J. Agrobiotech. **Vol. 9** (1S), 2018, p. 283–293.

ISSN 1985-5133 (Press)
ISSN 2180-1983 (Online)

The Effect of Em-1® (*Lactobacillus Spp.)* On Growth Performance of Quails, *Coturnix Japonica*

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Keywords:
Quails
Coturnic Japonica
Growth performance
Effective microorganism

ABSTRACT

This study aims to determine the effectiveness of using effective microorganism as a supplement in diet on growth performance and hepatosomatic index of quails. A 14 days age quails were used in a trial in triplicate. The experiment were carried out by comparing the control (c) group with three group of effective microbe enrichment feeding in which ration was 1L EM: 250 L H2O (treatment 1), 1L EM: 550 L H2O (treatment 2) and 1L EM: 750 L H2O (treatment 3), respectively. Effective microorganism was supplemented via drinking water. Weight measurements of the quails were carried out every 7 days and growth performance parameters of the quails such as Body Weight Gain (BWG), Feed Intake (FI), Feed Conversion Ratio (FCR), Average Daily Gain (ADG) and Hepatosomatic Index (HSI) were also recorded. The data collected was analyzed for ANOVA one-way test using Statistical Package for Social Science (SPSS) software. The result shows there was significant different ($p<0.05$) between control group and three other treatment during week four in terms BWG, FCR, ADG and FI. The result of hepatosomatic index shows no significant different ($p>0.05$) between treatments. This may indicates that the effective microbe supplementation did not affect the liver metabolism.

Keyword: Quails, *Coturnic Japonica*, Growth performance, Effective microorganism, Hepatosomatic index

ABSTRAK

Kajian ini bertujuan untuk menentukan keberkesanan menggunakan efektif mikroorganisma sebagai suplemen dalam diet terhadap prestasi pertumbuhan dan indeks hepatosomatik burung puyuh. Tempoh percubaan makanan bermula pada hari ke 14 puyuh dilakukan secara tiga kali ulangan dengan membandingkan kawalan (c) kumpulan dengan tiga kelompok mikroorganisma yang berkesan dengan nisbah adalah 1L EM: 250 L H2O (rawatan 1), 1L EM: 550 L H2O (rawatan 2) dan 1L EM: 750 L H2O (rawatan 3). Efektif mikroorganisma diberikan kepada puyuh melalui air minuman. Berat puyuh ditimbang setiap 7 hari dan parameter prestasi pertumbuhan yang digunakan untuk membandingkan prestasi pertumbuhan puyuh seperti Kenaikan Berat Badan (BWG), Pengambilan Makanan (FI), Nisbah Penukaran Makanan (FCR), Purata Kenaikan Harian (ADG) dan Indeks Hepatosomatik (HSI) telah direkodkan. Data yang dikumpul dianalisis untuk ANOVA satu hala menggunakan Pakej Statistik untuk Sains Sosial (SPSS). Keputusan menunjukkan terdapat perbezaan yang signifikan ($p <0.05$) antara kumpulan kawalan dan tiga rawatan lain dalam empat minggu pengajian dalam istilah BWG, FCR, ADG dan FI. Hasil indeks hepatosomatik menunjukkan tiada perbezaan yang signifikan ($p>0.05$) antara rawatan kerana ia menunjukkan bahawa puyuh yang diberikan efektif mikroorganisma dengan nisbah yang berbeza tidak memberi kesan buruk pada metabolisme hati mereka.

Kata kunci: Puyuh, *Coturnic Japonica*, Prestasi pertumbuhan, efektif mikroorganisma, indeks hepatosomatik

INTRODUCTION

The Japanese quails, *Coturnix japonica* is the most productive commercial strain in India but there is also a great deal of confusion concerning the taxonomic status and a variety of vernacular names persist even in the current literature with the origin of the Coturnix quails (Crawford, 1990). Thear (1998) and Mizutani (2003) reported that the scientific designation of the Japanese quails, *Coturnix japonica* differed with the common quails *Coturnix coturnix*. The distribution of Japanese quails, *Coturnix japonica* that spread throughout the large areas including Asia, Europe, and Africa and it is the most common quail species for the meat and eggs consumption. In 11th century, it was domesticated as the pet song bird (Kayang et al., 2004) and then, commercially used as the food consumption (Kayang et. al., 2004). Demand of the poultry product is expanding and growing rapidly in Malaysia. This growing industry can be developed well because most of the Malaysians prefer to consume poultry product especially meat as their daily consumption and market price of the poultry product are much cheaper rather than other protein source such as beef and mutton. Nowadays the largest quail meat producers are Europe and USA. Malaysian farmer are still lack of facilities, knowledge in quail production. And many farmers have negative perception on the quail meat quality. However, quail industry can become established in the industry and contribute to the demand of the poultry meat that increases every year worldwide. In addition, the usage of quail meat and its product over the last few years has grown rapidly (Awan et al., 2017).

