Preliminary Study of Anticoccidial Activity of Medium Chain Fatty Acids (MCFA) and their Corresponding Monoglycerides on Broiler Chicken Coccidiosis

G.H. Tan* and K. Long

Biotechnology Research Centre, Malaysian Agricultural Research and Development Institute, P.O. Box 12301, General Post Office 50774, Kuala Lumpur, Malaysia

Abstract: The effect of anticoccidial from oil containing medium chain fatty acids and their corresponding monoglycerides, which known as enhanced virgin coconut oil (EVCO) was investigated. A total of 100 broiler chicks were assigned to four treatment groups. Group A, B and C were infected with 8 species of live coccidia. Group A was then treated with 30% EVCO; Group B with antibiotic (Coccisul-Q) and no treatment for Group C. Group D as control. The birds infected with live coccidia (Group A, B and C) had significantly higher in the oocyst count and showed blood in droppings appeared after 7-day post-infection (DPI), except Group D. In Group A2 at 11-day post infection/4-day post treatment (DPI 11/ DPT 4), the number of oocyst count was dropped from 132,400 o/g to 600 o/g; and in Group B2 is about 75,600 o/g to 0 o/g. However, there was no significant reducing in Group C2. A significant deduction of oocysts level in faecal and cecal dramatically after 4 days post-treatment in group treated with EVCO. However, from the observation, the performance of EVCO was not as good as in the antibiotic treated group. Nevertheless, the EVCO is a plant-based compounds, it is good and safe to be used as alternative way to control coccidiosis in poultry industries. Therefore, for EVCO consumption, the treatment must be provided weekly for long lasting protection from coccidiosis.

Keywords: Anticoccidial, medium chain fatty acids, monoglycerides, broiler chicken, coccidiosis.

INTRODUCTION

Coccidiosis in young chicken is recognized as one of the parasitic disease that has the greatest economic impact on poultry meat and eggproduction. This disease needs to be controlled by coccidiostats or vaccination. Coccidiosis is not considered as a major problem in smallholder poultry due to the freerange habits of village chickens those have accessed to larger land areas. [9]. However, if smallholder poultry are restricted to a confined area, coccidiosis maybuild up to a level that would need to be controlled, and a single report on highprevalences (>75%) in smallholder poultry of Thailand seems to support this view [10]. The mechanism of parasite infection takes place by multiplying in the intestines and cause tissue damaged, lowered feed intake, poor absorption of nutrientsfrom the feed, dehydration, and blood loss. Birds are also more likely to get sick from secondary bacterial infections. However, n the low density production or with the use of preventative medication, coccidiosis generally remains a subclinical disease that only affects performance without the alarming losses of the past.

Coccidiosis is caused in poultry by a one-celled parasite of the genus called *Eimeria*. There are nine species of chicken coccidia, but only seven was valid to be pathogenic, such as *E. acervulina*, *E. brunetti*, *E. maxima*, *E. mitis*, *E. necatrix*, *E. praecox*, *andE. Tenella* [13]. Each species developing in a particular location within the chick digestive tract. Their pathogenicity ranged from moderate to severe. The life cycle of *Eimeria* is directly involving a sexual phase and a non-sexual phase. The life cycle is completed in 5 - 7 days, which ensures a rapid spread of infections in flocks. Clinical signs are highly variable in flocks and range from decreased growth to a high percentage of visibly sick animals with diarrhoea and high mortality. Infections can be seen in all age groups. Usually there is decreased feed and water consumption. Weight loss and decreased egg production are observed. Survivors of severe infections recover in 10 - 14 days, but may require even more time to recover to normal production. The degree of immunity acquired prior to the development of clinical disease may influence the severity and developmentof flock infection [9].

