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Research Article

EFFECTS OF DIETARY PROTEIN LEVELS ON OOCYSTS SHEDDING IN BROILERS DURING SUBCLINICAL COCCIDIOSIS

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ABSTRACT

We aimed to study the interaction between different dietary protein levels intake and the evaluation of response to a coccidial challenge by counting oocysts per gram (OPG) in broiler chicks.

70 chicks were divided into 7groups of ten chicks each. An uninfected control group (group 1) was fed a diet based on standard protein content (20%) and others (group 2 to 7) were fed diets based on dietary protein levels of 16%, 18%, 20%, 22%, 24% and 26% protein, respectively. At 15 d of age, treatments 2 to7 were subjected to a single dose infection of 50000 oocysts of Eimeria. OPG of all groups was counted at days 5 and 14 before inoculation and days 4, 5, 8 and 10 post infection.

The results indicated the mean oocyst index in all days post infection in groups 2 and 3 was a little more than the fourth group with diet based on standard protein content, whereas groups 5, 6 and 7 with diet protein of 22%, 24% and 26% shed oocysts lesser than group four. In fact an increase in dietary protein led to a decrease ($P<0.01$) in oocysts shed by infected chicks. Also the highest OPG (worst) was belong to group 2 in day 10 after infection while the lowest OPG (better) was calculated for the seventh group in day 3 after inoculation. We realized protein increased intake causes considerable decrease in OPG and makes the changes rising in OPG slow in alternative sampling post infection.

Keywords: *Coccidiosis, Oocysts, Broilers, Dietary Protein, Eimeria*

INTRODUCTION

Coccidiosis, a parasitic disease which causes a huge deal of stress to chicken owners, affecting performance of poultry reared under intensive production systems is caused by protozoan parasites of the genus *Eimeria*, infect poultry when ingested by the chicken and can lead to severe losses in poultry production (Williams, 1999).

Although coccidiosis is a disease known for many years, due to its short life cycle and fast dissemination, is one of the major constraints for the production of broilers and it is still considered as the most economical important parasitic condition affecting poultry production worldwide. It costs the world's commercial chicken producers at least US$ 1.5 billion every year (Williams, 1999; Yadav and Gupta, 2001). It has been shown that the disease has brought about great economic losses in the poultry industry of Iran like the other parts of the world (kheirabadi *et al.*, 2008).

Velker (2011) reported that nine species of *Eimeria* have been described in chickens, of which at least seven species are relevant for the poultry industry (*E. acervulina, E. maxima, E. brunetti, E. necatrix, E. mitis, E. praecox and E. tenella*)

However, there are only few available diagnostic methods for the diagnosis of subclinical coccidiosis, oocysts per gram (OPG) of faeces which can be counted by using a practical technique, is considered as the most common one (McMaster). It is a method that can be useful and applicable at farm level and only demands simple equipment and a trained person.

The method can be used for detecting and measuring infection dynamics of individual broilers (Roepstorff and Nansen, 1997) or a group of individuals by pooled samples or by litter analysis. Analysis of OPG in farmed chickens may identify the epidemiology of the disease and thus the population dynamics. However, methods should be used in combination if determination of the role of the subclinical disease on productivity is the target (Long and Rowell, 1975).

In broilers, coccidiosis control should not only address the prevention of clinical disease and mortality but also mild and subclinical infections, as even minor intestinal lesions can interfere with growth, feed conversion and therefore profitability. In addition to management measures, (litter condition, bird population in the poultry farm); the disease has largely been controlled by directly adding anticoccidial drugs to the chicken feed (Arabkhazaeli *et al.*, 2011).

Medication by anticoccidial drugs in chicken had been started about 30 years ago in Iran and numerous products were introduced, which are readily available and in use (Pirali kheirabadi *et al.*, 2008) but the intensive use of anticoccidial drugs has led to the development of resistance (Chapman, 1997).