There are many supplements or feed additives that are used as growth promoter in the poultry industry. The changes in the diet can substantially affect the growth and health of the animals by improving weight, flavor and body part proportions of animal. The practice of the antibiotic as the feed supplement is not relevant to be use in poultry industry due to it probable relationship with the antibiotic resistance bacteria in human (Baurhoo et al., 2009) and the presence of the antibiotic residues in product of the animal origin intended for human consumption. Europe and United States countries have banned the sub-therapeutic antibiotic usage that encourages more studies onto alternatives of antibiotic for poultry production. Probiotics is one of the promising approaches that can be used as feed supplement. Probiotic has been used for centuries; contain a component that can affect the body function and give a positive impact on health (Bellisle et al,. 1998). It is efficient to use probiotic to improve the animal production by reducing impact to the environment and effectively to lowers the prevalence of pathogen (Yirga, 2005).

Probiotic are microorganisms that are usually assumed to be non-pathogenic component of the normal microflora such as lactic acid bacteria. However, there are studies that show some of the pathogenic species can be used as probiotic and can protect against infection by respective virulent parent strain such as a virulent mutants of *Escherichia coli, Clostridium difficile, and Salmonella Typhimurium* (Fuller, 1995). It is important to maintain the intestinal microflora balance in animals as it will help to prevent disease by controlling the overgrowth of the pathogenic bacteria in the gut. As the probiotic that act as the living organism that contain certain number of exert health effect beyond inherent basic nutrition (Guarner & Schaafsma, 1998). Probiotic microorganism that proposed mechanism of pathogen inhibition include the production of antimicrobial conditions and compound such as volatile fatty acid, low pH, and bacteriocins , competition for binding sites on the intestinal epithelium and nutrition and stimulation of immune system (Rolfe, 2000). The acidic environment and the high bile concentration in gastrointestinal tract will help to make probiotic to work efficiently by work together with the primary barrier of the gut. Probiotic is widely used in the industry as the feed supplement or additive (Cromwell, 2002). To get the effective probiotic to the animal, it will need a specific probiotic species and correct dosage to form strain. Probiotic that were released as feed additive to animal in the market need specific strains, standardization of the dosage, viability and biosafety (Vohra & Satyanarayana, 2012).

This recent study was designed to analyze the effect of probiotic enrichment in the feeding regime of quails. Since any enrichment feeding might have positive or negative effect, we are also measure the hepatosomatic index of the quails, to see if the enrichment is burdening the liver process.

MATERIALS AND METHODS

Experimental design

A total of 240 broiler quails were selected and separated into four treatment groups with 60 quails per group. The experimental study was conducted after Day Old Quail (DOQ) were brooded for 14 days and bred until age 42 days. The quails in treatment groups were randomly arranged in the cages with the size of 150 g to 170 g. Quails were fed with commercial diet *ad libitum*. Experimental probiotic enrichment solution was given twice daily according to availability of the water in the water trunks. Three treatments with different concentration of probiotic were tested in this study. The experimental probiotic enrichment was prepared as below:

Control (C) = 100% water
Treatment 1 = 1 litre Pro EM-1 + 250 litre water
Treatment 2 = 1 litre Pro EM-1 + 500 litre water
Treatment 3 = 1 litre Pro EM-1 + 750 litre water

Data collection

Initial weights of the quails were recorded on day of arrival using weighing scale. The body weight of the quails were measured every week starting from day 1 (arrival day), 7, 14, 21, 28, 35 and 42 day respectively.

