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The effect of feeding a commercial essential oil product to broilers challenged with *Clostridium Perfringens*

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The aim of the present study was to investigate the influence of a commercial essential oil blend (EO), CRINA® Poultry (CP), as an alternative to in-feed antibiotic, enramycine®, on growth parameters of broilers during pre- and post-challenge with C*lostridium perfringens* (*C. perfringens*). The birds received treatments from 0 to 30 days as follows: T1 = positive control (+CONT): without medication or bacterial challenge; T2 = negative control (-CONT): without medication but with *C. perfringens* challenge; T3 = antibiotic with addition of enramycin with *C. perfringens* challenge (ENRA); and T4 = with addition of CRINA® Poultry to feed with *C. perfringens* challenge (CP). Overall, feed intake (FI), body weight gain (BWG) and feed conversion ratio (FCR) was not significantly different among the four treatments (P>0.05). On the other hand, EO supplementation caused some changes in the intestinal mucosa morphometrics; birds which had received EO had longer ileal villi as compared to +CONT or ENRA groups (P<0.05). The performance of the birds which had consumed CP was similar to all other treatments. The results from this study indicate that CP under the condition of this experiment had no influence on broilers performance but it had a positive effect on mucosal morphometrics.

Key words: Broiler, Crina®Poultry, enramycine, intestinal mucosa morphometrics, performance.

INTRODUCTION

The advantages of antibiotic as antimicrobial growth promoters (AGPs) for poultry when used at sub therapeutic levels are well documented (Hume et al., 2011). The AGPs have been used in animal nutrition since 1946, when their positive effects were observed for the first time (Moore et al., 1946). However, recently there are increasing concerns about the risk of developing cross-resistance and multiple antibiotic resistances in pathogenic bacteria, which could result in proliferation of antibiotics-insensitive bacteria; this could lead to a decrease in the therapeutic effectiveness of antibiotics used by humans (Castanon, 2007).

Clostridium perfringens can cause a subclinical disease associated with necrotic enteritis (NE) which is characterized by damage to the intestinal mucosa that decreases digestion, absorption and reduces weight gains which can result in great economic losses in poultry production (Stringfellow et al., 2009; Wu et al., 2010, 2011).

Current trends in poultry production point to reduce the use of AGPs and increase the use of non-antibiotic feed additives. Essential oils (EO) are among the alternative growth promoters that are already in use in practice (Mitsch et al., 2004). Essential oils are extracted from

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herbs and spices by steam distillation; most of these compounds are phenolic (thymol (*thymus vulgaris*), eugenol (*Syzygiumaromaticum*), curcumin (*Curcuma peppermint*) and piperin (*Piper nigrum*) with varying antimicrobial activity (Lee et al., 2004). Several reports showed that specific components of EO inhibit growth of many bacteria, including *C. perfringens in vitro* (Briozzo et al., 1988; Dorman and Deans, 2000) and *in vivo* (Mitsch *et al*., 2004). On the other hand, dietary concentrations of 100 and 200 mg/kg feed of CP failed to reduce the intestinal numbers of *C. perfringens* compared to a control group (Abildgaard *et al.,* 2010).

The effect of EO on broilers performance is controversial and depends on the active material of the EO. Tiihonen et al. (2010) reported that a blend of EO (thymol and cinnamaldehyde) added to soybean meal (SBM)-wheat-based diet increased body weight gain (BWG) of broilers from 0 to 42 days by 45%. Likewise, Francesch et al. (1999) noted that CRINA poultry supplementation to wheat and-barley-based diet improved feed conversion ratio (FCR) in broilers. On the other hand, the supplementation of CP blend to coccidia vaccinated broilers did not show any benefits in older birds (Oviedo-Rondón et al., 2006). Likewise, CP failed to improve BWG and FCR of broilers which were vaccinated against coccidiosis (Abildgaard et al., 2010). Further, Shanmugavelu et al. (2004) concluded that EO from thyme and garlic did not improve the nutritional value of the diet.

Therefore, this study was conducted to investigate the efficacy of a commercial EO (CRINA® Poultry) to replace typical AGPs (enramycine) and compare their effects on growth parameters, histomorphology and ileal bacterial count of broilers raised in cages under *C. perfringens* challenge.