Apart from anticoccidial compounds, a wide range of reports have studied the correlation between feed restriction and disease resistance (Yaissle *et al.*, 1999; Zulkifli *et al.*, 1993; Praharaj *et al.*, 1995; Nir *et al.*, 1996). It has been indicated that compared with ad libitum-fed controls, chickens on feed limitation were less vulnerable to a coccidial challenge (Zulkifli *et al.*, 1993). However, information on effects of the interaction of dietary protein level and coccidiosis in chicken is partly scanty.

The effect of dietary protein levels on resistance or susceptibility to infections has been found to be unpredictable. In some instances increasing protein levels increased resistance (Dubos, 1958) in others, increased susceptibility (Hill and Garren, 1961); and in at least one instance (Hill *et al.*, 1962) was found to have both effects and neither depending upon the presence of other dietary factors. Coccidiosis has been studied in relation to dietary protein levels by Jones (1934) who reported no marked effect on coccidiosis of chickens.

The objective of the present study was to study the interaction between different dietary protein levels intake and the evaluation of response to a coccidial challenge by counting oocysts per gram in feces in Ross broiler chicks.

MATERIALS AND METHODS

The experiment comprised 70 male one-day-old Ross broiler chicks which were randomly assigned to 7 groups containing 10 chicks in each treatment and allocated in plastic-floored battery cages in a previously *Eimeria*-free environment.

During the experiment the first group as uninfected control group was fed a diet based on standard protein content (20%) and others (group 2 to 7) were fed diets based on dietary protein levels of 16%, 18%, 20%, 22%, 24% and 26% protein, respectively. All diets contained an equal energy concentration and no antibiotic growth promoter or additives were included in the feed. Water was provided ad-libitum and the temperature was maintained at 30-32 °C, meanwhile the environment was equipped with an appropriate ventilation system.

Lighting was provided in the same condition for all groups including 23 hours light and one hour dark. Moreover no vaccine was used during the test period.

In order to purification and standardization of dose of the inoculum for experimental infection, four selected species including *E. acervulina, E. maxima, E. tenella* and *E. necatrix* were propagated in 8-wk-old *Eimeria*-free chickens by oral inoculation and the oocysts were recovered from feces which were collected for up to 8 days post inoculation, sporulated and stored in 2% potassium dichromate at 4°C before inoculation (Conway and McKenzie, 2007). The appropriate dose had been estimated in a preceding test inoculation from the results of these initial tests (Shirley, 1995).

At 15 d of age, 60 chickens (treatments 2 to7) were individually inoculated per os with an inoculum consisting of a mixture of 4 mentioned Eimeria species.

The infectious dose (50000 oocysts) contained sporulated oocysts of E. acervulina (20%), E. maxima (40%), *E. tenella* (25%) and *E. necatrix* (15%).

The group one was kept separately as uninoculated control, and fed basal diet. To avoid cross-infection, the birds were leg tagged with numbers and empty pens and walls of plastic sheet were placed between inoculated and uninoculated pens. Separate working clothes, footwear, and equipment were used for each area.

Twenty-four hour collection of feces was done on days 4, 5, 8 and 10 days post infection. With regard to control of probable infection, feces collection was performed on days 5 and 14 before inoculation, as well. Samples were analyzed for number of oocysts per gram of feces (OPG) using a modified McMaster technique as follows: a 20 to 30 g portion of the sample was mixed with tap water at a 1:15 ratio.
After homogenization, the suspension was filtered through gauze and five 2-mL aliquots were withdrawn and pooled in a tube. After centrifugation, the pellet was resuspended in 10 mL saturated sodium chloride solution and the OPG determined as described by Taylor et al (1995) (Waldenstedt *et al.*, 1999).
For ensuring the successful establishment of experimental coccidiosis, two chick was selected randomly in each groups and were examined Eimerial intestinal lesions at day 10 post Eimerial inoculatioan.
Statistical analyses were computed using SPSS software and results obtained from OPG counting were analyzed based on a one-way ANOVA and repeated major to see whether the differences between groups were significant. Differences between means were considered significant at $p < 0.05$. When significant differences were found between different treatments, the least significant difference (LSD) was calculated (Snedecor and Cochran, 1968) with Posthoc test.