In the experiment, the average body weight (ABW), daily feed intake (FI), feed conversion ratio (FCR), average daily gain (ADG) and Hepatosomatic Index (HSI) were calculated. All of the calculations were conducted based on the formula below (Malik et al., 2012; Manafi et al., 2016):

Body Weight Gain (BWG) = Average final weight - average initial weight

$$\text{Feed Conversion Ratio (FCR)} = \frac{Total\ feed\ intake}{Total\ weight\ gain}$$

$$\text{Feed intake} = \text{initial weight amount of feed (g)} - \text{remaining weight amount of feed (g)}$$

$$\text{Average daily gain} = \frac{Average\ final\ weight - average\ initial\ weight}{days\ of\ experiment}$$

$$\text{Hepatosomatic Index (HSI)} = \frac{drained\ mass\ of\ liver\ (g)}{total\ weight\ of\ the\ liver\ (g)} \times 100$$

Statistical analysis

Data obtained from study was analyzed for One-way Anova using SPSS Statistical Analysis version 20.0 to determine the significance of the result ($p<0.05$).

RESULTS

Effect on body weight gain and average daily gain

Figure 1 showed the increasing pattern of body weight gain (BWG) of the quail throughout the experiment. BWG of the quail in control and Treatment 2 group are always the highest in four consecutive feeding week. Result shows that there is significance different ($p<0.05$) between C and treatment 1 as weight of the quail for both treatment were 261 g and 248 g respectively at week 4. Treatment 1 has the lowest BWG in the end of study that is 248 g compare to the other group. However Treatment 2 show positive result compared to Treatment 1 and Treatment 3 as it almost get high body weight for four weight of study. Throughout four week of study, control group showing highest BWG in comparison to the treatment groups. This rise many question to our team. Is normal feeding without the probiotic enrichment is better than the enrichment feeding?

Figure 2 shows that Average Daily Gain (ADG) of quails within 4 weeks period of study fed with four different experimental diets demonstrated a decline in all parameters reading. At week 1 of the experiment, Control has the highest ADG at 13.43 g and followed by Treatment 2 and treatment 3 with same value at 10.85 g, and Treatment 1 with 10.57 g respectively. The quail gained the peak ADG at week 1 as the probiotic was start given to the quail. Throughout four weeks of study, the ADG of the quail gradually decreases as the age of the quails increased. The result shows that Control group has the highest ADG for four weeks of study as compared to the other three treatments. However, Treatment 3 shows the positive effect on the ADG of the quail throughout the period of study compare with Treatment 1 and 3.

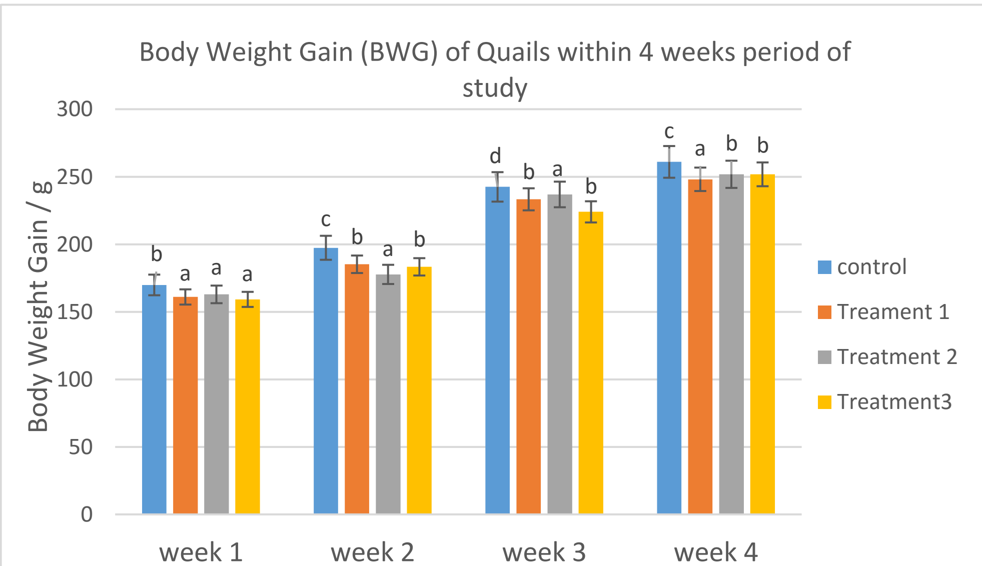

Figure 1 Body Weight Gain (BWG) of quail within 4 weeks of study period. a, b c and d in the graph means having different superscripts within the same column differ significantly ($p<0.05$)

