Impact on Non-Antibiotic Treatments for Prevention of Coccidiosis on Gut Inflammation and Integrity in Broilers

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Introduction

Coccidiosis is a disease in poultry flocks with widespread incidence in the US. It results in clinical and subclinical syndromes such as impaired feed conversion, poor flock uniformity and poor performance (Brake et al. 1997). Coccidiosis is also a predisposing factor for diseases such as enteritis and diarrhea, and may cause significant flock mortality. Thus coccidiosis has severe economic impact on the poultry industry with worldwide total losses of about $3 billion annually (Dalloul and Lillehoj, 2006) and at least $300 million to the US poultry farmers (Manger et.al, 1991). Currently, adding anti-coccidials to feed is the major way to control coccidiosis. However, there is a potential for development of drug-resistant strains of Eimeria and other bacteria species that may have negative consequences on human health. Although several products such as essential fatty acids, yeast, and direct fed microbials are currently being marked as alternatives to ionophores for prevention of coccidiosis and its complications, there is little understanding of their mechanism of action in preventing this disease and in promoting gut health. A more complete understanding of effect of these alternative treatments on the gut will allow the development of new therapies to treat enteric diseases in broilers and other poultry species. It will also allow us to understand how these alternative treatments differ from current treatments against coccidiosis.

The objectives of this study were to determine the effect of antibiotic alternatives on gut inflammation and integrity in the broilers challenged with Eimeria species vaccine and to determine the impact of these treatments on growth performance and nutrient utilization by broilers.

Materials and methods

A total of 672 male Ross 708 day-old broiler chicks were used for a 6-week study. Birds were randomly allocated to 6 dietary treatments (14/pen; 8 replicates/ treatment) on floor pens in randomized complete block design. The 6 treatments tested were Salinomycin (60g/ton, positive control; essential oil treatment (Orego-stim^{R}, 300g/ton); yeast extract (Alphamune^{TM}, 500g/ton); direct fed microbial (Avicorr, 500g/ton) and crude yeast (500g/ton) and a negative control (NC) without any supplementation. All diets were formulated to meet or exceed NRC (1994) nutrient requirements and made from the same basal diet. Birds had ad libitum access to water and feed. Birds were subjected to the dietary treatments from day 1. On day 21, birds were switched to the grower diets. All birds were orally vaccinated with Eimeria species using Coccivac B vaccine at 2 weeks (day 14) and at 5 weeks (day 35) at 10X dose per bird. Body weight and feed intake were recorded on days 21 and 42, and the calculated feed efficiency was corrected for mortality on a bird day basis.

Sample Collection: On days 21 and 42, 1 bird of body weight close to the pen average was sampled by collecting intestinal mucosal from mid-duodenum, mid-jejunum, mid-ileum and cecal tonsils. The section to be sampled was rinsed with water before scrapping with clean glass slides into micro centrifuge tubes containing RNA later reagent (Ambion) and stored at -80^{o}C until processed. RNA was extracted from samples following the Trizol (Invitrogen) protocol. Two micrograms of total RNA from each sample was reverse transcribed into cDNA using Reverse Transcription system of Promega.

Quantitative Real-Time PCR: PCR was performed on the Bio-Rad iCycler. PCR reaction mix consisted of 0.5 µg of cDNA, 0.075 nmol of each of the forward and reverse primers, and Faststart SYBR Green Master (Roche, Basel, Switzerland). Nuclease-free water (Ambion) was added to reach a total reaction volume of 20 µl. Reactions were incubated at 95 °C for 5 min. Afterwards, reactions were cycled 50 times using the following protocol: 10 s at 95°C, 20 s at 55°C, 72°C. Gene expression level of interleukin 1 (IL-1β), 6 (IL-6) and 10 (IL-10); tumor necrosis factor (TNF-α), interferon-gamma (IFN-γ), toll-like receptor 2 (TLR2) and 4 (TLR4) was analyzed using glyceraldehyde-3-phospate dehydrogenase (GAPDH) as an endogenous control.

Data analysis: Data were analyzed by SAS (SAS Institute Inc.) using PROC GLM, and mean separation using least squares (LS) means. Values were considered significantly different at $P<0.05$.

Results

Body weight gain, feed intake and feed efficiency (Gain/Feed intake) were calculated on days 21 and 42 (Table 1). There was no difference between treatments for body weight gain from d0-21. However, the Salinomycin treatment improved weight gain from d21-42 ($P < 0.05$). Overall (d0-42), there were no treatment differences in weight gain ($P > 0.05$). Similarly, feed efficiency was improved by Salinomycin from d21-42 and d0-42 compared with other treatments ($P<0.05$).

Several of the inflammatory genes were not affected by treatments on d21 and d42 in the ileum (data not shown). On d21, Orego-stim treatment as well as Salinomycin, Avicorr and Alphamune treatments resulted in lower IL-6 expression compared with NC ($P < 0.05$) (Table 2). In the cecal tonsils at d42, the Orego-stim^{R} treatment had lower TNF-α expression ($P<0.05$). Similarly, IFN-γ and TLR-4 and IL-10 expression levels were lower in the Orego-stim treatment ($P<0.05$) (Table 2).

At d21, lowest digestibility of DM, energy, N and P was obtained in the Avicorr treatment compared to the other treatments ($P < 0.05$) but there was no effect of treatments on Ca digestibility ($P > 0.05$). Except for the lower P digestibility in the NC treatment at d42, treatments did not affect nutrient digestibility at this stage.