RESULTS AND DISCUSSION

In this study 70 male one-day-old Ross broiler chicks were experimentally infected by four species of *Eimeria* and fed with different percentages of protein in the diet. We aimed that increasing amount of protein could decrease OPG.
For the pre-infection period of 14 days, OPG of all experimented groups were zero.
In all challenged groups, coccidial lesions in intestine of broilers were seen at day 10 post experimental challenge.
According to the results which has been summarized in Table 1, the mean oocyst index in all days post infection in groups 2 and 3 was a little more than the fourth group with diet based on standard protein content (20%) whereas groups 5, 6 and 7 with diet protein of 22%, 24% and 26% shed oocysts lesser than group four. This difference was significant between groups 5, 6 and 7 in compare to group 4 (P<0/01).
It can be seen obviously that the highest OPG (worst) was belong to group 2 in day 10 after infection while the lowest OPG (better) was calculated for the seventh group in day 3 after inoculation. In fact these two groups were fed with lowest and highest amount of protein, respectively (Table 1).

Table 1: Effects of dietary protein levels on Oocysts per gram(OPG) in all experimented groups at 4, 5, 8 and 10 d post infection

Groups	OPG in different days after inoculation			
	4 days	5 days	8 days	10 days
1 (20% protein)	0	0	0	0
2 (16% protein)	10100	67000	115000	123600
3(18% protein)	9900	58000	92500	101500
4(20% protein)	9500	56500	91000	98500
5(22% protein)	9450	50500	73000	75500
6(24% protein)	5770	23700	29500	29750
7(26% protein)	4650	17900	18750	18900

Figure 1 signifies that OPG was zero for the control group in all days of sampling such as we expected, which was representative of the suitable qualitative control of the experiment process.
In the challenged groups, there was a consecutive increasing trend of OPG in days 4-10 post challenging which was numerically more slightly in group 5, 6 and 7. Also the pick level of OPG in all groups was seen 10 days after infection (Figure 1).
The OPG of different challenged groups in the same days post infection along diet protein enhancing, declined consecutively from day 4 to day 10 (Table 1). In the other word, OPG of group 5 in days 8 and

10 post inoculation reduced 20% and 23% respectively in compare to OPG of group 4 in the same days after challenging.

This reduction in group 6 in compare to group 4 was respectively 68% (8 days after infection) and 70% (10 days post inoculation). While this decline in group 7 in compare to group 4 was substantially higher than other challenged groups which included 79% and 82% in days 8 and 10 post infection.

So, it can be realized that the increasing trend of OPG in four consequent times sampling against diet protein enhancing, became considerably slight. In addition inter-groups comparison showed that OPG versus increasing diet protein percentage lessened in all days post infection and according to ANOVA test this decrease was significant ($p<0.01$).

The above-mentioned criteria reveal that diet protein enhancing highlights this factor to decrease coccidial infection in poultry industry. In fact if a diet included 20% protein is considered as a normal diet, increasing of 2% protein in daily chicken diet could decrees OPG about 20-23% in compare to a normal diet.

While if this increasing amount reaches to 4%, then OPG will go down to approximate 30-32% and also increasing 6% of protein could progressively diminish OPG up to 79-82%, however in developing countries such Iran providing such diet is almost impossible.

Nevertheless, if diet including 16% and 18% protein considered as normal diet, diet with 20% protein can decrease OPG 21-28%.

In this study, OPG counting repeated few times for each treatment, then for OPG evaluation comparison in different experimented groups, Repeated Major as a parametric test was used which has a high testing value. Regarding to this, there was a significant difference between OPG of experimented groups ($P<0.01$).

