Effect of Dietary Inclusion of Probiotic, Prebiotic and Butyric Acid Glycerides on Resistance against Coccidiosis in Broiler Chickens

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This research was conducted to investigate the effects of single or combined use of probiotic (Primalac), prebiotic (Fermacto) and butyric acid glycerides (Baby C4) compared to salinomycin on resistance against coccidiosis in broiler chickens challenged with Eimeria. One day-old male Ross 308 broiler chicks were divided into 9 cages with 15 birds per each. Birds of different treatments received a regular corn-soybean meal basal diet supplemented with feed additives; none of them in negative control (C−), salinomycin in positive control (C+), probiotic in PRO group, prebiotic in PRE group, butyric acid glycerides in BAG group, probiotic+prebiotic in PRO+PRE group, probiotic+butyric acid glycerides in PRO+BAG group, probiotic+prebiotic+butyric acid glycerides in PRE+BAG group, and probiotic+prebiotic+butyric acid glycerides in PRO+PRE+BAG group. At 28 d of age, 8 birds from each treatment were removed and kept separately in other cages (2 birds/cage) to challenge with Eimeria. These separated chicks were orally challenged with $1 \times 10^5$ E. acervulina and $9 \times 10^4$ E. tenella. Positive effect ($P<0.05$) was observed in the duodenal and cecal lesion scores in any of the supplemented groups when compared to that of the C−. There was no significant difference between all supplemented groups and C+ for cecal lesion score. PRO, PRE+BAG, PRO+PRE, PRO+BAG and PRO+PRE+BAG reduced ($P<0.05$) oocyst shedding in birds challenged with Eimeria oocysts compared to the C−, and there was no significant difference between PRO+PRE, PRO+BAG, PRO+PRE+BAG and C+. These results demonstrate that prebiotic, butyric acid glycerides and especially probiotic or its combination with prebiotic or butyric acid glycerides enhance the resistance of birds and partially protects against coccidiosis. Therefore, it seems some of these supplements, in comparison to salinomycin, can diminish partially the adverse effects of coccidiosis.

Key words: broiler, butyric acid glycerides, coccidiosis, prebiotic, probiotic


Introduction

Coccidiosis is a disease that is caused by protozoan parasites of the genus Eimeria, developing within the intestine of birds. Although coccidiosis is a disease known for many years, it is still considered as the most economical important parasitic condition affecting poultry industry worldwide. This disease reduce efficacy of poultry production by increasing the mortality, feed conversion ratio, impaired growth rate in broilers, and declined egg production in layers (Lillehoj et al., 2004). Based on a compartmentalized model (Williams, 1999), worldwide cost of coccidiosis in poultry annually was estimated to be more than 2.3 billion € (Sorensen et al., 2006). Due to increasing problems with prolonged drug usage and prophylactic effects, and the high cost and subclinical disease effect of vaccines, alternative strategies are needed for more effective and safer control of coccidiosis in chickens (Dalloul et al., 2006; Williams, 2006).

A Supplement that could stimulate gut immune system, and reduce pathogenic bacteria by production of organic acid and other substances might be a candidate for substituting the anticoccidial drugs. Hence probiotic, prebiotic and butyric acid glycerides may be able to enhance the resistance of birds against coccidiosis. A few reports have examined the anticoccidial effects of these feed additives (Dalloul et al., 2003a; Leeson et al., 2005; Lee et al., 2007); however, there is no study to compare them and investigate the effects of using their combination. Therefore this study was conducted...
to investigate the effects of single or combined use of probiotic (Primacal), prebiotic (Fermacto) and butyric acid glycerides (Baby C4) compared to salinomycin on resistance against coccidiosis in broiler chickens challenged with *Eimeria*.

**Materials and Methods**

**Birds and Dietary Treatments**

In this study, one-day-old male Ross 308 broiler chicks were divided into 9 cages with 15 birds per each. All birds had access *ad libitum* to their particular supplemented diets in all growth periods. The basal diet was a typical mash corn-soybean meal diet that was formulated to meet Ross 308 broiler nutrient requirements. Birds of different treatments received a regular basal diet supplemented with feed additives; none of them in negative control (C−), salinomycin in positive control (C+), probiotic in PRO group, prebiotic in PRE group, butyric acid glycerides in BAG group, probiotic + prebiotic in PRO + PRE group, probiotic + butyric acid glycerides in PRO + BAG group, prebiotic + butyric acid glycerides in PRE + BAG group, and probiotic + prebiotic + butyric acid glycerides in PRO + PRE + BAG group. Probiotic was a commercial product of Primacal contained of 1×10⁸ cfu of *Lactobacillus*. The commercially available fermentation product of *Aspergillus orizae*, Fermacto referred to as *Aspergillus* meal, has no live cells or spores (Harms & Miles, 1988). This commercial feed additive of Fermacto was used as a prebiotic supplement. According to the manufacturer’s recommendations, probiotic, prebiotic and butyric acid glyceride (Baby C4) in groups at the levels 900, 1800, 3000 (on d 1 to 10) and 450, 1800, 2000 (on d 11 to 28), and 225, 1000, 0 (on d 29–42) mg/kg of the diet, respectively. The positive control group (C+) received salinomycin of 1 g/kg of the diet. At 28 d of age, 8 birds from each treatment were removed and kept separately in other cages (2 birds/cage) to challenge with *Eimeria*. These separated chicks were orally challenged with 1×10⁵ *E. acervulina* and 9×10⁴ *E. tenella*. The oocysts doses were prepared by the section of parasitology at Veterinary College, University of Tehran, Iran. Due to chicks mortality at first week of life and having enough health birds with almost similar body weight for challenging, 15 birds/treatment, more than required number at 28 d of age, were used from 1 to 28 d.