Discussion

Antibiotics have been widely used in animal production since 1940s. In the fifties it was also demonstrated that antibiotics could improve animal performance and gut health (Coates et al., 1955) and this led to widespread use of antiobiotics in poultry feed. In this study, the improvement of BWG and feed efficiency by salinomycin treatment was as expected. These data confirmed that Salinomycin improves animal performance (Duffy et al., 2005). Also, Salinomycin significantly reduced interleukin-6 expression in the early stage of the birds in the ileum. Direct fed microbials (DFMs) or probiotics improve animal performance partly by maintaining a beneficial gut microflora (Callaway et al., 2008). Although studies have shown that DFMs enhance growth of birds (Lee et al. 2010) we did not observe any growth improvement in Avicorr treatment in this study. Indeed lower digestibility of DM, energy, N and P was obtained in the Avicorr treatment compared to the other treatments at d21 suggesting that it might depress nutrient utilization in the early stage of the birds. However, depressed utilization of these nutrients did not reflect in reduced animal performance.

Alphamune is a yeast extract containing mannan-oligosaccharides and *β*-glucans. It is reported to increase the BW and feed efficiency, improve immune response and reduce salmonella colonization in chickens (Van Immerseel et al., 2000). However, we did not find any growth performance improvement with Alphamiune use in this study. However, alphamune may reduce IL-6 expression in ileum compared with control group. Although Orego-Stim had no effect on the growth performance, it suppressed the expression of multiple inflammatory cytokines in the cecal tonsils. The reduction of most inflammatory cytokines by Orego Stim in the cecal tonsils at d42 indicates it might have significant anti-inflammatory property, perhaps due to its essential oils content.

In conclusion, although several studies have shown the beneficial effects of DFMs, yeast and essential oils on poultry performance, results of this experiment indicates that under experimental challenge with coccidian vaccine, these benefits may not be achieved. Additional experiments using field isolates of Eimeria may better replicate field conditions of coccidiosis incidence and may allow better capture the benefits of these anti-coccidial alternatives.

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Table 1: Performance of birds supplemented with antibiotic alternatives from d0-42

	d0-21			d21-42			d0-42		
	BWG g/bird	FI g/bird	Gain/FI g/kg	BWG g/bird	FI g/bird	Gain/FI g/kg	BWG g/bird	FI g/bird	Gain/FI g/kg
NC	731	1024^{ab}	717	1965^{ab}	3405	578^{b}	2693	4397	612^{b}
Salinomycin	736	984^{ab}	748	2058^{a}	3408	605^{a}	2806	4372	642^{a}
Avicorr	759	1051^{a}	721	1919^{b}	3268	589^{ab}	2690	4315	623^{ab}
Alphamune	716	970^{b}	734	1947^{ab}	3299	592^{ab}	2670	4261	628^{ab}
Orego-stim	785	1051^{a}	748	1933^{b}	3357	578^{b}	2723	4396	620^{b}
Crude yeast	761	1050^{a}	726	1955^{ab}	3305	592^{ab}	2729	4340	629^{ab}

Table 2: Expression of inflammatory genes in the ileum and cecal tonsils

	NC	Salinomycin	Avicorr	Alphamune	Orego stim	Crude yeast	P Value
IL-6 (d21)*	2.67^{a}	0.98^{b}	0.86^{b}	0.80^{b}	0.89^{b}	2.47^{a}	P < 0.05
TNF-a	2.40^{a}	1.66^{ab}	1.47^{ab}	1.60^{ab}	0.54^{b}	1.80^{ab}	P < 0.05
IL-10	1.51^{ab}	2.40^{ab}	1.60^{ab}	1.37^{ab}	0.82^{b}	3.12^{a}	P < 0.05
IFN-γ	1.26^{ab}	2.54^{a}	1.77^{ab}	1.27^{ab}	0.66^{b}	2.54^{a}	P < 0.05
TLR4	1.16^{ab}	1.28^{ab}	1.65^{ab}	2.20^{a}	0.56^{b}	2.44^{a}	P < 0.05

*: Expression in the ileum at d21.

Table 3: Apparent ileal digestibility at d21

d21	NC	Salinomycin	Avicorr	Alphamune	Orego-stim	Crude yeast	P Value
Apparent ileal digestibility							
DM	0.58^{ab}	0.65^{a}	0.57^{ab}	0.52^{b}	0.64^{a}	0.664^{a}	P < 0.05
Energy	0.63^{ab}	0.69^{a}	0.62^{ab}	0.57^{b}	0.69^{a}	0.71^{a}	P < 0.05
Ca	0.74	0.82	0.78	0.71	0.80	0.818	P > 0.05
N	0.82^{ab}	0.84^{a}	0.73^{d}	0.77^{c}	0.79^{bc}	0.825^{ab}	P < 0.05
P	0.32^{bc}	0.42^{a}	0.19^{d}	0.28^{c}	0.34^{b}	0.44^{a}	P < 0.05

Table 4: Apparent ileal digestibility at d42

d42	NC	Salinomycin	Avicorr	Alphamune	Orego-stim	Crude yeast	P Value
Apparent ileal digestibility							
DM	0.65	0.59	0.65	0.64	0.64	0.651	P > 0.05
Energy	0.67	0.62	0.66	0.70	0.65	0.72	P > 0.05
Ca	0.77	0.77	0.79	0.79	0.79	0.800	P > 0.05
N	0.79	0.74	0.78	0.74	0.78	0.780	P>0.05
P	0.30^{c}	0.35^{bc}	0.42^{ab}	0.39^{bc}	0.42^{ab}	0.51^{a}	P < 0.05