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Short communication

Effect of Effective Microorganisms (EM1®) on Microflora Population and Carcass Yield of Quails

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Abstract

A total of 240 day-old Japanese quails were used to investigate the effect of EM1® on the microflora population and carcass quality of the quails in day 42 through drinking water. They were assigned randomly into four groups with 60 quails per treatments. The four treatments groups were given drinking water (DW) supplemented with EM1® (EM) with different rations which were: i) Drinking water (control group), ii) 1L EM + 250mL DW, iii) 1L EM + 500 mL DW and, iv) 1L EM + 750 mL DW. The result shows the population of *Escherichia coli* in the jejunum and faeces of the quails decrease significantly ($p<0.05$). It was also significantly increase the weight of eviscerated carcass with the value 183.17g, 210.0g, 221.0g and 225.0g and thigh weight of the quails with the value 26.57%, 31.25%, 32.01% and 33.68% for Control, T1, T2 and T3, respectively. However, the additional of EM1® into the drinking water does not significantly increase the *Lactobacilli* population in the jejunum and faecal sample of the quails. There were also no significant differences in the body weight and breast weight of the quails. At the end of this experiment, there is no *Salmonella* sp. were detected. In conclusion, quails given EM1® are suggested to reduce the number of *E. coli* population in intestine and produce better carcass quality.

Keywords: *Effective Microorganism (EM1®), Coturnix japonica, Gut Microflora, Carcass Quality*.

1. Introduction

Poultry industry is one of the most dynamic agribusiness trades. According to Baharumshah & Mohamed [1], the demanding of poultry industry is higher in Malaysia as it has been recognized as one of the highest poultry consumers in the world. Besides, poultry meat is the largest source of protein for human nutrition. Low retail price and the use of modern technology in production also contribute to the demand of poultry meat [2]. Consumption per capita of poultry meat in Malaysia is about 40 kg per year [3]. Although the production of poultry in Malaysia obtains self-sufficiency levels which are 98.4% poultry meat, it is still insufficient to meet the demand of consumers in the future due to rapid increase of Malaysian population.

The efficiency of poultry in converting the feed into meat plays an important role in broiler industry. Thus, feed efficiency for poultry need to be improved in order to produce economically feed that also safe to the consumer. Sub therapeutic antibiotic has been used in broiler diets for growth improvement and control of intestinal pathogens. However, this antibiotic given to the broiler will caused the antibiotic-resistant develop in human when they consumed it [4]. The long term use of antibiotic may have a side effect with the residue in meat. It is important to search for alternative sources to replace the antibiotic which trigger the same positive result without affecting bird growth, feed utilization and quality of final product. Therefore, the use of probiotic and prebiotic has been considered as an alternative approach for animal production and health worldwide [5]. In order to increase the production of quail, EM has been introduced in quail farming. EM stands for "Effective Microorganism", a liquid which contain of lactic acid bacteria (Lactobacillus and Pedicoccus), yeast (Sacharomyces) and small amounts of phototrophic bacteria, actinomyces and other organism with a pH of 3.5 [6]. EM is mixed cultures of beneficial microorganism that are not genetically modified or harmful to both humans and animals. EM that is known as probiotic is one of the direct-fed microbial (DFM) has been widely used in agriculture and animal husbandry because of its ability to enhance the performance of farm animals and replacing the use of antibiotics for growth promoter in animal feed. The use of EM in animal feeds are claimed to be beneficial to the animal as it improves meat and manure quality, animal health, reduction of manure odour and absence of toxic that affect bird growth [5].

The objective of this study was to determine and compare the carcass quality and microflora population in intestinal tract and faecal sample of quails supplemented with EM1® and without EM1®.

2. Methodology

2.1. Experimental design

The study was conducted in a clean or sanitary environment (experimental facilities were properly disinfected, clean pine wood shavings used and good management practice). Quails in brooding

period were housed in an environmentally controlled room, following a standard brooding temperature and good ventilation. Concrete floor pens were covered with a clean canvas following pine wood shavings and equipped with 1 feeder container and 1 drinking container. This experiment was done at the Faculty Bioresources and Food Industry in Universiti Sultan Zainal Abidin, Besut, Terengganu from November to January. October to March is a monsoon season in Besut, where heavy rain and low temperature (19 °C to 23 °C) occurred. After 2 weeks of brooding period, they were transferred into another pens with each pen consist of 20 quails.