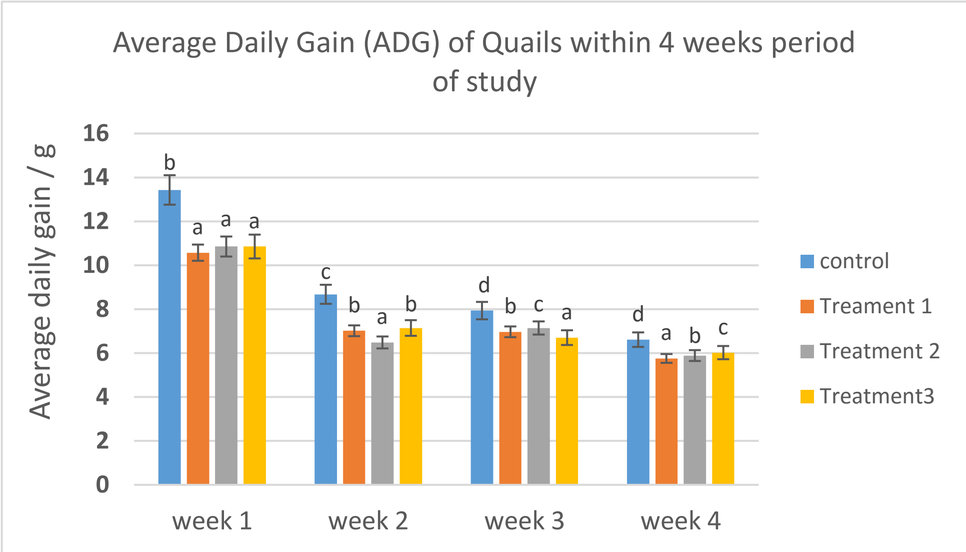

Figure 2 Average Daily Gain (ADG) of quails within 4 weeks of study period. a, b, c and d in the graph means having different superscripts within the same column differ significantly ($p<0.05$)

Effect on feed intake and feed conversion ratio

Figure 3 showed the feed intake (FI) increased significantly from week 1 until week 4. From week 1, the data show the feed intake pattern was quite similar for every week. During the first week of the experiment, there were significant different ($p<0.05$) between the control group, Treatment 1 and Treatment 2. Quails in Treatment 2 group consume less feed at 185.8 g while treatment 1 have higher feed intake at 197.2 g compare to other group. From week 2 until week 4, there were significant difference ($P<0.05$) between Control and the treatment group. Figure 4 showed feed conversion ratio (FCR) for each respective treatment was gradually decline from week 1 until week 4 and week 1 demonstrated highest FCR. In week 1, C, Treatment 1, Treatment 2 and Treatment 3 showed FCR ranging from 1.14 to 1.22. In week 2, Control is the lowest FCR at 1.16 in comparison to another three groups which show similar FCR. In comparison, Treatment 2 had the lowest FCR (0.86), followed by Control (0.87), Treatment 3 (0.93) and Treatment 1 (0.95) in week 4 respectively. In week 4, the value of treatment 2 is lower compare to the Treatment 1 and 3 in term of FCR, suggesting that growth performance of the quail improved with inclusion 1 litre Pro EM-1 + 500 litre water.

