human consumers. Thus the European Union banned the uses of antibiotics as a growth promoter since January 1, 2006 also it was been banned except with a veterinary prescription by FDA in 2012 (Laxminarayan et al., 2016).

Therefore, these prompted researchers to search for a new trend for preventing the infection by pathogenic bacteria and act as a growth promoter. Thus, using a natural antimicrobials like plant extracts as an alternative to the traditional antimicrobial (Rampadarath and Puchooa, 2016). Most of plant extracts still be studied to adding in animal nutrition instead of antibiotic as a growth promotion, especially after increase the restriction of using antibiotic in animal diets, which is induce resistance and persistence in addition to environmental contamination (Ventola, 2015). Natural feed additives could be used as a growth promoter to enhance broilers performance instead of antibiotic (Hassan et al, 2016 and 2018).

Also some of plant extracts have highly effect to eliminate bacteria or stunt bacterial growth such as *Salmonella* when addition these to animal diets (Castillo-López et al., 2017). Castor bean (*Ricinus communis* L.), growing extensively in both tropical and subtropical regions of the world (Zarai et al., 2012). It has an importance crop because it has highly active compounds like castor oil, which reach in ricinoleic acid and mixtures of volatile secondary metabolites, that support uses of it as antimicrobial (Kiran and Prasad, 2017; Salih et al., 2019). Castor extract showed the highly antimicrobial activity against most pathogenic bacteria included both gram positive and negative bacteria for example *E. coli*, *Bacillus cereus*, *P. aeruginosa*, and *Listeria innocua* (Rampadarath and Puchooa, 2016). In addition, it has approximately 75% antifungal activities (Das and Satyaprakash, 2018). Bess et al. (2012) found that the addition of castor oil-CNSL mixture (0.15%) to the broiler diets from 1-21 d of age lead to enhanced the broiler performance, which might caused by the antimicrobial effect of these functional oil. Using of castor oil and cashew shell extract in the diet of coccidiosis challenged broilers resulted in enhanced body weight gain and feed conversion ratio (Murakami et al., 2014).

Monensin (conventional antibiotic) could be replaced with essential oils of castor bean in beef cattle diets without influencing feed intake, digestibility, ruminal fermentation or microbial proteins synthesis. (Coneglian et al., 2019). Valero et al. (2016) concluded that castor oil could be used instead of antibiotic in diets of cattle to improve performance and feed efficiency.

Moraes et al. (2019a) reported that adding of the castor and cashew extracts to the broiler diets (0.15%) improved the BWG in the first week, but BWG did not significantly change compared to the other treatment groups in the second week after infection with *Eimeria* spp. The castor and cashew extracts groups exhibited increased gene expression of interferon (IFN), interleukin 6 (IL-6) and tumor necrosis factor (TNF), while the control group ex-

hibited increased expression of cyclooxygenase (COX) and IL-1. Which these modulated the inflammatory response against *Eimeria* spp., thus the functional oil is an effective tool in specifically modulating the immune system of birds afflicted with coccidiosis.

Moraes et al. (2019b) concluded that the use of functional oil (castor and cashew shell extracts) in broiler diets (0.15% inclusion) from 1-28 days improved the performance in similar to those receiving the ionophore monensin. The castor and cashew shell extracts acting as a modulator of the intestinal microbiota, with antimicrobial action against gram-positive bacteria, mainly *C. perfringens* and *S. aureus*.

Propolis (bee glue) is a dark resinous material that honey bees (*Apis mellifera* L.) collect from tree buds and the exudates of plants and then chew and digest through oral enzymes (Seven et al., 2010).

Propolis is a dim resinous material that bees gather from tree buds and the plants exudates and afterward digest it by enzymes of oral (Seven et al., 2010). Propolis could improve the poultry performance, via enhancing nutrient digestibility and absorption; which stimulating saccharase, amylase and phosphatase activities (Marieke et al., 2005).