There area numbers of natural products such as sources of fats containing high concentrations of n-3 fatty acids or feedstuffs have been tested as anticoccidial dietary additives [1-3]. The fish oil and flaxseed oil diets significantly reduced the degree of parasitization of *E. tenella* [1] and caused ultrastructural degradation of both asexual and sexual stages in broiler. Works o by Long in 2006 showed that this EVCO inhibit growth of gram positive bacteria i.e**.** *Staphylococcus aurous, Listeria monocytogenes,*

*Address corresponding to this author at the Biotechnology Research Centre, Malaysian Agricultural Research and Development Institute (MARDI), P.O. Box 12301, General post Office 50774 Kuala Lumpur, Malaysia; Tel: 603-89431797; Fax: 603-89456037; E-mail: tangeokhun@gmail.com

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Sterptococcus pyogene, gram negative bacteria i.e. *Vibrio cholerae, Escheria coli* and yeast i. e**.** *Candida albicans*. This EVCO contains about 57.48 % triglycerides, 26.88 % diglycerides, 1.51 % monoglycerides and 14.13 % of free fatty acids and prepared by enzymatic reaction using lipase.. Enzymatic reaction of virgin coconut oil was carried out using 2.5 g of 1,3 positional specific lipase with 250g coconut oil and 2.5 ml distilled water by stirring at 250 rpm, 45 °C for 24 h [8]. The medium chain fatty acids (MCFA) and their corresponding monoglycerides have been found to have anti-coccidia activity in calve [11,12], and anti-protozoa in dairy cattle, swine, sheep, goat and infant animals [6]. Thus, the objective of this study is to determine the potential use of this enhanced virgin cocnutoil (EVCO) for controlling coccidial in broiler chicken.

MATERIALS AND METHODS

Birds and Experimental Design

One hundred day-old COBB broiler chicks which obtained from Linggi Poutry Farm, Negeri Sembilan, Malaysia were reared in a coccidia-free environment. The birds were challenged with 8 species of live coccidia (ATD-3 VAC, Coccivac, USA) at the concentration of 5X higher than recommended doses. The birds were then randomly assigned to 4 different treatments 1 week later: A: treatment with EVCO (30% in 0.01% Tween 20); B: treatment with antibiotic (Coccisul-Q, USA); C: no treatment and D: as undisturbed control group. The experiment was repeated for twice.

Bird Diets and Husbandry

Maize and soybean based starter (0 to 7 d of age), and grower (8 to 34 d of age) diets, without coccidiostat additives, were provided to the birds. Birds were reared under coccidia-free, and five birds were housed in each cage. The formal experiment was conducted from 15 to 37 d of age. The temperature was set at 30 to 33°C during the first week and was reduced by 2°C per week until 20°C was reached. Relative humidity was about 60 to 80%. The lighting program was 23L: 1D.

Preparation of Enhanced Virgin Coconut Oil from Coconut Oil

The enzymatic reaction was carried out according to Long [8], 2.5 g of 1, 3 positional specific lipase with 250g coconut oil and 2.5 ml distilled water by stirring at 250 rpm, 45 °C for 24 h. Samples were then passed through funnel containing sodium sulfate powder to remove water. The reaction mixture was centrifuged to separate the oil phase and lipid classes and fatty acids composition were determined by High Performance Thin Layer Chromatography (HPTLC) and Gas Chromatography (GC) techniques [8].

Parasite Preparation

The live vaccine (ATD-3 VAC, Coccivac, USA) were purchased from Pahang Pharmacy Sdn Bhd. It contains live oocysts of *Eimeriatenela*, *E. mivati*, *E. acervulina*, *E. maxima*, *E. brunette*, *E. hagani*, *E. necatric* and *E. praecox*. The birds were fasting before challenged with Coccivac at the concentration of 5X higher than recommended doses by force feeding method. The 30% EVCO in 0.01% Tween 20 was mixed with the bird diet and drinking water according to the experimental designed, meanwhile the anti-coccidia were supplemented as drinking water.

Sampling

At 7, 11, 15 18 and 22 days of post-infection (DPI) and 0, 4, 8, 11 and 15 days of post-treatment (DPT), 5 birds from each treatment were selected for killed. The cecal and faecal samples were collected for oocysts examination. The body weights of birds were measured once arrived and before killed for each bath of sampling.

Faecal Egg Counting

Faecal egg cont of *Eimeriasp* was examined using modified McMaster Technique [15]. Approximately 2 g of faeces from each sample was weighed into 60 ml square glass bottle and replaced each on their respective position. The saturated salt solution was mixed with the sample until the faeces is broken up and the particles were evenly distributed (3-5 sec/sample is sufficient). The samples were then transferred into a chamber of a Whitlock McMaster slide and examined under microscope [15].