**Lesion Scoring**

On d 34, one bird/cage (4 birds/treatment) of infected group was selected, and sacrificed and examined for degree of presence of coccidial lesions. Lesion scores were observed and recorded according to the system of Johnson and Reid (1970), where in 0 is normal and 1, 2, 3, or 4 indicate increasing severity of infection. The ascending + descending portion of duodenum and cecum of the intestine were examined for lesions.

**Oocyst shedding**

Oocyst shedding was assessed as described by Dalloul et al. (2002). Briefly, dropping from 4 birds/treatment (one bird/cage) were collected for 6 d starting on 7 d postinoculation, total fecal material was homogenized, and samples were taken, diluted, and the oocysts were counted microscopically using a McMaster counting chamber.

**Statistical Analysis**

Differences among experimental treatments were tested by analysis of variance using the ANOVA procedures of SAS (SAS Institute, 1999), and the mean values of fecal oocyst shedding, and lesion scoring were compared among groups by Duncan’s Multiple Range Test.

**Results and Discussion**

To prevent and control coccidiosis, the poultry industry has relied heavily upon prophylactic chemotherapy resulting in the development of resistant strains of *Eimeria* to all introduced anticoccidial drugs (Chapman, 1997). Therefore, recent research has focused on the development of alternative disease control strategies including the introduction of alternative prevention or treatment measures such as nonchemical feed supplements (Dalloul et al., 2003a,b), and novel and effective vaccines like recombinant (Ding et al., 2004; 2005; Lillehoj et al., 2005) and new live (Weber et al., 2004) vaccines, and other immunization strategies such as the use of CpG oligodeoxynucleotides (Dalloul et al., 2004, 2005). We therefore tested the potential protective effects of different feed additives in comparison with the salinomycin on susceptibility of chickens to coccidiosis. Protection against coccidiosis was reflected by reduced lesion scores and oocyst shedding, in birds infected with *Eimeria* oocysts. Positive effect (*P*<0.05) was observed in the duodenal (Fig. 1) and cecal (Fig. 2) lesion scores in any of the supplemented groups when compared to that of the C−. There was no significant difference between all supplemented groups and C+ for cecal lesion scores. PRO, PRE + BAG, PRO + PRE, PRO + BAG and PRO + PRE + BAG reduced (*P*<0.05) oocyst shedding in birds challenged with *Eimeria* oocysts compared to the C−, and there was no significant difference between PRO + PRE, PRO + BAG, PRO + PRE + BAG and C+ (Fig. 3).

It seems lowering lesion scores and oocyst shedding by feed additives may be related to reducing pH and inducing the condition to increase the beneficial microflora. Van der Wielen et al. (2000) showed a correlation between the presence of undissociated butyrate (and acetate and propionate) and pathogen control in the cecum of young birds. Taheri et al. (2009) showed a general relationship between pH and antibacterial effects of lactic acid bacteria *in vitro*. Van Immerseel et al. (2004a, b) have indicated significantly reduced levels of *Salmonella* in the cecum of birds fed organic acids, whereas Cox et al. (1994) showed butyric acid in particular was effective in reducing *Salmonella* colonization of the intestine. Chaveerach et al. (2002) indicate that this efficacy of organic acids in controlling microbes such as *Campylobacter* is influenced by concentration, form of the acid, and the degree of any dissociation. Leeson et al. (2005) reported that with *Eimeria* oocysts challenge, birds that had received butyric acid before challenge showed higher growth rate following the challenge compared with birds that received non-medicated feed; however, they did
not examine the lesion scores, oocyst shedding.

Although all feed additive treatments could reduce lesion scores, using the probiotic or its combination with other feed supplements was more effective for decreasing the oocyst shedding; PRO had significant difference with C− but PRE and BAG not, and also its combination with PRE or BAG (PRO + PRE, PRO + BAG and PRO + PRE + BAG) had no significant difference with salinomycin supplemented group but PRE + BAG did. Such beneficial effect of the dietary inclusion of probiotic may be related to the competitive exclusion and antimicrobial production of lactic acid bacteria. However, Eimeria, being an intracellular parasite, must invade the host cells in order to replicate. First, it must adhere to epithelial surfaces. Gut-adapted probiotic bacteria may compete for adhesion sites and occupy common receptors on the epithelial cells. This would retard penetration and infiltration by Eimeria oocysts and consequently, their replication and shedding. Also probiotic may be more effective to enhance both cellular and humoral immune responses against Eimeria in chickens. Dalloul et al. (2003a) showed that administration of a Lactobacillus-based probiotic induced protective immunity against E. acervulina infection. They demonstrated an immunoregulatory effect of dietary Lactobacillus-based probiotic on the local immune system and improved resistance to E. acervulina (e.g., reduced oocyst shedding) in broiler chickens. Lee et al.
(2007) showed higher antibody production against *E. tenella* by *Pediococcus*-based probiotic.

In some characteristics, only the combination use of supplements had significant difference compared to the C−; PRE+BAG in compare to PRE or BAG on oocyst shedding. Also only combined treatments of PRO in compare to its single use had no significant difference to SAL group. Lower oocyst shedding of the combination treatments than those of the mono-supplementation treatments might be the result of synergistic effects of using these feed additives together. With regard to similar effects of the combination treatments of PRO to salinomycin, these supplements (PRO +PRE, PRO+BAG, PRO+PRE+BAG) can probably be used as protective feed additives against coccidiosis in poultry farms whereas they can improve performance as well.

In conclusion, these results demonstrate that prebiotic, butyric acid glycerides and especially probiotic or its combination with prebiotic or butyric acid glycerides enhance the resistance of birds and partially protects against coccidiosis. Therefore, it seems some of these supplements, in comparison to salinomycin, can diminish partially the adverse effects of coccidiosis.

**References**


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