Kacaniova et al. (2013) and Krocko et al. (2012) found that increased of the beneficial bacteria count (such as Enterococci (8.65 cfu/g) and Lactobacilli (8.83 cfu/g)) while decreased the pathogenic bacteria count in the intestine of the broilers fed a diet containing propolis (0.2 or 0.6 g/kg feed). concluded that the addition of propolis and bee pollen to the broiler diets enhanced this intestinal microbiota. Kleczek et al. (2014); Hascik et al. (2016); Khan, (2017) and Zafarnejad et al. (2017) found that the broiler fed diets supplemented with propolis was enhanced the performance comparable to those supplemented these diet with antibiotic. Shaddel-Tili et al. (2016) and Shreif and El-Saadany (2017) found that weight gain is directly proportional to the increase in the percentage of propolis addition to the broiler diets.

Therefore, this study was conducted to investigate the influence of Castor extract, propolis extract and the mixture of them as antibiotic alternatives for growth promoter in broiler diets on performance, intestinal parameters, intestinal microbiota and the immune response.

## MATERIALS AND METHODS

### BIRDS, HOUSING AND MANAGEMENT

The experiment was conducted at Poultry Nutrition Research Unit (PNRU), Agriculture College, Cairo University, Giza, Egypt. A total 250 one day old male Arbor

acres broiler chicks with initial weight about 40 g were randomly allocated at semi close house in clean three deck batteries and divided into 5 groups, 50 chicks each with 5 replicates. The cages diameter was 0.50\*1.0m with nipple drinkers. Birds were raised in a heated house during starter (1-14 day) and the temperature was decreased gradually to meet grower (14-28 day) and finisher (28-35 day) period's needs. Light was given 23 h every day all through the experimental period. Feed and water were given ad libitum. An immunization program against avian influenza, New Castle, IB and IBD was carefully embraced all through the experimental period.

### EXPERIMENTAL MATERIALS

**I) Castor extract (CE):** Extraction of castor beans method were, heat reflux extraction by 70% ethanol for 3h with vigorous stirring after that separation of the extract by rotary evaporator at 40°C.

**II) Ethanolic propolis extract (PE)** was purchased from the Economic Entomology Department, Faculty of Agriculture, Cairo University, Egypt.

**III) Antibiotic:** Terramycin (oxytetracycline 40%) IM® from local market.

### EXPERIMENTAL DESIGN AND DIETS

A total 250 one day old male Arbor acres broiler chicks were partitioned into five gatherings, five replicates of ten chicks each. The group one (control) was received the basal starter, grower and finisher diets according to Arbor acres recommended levels (Table 1). The groups from 2 to 5 were control diets supplemented with 1g antibiotic/Kg diet, 1.5 g CE/Kg diet, 1.5 g PE/Kg diet and mixture of 0.75 g CE and 0.75 g PE/Kg diet, respectively. Addition levels of castor and propolis extracts according to Bess et al. (2012); Murakami et al. (2014) and Moraes et al. (2019a,b).

### MEASURED PARAMETERS

**(I) Growth performance parameters:** At the end of different stages (starter,14d; grower,28d and finisher,35d) all chicks were weighted and recorded the feed consumption (g/chick), then calculate the average weight gain and feed conversion ratios (g feed/g weight).

### (II) Slaughter test:

At the 35<sup>th</sup> day old, five birds with weight near to the gathering average for every treatment were chosen to examine the carcass, weight of immune organs, intestinal parameters, intestinal microbiota and immune response. After birds overnight fasting, the live body weights were recorded, also the carcass weight, liver, spleen, heart, thymus, bursa and gizzard were recorded after slaughtered and feathered. Calculated the percentages of live body weight for liver, gizzard and heart for each bird individually (Mo-

hamed et al., 2016). Immune organs index were calculated by the equation (weight of organ/body weight) ×1000.

### (III) Intestinal parameters:

**Length and diameter:** Measured the length of intestine (duodenum, jejunum and ileum) and diameter in the center of the ileum on five birds from each treatment, at 35<sup>th</sup> day of age (Dahiya et al., 2005).