Calculations and Recording

The total number of eggs per gram of faeces is calculated using the following equation:

$$\text{Number of eggs/g faeces} = \frac{\text{Number of eggs counted} \times \text{(60) total volume (mL)}}{\text{(0.3) Volume of counted chamber (mL)} \times \text{(2) weight of faeces (g)}}$$

(Shortcut for conversion = Number of eggs actually counted x 100)

Coccidia record: + (low) <10 per x100 field

++ (moderate) 10-30 per x100 field

+++ (high) >30 per x100 field

Simple Flotation Method

This is a qualitative technique for separating and concentrating of eggs/larvae. Approximately 3 g of faeces were weighed and added into the first container. Then, 50 ml of floatation fluid was poured into the container and mixed well. The resultant faecal suspension was poured through double-layer cheesecloth into second container and leaved it for 10

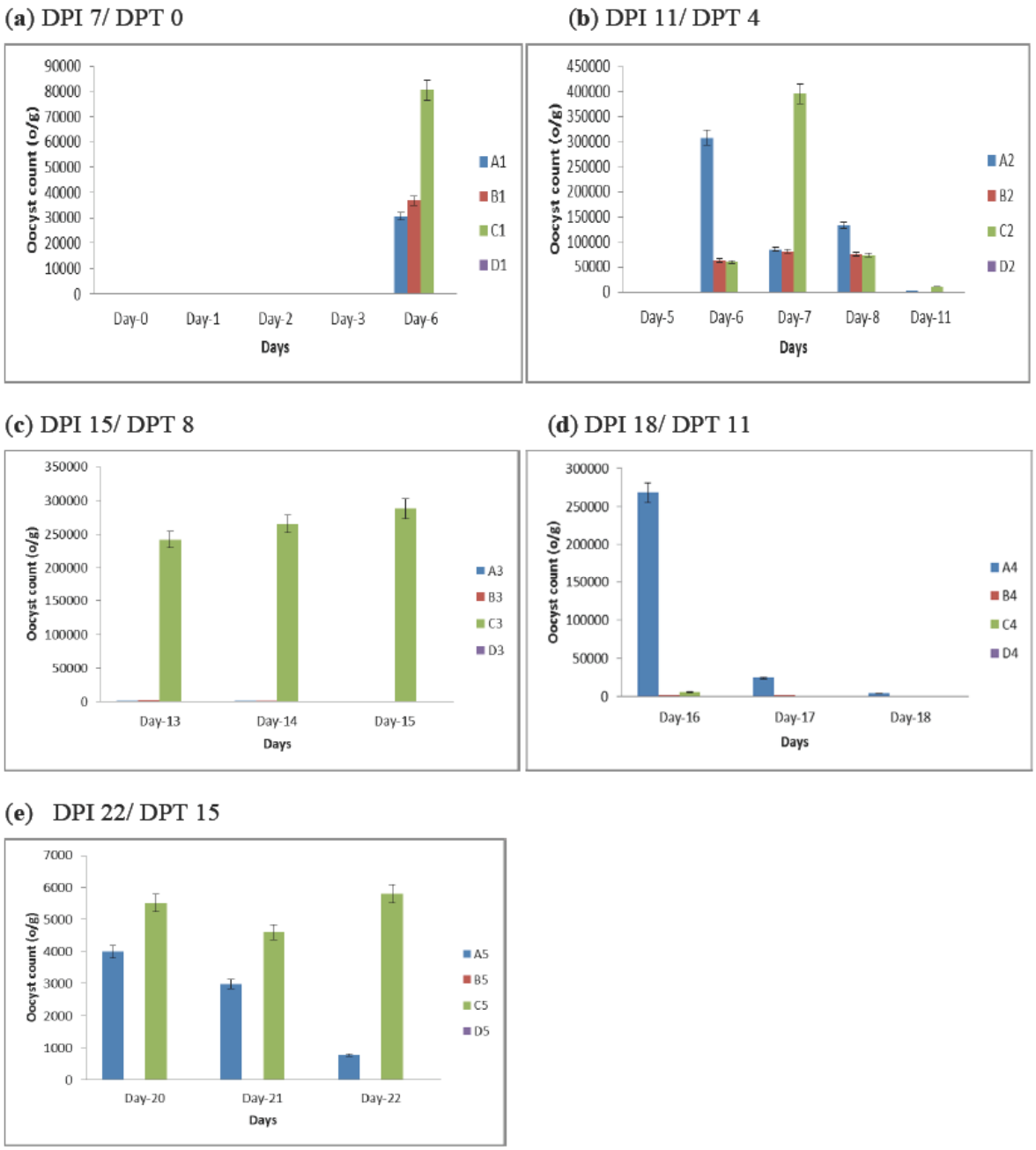


