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Use of a Competitive Exclusion Product (Aviguard®) to Prevent *Clostridium perfringens* Colonization in Broiler Chicken under Induced Challenge

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Abstract.- The effect of Aviguard (AG) on growth parameters, gut health and control of pathogenic colonization of broilers was examined under C*lostridium perfringens* (*C. perfringens*) challenge. Chicks (1-d old) received one of the following treatments: 1. positive control (CONT): with no additions; 2. negative control + *C. perfringens* challenge ($CONT_{cp}$); 3. coarse spray of AG at d 1 + *C. perfringens* challenge (AG_{spray}); or 4. AG in drinking water at d 8 + *C. perfringens* challenge (AG_{water}). Overall, feed conversion ratio (FCR) in treatment AG_{water} was significantly better ($P < 0.05$) than the $CONT_{cp}$ or AG_{spray} treatments, whereas CONT was intermediate and not different from AG_{water}. The improvement in FCR was due to reduction in feed intake (FI) in AG_{water}, while body weight gain (BWG) was not influenced by treatment. AG_{water} was very effective in eliminating *C. perfringens* that was induced by necrotic enteritis infection model. The changes in *C. perfringens* count appear in parallel to observed changes in villi width in the small intestine. The results from this study indicated that AG_{water} can be considered as an effective nonantibiotic alternative *for C. perfringens* prevention in broilers.

Key words: Competitive exclusion, Aviguard, performance, broiler, C*lostridium perfringens*.

INTRODUCTION

There are increasing concerns about the risk of developing cross-resistance and multiple antibiotic resistances in pathogenic bacteria in both humans and poultry linked to the therapeutic and subtherapeutic use of antibiotics in livestock (Castanon, 2007). Current trends in poultry production point to reduce use of antimicrobial growth promoters (AGPs) and increase use of non-antibiotic feed additives such as direct-fed microbials (DFM) (Mountzouris *et al*., 2007).

Many researchers are now focused on identifying viable alternatives to antibiotics that offer similar benefits, such as to improve the growth of broilers, improve the utilization of feed, and in this way realize better production and financial results. Several groups of these additives are in use such as AG, a competitive exclusion product (CE), which contains a mixture of live bacteria that represent the bacterial populations in the cecum of adult chickens. Generally, CE products are competing for locations to adhere to the intestinal mucous membranes, preventing pathogenic microorganisms from inhabiting the intestinal tract and competition for nutritious substances (Line *et al*, 1998; Patterson and Brukholder, 2003).

Aviguard (AG) is commercially available for use in chickens and turkeys as spray treatment or drinking water application (Schering-Plough Animal Health, Kenilworth, NJ, USA). AG effect on broiler production can be expressed in reduction of *Salmonella* colonization (Nakamura *et al*., 2002), reducing gross lesions and mortality caused by *C. perfringens* associated necrotic enteritis (Hofacre *et al*., 1998a), improving the immune system (Nakamura *et al*., 2002). These effects lead to better growth and FCR in broilers and improvement in livability of the flock (Hofacre *et al*., 1998b).

The objective of this study was to determine the efficacy of AG administered in drinking water or as a coarse spray on growth performance, histomorphology and bacterial count of small intestinal mucosa in broilers raised in cages under subclinical C. *perfringens* model.

MATERIALS AND METHODS

Animals, husbandry and treatments

Ross 308 chicks were obtained from a

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commercial hatchery (Al-Wadi Poultry Farm Co., Riyadh, Saudi Arabia). Chicks were sexed, grouped by weight in such a way as to reduce variation in mean body weight, a total of 120, 1-d old female broiler chicks allotted to 20 cages (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. The chicks had been vaccinated for Marek's disease, Newcastle and Infectious Bronchitis. Birds were maintained at 24h light schedule.