MATERIALS AND METHODS

Animals, husbandry and treatments

A total of one hundred (100), 0-day old male broiler chicks (Ross 308) were obtained from a commercial hatchery where they were vaccinated for Marek's disease, Newcastle disease and Infectious Bronchitis. At arrival, chicks were sexed, grouped by weight in such a way as to reduce variation in mean body weight, and then were allotted to 20 cages according to the treatment. Five chicks were placed in each cage (50 cm length, 60 cm width and 36 cm depth) in a four-deck cage system and received the experimental diets in electrically heated battery brooders with raised wire floors.

Birds received one of the following four treatments: T1 = Control diet (+CONT): without medication or bacterial challenge; T2 = T1 + *C. perfringens* challenge (-CONT): without medication but with bacterial challenge; T3 = 0.1 g/kg of feed of enramycin (ENRA) with bacterial challenge; T4 = 0.01% of feed as dry CRINA® Poultry (CP) with bacterial challenge. The EO component used in this studies (CRINA® Poultry, DSM Nutritional Products Ltd. Basel, Switzerland), is commercially available and contains primarily 29% of active components, including thymol, according to the manufacturer. On day 16, birds in treatments two to four were challenged with *C. perfringens* using 10-fold dose of anticoccidial vaccine (Paracox-8), on days 18 and 20 chicks were gavaged with 1 ml of a cocktail containing *C. perfringens* inoculations (4 x 10^8 CFU) which was prepared from culture of *C. perfringens* that was obtained commercially (MicroBiologics, Cloud, MN-U.S.A) (Jayaraman et al., 2013).

A typical isocaloric and isonitrogenous starter (0-16 days) and finisher (17-30 days) diets based on corn-soybean meal diets were formulated in mashed form which met or exceeded the recommendations in commercial practice in Saudi Arabia (Table 1). Ambient temperature and relative humidity were concurrently and continuously recorded at 3 h interval using two data loggers (HOBO Pro Series Data Logger, Model H08-032-08, Onset Co., USA) placed inside the chamber. The average temperature and relative humidity for the whole period were 24.95°C ± 0.26 (SD) and 26.63% ± 3.30 (SD), respectively. The study was conducted under a protocol approved by King Saud University and complied with the current laws of Saudi Arabia.

Measurements

Feed intake and BWG were recorded weekly by pen and FCR computed at 16 and 30 days of age. Mortality was checked daily and weights of dead birds were used to adjust FCR. At the conclusion of the trial (30 days), five birds per treatment were selected, after euthanasia, feather and skin, heads, necks, and shanks were removed, and the remaining carcasses were dissected to breast and leg quarter and were weighed. The percentage of yield of each part was calculated on the basis of dressed weight. At 16 and 30 days of age, the entire small intestine tract from five birds per treatment was removed aseptically, weighed and the total length was measured then was separated into duodenum, jejunum and ileum and for each part measurements of length and weight were taken. A 2-cm-long sample from each portion of the small intestine was collected for histology measurements. Samples were fixed in phosphate-buffered formalin for at least 48 h, after which they were embedded in paraffin. Sections of 5 mm were cut and stained with haematoxylin and eosin for measurements of height and width which were based on at least 4 well-oriented villi per section per broiler. The measurements were done by using an IX71 Inverted Olympus Microscope (Eyepiece: WH10X, Objective Lens: 4X). Cellsens Digital Imaging Software for Research Application software was used for calculations.

Ileal digesta contents were aseptically emptied in a new sterile bag and kept in ice until time of analyses. Samples were diluted in 0.9% saline and 0.1 ml of each sample was plated on duplicates by using selective agar media for enumeration. Tryptose sulfite-cycloserine (TSC) agar media was used for *C. perfringens* enumeration (Oxoid CM587 with the addition of SR88 and SR47). Colonies on TSC agar that were suspected to be *C. perfringens* were plated secondarily on blood agar (Garridol et al., 2004). *Enterobacteriaceae* and *Salmonella* were identified by API 20E. The API 20E strips (bioMérieux, Craponne, France) were inoculated, incubated at 37°C for 24 h and interpreted as recommended by the manufacturer. Reactions were recorded and identifications were determined by using a computer program [API Lab Plus software version 3.2.2 (bioMérieux)]. Results were expressed as $\log_{10}$ colony-forming units per gram of ileal digesta (log CFU/ g).

Statistical analysis

Data were analyzed by using the general linear model procedure of

Table 1. Composition of the experimental diets.

