



## EFFECT OF DIFFERENT LEVELS OF POSTBIOTIC ON GROWTH PERFORMANCE, INTESTINAL MICROBIOTA COUNT AND VOLATILE FATTY ACIDS ON QUAIL

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### Abstract

Recently postbiotics used as a new feed additive. In the current study, effect of postbiotic examined on growth performance, intestinal microbiota count and volatile fatty acid of quail. This experiment has been conducted in Qwail field in Qushtapa for six weeks. Two hundred and forty one week old quails were distributed into four treatment groups, basal diet (control groups), T1= basal diet + 0.2% postbiotic, T2= basal diet + 0.4% postbiotic, T3= basal diet + 0.6% postbiotic and fed for five weeks. The results indicate that the highest ( $P<0.05$ ) body weight and body weight gain showed with T2 and significantly higher than other treatments. On the other hand, the results indicated that birds fed different levels of postbiotics (0.2 and 0.6 %) had no significant differences of growth performance in comparison with control group. Quails that were nourished with different levels of postbiotic had significantly higher ( $P<0.05$ ) lactic acid bacteria count than control group. At the same time, the lowest level of *E. coli* had shown with treatment groups and they significantly lower ( $P<0.05$ ) than control. No significantly different ( $P>0.05$ ) had shown to organic acids among treatments. It can be concluded that postbiotic as a new feed additive is potential replacements for antibiotic growth promoters in quails feed.

**Keywords:** Postbiotic, Intestinal microbiota, Volatile fatty acid, New feed additive, Quail.

### Introduction

It is generally recognized that the diverse gut microbiota shows an imperative role in host metabolism, digestion of nutrients, growth productive and health of the host (Thacker, 2013; Falcinelli *et al.*, 2015). Nevertheless, during the poultry rearing using antibiotics not only changes microsystem of gut but also causes resistant of pathogenic bacteria against antimicrobials, which has extremely exposed human health and animal husbandry (Gong *et al.*, 2014). Consequently, researchers have been tried to find natural alternative to antibiotics to improve nutrient utilization, growth performance and immunity of poultry.

Postbiotics are new additives used as an alternative to antibiotics in recent years and defined as metabolites produced by lactic acid bacteria groups that beneficially affect host by altering microbiota in poultry and post-weaning lambs (Kareem, *et al.*, 2016a; Izuddin, *et al.*, 2019).

Postbiotics are strongly used as non-antibiotic growth promoters such as probiotics and prebiotics which are already recognized in animal nutrition (Markowiak and Slizewska, 2018). Some studies have revealed that postbiotics consist of many energetic substances which have a beneficial impact on the performance of livestock (Kareem *et al.*, 2016b; Loh *et al.*, 2014). Besides and Mookiah *et al.* (2014) who revealed that using prebiotic and probiotic in poultry feed leads to increase organic acids such as acetic acid, propionic acid and butyric acid. A significant increase of lactic acid bacteria and decrease of pH and *Enterobacteria* population in fecal by adding 0.6% liquid metabolite combinations to poultry feed reported by Choe *et al.* (2012).

It believes that the antimicrobial and short-chain fatty acids contain in postbiotics could help balance gut microbiota, which eliminates pathogenic bacteria that positively affect growth performances of quails. Hence, the present study aimed to examine the influence of different

levels of postbiotic growth performance, intestinal microbiota count and VFA on Quail.

### Materials and Methods

#### Postbiotic

The stock culture of *Lactobacillus animalis* (Plymouth University, United Kingdom) and postbiotic were prepared as described by Kareem *et al.* (2016a).

#### Bird and Experimental design

Two-hundred-and-forty quails at one week old were purchased from a commercial hatchery (Qwail farm). Quails were allocated randomly into four treatments. Each treatment group had three replicates, twenty birds per replicate. The treatment groups comprised basal diet (control), basal diet + 0.2% postbiotic (T1), basal diet + 0.4% postbiotic (T2), basal diet + 0.6% postbiotic (T3). Birds were offered water and feed *ad libitum* until 42 days of age.

#### Timing of sample collection

Body weight (BW) and feed intake (FI) were recorded and body weight gain (BWG) and feed conversion ratio (FCR) were calculated. At the end of the experiment period, six quails were taken from treatments and their caecal digesta were fully aseptically separated to investigate the intestinal microorganisms.

