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Original Research

# Effect of Microencapsulated Multispecies Probiotic Supplementation in the Control of Coccidiosis

**K. Divya Manjari[1]*, M. Parthiban[2], T. S. Saravanan[3], M. Raman[4] and A. Palanisammi[5]**

[1]Department of Animal Biotechnology, Madras Veterinary College, TANUVAS, Chennai, Tamil Nadu, INDIA
[2]Department of Animal Biotechnology, Madras Veterinary College, TANUVAS, Chennai, Tamil Nadu, INDIA
[3]Institute of veterinary preventive medicine, Ranipet, Tamil Nadu, INDIA
[4]Translational Research Platform for Veterinary Biologicals, Centre for Animal Health Studies, TANUVAS, Madhavaram Milk Colony, Chennai, Tamil Nadu, INDIA
[5]Dean, Veterinary College and Research Institute Tirunelveli, TANUVAS, Chennai, Tamil Nadu, INDIA

*Corresponding Author: manjubrindha@gmail.com

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## Abstract

*The annual financial loss to the poultry industry as a result of coccidiosis has been estimated at about US dollar 3 billion. The objective of the study to evaluate the effect of microencapsulated multispecies probiotic supplementation in the control of coccidiosis. In vivo trial, the birds were divided into 6 different groups with 10 birds in each group. Group1: Birds fed with microencapsulated immunomodulatory probiotic consortium1; Group2: Birds fed with microencapsulated immunomodulatory probiotic consortium2; Group3: Birds fed without probiotic organism and coccidiostat alone; Group4: Birds fed with commercial probiotic Group-5 and 6: Positive and negative control groups: Birds fed without probiotic organisms. On 18th day, all the groups except group 6 (negative control) were challenged with mixed oocysts of Eimeria maxima 60 % and Eimeria tenella 40 % at 15,000 oocysts/ chick by oral gavage method. Coccidiostat (12% salinomycin) was given at the concentration of 5 g/ 10 kg of feed from 14th day to 35th day age of birds in groups 3. The oocyst count, lesion score, histopathology of intestinal and body weight and feed conversion ratio were analyzed between probiotic treated and untreated groups. There is a reduction in shedding of oocyst count and severity of lesion between probiotic treated and untreated groups clearly indicated that the probiotic could be used to control the coccidiosis in broiler chicken.*

**Keywords:** Broiler, Coccidiosis, Performance, Probiotics, Salinomycin

## Introduction

Coccidiosis is an economically important disease with an estimated loss of 3.2 billion USD per year to world poultry industry (Dalloul and Lillehoj 2006). Eimeria species are primarily affects intestinal epithelium, naturally leads to the diminished ability of the intestine to absorb nutrients, resulting in reduced performance and higher susceptibility to other bacterial diseases. (Metzier – Zebeli *et al.*, 2009). The control of coccidia has been greatly dependent on the use of chemotherapeutic drugs. More recently, the poultry industry has been under pressure to reduce the usage of antimicrobials including anticoccidial drugs, despite the global acceptance and success of these drugs. The pressure comes primarily from the high costs of these antimicrobials, which contribute to the cost of disease control, besides public health concerns and demands for drug residue free products for consumption. Moreover, the development of resistance of Eimeria species to chemotherapeutic agents has been reported for several years from different parts of the world, and this resistance has caused significant reductions in drug effectiveness. Although some coccidia develop less resistance to some drugs, long-term exposure eventually leads to a loss of sensitivity and development of resistance to the drug (Stringfellow *et al*., 2011). Hence, researchers have recently focused on more 'natural' means of controlling and managing coccidiosis. Probiotic supplementation is currently being explored as a means of reducing the amount and severity of enteric diseases in poultry and subsequent contamination of poultry products for human consumption. The probiotics have great potential to enhance host defenses and affect the digestive microbiota positively, while protecting against colonization by harmful bacteria and maintaining intestinal integrity (Pender *et al*., 2016). Hence in this study, the effect of microencapsulated multispecies probiotic supplementation in the control of coccidiosis was investigated.