Figure 1: Oocyst count in the faecal samples from Group A, B, C and D for 3 replicates. (**a**) Oocyst count (o/g) at 7-days of post infection and 0-day of post treatment. (**b**) Oocyst count (o/g) at 11-days of post infection and 4-day of post treatment. (**c**) Oocyst count (o/g) at 15-days of post infection and 8-day of post treatment. (**d**) Oocyst count (o/g) at 18-days of post infection and 11-day of post treatment. (**e**) Oocyst count (o/g) at 22-days of post infection and 15-day of post treatment. Assays were performed in triplicates and the error bars represent the standard deviation from the arithmetic mean.

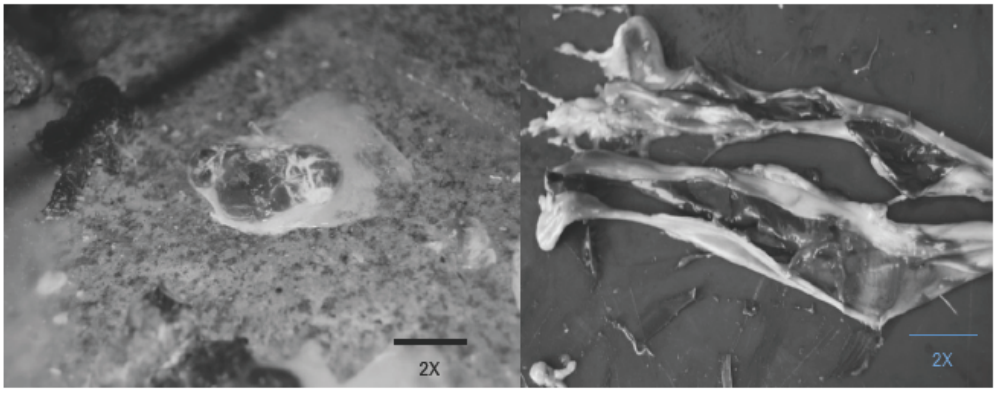


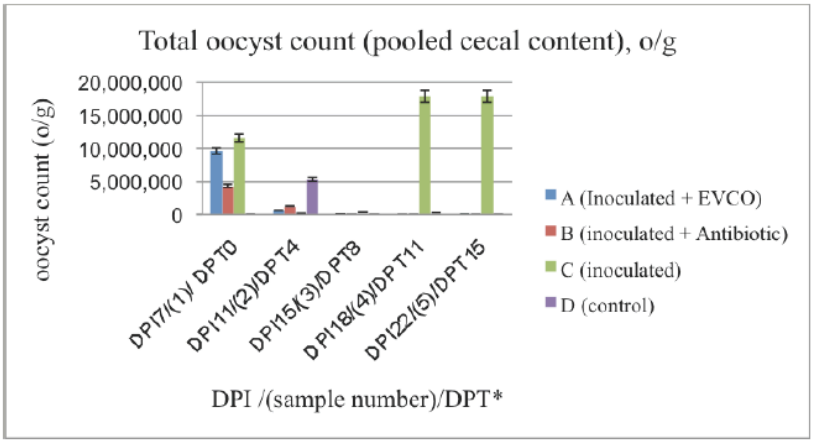
Figure 2: The faecal (**a**) and cecal (**b**) samples infected with coccidia.

minutes. After that, the faecal suspension was filled into the test tube and covered with cover slip on top. Finally, the cover slip was mounted on micro slide for microscopic examination for egg /larvae identification.