Birds received one of the following four treatments: 1) Control, without any addition or challenge (CONT); 2) Control + *C. perfringens* challenge ($CONT_{cp}$); 3) Aviguard was sprayed to 1 d old chicks + *C. perfringens* challenge (AG_{spray}); 4) Aviguard was administered in drinking water for 4 h at d 8 + *C. perfringens* challenge (AG_{water}). The doses used in treatments 3 and 4 were according to the manufacturer recommendations (Schering-Plough Animal Health, Kenilworth, NJ, USA). On d 16, birds in treatments 2 to 4 were challenged by *C. perfringens* using overdose of anticoccidial vaccine namely Paracox-8 (10-fold dose), and on d-18 and 20 chicks were gavaged with 1 ml of a cocktail containing *C. perfringens* inoculations (4×10^8 CFU) according to the procedure described by Gholamiandehkordi *et al*. (2007). Culture of *C. perfringens* was obtained commercially (MicroBiologics, Cloud, MN, USA) and was propagated under anaerobic conditions for 16 hours at 37°C in screw cap tubes; cells were harvested by centrifugation (4500 g at 4°C) and diluted in physiological saline. A typical isocaloric and isonitrogenous starter (0 to16 d) and finisher (17 to 30 d) diets based on corn-SBM diets were formulated in mashed form according to Table I which met or exceeded the recommendations in commercial practice in Saudi Arabia. Ambient temperature and relative humidity were concurrently and continuously recorded at 3 hours 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±0.26°C (SD) and 26.63±3.30% (SD), respectively. The study was conducted in May and June, 2012 under a protocol approved by King Saud University and complies with the current laws of Saudi Arabia.

Table I.- Dietary composition and calculated analysis of starter and finisher diets.

Ingredients	Starter	Finisher
Yellow corn	56.23	57.75
Soybean meal	36.10	34.0
Palm oil	3.80	4.80
Dicalcium phosphate	2.30	2.0
Ground limestone	0.72	0.64
Choline chloride	0.10	0.05
DL-methionine	0.23	0.16
L-lysine	0.15	-
Salt	0.30	0.30
Vitamin premix[1]	0.25	0.25
Trace mineral mix[2]	0.05	0.05
Total	**100**	**100**
Calculated analysis		
ME (kcal/kg)	3000	3100
Crude protein (%)	22.0	21.0
Non phytate P (%)	0.45	0.40
Calcium (%)	1.0	0.9
Lysine (%)	1.25	1.1
Methionine (%)	0.55	0.47

[1]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; panthotenic acid, 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.

[2]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.

Carcass measurements

At d 30, five birds per treatment were selected, after euthanasia, feather, heads 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.

Histopathology and morphometric measurements

At d 30, the entire gastrointestinal tract from five birds per treatment was removed aseptically, small intestine was weighed and the total length was measured then was separated into duodenum, jejunum (proximal to Meckel's diverticulum) and ileum (proximal to ileo-caecal junction) and for each part measurements of length and weight were

taken. A 2-cm-long samples from each portion of the small intestine were 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. Measurements of height and width were based on at least 5 well-oriented villi per section per broiler using an IX71 Inverted Olympus Microscope (eyepiece: WH10X, objective lens: 4X) and a PC-based image analysis system (Olympus DP72 Microscope Digital Camera; Olympus NV, Aartselaar, Belgium) with software Analysis (Cellsens Digital Imaging Software for Research Application).

Enumeration and identification of bacterial cells

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 of target bacterial groups. *C. perfringens* was counted on tryptose sulfite-cycloserine (TSC) agar for *C. perfringens* (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 were isolated on MacConkey agar (Oxoid CM7) after an incubation time of 24 h in an aerobic atmosphere at 37° (Garridol *et al.*, 2004). Isolates of Enterobacteriaceae and *Salmonella* were identified by API 20E. The API 20E strips (BioMerieux Vitek) were inoculated, incubated at 37°C for 18 to 48 h, and interpreted as recommended by the manufacturer. Results were expressed as log10 colony-forming units per g of ileal digesta (log_{10} CFU/ g).

Statistical analysis

All statistical analysis was performed using the Statistical Analysis System (SAS, 2002-2003). A cage constituted the experimental unit. Three treatments were replicated 5 times in a randomized complete block design. Means for measurements showing significant differences in the analysis of variance were tested using the PDIFF option. Means±standard error of the mean (SEM) are presented in the tables and differences were considered statistically significant at $P < 0.05$.