## Materials and Methods

### Maintenance of Experimental Chicken

Sixty numbers of Vencobb chicken of both male and female sex were maintained form day post hatching and vaccinations were given as per protocol. The day-old broiler chicken were wing tagged for identification The birds were maintained in a well aerated condition in cages. The daily ration for the birds was 30g, 50g, 80g, 100g and 120g during 1$^{st}$, 2$^{nd}$, 3$^{rd}$, 4$^{th}$ and 5$^{th}$ weeks respectively.

### Probiotic Consortium

The following multispecies probiotic consortium was made based on the immunomodulatory potential probiotic bacteria based on our previous work (Divya *et al.*, 2019).

Consortium I: *Lactobacillus plantarum , Enterococcus hirae, Weissella Cibaria*
Consortium II: *Enterococcus faecium, Pediococcus acidilacti, Weissella paramenteroides*

### Birds Challenged with Eimeria Oocyst

The birds were divided into 6 different groups with 10 birds in each group (equal ratio of male and female birds in each groups) Group1: Birds fed with microencapsulated immunomodulatory probiotic consortium 1; Group2: Birds fed with microencapsulated immunomodulatory probiotic consortium 2; Group3: Birds fed without probiotic organism and coccidiostat alone; Group4: Birds fed with commercial probiotics Group5 and 6: Positive and negative control groups :Birds fed without probiotic organisms.

On 18$^{th}$ day, all the groups except group 6 (negative control) were challenged with mixed oocysts of *Eimeria maxima* 60 % and *Eimeria tenella* 40 % at 15,000 oocysts/ chick by oral gavage method. Coccidiostat (12% salinomycin) was given at the concentration of 5 g/ 10 kg of feed from 14$^{th}$ day to 35$^{th}$ day age of birds in groups 3 (Dalloul *et al*., 2002).

### Oocyst Count

Forty-eight hours after the parasitic challenge, the fresh droppings were collected from birds of all groups (n=60). Oocyst counting was carried out based on McMaster counting method. Counting was done on the 3$^{rd}$, 4$^{th}$ and 5$^{th}$ day post challenge (Dalloul *et al*., 2002).

**Lesion Scores**

From each trial group three chicks were sacrificed on 9th day post challenge, lesions scoring of Johnson and Reid (1970) were made as baseline for assessing the gut lesions in the middle part of small intestine.

**Histopathology of Intestine**

Middle part of small intestine were collected from 3 chicks of each in all trial groups after 9 days post challenge and preserved in 40% formalin for further histopathological examination (Kettunen *et al*., 2001).

**Body Weight and Feed Conversion Ratio**

The body weight of each bird in all the six groups (n=60) were recorded on weekly basis till 5th week. Based on the body weight gain and cumulative weekly feed consumption, Feed Conversion Ratio (FCR) was calculated (Zhao *et al*., 2013).

## Results

The oocyst of Eimeria collected from droppings of all the six groups (n=60) were statistically analyzed and presented in Table 1. There is 52% and 42 % reduction in the shedding of oocysts in the probiotic supplemented groups 1 and 2 respectively in comparison to positive control group 5.

**Table 1:** Comparison of oocysts count in faeces between probiotic treated and untreated groups

| Group | Oocyst count |
|---|---|
| **1** | $21,533.33^{d}$±334.53 |
| **2** | $19,400.00^{d}$±375.94 |
| **3** | $9,766.67^{b}$ ±130.46 |
| **4** | $18,533.33^{c}$±312.69 |
| **5** | $46,466.67^{e}$±329.33 |
| **6** | $1183.33^{a}$ ±90.87 |

*Values of different superscripts are significant within the group*

The feed conversion ratio and body weight at weekly intervals between multispecies probiotic consortium, commercial probiotic treated and untreated control groups were presented in Table 2 and Fig.1 respectively.