**Intestinal lesion score:** opened and scored the small intestine from a five birds of each treatment on a scale from zero to four, Zero is a normal intestinal appearance with no lesion, One half is severely congested serosa and mesentery engorged with blood, One is a thin walled and friable intestines with small red petechiae (>5), Two is a focal necrotic lesions, Three is patches of necrosis (1 to 2 cm-long), and Four refer to diffused necrosis typical of field cases (Dahiya et al., 2005).

### (IV) Intestinal microbiota:

**Enumeration of Lactic acid bacteria:** Transferred one gram of fresh digesta samples from the ileum and caecum to 9ml MRS broth and serially diluted in 10-fold increments. Plated 0.1 ml from the last three diluted samples individually on MRS agar (Oxoid, CM0361) then incubated at 39°C for 48 h (Micro aerobic). (Dahiya et al., 2005).

### :Intestinal colonization of *C. perfringens*

Taken 0.2 g of intestinal contents from five birds of each treatments individually the make a serial dilution in sterile PBS to 1:100, 1:1000, and 1:10000 after that 0.1 ml of each dilution was poured on the surface of sheep blood agar plates and tryptose sulfite-cycloserine (TSC) agar (supplemented by D-cycloserine) with egg yolk emulsion. After 24h of anaerobic incubation at 37°C, typical *C. perfringens* colonies (black colonies) on TSC agar or large dome-shaped colonies with a double zone of hemolysis on blood agar plates were counted as CFU (colony-forming units) per gram. (Carrido et al., 2004).

### (V) Immune status assessment:

For determination of the effect on immunity. The blood serum samples used in hemagglutination inhibition (HI) test for determining antibody titers against ND and H5 vaccine. Expressed all titers as log of the reciprocal of highest dilution indicating hemagglutination (Swayne et al., 1998).

### (VI) Statistical analysis:

General Liner Model of SAS (one-way analysis of variance) was used to statistical analyzed of data (SAS, 2004). Duncan's new multiple range test at (P<0.05) was used to separate the significant differences among treatment means (Duncan, 1955).

**Table 1:** Formulation and nutrients composition of the starter, grower and finisher diets.

Ingredients %	Starter (1-14 days)	Grower (14-28 days)	Finisher (28-35 days)
Yellow corn	55.60	58.73	62.80
Soybean meal (44%)	34.00	30.00	25.86
Corn gluten meal (60%)	4.50	4.40	4.50
Soybean oil	1.50	2.80	3.00
Mono calcium phosphate	1.00	0.90	0.85
Limestone	1.80	1.57	1.46
Vitamin & Mineral mix <sup>(1)</sup>	0.30	0.30	0.30
Nacl	0.30	0.30	0.30
L-lysine HCl	0.30	0.33	0.33
DL-methionine	0.26	0.26	0.20
Threonine	0.15	0.12	0.11
NaHCO <sub>3</sub>	0.17	0.17	0.17
Choline chloride	0.10	0.1	0.1
Phytase	0.01	0.01	0.01
Energy enzymes	0.01	0.01	0.01
<b>Total</b>	<b>100</b>	<b>100</b>	<b>100</b>
Calculated composition <sup>(2)</sup>			
Crude protein %	23.02	21.47	20.01
ME (Kcal/Kg)	2949	3076	3138
Ether extract%	3.99	5.38	5.71
Crude fiber%	3.66	3.45	3.25
Lysine %	1.34	1.26	1.16
Methionine %	0.64	0.61	0.54
Methionine + Cystine %	1.01	0.97	0.87
Threonine %	0.97	0.88	0.81
Calcium %	0.98	1.00	0.81
Nonphytate P %	0.38	0.35	0.33
Sodium%	0.18	0.18	0.18
Chlorine%	0.22	0.22	0.22

<sup>(1)</sup> Vitamin - mineral mixture supplied per Kg of diet: Vit A, 12000 IU; Vit D<sub>3</sub>, 2200 IU; Vit E, 10 mg; Vit K<sub>3</sub>, 2 mg; Vit B<sub>1</sub>, 1mg; Vit B<sub>2</sub>, 4mg; Vit B<sub>6</sub>, 1.5mg; Vit B<sub>12</sub>, 10g; Niacin, 20 mg; Pantothenic acid, 10 mg; Folic acid, 1 mg; Biotin, 50 g; Choline chloride, 500 mg; Copper, 10 mg; Iodine, 1mg; Iron, 30 mg; Manganese, 55 mg; Zinc, 50 mg and Selenium, 0.1 mg.