Ingredient	Starter			Finisher		
	Treatment					
	1 & 2	3[A]	4[A]	1 & 2	3[A]	4[A]
Yellow corn	56.00	55.99	55.99	57.75	57.74	64.12
Soybean meal	36.10	36.10	36.10	34.0	34.0	34.0
Palm oil	3.80	3.80	3.80	4.80	4.80	4.80
DCP	2.30	2.30	2.3	2.0	2.0	2.0
Ground limestone	0.72	0.72	0.72	0.64	0.64	0.64
Choline chloride	0.10	0.10	0.10	0.05	0.05	0.05
DL-methionine	0.23	0.23	0.23	0.16	0.16	0.16
L-lysine	0.15	0.15	0.15	-	-	-
Salt	0.30	0.30	0.30	0.30	0.30	0.30
Vitamin premix[B]	0.25	0.25	0.25	0.25	0.25	0.25
Trace mineral mix[C]	0.05	0.05	0.05	0.05	0.05	0.05
Enramycin	-	0.01	-	0.00	0.01	0.00
Crina Poultry	-	-	0.01	-	-	0.01
Total	100	100	100	100	100	100
Calculated analysis						
ME (kcal/kg)	3000	3000	3000	3100	3100	3100
Crude protein (%)	22.0	22.0	22.0	21.0	21.0	21.0
Non phytate P (%)	0.45	0.45	0.45	0.40	0.40	0.40
Calcium (%)	1.0	1.0	1.0	0.9	0.9	0.9
Lysine (%)	1.25	1.25	1.25	1.1	1.1	1.1
Methionine (%)	0.55	0.55	0.55	0.47	0.47	0.47

[A]Diet 3 had 0.01% Enramycin, diet 4 had 0.01% Crina Poultry on the expense of corn during starter and finisher; [B]vitamin mix is supplied in the following per kg of diet: Retinyl acetate, 3.41 mg; cholecalciferol, 0.07 mg; DL-α-tocopheryl acetate, 27.5 mg; menadione sodium bisulphate, 6 mg; riboflavin, 7.7 mg; niacin, 44 mg; pantothenic acid, 11 mg, cyanocobalamin, 0.02; choline 496 mg; folic acid, 1.32 mg; pyridoxine HCl, 4.82 mg; thiamine mononitrate, 2.16 mg; D-biotin, 0.11 mg; [C]Mineral-mix is supplied in the following per kg of diet: manganese, 67 mg; zinc, 54 mg; copper, 2 mg; iodine, 0.5 mg; iron, 75 mg; and selenium, 0.2 mg.

SAS (SAS, 2002-2003). Four treatments were replicated five times in a randomized complete block design; a cage constituted the experimental unit. Means for measurements showing significant differences in the analysis of variance were tested using the PDIFF option. Means ± SEM are presented in the tables and differences were considered statistically significant at $P<0.05$.

RESULTS AND DISCUSSION

Feed intake, BWG and FCR of male broiler at different ages are shown in Table 2. During the starter (0-16 days) and the finisher (17-30 days) periods, BWG, FI and FCR were not influenced ($P>0.05$) by treatment. As a result, cumulative (0-30 days) FCR was not significant ($P>0.05$), even though there was a numeric improvement in cumulative FCR for birds which had received ENRA or CP as compared to -CONT (1.511, 1.535 and 1.611 g: g, respectively). The mean percentage of carcass parts in different treatments is documented in Table 3. Treatment had no effect on dressing percentage breast muscle yield, leg quarter yield, abdominal fat and relative liver weight ($P>0.05$).

The results of this study are in general agreement with previous reports (Abildgaard et al., 2010; Oviedo-Rondón et al., 2006). It is worth mentioning that both previous reports used CP as EO and the birds were vaccinated against coccidiosis. However, in the current study, a mega dose of anticoccidial vaccine (Paracox-8®, Schering-Plough Animal Health, England) was used as a part of the protocol to induce the *C. perfringens* challenge. The inclusion level of CP recommended by the manufacturer was used in this trial, so the lack of effect of

Table 2. Body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR) of broiler chickens given experimental diets at different ages.