RESULTS AND DISCUSSION

The active compounds in the enhanced virgin coconut oil (EVCO) were evaluated for their anti-protozoa property against *Ermeriasp* in broiler chickens. The oocysts level from faecal samples were measured at day post infection/day post treatment (DPI 7/ DPT 0; DPI 11/ DPT 4; DPI 15/ DPT 8; DPI 18/ DPT 11 and DPI 22/ DPT 15) as showed in Figure (**1a-e**). The birds infected with live coccidia (Group A, B and C) had significantly higher in the oocyst count and showed blood in droppings appeared some after 7-d post-infection (Figure **1**), except Group D which set as undisturbed control. At 11-d post infection/4-d post treatment, the number of oocyst count was dropped from 132,400 o/g to 600 o/g in Group A2, and 75,600 o/g to 0 o/g in Group B2. However, there was no significant reducing in Group C2. The oocysts level continues to drop at 15-d post infection/8-d post treatment. Nevertheless, at DPI 18/DPT11, the oocysts count in Group A increased dramatically as compared to the other groups. This may be due to the secondary infection whereby the parasites (oocysts) excreted in faeces during the previous cycle, especially species with short pre-patent and sporulation period. Therefore, causing the fluctuation of total oocysts count as seen from the results. However, the continuously supply of EVCO treatment helped to reduce the oocysts level at 15-d post treatment. Meanwhile,t he antibiotic (Coccisul-Q) provides longer lasting protection towards recurring infection.

Many different types of substances have been investigated in the search for alternatives controls of coccidiosis [1-4]. The results reported in this study



* DPI refers to "days of post infection". DPT: days of post treatment.

Figure 3: Total oocyst count (pooled cecal content), o/g. Assays were performed in triplicates and the error bars represent the standard deviation from the arithmetic mean.

have shown the potential of this mixture of oil to control the sporulation of coccidia in the broiler chickens. Coccidia are parasites that get their nutrients from the chicken host, and the infection is different from bacterial and viral infection because coccidia are self-limiting and usually stop multiplying before killing the bird [5].

The sampling of faecal and cecal was examined concurrently because it is presumed that most of the