**Table 2:** Feed conversion ratio comparison among probiotic treated and control groups

| Trial groups | Weeks | | | |
|---|---|---|---|---|
| | **1** | **2** | **3** | **4** |
| **I** | $1.47^{ab}$ ±. 0.02 | $1.76^{a}$±0.02 | $1.94^{a}$± 0.01 | 2.07±$0.01^{b}$ |
| **II** | $1.46^{ab}$±0.19 | $1.76^{a}$±0.01 | $1.92^{a}$±0.02 | 2.01±$0.01^{b}$ |
| **III** | $1.45^{a}$±0.03 | $1.81^{bc}$±0.02 | $1.93^{a}$±0.01 | 2.03±$0.02^{b}$ |
| **IV** | $1.43^{a}$±0.19 | $1.82^{c}$±0.02 | $1.93^{a}$±0.01 | 2.03±$0.03^{b}$ |
| **V** | $1.44^{b}$±0.35 | $1.82^{bc}$±0.02 | $1.9^{a}$±0.06 | 2.01±$0.02^{b}$ |
| **VI** | $1.63^{c}$ ±0.34 | $1.81^{bc}$ ±0.01 | $2.05^{b}$ ±0.01 | 2.26±$0.02^{a}$ |

*Significant difference is noticed in feed conversion ratio between probiotic fed groups and other groups during starter phase (first two weeks) of broilers.*

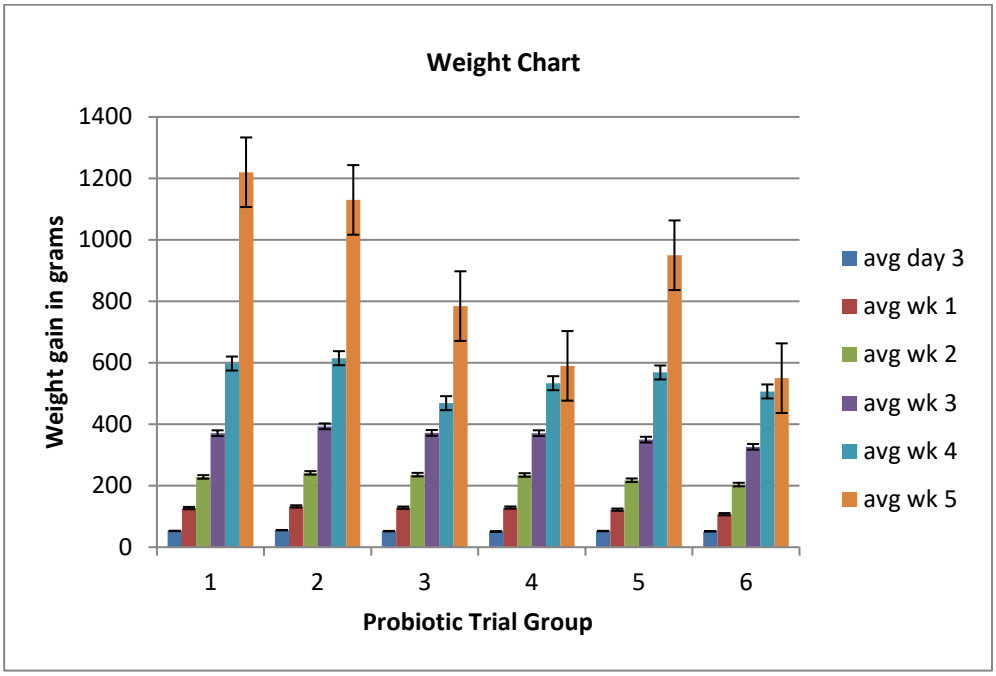



**Fig. 1:** Body weight and comparison among probiotic treated and control groups

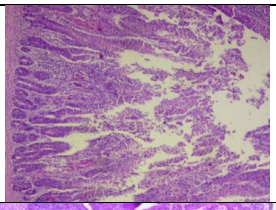
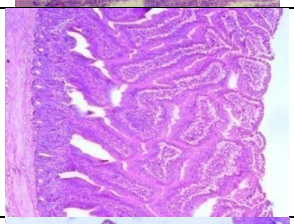
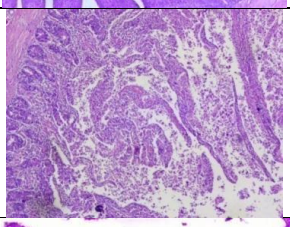
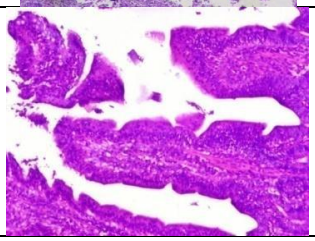
**Table 3:** Leision scoring of coccidia challenged groups