Parameter	Treatment				SEM	P
	T1	T2	T3	T4		
Performance at 16 days						
BWG (g)	479.2	481.2	515.2	486.2	±14.0	NS
FI (g)	638.0	645.6	652.6	631.0	±20.8	NS
FCR(g: g)	1.333	1.342	1.267	1.300	±0.03	NS
Performance at 30 days						
BWG (g)	886.3	832.1	869.5	880.4	±26.7	NS
Feed (g)	1489.6	1468.9	1432.5	1464.6	±36.4	NS
FCR (g: g)	1.718	1.770	1.656	1.666	±0.04	NS
Cumulative						
BWG (g)	1345.3	1313.3	1384.6	1366.5	±34.5	NS
Feed (g)	2127.4	2114.5	2084.8	2095.8	±36.1	NS
FCR(g: g)	1.583	1.611	1.511	1.535	±0.03	NS
Livability (%)	100.0	92.0	92.0	92.0	±6.939	NS

Table 3. Effect of different treatments on parts yield as percentages of broiler dressed weight at 30 days of age.

Parameter	Treatment				SEM	*P*
	T1	T2	T3	T4		
Dressed yield (%)	60.9	60.7	62.1	58.9	±1.38	NS
Breast (%)[1]	35.6	35.2	34.2	34.3	±0.56	NS
Leg quarter (%)[1]	40.4	40.3	40.2	41.2	±0.61	NS
Abdominal fat (%)	1.05	0.80	1.26	1.14	±0.20	NS
Liver (g/100 g)	0.31	0.24	0.28	0.31	±0.02	NS

[1]Breast and leg quarter were expressed as percentage of the carcass weight.

the supplements on performance could be related to the anticoccidial vaccine. Other reports show that dietary essential oil components improve performance of broiler chickens through different mechanisms such as stimulating the activity of endogenous digestive enzymes and enhancing nutrient digestibility (Francesch et al., 1999; Tiihonen et al., 2010; Williams and Losa, 2001).

The morphometric measurements of the intestinal epithelium samples at 16 days of age are given in Table 4. Percentage of duodenal, jejunal and ileal length showed significant differences due to treatments (P<0.01, 0.001, 0.001, respectively). Duodenum and jejunum from birds which had received +CONT were the longest among all treatments while the ileum from the same group was the shortest. Intestine weight and relative weight were not affected by any treatment (P>0.05). Ileal villus height was significantly shorter for birds which had received ENRA.

On the other hand, ileal villus width was not influenced by treatment (P>0.05). At 30 days of age, lower duodenum length percent was obtained from bird which had received ENRA treatment (P<0.05). Similar result was reported by Oliveira et al. (2008) who found that antibiotic supplementation caused low villi height; they explained that by the suppressing effect of the antibiotic for the beneficial bacteria in the gut, as *Lactobacillus* and *bifidobacteria*. On the other hand, birds which had received CP had longer ileal villi (P<0.05); while those received ENRA had wider duodenal villi as compared to all other treatments (P<0.05). Long villi are usually equated with excellent gut health, high absorptive efficiency and healthier intestinal tract of chickens (Alfaro *et al.,* 2007). According to Cera et al. (1988), maximum absorption and digestion capacity is provided by a large luminal area with villus height and mature enterocytes and is essential to animal development. Jejunal villus height and width were similar among all treatments (P>0.05).

Table 4. Intestinal morphology and histology of broilers at 16 days of age

Parameter	Treatment				SEM	*P*
	T1	T2	T3	T4		
Intestine length (cm)	129.7	132.0	127.0	130.3	±7.20	NS
Duodenum length (%)	19.5^{a}	17.4^{b}	16.8^{bc}	15.9^{c}	±0.44	**
Jejunum length (%)	47.8^{a}	41.6^{b}	41.5^{b}	39.9^{b}	±0.71	***
Ileum length (%)	32.7^{c}	41.1^{b}	41.7^{ab}	44.3^{a}	±0.86	***
Intestine weight (g/ cm)	0.28	0.21	0.27	0.24	±0.02	NS
IRW^{A} (g/100g BW)	7.4	7.6	7.2	8.0	±0.74	NS
Ileal *Villus height*^{B} *(µm)*	4509^{a}	4296^{ab}	3804^{b}	4378^{a}	±155.7	*
Ileal *Villus width*^{B} *(µm)*	693	766	653	879	±59.9	NS

^{A}IRW: intestine relative weight; ^{B}Measurements of height and width were based on at least 5 well-oriented villi per ileum per broiler for a total of 5 birds per treatment; ^{abc}means in the row with different superscripts differ significantly (*P<0.05, **P<0.01, ***P<0.001).