This study was carried out on 240 of 1-day old Japanese quails until they reached 6 weeks old. The quails were obtained from local commercial hatchery (Surada Jaya Sdn. Bhd, Kemaman, Terengganu) and were set up for brooding period for 2 weeks. After the brooding period, they were randomly assigned into 4 treatments group, in which 60 quails represent for each treatment and each treatment was divided into three replicates pen (20 quails per pen). The four treatments group were: i) control drinking water (C, 100% drinking water), ii) T1 (1 L of drinking water + 250 mL of EM1®), iii) T2 (1 L of drinking water + 500 mL of EM1®) and iv) T3 (1 L of drinking water + 750mL of EM1®). The treatment period started after the brooding period and was done within 28 days. The feed and water was given ad libitum to the quails within the rearing period. This treatment was given to the quails in order to determine theirs microflora populations in their intestine, faecal sample and their carcass quality.

2.2. Microflora Population Analysis

At day 42, six birds from each treatment group were selected randomly and sacrificed by severing the jugular vein. The intestinal content was then taken off immediately in order to avoid contamination. 1 gram of jejunum was cut off from the small intestine and placed into the sterilized tubes and diluted with saline water at the ratio of 1:10. The mixture was serially diluted up to 6 times. Using a micropipette, 0.1 µl of dilution of 10^{-4} ,10^{-5}, and 10^{-6} were pipetted and streaked on the appropriate selective media in which were MRS agar to enumerate *Lactobacillus sp.*, XLD agar for *Salmonella* spp., and EMB agar for *E. coli* [7, 8] by using L shape glass rod. Then, the streaked agar was incubated according to the appropriate condition (48 H at 37 °C in anaerobic condition for *Lactobacilli*, and 24 H at 37 °C in aerobic condition for *Salmonella* spp. and *E. coli*). Colonies on the plates were counted using Galaxy 230 colony counter and the result was expressed as $\log_{10}$ CFU g^{-1}. The same method was applied to the faecal sample analysis in which the fresh faeces were collected at each treatment.

2.3. Carcass Quality Analysis

At day 42, two birds per pen (n = 6/treatment) were randomly selected and slaughtered after 4 hours of the feeding. Then, quails were scalded at 65 °C for 15-30 seconds and deplumed in a rotary plucker to remove the feathers. After the removal of feathers and blood from slaughtering, the carcass weight was measured. The carcasses were then eviscerated by removed the head, feet, abdominal fat pad and viscera except the lung and kidney. The weights of eviscerated quails were recorded. After the eviscerated process, the carcasses were cut according to the Hudspeth et al., [9], which were i) anterior posture cuts (forequarter) – a longitudinal cut made at beginning of first thoracic vertebra cutting keel in half; and ii) posterior portion cuts (hindquarter) – cut at beginning of seventh thoracic vertebra and extending posteriorly splitting the lumbar-sacral vertebra in half. The forequarter and hindquarter part is then weighed and recorded. The result was expressed in percentage (%) based on their carcass weight.

2.3. Statistical Analysis

The obtained data on the microflora population analysis and carcass quality were subjected to one-way analysis of variance (ANOVA) using the SPSS 14.0 analysis software followed by Tukey Test for determining significant different level at $p<0.05$. All microbiological concentrations were subjected to $\log_{10}$ transformation before statistical analysis.

3. Results and Discussions

Probiotics are known to be beneficial to the animals by improving the balance of their microflora population [10]. In this experiment, Effective Microorganism (EM1®) was used as the probiotic to study their effect towards the gut microflora, faecal examination and carcass quality of the quails. This EM1® contains Lactic Acid Bacteria (LAB) such as Lactobacillus plantarum, Lactobacillus casei, Lactobacillus fermentum, and Lactobacillus delbrueckii, Saccharomyces cerevisiae (yeast) and Rhodopseudomonas palustris (phototrophic bacteria) as their active ingredients [11].

From the result of this study, there was significant decrease ($p<0.05$) in the population of intestinal E. coli which relatively higher in control group as compared to the group supplemented with EM1® with the value of log10 (CFU/ml) for Control, T1, T2, T3 were 6.88, 6.45, 6.32 and 6.31, respectively (Table 1). Birds supplemented with activated EM1® with inclusion on 1:750 and 1:500 ratios in drinking water shows there was significant different ($p<0.05$) than those control quails. On the other hand, the population of Lactobacillus sp. in the jejunum of quails supplemented with EM1® was not significantly higher between the treatments and the control quails, however, the number of Lactobacilli population exceed the population of E. coli which mean the addition of the probiotic used suppress the E. coli and induced Lactobacilli population in their jejunum after 42 days of feeding. This was in agreement findings of Suzuki et al., [12] and Jin et al., [13] which also supports the hypothesis which Lactobacilli contain in the feed will compete with coliform for site of adherence on the surface of the intestine.