RESULTS

Performance observations at 16 and 30 d of age are listed in Table II. During the starter period, body gain weight (BWG) was not influenced ($P>0.05$) by treatment. However, feed intake (FI) and feed conversion rate (FCR) showed differences due to treatment, birds which had received AG_{water} consumed less amount of feed as compared to all other treatments as a result FCR for this group was better ($P<0.001$). During the finisher period, no significant differences in BWG, FI or FCR were observed. Cumulative data (d 1 to 30) showed similar trend to the starter period, birds which had received AG_{water} consumed less amount of feed from 1 to 30 d of age and had the best FCR as compared to all other treatments ($P<0.05$). The mean percentage of carcass parts in different treatments is documented in Table III. No difference in dressing percentage, breast muscle yield, leg quarter yield or abdominal fat was noticed between treatments ($P<0.05$).

The morphometric measurements of the intestinal epithelium samples at 30 d of age are given in Table IV. No significant differences were observed in intestine length, weight or IRW due to treatment ($P>0.05$). Duodenal villus from birds which had received CONT was shorter than those in birds received treatments $CONT_{cp}$ or AG_{spray} ($P < 0.001$). Jejunal villus from birds which had received CONT was shorter than those received treatment $CONT_{cp}$ or AG_{water} ($P<0.05$). Ileal villus height was significantly longer for birds which had received treatments AG_{spray} or AG_{water} as compared to treatments CONT or $CONT_{cp}$ ($P<0.05$). On the other hand, ileal villi were wider in the birds received AG_{water} as compared to those received $CONT_{cp}$ ($P<0.01$). Ileal villus width in AG_{water} was higher than CONT or $CONT_{cp}$ groups ($P<0.05$).

Data related to ileal bacterial counts in broilers at 16 and 30 d 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). At the end of the

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

	Treatment				SEM	P
	CONT	$CONT_{cp}$	AG_{spray}	AG_{water}		
Performance at 16 d						
BWG (g)	443.2	453.0	444.2	437.9	±8.9	NS
FI (g)	609.9[a]	612.0[a]	631.1[a]	523.9[b]	±74.3	***
FCR(g: g)	1.379[a]	1.351[a]	1.422[a]	1.197[b]	±0.03	***
Performance at 30 d						
BWG (g)	807.8	753.1	739.2	725.3	±29.49	NS
Feed (g)	1262.1	1286.9	1277.1	1216.7	±74.3	NS
FCR (g: g)	1.564	1.712	1.720	1.680	±0.09	NS
Cumulative						
BWG (g)	1250.9	1260.1	1183.4	1163.1	±30.8	NS
Feed (g)	1880.0[a]	1906.1[a]	1896.3[a]	1624.2[b]	±68.7	*
FCR(g: g)	1.504[ab]	1.581[a]	1.599[a]	1.397[b]	±0.05	*
Livability (%)	100.0	92.0	92.0	92.0	±6.939	NS

[abc]means in the row with different superscripts differ significantly (* $p < 0.05$, N.S: Not significant).
CONT, without any addition or challenge; $CONT_{cp}$, control + *C. perfringens* challenge; AG_{spray}, Aviguard sprayed to 1 d old chicks + *C. perfringens* challenge; AG_{water}, Aviguard was administered in drinking water for 4 h at d 8 + *C. perfringens* challenge. BWG, body weight gain; FRC, feed conversion rate; F1, feed intake.

Table III.- Effect of different treatments on parts yield as percentages of broiler dressed weight at d 30.

	Treatment				SEM	P
	CONT	$CONT_{cp}$	AG_{spray}	AG_{water}		
Dressed yield (%)	70.1	69.5	69.9	70.1	±0.21	NS
Breast (%)[1]	33.7	34.1	33.4	35.8	±0.91	NS
Leg quarter (%)[1]	43.0	41.1	41.3	40.1	±0.93	NS
Abdominal fat (%)	1.87	1.03	1.67	1.63	±0.31	NS
Liver (g/100 g)	0.43	0.50	0.40	0.43	±0.05	NS

[1]Breast and leg quarter were expressed as percentage of the carcass weight
N.S: Not significant.
For details of treatment, see Table II.

experiment, the ileal *C. perfringens* count in birds which had received AG_{water} was not detectable and was the lowest among all groups ($P < 0.001$). No differences in gram negative Bacilli were found because of the treatment.