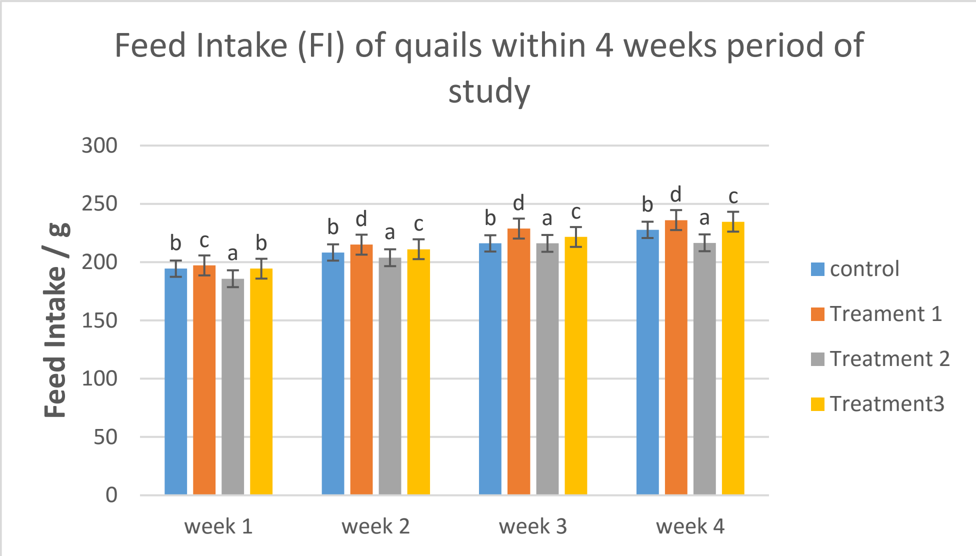

Figure 3 Feed Intake (FI) of quails within 4 week of study period. a, b, c and d in the graph means having different superscripts within the same column differ significantly ($p<0.05$)

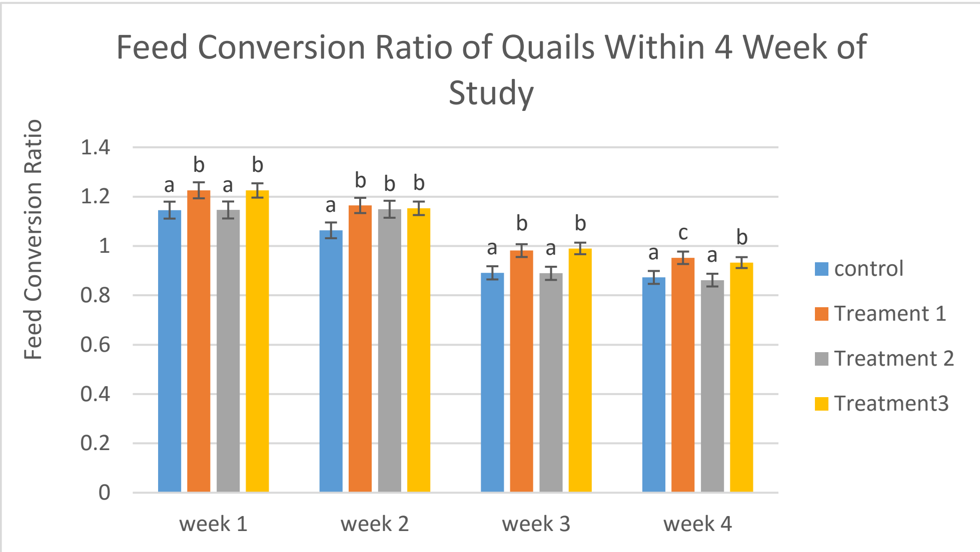

Figure 4 Feed Conversion Ratio (FCR) of quails within 4 week of study period. a, b, c and d in the graph means having different superscripts within the same column differ significantly ($p<0.05$)

Table 1: Mean value summarization of BWG, ADG, FI and FCR

	Week 1				Week 2				Week 3				Week 4			
	C	T1	T2	T3	C	T1	T2	T3	C	T1	T2	T3	C	T1	T2	T3
BWG	169.92* ± 0.75	161.02 ± 0.62	162.93 ± 1.56	159.23 ± 1.29	197.40* ± 2.38	185.17 ± 1.29	177.73* ± 1.07	183.26 ± 0.62	242.53* ± 0.36	233.27 ± 0.63	236.97* ± 0.95	224.03 ± 0.95	261.04* ± 0.62	248.10* ± 0.71	251.80 ± 1.13	251.83 ± 0.95
ADG	13.43* ± 0.08	10.57 ± 0.10	10.85 ± 0.10	10.85 ± 0.26	8.68* ± 0.14	7.01* ± 0.11	6.48* ± 0.14	7.14* ± 0.08	7.93* ± 0.03	6.97* ± 0.04	7.14* ± 0.05	6.70* ± 0.03	6.61* ± 0.03	5.76* ± 0.04	5.87* ± 0.01	6.02* ± 0.01
FI	194.10* ± 0.06	197.20* ± 0.06	185.80* ± 0.06	194.40 ± 0.07	208.30* ± 0.07	215.00* ± 0.08	203.80* ± 0.06	211.10* ± 0.06	216.10* ± 0.08	228.80* ± 0.06	216.10* ± 0.06	221.60* ± 0.06	227.70* ± 0.09	236.10* ± 0.07	216.60* ± 0.06	237.70* ± 0.06
FCR	1.15* ± 0.01	1.23* ± 0.01	1.15* ± 0.01	1.22* ± 0.1	1.06* ± 0.09	1.16 ± 0.01	1.14 ± 0.01	1.15 ± 0.00	0.89* ± 0.01	0.98* ± 0.02	0.88* ± 0.01	0.99* ± 0.02	0.87 ±0.01	0.95* ±0.01	0.86 ±0.01	0.93* ±0.0