<sup>(2)</sup> According to NRC 1994.

## RESULTS

**Growth performance:** Results of growth performance of birds fed different dietary treatments (Control, Antibiotic, Castor extract (CE), Propolis extract (PE) and Castor and Propolis extracts mix (CE+PE)) during different stages and overall period are shown in Table 2.

At starter period data showed that no significant differences in birds performance were detected among all treatments compared to the control.

While in the grower period, only body weight gain (BWG) is significantly (P<0.05) improved in chicks fed diets containing CE and CE+PE compared to the control. While no significant differences were recorded in both, feed intake (FI) and feed conversion ratio (FCR).

At finisher period, Birds fed diet containing CE+PE

showed significantly (p<0.05) enhanced BWG compared to the control. While significantly (P<0.05) improvement in FCR recorded in all treatments compared to control.

At overall period (1-35 days of age), the best BWG significantly (P<0.05) recorded from the chicks fed diets containing CE and CE+PE. In addition, the similarity of improvement of BWG recorded between the birds fed diets containing PE and antibiotic compared to the control. Also, significantly (P<0.05) improvements in FCR for birds fed diets containing CE, CE+PE or PE compared to control. No significant differences in FI was detected among all treatments compared to the control.

## CARCASS CHARACTERISTICS

Effect of dietary treatments on dressing percent, giblets and weight of the immune organs at 35-day of age are shown in Table 3.

**Table 2:** Effect of dietary treatments on growth performance {Weight gain, g (WG); Feed intake, g (FI) and feed conversion ratio, FI g/ WG g (FCR)} of broiler chicks during starter, grower, finisher and overall periods.

Item	Starter (1-14)*			Grower (14-28)			Finisher (28-35)			Overall period (1-35)		
	WG	FI	FCR	WG	FI	FCR	WG	FI	FCR	WG	FI	FCR
Control	309	389	1.26	894 <sup>b</sup>	1149	1.27	469 <sup>b</sup>	981	2.10 <sup>a</sup>	1672 <sup>c</sup>	2541	1.52 <sup>a</sup>
Antibiotic	312	368	1.18	945 <sup>ab</sup>	1155	1.23	505 <sup>ab</sup>	963	1.92 <sup>b</sup>	1762 <sup>b</sup>	2520	1.43 <sup>ab</sup>
Castor extract	335	382	1.14	961 <sup>a</sup>	1162	1.21	506 <sup>ab</sup>	945	1.87 <sup>b</sup>	1807 <sup>a</sup>	2512	1.39 <sup>b</sup>
Propolis extract	317	355	1.12	945 <sup>ab</sup>	1131	1.20	502 <sup>ab</sup>	932	1.87 <sup>b</sup>	1764 <sup>b</sup>	2417	1.37 <sup>b</sup>
Castor+Propolis	318	369	1.16	981 <sup>a</sup>	1169	1.19	546 <sup>a</sup>	957	1.76 <sup>b</sup>	1845 <sup>a</sup>	2528	1.37 <sup>b</sup>
SE of means	±3.79	±3.74	±0.02	±9.31	±11.51	±0.01	±8.75	±10.82	±0.03	±14.33	±23.24	±0.02
Significances	NS	NS	NS	**	NS	NS	**	NS	***	***	NS	**

Means designated with the same letter within the same column are not significantly different at 0.05 level of probability, \*\*p<0.01, \*\*\*P<0.001, NS: Not significant (P>0.05). \* The beginning weight is 40g.

**Table 3:** Effect of dietary treatments on dressing percent, giblets (as percent of live body weight) and immune organs at 35-day of age.

