

## Scrutinizing of a Liquid Prebiotic on Growth Performance and Internal Organs of Japanese Quail

### Research Article

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### ABSTRACT

An experiment has been conducted to evaluate the effects of a liquid prebiotic (LP) on the carcass, digestive tract, and productive traits of Japanese quail. Eighty 7-day-old Japanese quails were randomly divided into 2 treatments. Each treatment consisted of 4 replicates (pens) of 10 birds each. The first treatment (control) contained a standard recommended diet with no added prebiotic. The second treatment was similar to the first diet accompanied by LP in the drinking water (0.5 milliliter LP per liter of water through the entire study). Feed and water were offered *ad libitum* from days 7 to 42. Feed intake (FI), body weight gain (BWG), and feed conversion ratio (FCR) were measured weekly. Utilization of LP did not have a significant effect on the relative weight of internal organs and carcass. The mortality rate was not influenced by treatments. In overall, the FI of birds receiving LP was significantly decreased, while BWG was considerably increased as compared to the control group. Consequently, overall FCR was dramatically improved.

**KEY WORDS** carcass, liquid prebiotic, production efficiency, quail.

### INTRODUCTION

For several decades, antibiotics in sub-therapeutic dosage have been added to poultry feed to improve bird health and to attain the economic benefits of enhanced performance. However, concerns have been increased about the risk of multiple antibiotic resistance of pathogenic bacteria in both humans and livestock. Thus, antibiotic replacements such as prebiotics and other products have become highly important feed additives in recent years (Hajati and Rezaei, 2010; Nosrati *et al.* 2017; Pournazari *et al.* 2017).

Gibson and Roberfroid (1995) stated that prebiotics is defined as a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and activity of one or a limited number of bacteria in the gut. They provide a substrate for beneficial gastrointestinal mi-

crobes. Most commercial prebiotic supplements are usually obtained by processing the yeast *Saccharomyces cerevisiae*. The main components, including  $\beta$ -glucan and mannan oligosaccharides (MOS), are derived from the yeast cell wall. Moreover, the yeast extract is obtained after removal of the cell wall of yeast by processing with proteolytic enzymes (Kollar *et al.* 1997). It is hypothesized that such supplements influence the intestinal microbiome and improve intestinal absorption, which altogether promotes animal performance (Sohail *et al.* 2011).

The poultry industry has responded to the development of prebiotic products that are claimed to have beneficial impacts. Although many of these products have reasonable levels of field efficacy; however, considerable fluctuation still exists in their effectiveness in comparison with antibiotics (Ajuwon, 2016). Interestingly, it has been shown that

these feed additives are more beneficial under suboptimal circumstances such as infection by pathogenic bacteria (Haldar *et al.* 2011), heat stress (Fowler *et al.* 2015), lipopolysaccharide challenge (Alizadeh *et al.* 2016b), and in immunosuppressed chickens (Zhang *et al.* 2012). No report about the effects of liquid prebiotic (LP) on quail was found among numerous published papers. Therefore, this trial was conducted to assess an enzymatically hydrolyzed yeast (EHY) as a novel LP on growth traits and carcass merits of Japanese quail.

## MATERIALS AND METHODS

### Experimental design and birds' housing

Eighty 7-day-old Japanese quail (*Coturnix Coturnix japonica*) were randomly divided into 2 treatment groups as completely randomized design. Each treatment consisted of 4 replicates (pens) of 10 birds each. The first treatment (control) contained a standard recommended diet with no added prebiotic. The second treatment was a basal diet, similar to that of control, with an LP added to the drinking water (0.5 milliliter LP per liter of water, vol/vol). CEL-MANAX™ liquid is a preparation of components derived from an enzymatically hydrolyzed yeast cell wall blended with yeast extract, as well as a culture of *Saccharomyces cerevisiae* on special nutrient media. This yeast product is produced as an LP by Arm and Hammer Animal Nutrition (Princeton, NJ, USA).

The diets (Table 1) were formulated to meet the nutrient requirements of quail as recommended by the NRC (1994). Pen dimensions were 40 × 40 cm so that each bird had 160 cm<sup>2</sup> floor space. The initial house temperature was set at 36 °C and was gradually decreased to 22 °C at day 35. Average relative humidity was kept at 60% during the experimental period. A lighting schedule of 24 h illumination with approximately 20 lx was in place for the entire period. Mash feed and water were offered *ad libitum* throughout the trial. The experimental protocol was approved by the Animal Care Committee of Amol University of Special Modern Technologies, Amol, Mazandaran Province, Iran.

### Growth performance

Feed intake (FI), body weight gain (BWG), and feed conversion ratio (FCR) were measured weekly and overall. The FI was determined from the difference between supplied and residual feed in each pen. The FCR was calculated as the ratio between FI and BWG of quail in each pen and was adjusted for mortality. Mortality was recorded daily.

### Internal organs

Four birds from each treatment were randomly selected and slaughtered in Islamic (Halal) method at day 42.

Then the weight of the liver, thighs, breast, heart, spleen, proventriculus, and gizzard was measured. Relative organ weights were calculated as [organ weight (g) / live body weight (g)] × 100. The small intestine of the birds was opened immediately after killing and the relative length of intestine to live body weight as a percentage (cm/g) was measured.