*mean significant different ($p<0.05$), BWG = Body Weight Gain, ADG = Average Daily Gain, FI = Feed Intake, FCR = Feed Conversion Ratio, C = control, T1 = 1 litre Pro EM-1 + 250 litre water, T2 = 1 litre Pro EM-1 + 500 litre water, T3 = 1 litre Pro EM-1 + 750 litre water.

Effect on hepatosomatic index

HSI is a parameter used to examine the ratio of liver per weight of the carcass of the animals. The hepatosomatic index (HSI) off all treatments in this study were not significant difference ($p>0.05$). According to the result, Treatment 1 had the highest HSI at about 1.71 percent while control had the lowest HSI at 1.61.

Table 2 Hepatosomatic index (HSI) value between treatments

	C	T1	T2	T3
HSI	1.62±0.37	1.71±0.67	1.61±0.68	1.76±0.68

C = control, T1 = 1 litre Pro EM-1 + 250 litre water, T2 = 1 litre Pro EM-1 + 500 litre water, T3 = 1 litre Pro EM-1 + 750 litre water.

DISCUSSION

The main role of feed is to provide enough nutrients that are required by metabolic function of the body but it also needs to modulate the various function of the body. Effective microbes are the beneficial microorganisms or the substrate that facilitate the growth of this microorganism which can be suitable harnessed by the food manufacture and considerable for the health care industry. The improvement of broiler chicken growth performance and feed efficiency that fed with probiotic (Zulkifli et al., 2000; Kabir et al., 2004; Mountzouris et al., 2007; Samli et al., 2007) need to be induced by the total effect of the probiotic action including maintenance the microbial population (Fuller, 1989), improving digestion and feed intake (Nahanshon et al., 1993), and altering bacterial metabolism (Jin et al., 1997).

The effective microbe strains, *Lactobacillus* spp. on the growth performance of the quails was evaluated in this study. All the birds were in a good health condition during the experimental period of 4 week. The result of this study showed that there is increment in body weight gain of the quail throughout the experiment. However, the Control group showing higher BWG in comparison to all treatment groups. This result was contradicted with our early hypothesis where we forecasting that the probiotic enrichment might increase the BWG of the treatment groups. However, several authors also reporting the same situation with broiler chickens. Huang et al. (2004) reported that enrichment of *Lactobacillus casei* or *L. acidophilus* in the diets of broiler chicks does not show any significant increment of BWG. While Panda et al. (2000) reported that the BWG of chicken supplemented with host-specific (KTM, 74/1 and 59), *L. acidophilus* and *Streptococcus faecium* does not show any significant difference compared with those given non-supplemented diets.

On the other hand, there are numerous studies that report the positive effect of using probiotic on bird performance. Jin et al., (2000) reported that BWG of broiler was improved by using *L. acidophilus* and by single strain of Lactobacillus (*E. faecium*) (Cao et al., 2013) or a mixture of Lactobacillus (Kalavathy et al., 2003). Type of the probiotic and the condition of the probiotic that were used can affect the magnitude to improve the efficiencies. The range of the BWG could be from 5% to 9% higher and reduce the FI 2% lower when supplemented with probiotic (Mohan et al., 1996).