Item	Carcass weight (g)	Dressing %	Giblets % (of LBW)			Immune organs index		
			Liver	Heart	Gizzard	Spleen	Bursa	Thymus
Control	1305 <sup>c</sup>	72.85	2.27	0.60	2.11	1.01	1.66	1.76
Antibiotic	1352 <sup>bc</sup>	72.39	2.13	0.55	2.16	0.95	1.55	1.65
Castor extract	1393 <sup>ab</sup>	73.73	2.12	0.56	1.98	0.97	1.63	1.96
Propolis extract	1363 <sup>bc</sup>	72.78	2.10	0.58	2.28	1.06	1.63	1.83
Castor+Propolis	1426 <sup>a</sup>	74.81	2.07	0.52	2.27	0.98	1.64	1.73
SE of means	±14.42	±0.47	±0.09	±0.01	±0.07	±0.06	±0.04	±0.07
Significances	***	NS	NS	NS	NS	NS	NS	NS

Means designated with the same letter within the same column are not significantly different at 0.05 level of probability, \*\*\*P<0.001, NS: Not significant (P>0.05).

**Table 4:** Effect of dietary treatments on intestinal length, diameter and lesion score of broiler chicks at 35 day of age.

Item	Intestinal length	Intestinal diameter	Intestinal lesion score
Control	145 <sup>c</sup> cm	0.3 <sup>c</sup> cm	1 <sup>a</sup>
Antibiotic	152 <sup>c</sup> cm	0.4 <sup>c</sup> cm	1 <sup>a</sup>
Castor extract	188 <sup>a</sup> cm	1.2 <sup>a</sup> cm	0 <sup>b</sup>
Propolis extract	165 <sup>b</sup> cm	0.4 <sup>c</sup> cm	1 <sup>a</sup>
Castor+Propolis extracts	192 <sup>a</sup> cm	0.9 <sup>b</sup> cm	0 <sup>b</sup>
SE of means	±5.12	±0.09	±0.13
Significances	***	***	***

Means designated with the same letter within the same column are not significantly different at 0.05 level of probability, \*\*\*P<0.001.

No significant differences in dressing, giblets and immune organs weight were detected among all treatments compared to the control.

Carcass weight significantly (P<0.05) to be improved in birds fed CE and CE+PE compared to the control. The results indicated that no adverse effect of the CE or/and PE extracts on dressing %, giblets and immune organs index.

**EFFECT OF DIETARY TREATMENTS ON THE INTESTINAL LENGTH, DIAMETER AND LESION SCORE OF BROILER CHICKS AT 35 DAY OF AGE.**

**Intestinal length and diameter:** The effect of different dietary treatments on intestinal length (duodenum + jejunum + ileum) and diameter (in the middle of ileum) of broiler chicken at 35 day of age are shown in Table 4.

The results showed the significantly (P<0.05) improvement of the intestinal length in the birds fed diets containing CE, PE and PE+CE are being 188 cm, 165 cm,

and 192 cm respectively compared to the 152 cm for antibiotic treatment and 145 cm for the control. In addition, the enhanced intestinal diameter recorded for the birds fed diets containing CE, and CE+PE 1.2 cm and 0.9 cm respectively. While the similar intestinal diameter recorded for the birds fed diets containing PE and antibiotic 0.4 cm compared to the control 0.3 cm.

**Intestinal lesion score:** The results of the intestinal lesion score showed in Table 4. The birds fed diets containing CE and CE+PE recorded zero on the scale of intestinal lesion score, which mean that the intestinal is normal appearance with no lesion. While the treatments for PE, antibiotic and control recorded one on the scale which mean that the intestinal is thin walled and friable intestines with small red petechiae (>5).

**EFFECT OF DIETARY TREATMENTS ON THE INTESTINAL MICROBIOTA OF BROILER CHICKS AT 35 DAY OF AGE.**

The results of the enumeration of lactic acid bacteria and intestinal colonization of *C. perfringens* are shown in Table 5. The results showed that the significantly (P<0.05) increased of the number of beneficial intestinal bacteria (lactic acid bacteria) in the birds fed diets containing CE and CE+PE are being 4X10<sup>8</sup> and 3X10<sup>7</sup>, respectively. While the similarity of beneficial intestinal bacteria count in birds fed diets containing PE and control are being 4X10<sup>4</sup> and 5X10<sup>4</sup>, respectively. However, the worst lactic acid bacteria count recorded for birds fed diets containing antibiotic 5X10<sup>3</sup>. On the other hand the number of the intestinal colonization of *C. perfringens* (Pathogenic intestinal bacteria) recorded nil for the birds fed diets containing CE and CE+PE. Whereas birds fed diets containing PE and antibiotic recorded the pathogenic bacteria number similar to the control.