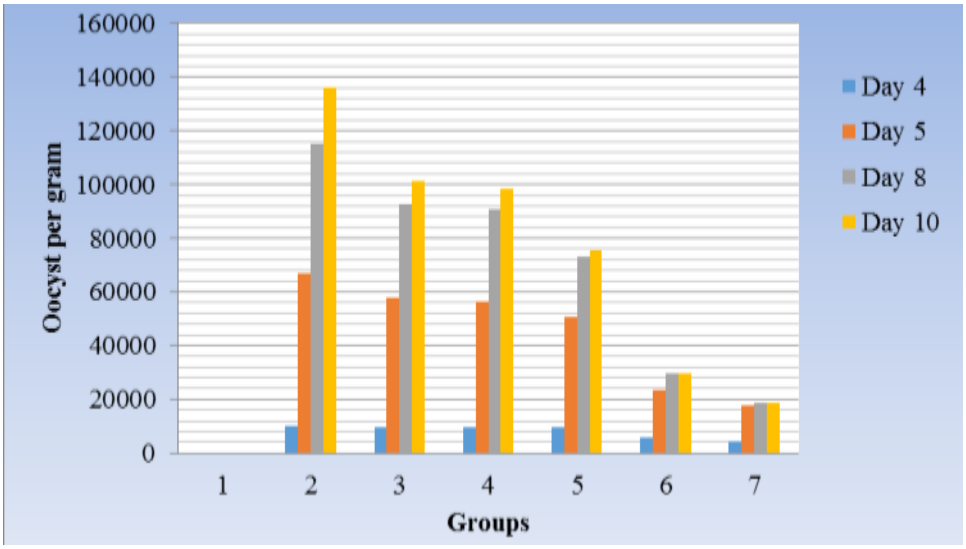

Figure 1: Oocysts per gram feces in different days post infection by *Eimeria* species in groups 2 to 7 and uninfected group 1. OPG on vertical axis has been plotted against different experimented groups on horizontal axis. Different colors indicate different days post inoculation

Discussion

A variety of studies have reported the impact of dietary protein on the severity of the symptoms related to coccidiosis (Britton *et al.*, 1964; Harms *et al.*, 1967; Welch *et al.*, 1986; Ruff, 1993). Dietary protein retention has been reported to decrease during a coccidial challenge (Sharma *et al.*, 1973) and other studies have reported that feeding high levels of dietary protein enables birds to better cope with a coccidial challenge (Harms *et al.*, 1967; Welch *et al.*, 1986). Yaissle (1999) examined the effects of dietary protein on restrict-fed broiler breeder pullets during a coccidial Challenge. He showed that

increased protein intake can have a positive effect on BW during feed restriction and a coccidial challenge. In fact protein did have beneficial effects on BW (protective against weight reduction) even during a coccidial challenge.

In another study the effect of protein level in the diet and chicks infected experimentally with Eimeria sp was investigated (Sharma *et al.*, 1973). Different groups were fed one of the three diets based on dietary protein level of 16%, 20% and 24%. They indicated that an increase in dietary protein led to a linear ($P<0.01$) increase in daily weight gains and feed efficiency. This increase which resulted from feeding graded levels of crude protein was in agreement with the results of other workers as summarized by Velu et al (1971). Although they showed that increased dietary crude protein levels were protective against weight reduction which normally occurs during coccidiosis, the mortality rate was increased by feeding a diet containing 24% crude protein. Of course Britton et al (1964) showed the same results that increased severity of cecal coccidiosis was associated with increased dietary protein level and was attributed to a greater rate of trypsin secretion. The increased trypsin secretion caused by an increased dietary protein level was considered responsible for excystation of a larger proportion of the infective dose of oocysts.

In an earlier study, Allen (1932) observed lowered mortality rate and a lowered oocyst production in infected chickens on diets containing high protein.

Actually the influence of dietary crude protein in infectious diseases of chickens seems to differ with different pathogens. Hill and Garren (1961) reported an increased mortality rate associated with feeding increased dietary crude protein in chicks infected with Salmonella gallinarum, whereas Boyd and Edward (1963) observed greater mortality in *E. coli* infection in chicks fed low protein diets. The effect of the interaction of dietary crude protein and cecal coccidiosis in chicks is, therefore, similar to that reported for *E. coli* (Boyd and Edward, 1963).

In conclusion we realized protein increased intake causes considerable decrease in OPG and makes the changes rising in OPG slow in alternative sampling post infection. But it should be considered that increasing of protein in poultry feed costs a lot, therefore it should be applied if the bed OPG of poultry houses are in extensive levels (usually more than 5000) and also main indexes such FCR (food convert rate) could justify the necessity of this act.

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