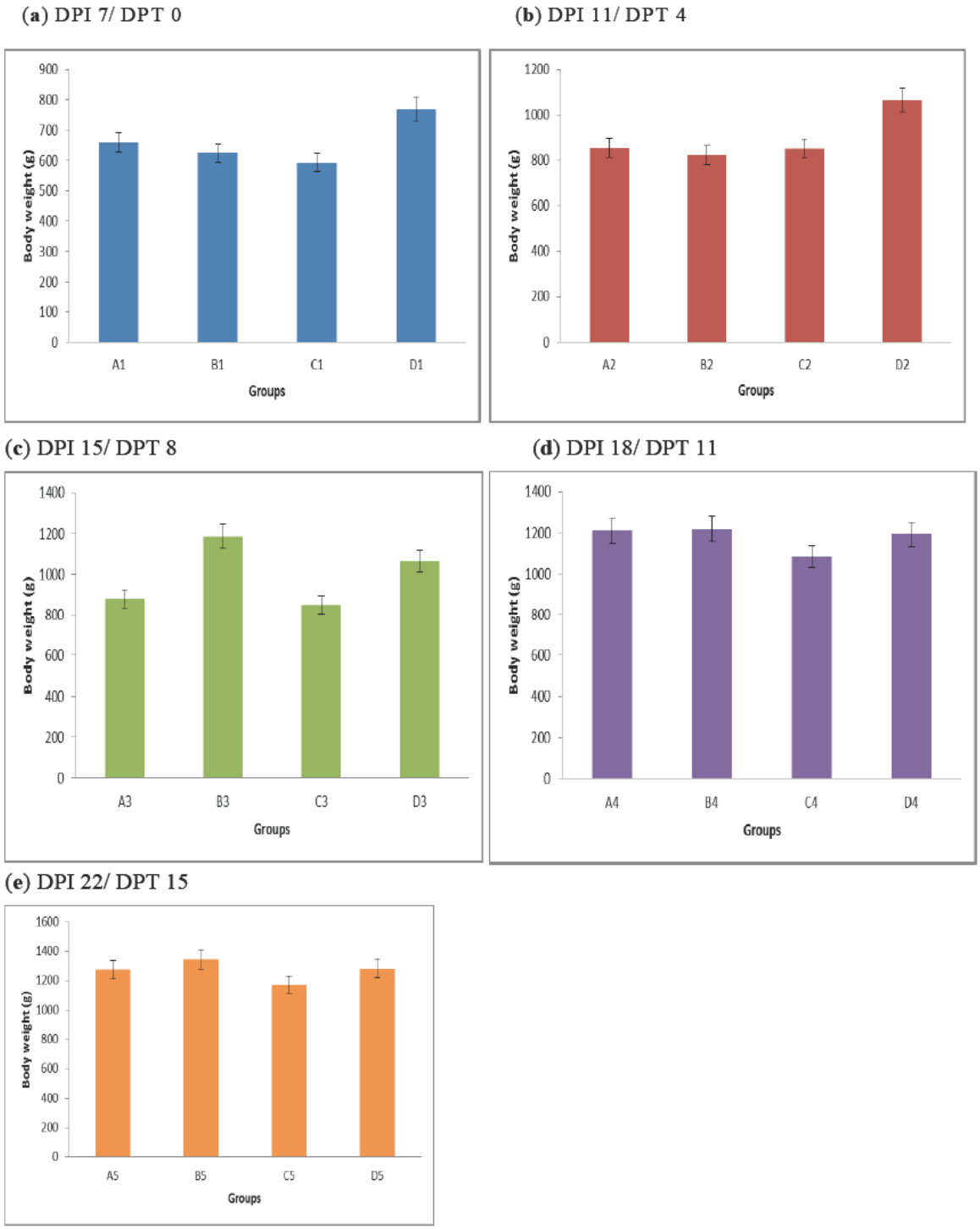


Figure 4: Body weight (g) measurement of birds in Group A, B, C and D for 3 replicates. (**a**) The body weight (g) at 7-days of post infection and 0-day of post treatment. (**b**) The body weight (g) at11-days of post infection and 4-day of post treatment. (**c**) The body weight (g) at15-days of post infection and 8-day of post treatment. (**d**) The body weight (g) at18-days of post infection and 11-day of post treatment. (**e**) The body weight (g) at 22-days of post infection and 15-day of post treatment. Assays were performed in triplicates and the error bars represent the standard deviation from the arithmetic mean.