DISCUSSION

AG administration to drinking water resulted in 20 points improvement in FCR as compared to the spray method and 18 points improvement in FCR as compared to $CONT_{cp}$. This could be explained by the significant reduction in FI which was associated with the group AG_{water}, while BWG was similar among all groups. Birds which had received AG_{water} consumed 256, 282 and 272 g of feed less than those received treatments CONT, $CONT_{cp}$ or AG_{spray}, respectively. There was no significant difference in FCR in birds which had received AG_{spray} to those which had received CONT. Evidence has been presented that AG improves performance and FCR in broilers. The results of this study are in general agreement with results reported by Hofacre et al. (1998b) who reported that overall FCR for treatment AG which was administered orally at 1 d in *C. perfringens* challenged birds was

Table IV.- Intestinal morphology and histology of broilers at 30 d of age.

	Treatment				SEM	P
	CONT	$CONT_{cp}$	AG_{spray}	AG_{water}		
Intestine length (cm)	155.7	153.5	181.5	151.5	±9.3	NS
Duodenum length (%)	14.8	16.5	15.8	19.4	±1.2	NS
Jejunum length (%)	45.5	43.7	41.0	48.7	±3.3	NS
Ileum length (%)	39.7	39.8	43.2	31.8	±4.4	NS
Intestine weight (g/ cm)	0.33	0.40	0.47	0.53	±0.05	NS
IRW[1] (g/100g BW)	5.1	6.0	6.83	6.5	±0.45	NS
Villus height [2] (µm)						
Duodenum	7768^{b}	9964^{a}	9640^{a}	7989^{ab}	±373	***
Jejunum	7318^{b}	9392^{a}	8483^{ab}	8891^{a}	±515	*
Ileum	6245^{b}	5206^{c}	6849^{a}	7225^{a}	±133	*
Villus width[2] (µm)						
Duodenum	1179^{ab}	1080^{b}	1268^{ab}	1363^{a}	±67	**
Jejunum	1158^{b}	1104^{b}	1200^{ab}	1316^{a}	±45	*
Ileum	843	882	851	736	±108	NS

[1]IRW: intestine relative weight.
[2]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 (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, N.S: Not significant).
For details of treatment, see Table II.

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

	Treatment				SEM	P
	CONT	$CONT_{cp}$	AG_{spray}	AG_{water}		
Starter Mean (log_{10} CFU/g)						
C. perfringens	4.7	5.2	4.0	4.1	±0.37	NS
Gram negative Bacilli	3.9	4.2	4.6	4.5	±0.24	NS
Finisher						
C. perfringens	4.3^{a}	5.5^{a}	3.7^{b}	0.0^{c}	±0.25	***
Gram negative Bacilli	4.7	5.3	5.3	5.1	±0.15	NS

Measurements of were based on 5 broilers per treatment.
[abc]Means in the row with different superscripts differ significantly (*** $p < 0.001$, N.S: Not significant).
For details of treatment, see Table II.

not different from treatment antibiotic and superior to birds without challenge. Hajati *et al*. (2012) administered AG to drinking water and found that AG improved FI, BWG and FCR numerically.

The effect of CE products such as AG to reduce or prevent colonization of undesirable bacteria such as *C. perfringens* and *Salmonella* in the intestinal tract of poultry has been mostly positive (Hofacre *et al*., 1998b; Nakamura *et al*., 2002). The elimination of *C. perfringens* could be explained by CE and stimulation of the immune system of the birds. The total elimination of ileal *C. perfringens* in the birds which had received AG_{water} could explain the improvement in performance. In this experiment, birds were challenged with *C. perfringens*, and according to Lovland and Kaldhusdal (2001) *C. perfringens* infection of broilers may cause impairment of production performance and subclinical disease associated with necrotic enteritis which is characterized by damage

to the intestinal mucosa that decreases digestion, absorption and reduces weight gains (Kaldhusdal *et al*., 2001). Wilson *et al*. (2005) suggested that the growth suppressing effect of intestinal bacteria was due to the production of toxic metabolites that irritate the gut mucosa, thereby inhibiting nutrient absorption. None of the challenged birds produced overt clinical signs of necrotic enterities and there were no mortalities associated with oral exposure to high doses of *C. perfringens*. However, many of the 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.

In summary, AG in water was found to be effective in improving the performance by eliminating *C. perfringens* and changing the villi width in the duodenum and jejunum areas under necrotic enteritis infection model used in this experiment. Under conditions in the present study, AG administration in drinking water was superior to the spray application which was not effective in performance or *C. perfringens* reduction.

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