Table 1: Effect of E.M1® on microflora of jejunum in quails.

Item	Treatment			
	Control	T1	T2	T3
E. coli	6.87±0.16*	6.45±0.16	6.32±0.16*	6.31±0.16*
Lactobacillus sp.	7.42±0.40	7.51±0.40	7.50±0.40	7.45±0.40
Salmonella sp.	-	-	-	-

Value represent Mean ± SE; * Mean with this superscript shows there were significant difference in the value (Tukey Multiple Comparison Test, $p<0.05$)

The ability of their adhesion to the intestinal mucosa provides a competitive advantage in intestinal ecosystem and considered important in order to observed probiotic health effect [12, 13]. It interacts with the toll-like receptor which presents at the surface of the intestinal epithelium and trigger the host immune response to fight against the infections [14]. Nowadays, the number of researchers studied on the effects of probiotic towards the microflora of the poultry had increased rapidly. Their studies also showed that the population of gram-negative bacteria has been significantly decrease ($p>0.05$) in their intestinal microflora when supplemented with probiotic [8, 15, 16, 17, 18]. The result of microflora population of E. coli, Lactobacillus sp. and Salmonella spp. in quail faecal sample on day 42 was as shown on Table 2. The result shows negative for Salmonella population in quail faecal sample in all four different treatments at the end of the rearing period. At the end of this experiment, the population of E. coli in the faeces collection of the quails supplemented with the

inclusion of 1:500 and 1:750 ratios of activated EM1® were found to be significantly lower ($p<0.05$) than those in control quails with the value of log10 (CFU/ml) T2, T3 and C were 6.09, 6.11 and 7.05, respectively. Lower Lactobacilli count were recorded for all treatment groups and the results show no significant different ($p>0.05$).

Table 2: Effect of EM1® on faecal sample examination in quails.

Item	Treatment			
	Control	T1	T2	T3
E. coli	7.05±0.27*	6.39±0.27	6.09±0.27*	6.11±0.27*
Lactobacillus sp.	5.88±0.40	6.23±0.40	6.53±0.40	6.57±0.40
Salmonella sp.	-	-	-	-

Value represent Mean ± SE; * Mean with this superscript shows there were significant difference in the value (Tukey Multiple Comparison Test, $p<0.05$)

Supplementing of EM1® into quails drinking water in this experiment does not significantly ($p>0.05$) alter the population of lactobacilli count inside their jejunum wall as shown on Table 1. This result agreed with Jin et al., [19] who studies the effect of addition of L. acidophilus or a mixture of Lactobacillus to broiler which does not significantly influence the count of lactobacilli in their cecum and ileum. Other than that, Watkins and Kratzer, [20] also found chicks dosed with host specific Lactobacillus strains had slightly increased the number of Lactobacilli in their intestine. In contrast with this experiment, Jin et al., [13], Mookiah et al., [18], Peng et al., [21] and Shokryazdan et al., [8], shows that poultry supplemented with Lactobacillus will increased significantly the population of lactobacilli in their intestine. Other than that, according to Strompfova et al., [22], showed that administrating Lactobacillus fermentum AD1 to quails lead to significant increase of LAB.

Generally, it has been a great deal of interest to prevent Salmonella infection in poultry and other livestock as animals with Salmonella can serve as a source of infection to human and may cause illness or death [23, 24]. At the end of this experiment, there was no Salmonella observed on quails supplemented with EM1® as well as the control group in both intestinal microflora and faecal examination. This has shown that the experiment was carried out at the good hygiene environment.

In addition, the result of microflora population of E. coli, Lactobacillus sp. in quail faecal sample on day 42 was shown on Table 2. At the end of this experiment, the population of E. coli in the faeces collection of quails supplemented with the inclusion of 1:500 and 1:750 ratio of activated EM1® was significantly lower ($p<0.05$) than the control quails with the value of log10 (CFU/ml) T2, T3 and C were 6.09, 6.11 and 7.05, respectively. Lower Lactobacilli count was recorded for all treatments group and the result shows no significant different ($p>0.05$).