**Table 5:** Effect of dietary treatments on the Enumeration of Lactic acid bacteria and intestinal colonization of *C. perfringens* of broiler chicks at 35 day of age.

Item	Lactic acid bacteria	Colonization of <i>C. perfringens</i>
Control	5X10 <sup>4c</sup>	2X10 <sup>2c</sup>
Antibiotic	5X10 <sup>3c</sup>	5X10 <sup>2a</sup>
Castor extract	4X10 <sup>8a</sup>	Nil <sup>d</sup>
Propolis extract	4X10 <sup>4d</sup>	3X10 <sup>2b</sup>
Castor+Propolis extracts	3X10 <sup>7b</sup>	Nil <sup>d</sup>
SE of means	±4X10 <sup>7</sup>	±51.35
Significances	***	***

Means designated with the same letter within the same column are not significantly different at 0.05 level of probability, \*\*\*P<0.001.

**EFFECT OF DIETARY TREATMENTS ON THE HUMORAL IMMUNITY OF BROILER CHICKS AT 35 DAY OF AGE**

The immune status assessment; the serum samples were subjected to haemagglutination inhibition (HI) test for determining antibody titers against Newcastle (ND) and avian flu (H5) vaccine are shown in Table 6.

**Table 6:** Effect of dietary treatments on the immune status assessment of broiler chicks at 35 day of age.

Item	Haemagglutination inhibition test	
	Newcastle (ND)	Avian flu (H5)
Control	3.8 <sup>c</sup>	4.3 <sup>b</sup>
Antibiotic	4.1 <sup>c</sup>	4.8 <sup>a</sup>
Castor extract	5.5 <sup>a</sup>	5.2 <sup>a</sup>
Propolis extract	4.6 <sup>b</sup>	3.8 <sup>b</sup>
Castor+Propolis extracts	5.3 <sup>a</sup>	4.9 <sup>a</sup>
SE of means	±0.19	±0.18
Significances	***	*

Means designated with the same letter within the same column are not significantly different at 0.05 level of probability, \*p<0.05, \*\*\*P<0.001, NS: Not significant (P>0.05).

The results showed the significantly (P<0.05) improvement in the immune status against both of ND and H5 vaccine in the birds fed diets containing CE and CE+PE. While the addition of PE to diets have a good immune response against ND but gave the similar immune response against H5 to the control. Also, Birds fed diets containing antibiotic gave a good immune response against H5 but have the similar immune response against ND to the control.

**DISCUSSION**

These results clearly showed that the addition of CE and CE+PE to the broiler diets recorded the best improvements in growth performance; intestinal length; diameter and lesion also gave the best values in intestinal beneficial and pathogenic bacteria counts and finally determined the best immune response against the ND and H5 vaccine. On the other hand, these results showed that addition of PE gave the similar results to the addition of antibiotic to the diet. The enhanced occurred in the broiler performance could be due to the improvements in the intestinal length, diameter and microbiota which increase nutrient digestibility and absorption.

The intestinal development (length and diameter) is relative to the changes in growth performance, therefore, addition of the castor extract or the mixture of castor and propolis extracts may have positively influenced the growth performance by enhanced the development of the

small intestine. The changes in the intestinal development were in accordance with the changes in the growth performance (Yin et al., 2017).

Therefore, the use of antibiotic as a growth promoter in broiler diets could be replaced with the highly safety materials such as CE, PE or the mixture of them which have the superior effect on the growth performance, intestinal parameters, intestinal microbiota and immune response compared to the antibiotic effect.