The effect of the probiotic on the growth performance, feed conversion ratio or the production of the animal not in consistent to consider their uses due to the economic considerations although in the specific situation (Veldman, 1992). As the study was based on the laboratory or small scale experiment under a clean condition that may have masked any growth promoting effect of probiotic. The FCR show significant different ($p>0.05$) between control and treatment 2 in week 2. As in line with several studies that shows that FCR can be improve by supplement the feed with probiotic (Silva et al., 2000). However, there is no significant difference in week 2 between control and treatment group in week 2. Animal that supplement with probiotic does not influence the FCR significantly or no effect on FCR (Ahmad, 2004). Chatsavang and Watchangkul (1999) reported that FCR does not give any significant difference between the treatment groups. FCR can be defining as amount of the feed that being consumed by the animal in order to build up the muscle body. Thus, the lower the FCR is needed as the animal will consumed less amount of feed and converted into high weight gain.

It has been stated that supplementation of probiotics has no effect on the performance of broiler chicks (Ergun et al., 2000; Kumprechtova et al., 2000). Maiolino et al., (1992) reported that chicken that given diet with or without Lactobacillus culture does not show significance difference in weight gain. However, Baidya et al.,

(1993) reported supplement with probiotic will be the effective way for growth performance and the chicken can achieve more weight than other groups (Zulkifli et al., 2000).

The inconsistent of result when using effective microorganism in the growth performance have been a constraint to the promotion of their uses. The differences of microbial species or strains of microorganism that were used or the method of preparing the supplement may cause the variations in the efficiencies of probiotic. The benefit of the probiotic may be result when there are organism grows and contribute the beneficial function to the intestinal tract. Therefore, the most importation action in achieving the desired effect from using probiotic as growth promoter by ensure the organism of probiotic can survive passage through the stomach and proliferate the intestinal tract. The bacterial strains must be able to adhere the physically and multiply on the intestinal surface in order to successfully establish in intestinal tract. In addition, there is a tendency for probiotic supplement to be less effective in a high production number of quail. Timmerman et al., (2005) reported that the growth performance of the control group of veal calves does not influence by magnitude of the effect of probiotic. Administration of the probiotic to the quail might be another factor to influence the efficacy of the probiotic. Watkins and Kratzer (1984) reported that probiotic that supplemented by drinking water resulted in lower average daily gain compared to the probiotic that administered via feed (Jin et al.,2000; Zulkifli et al., 2000; Kalavathy et al., 2003). Another determinant of probiotic efficacy may be the timing of administration. Timing to administered become issue as the bacteria in effective microbe can be productive in what kind of condition.

Hepatosomatic index (HSI) is methods that examine the ratio of liver per weight of the carcass of the animals. HSI is a reliable indicator of hepatic growth and development according to age and physiological or physiochemical status of the liver. The result indicates that all treatments using different percentage of probiotic had no negative impact on the liver size of the quails as there were no significance differences ($p<0.05$) among the control group and treatment group. In contrast, the weights of liver, spleen, pancreas, bursa, gizzard and duodenum were not affected by the treatments (Chen et al., 2016). Awad et al. (2009) and Samli et al. (2007) reported that there are no significant differences in liver weights between the control and the probiotic supplemented groups. HSI important in order to describe the effect of the supplement that add in the feed and is a good indicator of the animal feeding activity. Liver is one of the main organs that collect and utilized all the toxin before excreted from the body. This study also shows that higher amount of probiotic that given to the quails in feed not give adverse effect toward quail health. This indicates that the liver maintain in normal size without effect the function of the amino acid catabolism.

CONCLUSION

In this study, feed incorporated with probiotic did not have significant effect on growth performance of quail especially in the average daily gain, weight gain, feed conversion ratio and feed intake. It shows control group have better result compare to the treatment group. However, 1 litre EM + 500 litre water (treatment 2) of probiotic inclusion had shown a positive effect on the growth performance of the quail starting from week 1 till week 4 while probiotic with inclusion of 1 litre EM + 250 litre water (treatment 1) of probiotic had the lower growth performance of the quail within the experimental period. The result of the Hepatosomatic Index also demonstrated that all the treatment had no negative effect on the quail.

ACKNOWLEDGEMENT

The authors wish to express their gratitude to laboratory staff at Faculty of Bioresources and Food Industry, Universiti Sultan Zainal Abidin Besut Campus for their invaluable assistance and hospitability throughout the study. The authors also grateful to Universiti Sultan Zainal Abidin for supporting this research under University Grant (UniSZA/2017/DPU/11).

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