#### Intestinal microbiota count

For determining the intestinal LAB and *E. coli* after taking samples from fecal directly kept in  $-20\text{ }^{\circ}\text{C}$ , and before using it was put for one hour at room temperature. After that Ten-fold dilution was prepared in sterile peptone water. Furthermore, for enumerate LAB and *E. coli*, Manan and Rogosa Sharp-agar and coliform selective agar was used respectively. Plates were incubated in anaerobic jars at  $30\text{ }^{\circ}\text{C}$  for 48 hours. The number of colony-forming unit (CFU) was expressed by 10 logarithm of CFU ( $\log\text{CFU}$ ) per gram (Foo *et al.*, 2003).

## Determination of VFA by HPLC

To determine the volatile fatty acids (VFA) in gut the modified method of Torii, *et al.*, (2010) was used. One gram digesta were weighed from each sample. Digesta samples were added to 5.0 mL of 70% ethanol contained in a weighed glass centrifuge tube with a screw cap, and the tube as weighed to determine the digesta weight, then mixing well by glass stick and centrifugation at 2500 rpm for 10 min, and the supernatant was collected as the analytical sample.

## Statistical Analysis

SAS Institute, Inc., (2014) program one-way ANOVA was used to analyses data. Duncan's multiple range tests performed to find differences between treatments and comparing them. Significant levels were considered as  $P < 0.05$ .

## Results and Discussion

### Effect of different levels of postbiotic on growth performance

Table 1 shows the effect of different levels of postbiotic on quail's growth performance. The supplementation of postbiotic 0.4 % in quails significantly ( $p < 0.05$ ) increased the BW and BWG in comparison with other treatments. There was no significant differences ( $P > 0.05$ ) among other treatments for BW and BWG. Moreover, there were no significant differences ( $p > 0.05$ ) detected for all treatments for FI and FCR.

The outcomes of these findings showed that the bird groups which were fed 0.4 % postbiotic improve the growth performance of quails. The outcomes of this study as presented were similar to our previous studies Kareem *et al.* (2016a) that showed birds fed postbiotics had higher ( $p < 0.05$ ) BW and BWG than control. Whereas, Cakir *et al.* (2008) revealed that there were no differences for BW and BWG by supplementation of probiotics in comparison with the control birds.

Improved performances were observed in birds nourished with diet supplemented with the postbiotic may be due to the organic acids, bacteriocins, and vitamins present in the metabolites Loh *et al.* (2006).

Moreover, it was observed in a companion *in vitro* trial that postbiotics produced by *Lactobacillus plantarum* showed inhibitory effect against various pathogens (Kareem *et al.*, 2014). This could be another reason for improved performance observed in birds nourished diets which include postbiotic.

This study in agreement with the report of Vali *et al.* (2009) which showed no beneficial effect of probiotic on feed efficiency. However, these findings disagree with the report of Liu *et al.* (2012) who found that probiotic, prebiotic and synbiotic significantly improve the feed efficiency in comparison with the negative control diets.

### Effect of different levels of postbiotic on intestinal microbiota

Table 2 shows the effect of different levels of postbiotics on intestinal microbiota of birds fed various treatment groups at 6 weeks old. The data obtained showed that the population of LAB significantly ( $P < 0.05$ ) increased in birds fed different levels of postbiotics compared to the control group. However, no significant ( $P > 0.05$ ) difference was observed among the different levels of postbiotic for LAB. All treatments which include postbiotic decreased ( $P < 0.05$ ) *E. coli* count in comparison with control. Nevertheless, there were no significant among levels of postbiotic. These findings are in agreement with Vali *et al.* (2013) who reported that supplementation with a probiotic increased *Lactobacillus*.

Furthermore, the current finding is also in line with study by Kareem *et al.* (2016b) who revealed that different levels of postbiotic leads to increase LAB and at the same time decrease pathogenic bacteria like *E. coli* and Enterobacteria in broiler's cecum. This result suggested that using postbiotic as a new feed additive have major suppressing effect on pathogenic bacteria in gut microbiota in the quail. The enhancement of population of beneficial bacteria by postbiotic could be due to the inhibition of pathogenic bacteria colonization caused by decreased intestinal pH. Moreover, high fermentation activity and high concentration of the VFA is correlated with a lower pH, which is associated with a suppression of pathogens and increased solubility of certain nutrients (Józefiak *et al.*, 2008). On the other hand, Siriken, *et al.* (2003) reported that the supplementation of probiotic did not effect on bacterial number in quail's cecum.