The results of enhanced performance observed in the present study by replacement of antibiotic by natural and safety extracts such as CE, PE and CE+PE are in accordance with many of previous studies. Mpraes et al. (2019 a,b) concluded that the use of functional oil (castor and cashew shell extracts) in broiler diets (0.15% inclusion) from 1-28 days improved the performance in similar to those receiving the ionophore monensin. The castor and cashew shell extracts acting as a modulator of the intestinal microbiota, with antimicrobial action against gram-positive bacteria, mainly *C. perfringens* and *S. aureus*. Valero et al. (2016) suggested that castor oil could be used instead of antibiotic in diets of cattle to improve performance and feed efficiency.

Propolis could improve the poultry performance, via enhancing nutrient digestibility and absorption; which stimulating saccharase, amylase and phosphatase activities (Marieke et al., 2005). The addition of propolis (0.05 g/Kg feed) to the broilers diets gave comparable performance to the other group, which fed a diet with antibiotic (Kleczeck et al., 2014; Hascik et al., 2016). Shaddel-Tili et al. (2016) found that weight gain is directly proportional to the increase in dose of propolis in broiler diets.

Shreif and El-Saadany (2017) observed that gain of body weight increased with the increase in propolis level in diet on Bandarah chicks during the experimental periods. Khan, (2017) suggested that added of propolis in diets of broiler enhanced the growth. Zafarnejad et al. (2017) found that the body weight, average daily weight gain, feed intake, carcass characteristics were significantly higher in broilers fed diets with propolis compared to the control group.

Bess et al. (2012) found that the addition of castor oil-CNSL mixture (0.15%) to the broiler diets from 1-21 d of age lead to enhanced the broiler performance, which might caused by the antimicrobial effect of these functional oil. Using of castor oil and cashew shell extracts (0.15% inclusion) in the diet of coccidiosis challenged broilers resulted in enhanced body weight gain and feed conversion ratio (Murakami et al., 2014).

Coneglian et al. (2019) who found that monensin (conventional antibiotic) could be replaced with essential oils of castor bean in beef cattle diets without influencing feed intake, digestibility, ruminal fermentation or microbial proteins synthesis.

As well, the present results of the effect of dietary treatments on the intestinal length, diameter, lesion, the Enumeration of Lactic acid bacteria, intestinal colonization of *C. perfringens* and the immune status assessment of broiler chicks are in agreement with many of the previous studies. Some of plant extracts have highly effect to eliminate bacteria or stunt bacterial growth (e.g. *Salmonella enterica* serovar Typhimurium) when added to animal diets such as Castor extract (Castillo-López et al., 2017). Castor bean (*Ricinus communis* L.) has an importance crop because it has highly active compounds like castor oil, which reach in ricinoleic acid and mixtures of volatile secondary metabolites, that support uses of it is extract as antimicrobial (Kiran and Prasad, 2017; Salih et al., 2019). Castor extract showed the highly antimicrobial activity against most pathogenic bacteria included both positive and negative gram for example *E. coli*, *P. aeruginosa*, *Bacillus cereus* and *Listeria innocua* (Rampadarath and Puchooa, 2016). In addition, it has antifungal activities about 75% (Das and Satyaprakash, 2018). The changes in the intestinal development were in accordance with the changes in the growth performance (Yin et al., 2017).

Krocko et al. (2012) found that the supplemented of propolis and bee pollen to the broiler diets enhanced this intestinal microbiota. Kacaniova et al. (2013) reported that increased the beneficial bacteria count (Enterococci and Lactobacilli) while decreased the pathogenic bacteria count in the intestine of the broilers fed a diet containing propolis (0.2 or 0.6 g/kg feed).

## CONCLUSION

The addition of either castor extract or castor and propolis extracts mix to the bird's diets gave superior in all measurements than the addition of antibiotic. Furthermore, using of propolis extract gave the comparable results to the antibiotic treatment. Therefore, this more effective and safety materials have to use instead of using antibiotic as growth promoter in broiler diets.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## AUTHORS CONTRIBUTION

All authors contributed equally to study design methodology, interpretation of results, and writing of the manuscript.

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