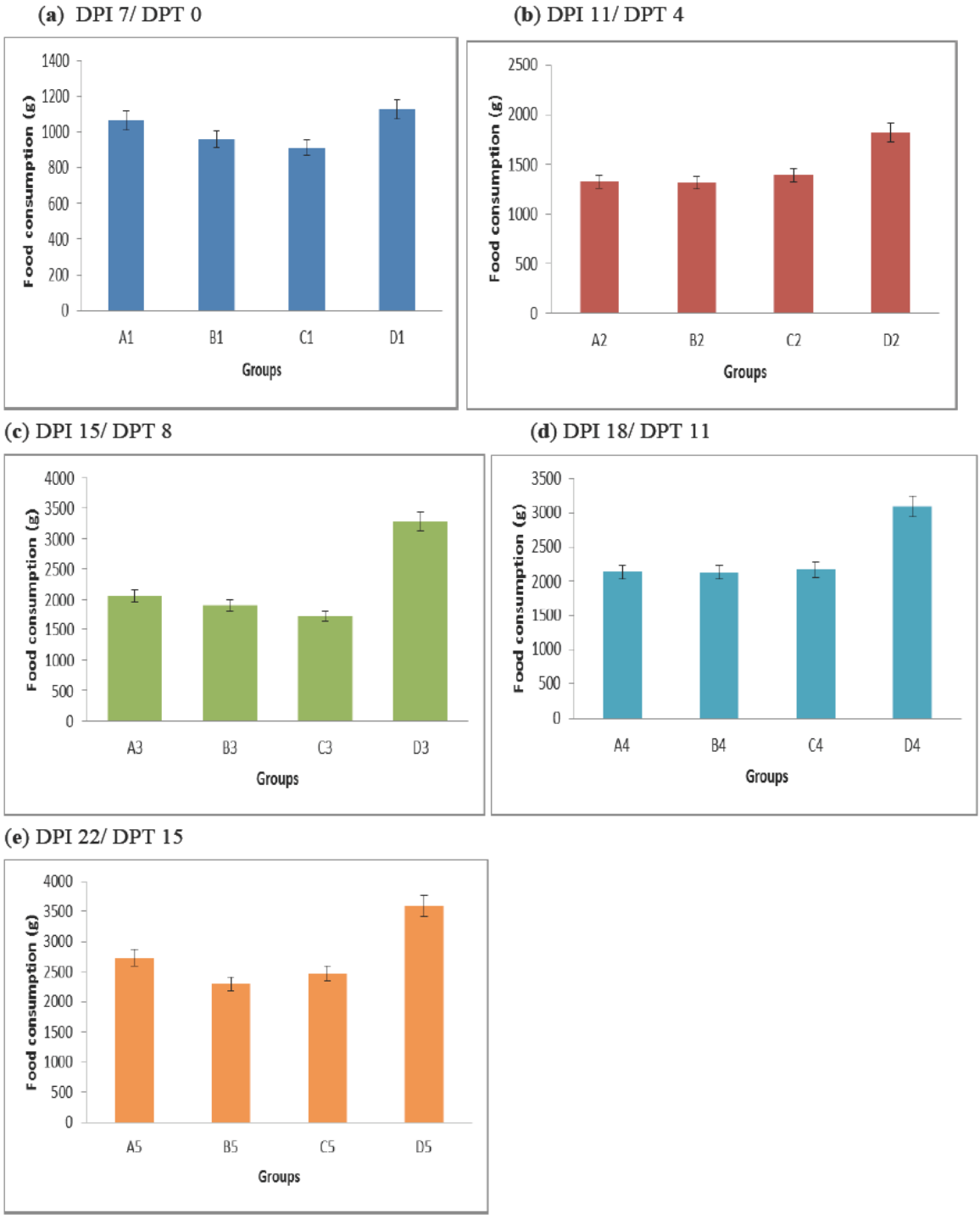


Figure 5: Food consumption (g) of birds in Group A, B, C and D for 3 replicates. (**a**) The food consumption (g) at 7-days of post infection and 0-day of post treatment. (**b**) The food consumption (g) at 11-days of post infection and 4-day of post treatment. (**c**) The food consumption (g) at 15-days of post infection and 8-day of post treatment. (**d**) The food consumption (g) at 18-days of post infection and 11-day of post treatment. (**e**) The food consumption (g) at 22-days of post infection and 15-day of post treatment.Assays were performed in triplicates and the error bars represent the standard deviation from the arithmetic mean.

Eimeria species will pass through the cecal before excreted out with the faeces. Therefore, the oocysts were collected from the cecal since they might represent the whole pooled "population" of *Eimeria*species that might infect that single host. Thus, the enumeration of oocysts collected from the fecal sample can also be used to confirm the result. The fluctuation of total oocysts count in Group A at the 11-d post treatment may be due to secondary infection and also the differences of pre-patent period of *Eimeriasp* in

the live coccidia vaccine. The pre-patent period and sporulation of *E. praecox* is 84 h and 24.8 h; *E. acervulina* is 89 h and 11.4 h; *E. maxima* is 120 h and 38.1 j; *E. brunette* is 120 h and 38.3 h; *E. tenella* is 132 h an 21.2 h; *E. necatrix* is 138 h and 19.7 h, and *E. mitis* is 91 h [13, 14]. This enhanced virgin coconut oil significantly reduced the degree of parasitization where the number of oocysts dropped dramatically after 4 days of post-treatment. This finding corroborates with study of Medium Chain Triglyceride (MCT) in calve where the oocysts had disappeared from the faeces after 3 to 11 days of post-treatment [11, 12]. Although the use of natural products were not as effective as antibiotics. On the other hand, at the downside of view, antibiotic stays longer in the host, and if this were commercial flocks sold to consumers, the antibiotic might ends up in human food chain as well. Therefore, for EVCO consumption, the treatment must be provided weekly for long lasting protection from coccidiosis.

The total oocysts count in pooled cecal (Figure **3**) also showed the similar results as obtained from the faecal samples. Anyway, there was some oocysts appeared in Group D, which supposed to be a control sample. This may be due to cross-infection or indirect infection may occur from airborne oocysts (from other infected samples) since there were no effective physical barrier between cages. The body weights of birds were significantly less in infected groups (A, B and C) than their respective control (Group D) (Figure **4**). Group A revealed higher body weight than Group C and the highest weights were observed in Group B after the treatment with antibiotic (coccisul-Q). The body weight gains were significantly less in infected groups than the respective control group. However, the food consumption is not significantly different in Group A, B and C, except D (Figure **5**).

CONCLUSION

The enhanced virgin coconut oil is found to exhibit anti-coccidia activity in chicken. As it is a plant-based compounds, there is no problem if its end up in human food chain. Therefore, it can be a good and safe alternative to coccidia antibiotic.

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