### Effect of different levels of postbiotic on VFA

Table 3 Demonstrate the effect of different levels of postbiotic on organic acids. The results of this study indicated that birds fed different levels of postbiotics had no significant ( $P > 0.05$ ) differences on FA. Organic acids such as acetic, propionic, isobutyric and lactic acids will be absorbed mostly in the large intestine to produce energy for the host (Franklin *et al.*, 2002). This finding in contrast with Mookiah *et al.* (2014) who described that probiotic and symbiotic increased the concentration of organic acids like acetic acid, propionic acid and total VFA of broiler's cecum in comparison with control group.

## Conclusion

It can be hypothesized that postbiotics derived from *Lactobacillus animalis* can improve performance and promote quails health by modulating gut microbiota. The feeding of postbiotic liquid to quails through feed can be used as an alternative to antibiotics to balance gut microbiota in Quails.

**Table 1: Effect of different levels of postbiotic on quail's growth performance at six weeks age**

Parameters	Dietary Treatments <sup>1</sup>				SEM <sup>2</sup>
	Control	T1	T2	T3	
Body weight (g)	189.68 <sup>b</sup>	195.63 <sup>b</sup>	210.71 <sup>a</sup>	200.88 <sup>b</sup>	3.22
Body Weight gain(g)	163.30 <sup>b</sup>	169.08 <sup>b</sup>	184.22 <sup>a</sup>	172.64 <sup>b</sup>	3.18
Feed Intake(g)	759.29 <sup>a</sup>	772.15 <sup>a</sup>	786.83 <sup>a</sup>	731.86 <sup>a</sup>	11.00
FCR	4.59 <sup>a</sup>	4.58 <sup>a</sup>	4.28 <sup>a</sup>	4.26 <sup>a</sup>	0.10

<sup>ab</sup> Means with different superscripts in the same row are differ significantly ( $P < 0.05$ ). <sup>1</sup>Control group includes basal diet feed, (T1) basal diet + % 0.2 postbiotic, (T2) basal diet + % 0.4 postbiotic, (T3) basal diet + % 0.6 postbiotic. <sup>2</sup>SEM, standard error of the means (pooled).

**Table 2:** Effect of diets containing different levels of postbiotic on quail's intestinal microbiota

Parameters	Dietary treatments <sup>1</sup>				<sup>2</sup> SEM
	Control	T1	T2	T3	
<b>Microbiota count log CFU/g</b>					
<b>LAB</b>	3.03 <sup>b</sup>	8.85 <sup>a</sup>	12.81 <sup>a</sup>	10.23 <sup>a</sup>	1.32
<b><i>E. coli</i></b>	2.01 <sup>a</sup>	0.70 <sup>b</sup>	0.58 <sup>b</sup>	0.67 <sup>b</sup>	0.18

<sup>a,b</sup> Means with different superscripts in the same row are differ significantly ( $P < 0.05$ ). <sup>1</sup>Control group includes basal diet feed, (T1) basal diet + % 0.2 postbiotic, (T2) basal diet + % 0.4 postbiotic, (T3) basal diet + % 0.6 postbiotic. <sup>2</sup>SEM, standard error of the means (pooled).

**Table 3 :** Effect of diets containing different levels of postbiotic on quail's gut digesta VFA (%)

Parameters	Dietary treatments <sup>1</sup>				<sup>2</sup> SEM
	Control	T1	T2	T3	
Acetic acid	0.95 <sup>a</sup>	1.5 <sup>a</sup>	1.5 <sup>a</sup>	1.12 <sup>a</sup>	0.14
Lactic acid	0.44 <sup>a</sup>	0.59 <sup>a</sup>	0.62 <sup>a</sup>	0.51 <sup>a</sup>	0.13
Propionic acid	0.03 <sup>a</sup>	0.02 <sup>a</sup>	0.02 <sup>a</sup>	0.02 <sup>a</sup>	0.02

<sup>1</sup>Control group includes basal diet feed, (T1) basal diet + % 0.2 postbiotic, (T2) basal diet + % 0.4 postbiotic, (T3) basal diet + % 0.6 postbiotic. <sup>2</sup>SEM, standard error of the means (pooled).

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