Feacal examination showed the same result as the intestinal microflora in the gut of the quails. Faecal flora of the quails supplemented with EM1® showed a significant difference ($p<0.05$) in E. coli and slightly increase ($p>0.05$) for Lactobacilli count. There are two proposed mechanisms that play an important role in reducing the population of E. coli and increase the number of Lactobacilli in intestinal tract and faeces of the quails. Firstly, probiotic can secrete antimicrobial substance such as organic acids, bacteriocins and hydrogen peroxide which act as inhibitory substance that might active against pathogens [25]. Second mechanism is the competition between pathogenic bacteria and Lactobacilli present in the EM1® which they compete for adherence site on the intestinal epithelial.

According to Fritts et al. [26], the inclusion of probiotic (Bacillus subtilis) in diet stimulated the favourable microbial balance in the intestinal tract and consequently resulting in a significant improvement ($p<0.05$) in 42-days body weight and feed conversion during the 21 and 42 day period. Despite that, in this experiment, the body weight of the quails does not showed significant value ($p>0.05$) among all the treatments including control. This can be related to the uneven distribution of probiotic given in the drinking water of the quails. Other than that, the possible factor causing the results not significant was there might be a contamination from their own faeces at the drinking container. It also can be due by the water provided were not being consume properly especially during rainy seasons. According to Winn and Godfrey [27], broiler that reared in low temperature drink less amount of water compared to the one reared in high temperature. According to Jin et al., [13], it is shown that addition of Lactobacilli in feed has a significant difference ($p<0.05$) than addition of Lactobacilli in the drinking water ($p>0.05$) as compare with control. Jin et al., [13] also stated that, reasons why the addition of Lactobacilli in drinking water failed to increase the growth performance of the broiler are still unknown.

Although the body weight of the quails does not differ between treatments, the eviscerated weight and dressing percentage of the quails supplemented with 1:750 ration of EM1® showed significant difference as compared to the control group. According to Winn and Godfrey [27], there are two factors in order to measure the effectiveness of probiotics which were the presence of stress to the chicken and correct number of living bacteria used. The result in this study showed that there were improvements in both eviscerated weight and dressing percentage of the quails supplemented with EM1® even when reared under the stressful temperature which the quails were reared during the monsoon seasons in Terengganu with the temperature range of 19-23°C. The stressful condition with the temperature above or lower than their thermo neutrality might give negative affect to their production and welfare [29]. Another factor which could contribute to the effectiveness of the probiotic EM1® on the carcass quality was the correct number of living bacteria used. In this case, the Lactobacilli contained in the EM1® were in a correct quantity to attach at the intestinal site of the quails.

Moreover, the dressing percentage (%) of the quails is significant, but the breast weight (%) of the quails did not show any significant difference ($p>0.05$) as shown in Table 4.3. This might be caused by the sex of the quails. When quails were being randomly picked for the slaughter, they were not sorted by appropriate sex accordingly. According to Olawumi [30], Japanese quails is a sexually dimorphic bird. Female birds having a larger body size than male unlike the other poultry species. Musa et al., [31] and Ilori et al., [32] reported that the differences in growth pattern and carcass weight of the quails could be due to feed metabolism, differences in hormonal profile, aggressiveness and dominance especially when both sexes are reared together. Walita et al., [33] also reported that the male quails has smaller body weight and decreased in live weight as this is associated with the higher metabolic rates and hormonal changes respectively. Other parameter, which was thigh (%) has also showed a significant difference ($p<0.05$) between quails supplemented with EM1® and the control groups. This showed that quails supplemented with higher ration of EM1® producing higher thigh (%).

Table 3: Effect of EM1® on weight and quality of quail carcass at day 42.

Item	Treatment			
	Control	T1	T2	T3
Body weight (g)	248.33 ± 10.58	252.50± 10.58	259.17± 10.58	269.17± 10.58
Eviscerated. Carcass (g)	183.17± 12.72*	210.00± 12.72	221.67± 12.72*	225.00± 12.72*
Dressing %	73.90± 2.76*	81.82± 2.76*	80.56± 2.76	82.97± 2.76*
Weight breast (%)	37.03± 1.17	35.66± 1.17	36.83± 1.17	34.75± 1.17
Thight (%)	26.57± 1.43*	31.25± 1.43*	32.01± 1.43*	33.68± 1.43*

*Value represent Mean ± SE; * Mean with superscript shows there were significant difference in the value (Tukey Multiple Comparison Test, $p<0.05$)*

4. Conclusion

Based on the experiment, drinking water supplemented with activated EM1® with the ratio of 1:750 was the best treatment to be given to the quails as it resulting in good CFUs result for both E. coli and Lactobacillus sp. toward the microflora of jejunum and fecal examination of the quails after 42 days of age. Other than that, this ration also resulted in positive result for most of the parameters tested for the carcass quality.

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