| Treatment | Intestinal lesions | Lesion score |
|---|---|---|
| Birds fed with multispecies probiotic consortium 1 and 2 without coccidiostat |  | Grade 2 lesions ballooning in the caecum |
| Birds fed with commercial probiotic |  | Grade 1 Lesions Mild thickening of the intestinal wall |
| Coccidiostat |  | No Lesion |
| Positive control |  | Grade 3 lesions ballooning of the caecum and severe congestion of the intestinal wall |

| | | |
|---|---|---|
| Negative control |  | No Lesion |

*Lesion score clearly indicated that in the probiotic fed with coccidiostat group reduces the severity of the infection and only Grade 1 lesions (slight redness of the intestinal wall) were observed.*

**Table 4:** Histopathology of intestine in coccidia experiment

| Treatment | Histopathology of intestine | Observation |
|---|---|---|
| Birds fed with multispecies probiotic consortium 1 and 2 without coccidiostat |  | Moderate necrotic enteritis |
| Birds fed with Commercial Probiotic with coccidiostat |  | No necrotic enteritis |
| Coccidiostat |  | No necrotic enteritis |
| Positive control |  | Severe necrotic enteritis |
| Negative Control |  | No necrotic enteritis |

## Discussion

The practice of supplying broilers with sub therapeutic doses of antibiotics to protect against coccidiosis has recently been under scrutiny. Nevertheless, anticoccidial drugs and vaccines are still the most powerful solution for controlling coccidiosis in poultry and reducing its negative economic impact. However, until these vaccines are available for commercial use, the industry has to consider probiotics as a alternative for control of coccidiosis (Ritz *et al*., 2016). Hence, probiotics can be recommended as a complementary approach in shuttle and rotation programs to reduce the incidence and severity of the disease as well as the development of anticoccidial drug resistance in

Eimeria species (Lee *et al*., 2010) reported that birds fed with Bacillus based probiotics had significantly lower lesion scores in the gut than birds fed without probiotic supplementation when challenged against *E. maxima* challenge. In this study also, probiotic supplementation has reduced the shedding of oocytes and severity of lesion. The less severe lesion in the intestinal epithelium leading to the coccidia infected birds having a greater chance of recovery from the disease.

The integrity of the digestive tract is essential for protecting the host against enteric diseases such as coccidiosis. With the ban of antibiotics as feed additives by the European Union and 'natural' alternatives need to be procured in the near future. The supplementation of probiotics enhances performance in poultry and provides protection against several economically important diseases including coccidiosis and necrotic enteritis as reported by many workers (Dalloul and Lillehoj, 2006). In this study, the significant difference was observed in terms of feed conversion ratio and body weight gain between probiotic supplemented groups in comparison to non-probiotic supplemented groups clearly indicates the early intestinal epithelial damage may be reduced with probiotic supplementation. An intact epithelium acts as a vital barrier for preventing the entry of dangerous pathogenic organisms and resulting in proper nutrient absorption and utilization leading to improved growth performance of the bird (Chapman *et al*., 2002). The probiotics as a feed additive in poultry has exhibited varying effects on the performance of the birds. Some researchers reported that probiotic supplementation in the feed can improve the body weight gain and FCR (Kabir *et al.,* 2004). Other workers stated that probiotic addition in the feed has beneficial effects in terms of body weight gain (Hashemzadeh *et al.*, 2013). Till date there is no universal available to exert all beneficial aspects of probiotics. Timmerman *et al.* (2006) demonstrated the positive impact of multispecies probiotic product available in the market. Hence, multiple species probiotic consortium was used to improve the overall performance of the chicken. However, to understand the interaction between probiotics and Eimeria species, a larger scale studies would be needed for further investigation to formulate the potential strategy to control the coccidiosis.

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## Conflict of Interests

There is no conflict of interest.

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