Table 5. Intestinal morphology and histology of broilers at 30 days of age.

Parameter	Treatment				SEM	P
	T1	T2	T3	T4		
Intestine length (cm)	152.0	178.0	176.2	174.3	±6.60	NS
Duodenum length (%)	15.7^{ab}	16.6^{a}	14.8^{bc}	16.6^{a}	±0.37	*
Jejunum length (%)	52.1	41.8	43.2	41.7	±4.18	NS
Ileum length (%)	32.2	41.7	42.1	41.7	±4.19	NS
Intestine weight (g/ cm)	0.36	0.44	0.49	0.49	±0.03	NS
IRW^{A} (g/100g BW)	4.7	5.7	5.5	6.4	±0.35	NS
Villus height ^{B} (µm)						
Duodenum	8964	8315	8480	10068	±445	NS
Jejunum	7208	8449	9467	9659	±597	NS
Ileum	5869^{bc}	6643^{ab}	4692^{c}	7664^{a}	±515	*
Villus width^{B} (µm)						
Duodenum	1245^{b}	1298^{b}	1647^{a}	1316^{b}	±139	*
Jejunum	1109	938	1088	1245	±81	NS
Ileum	843	882	851	736	±108	NS

^{A}IRW: intestine relative weight; ^{B}Measurements of height and width were based on at least 5 well-oriented villi per section per broiler for a total of 5 birds per treatment; ^{abc}means in the row with different superscripts differ significantly (*P<0.05).

Data related to ileal bacterial count in broilers at 16 and 30 days of age are presented in Table 6. Similar bacterial count of *C. perfringens* and gram negative *Bacilli* were found in the starter period (before the challenge) and finisher (after the challenge) (P>0.05). The results obtained from this trial clearly show that CP supplementation can failed to control the proliferation of *C. perfringens* in the intestine. Similar conclusion was obtained by Abildgaard et al. (2010) by using two levels of CP (recommended by the manufacturer and double the dose); in both cases the EO did not reduce the intestinal numbers of *C. perfringens* compared to a non-supplemented group. Several other groups used the same EO supplement as in this experiment and came to different findings. Losa and Kohler (2001) gave 50 ppm of CP; they found a reduction in the *C. perfringens* count in the intestinal content. Similarly, the findings of Mitsch*et al.* (2004) correspond to those of Losa and Kohler (2001). In this experiment, birds were challenged with *C. perfringens* to mimic a challenge that a bird could face in rearing facilities. *C. perfringens* is among the most gut-specific pathogens which is assumed to be the main health problem associated with removing the antibiotics from feed (Keyburn et al., 2013).*C. perfringens* infection of broilers may cause impairment of production performance (Stringfellow et al., 2009; Wu et al., 2011)

Table 6. Ileal bacterial count in broilers at 16 and 30 days of age.

Parameter	Treatment				SEM	*P*
	T1	T2	T3	T4		
	Mean (log_{10} CFU/ g)					
Starter						
C. perfringens	4.2	3.9	4.3	4.8	±0.20	NS
Gram negative Bacilli	3.8	4.3	4.3	3.8	±0.28	NS
Finisher						
C. perfringens	4.7	5.8	4.8	3.4	±0.91	NS
Gram negative Bacilli	4.8	4.7	5.0	5.0	±0.18	NS

Measurements of were based on 5 broilers per treatment.

which was explained by the high level of bile salt hydrolase activity in the *C. perfringens* which causes growth depression (Feighner et al., 1987).

Many challenged birds showed distinctly pronounced pathological changes in the intestinal tissue. The gross examination of the responses in birds challenged orally with *C. perfringens* showed sub-clinical inflammatory responses throughout various sections of gizzard, duodenum, jejunum, ileum, and ceca associated with intestinal lesions and hemorrhages. However, none of the challenged birds produced overt clinical signs of necrotic enteritis and there were no mortalities associated with oral exposure to high doses of *C. perfringens*. This finding may be due to the absence of stress related to other diseases, diets or environment; all these factors with the presence of *C. perfringens* could cause an outbreak of necrotic enteritis (Ficken and Wages, 1997).

Conclusion

In this experiment, CP failed to reduce ileal bacterial count as a result the performance was not improved significantly. Essential oil supplementation caused some significant changes in the intestinal mucosal morphometric; birds which had received EO had longer ileal villi as compared to +CONT or ENRA groups which is correlated to better absorption capacity.

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