### Statistical analysis

All data were analyzed using the statistical analysis system (SAS) software (SAS, 2004). Duncan's multiple range test was used to compare the means. All statements of significance were based on the probability of  $P < 0.05$ .

## RESULTS AND DISCUSSION

Results of FI, BWG, and FCR are presented in Tables 2, 3, and 4, respectively. The FI declined significantly through the addition of LP in the drinking water of quail during days 7-21 and overall. Generally, the body weight of birds receiving LP was significantly increased compared to the control group. The FCR of chickens that drank LP was considerably lower than that of birds in control treatment due to less FI and more BWG in most weeks. The mortality rate was not influenced by treatments; a few birds died in each group during the study. Relative weights of carcass and internal organs are shown in Tables 5 and 6. Neither relative weight nor length of gastrointestinal organs and carcass parts were significantly influenced by LP.

### Growth performance

Our hypothesis was that LP, an enzymatically hydrolyzed yeast (EHY) product, could improve productive traits in quail. In line with the current results, Kanagaraju and Subramanian (2016) reported that supplementation of MOS at levels of 0.15 and 0.20% significantly improved BWG of quail. Among the MOS-supplemented groups, FI was significantly lower in the 0.2% MOS group. Significantly, superior FCR was noted in 0.2% MOS supplemented group followed by the 0.15 and 0.1% groups, respectively. Livability was not affected by the MOS supplementation. Moreover, they mentioned that MOS supplementation significantly reduced *Escherichia coli* count in duodenal contents. Thus, the above-mentioned effects might be due to microflora changes. Additionally, Teshfam *et al.* (2011) reported that prebiotic supplementation in quail diet improved birds' BWG and FCR. Furthermore, higher BWG through the addition of MOS was observed in other research conducted on quail. Güçlü (2011) revealed that supplementing powder prebiotic to quail diet increased BWG and egg production. Eggshell thickness was improved by the prebiotic supplement.

**Table 1** The composition of experimental diets

Ingredients (%)	Starter (0-21 d)	Finisher (22-42 d)
Corn	47.00	60.00
Soybean meal	43.50	33.40
Corn gluten germ	3.00	1.00
Vegetable oil	2.70	2.00
Oyster shell	1.20	1.10
Dicalcium phosphate	1.50	1.45
Common salt	0.30	0.32
L-threonine	0.05	0.04
DL-methionine	0.25	0.20
Vitamin and mineral premix <sup>1</sup>	0.50	0.50
<b>Calculated contents (%)</b>		
Metabolizable energy (kcal/kg)	2925.00	3020.00
Crude protein	24.00	20.20
Calcium	0.95	0.86
Available phosphorus	0.44	0.41
Sodium	0.19	0.18
Lysine	1.31	1.06
Methionine + cystine	0.68	0.60

<sup>1</sup> Vitamin and mineral premix supplied per kilogram of diet: vitamin A: 10000 IU; vitamin D<sub>3</sub>: 9800 IU; vitamin E: 121 IU; B<sub>12</sub>: 20 µg; Riboflavin: 4.4 mg; Calcium pantothenate: 40 mg; Niacin: 22 mg; Choline: 840 mg; Biotin: 30 µg; Thiamin: 4 mg; Zinc sulfate: 60 mg and Manganese oxide: 60 mg.

**Table 2** Evaluation of a liquid prebiotic on feed intake (g) of quails

Treatments	7-14 d	15-21 d	22-28 d	29-35 d	36-42 d	Overall
Control	80.72 <sup>a</sup>	121.40 <sup>a</sup>	174.00 <sup>a</sup>	131.58	238.65	746.49 <sup>a</sup>
Liquid prebiotic	72.50 <sup>b</sup>	103.30 <sup>b</sup>	143.30 <sup>b</sup>	127.95	225.25	672.49 <sup>b</sup>
SEM	1.470	0.047	1.597	1.717	1.864	3.744
P-value	0.007	< 0.0001	< 0.0001	0.185	0.002	< 0.0001

The means within the same column with at least one common letter, do not have significant difference (P>0.05).  
SEM: standard error of the means.

**Table 3** Evaluation of a liquid prebiotic on body weight gain (g) of quails

Treatments	7-14 d	15-21 d	22-28 d	29-35 d	36-42 d	Overall
Control	31.30	37.50	55.14	29.47	24.09	185.76 <sup>b</sup>
Liquid prebiotic	35.10	45.20	59.29	36.30	23.01	192.86 <sup>a</sup>
SEM	1.200	2.240	2.225	2.495	1.940	1.782
P-value	0.064	0.052	0.236	0.100	0.706	0.030

The means within the same column with at least one common letter, do not have significant difference (P>0.05).  
SEM: standard error of the means.

**Table 4** Evaluation of a liquid prebiotic on feed conversion ratio (FCR) of quails

Treatments	7-14 d	15-21 d	22-28 d	29-35 d	36-42 d	Overall
Control	2.59 <sup>a</sup>	3.29 <sup>a</sup>	3.15 <sup>a</sup>	4.16	10.22	4.02 <sup>a</sup>
Liquid prebiotic	2.06 <sup>b</sup>	2.29 <sup>b</sup>	2.44 <sup>b</sup>	3.55	9.87	3.48 <sup>b</sup>
SEM	0.118	0.206	0.100	0.377	0.822	0.051
P-value	0.018	0.014	0.0023	0.092	0.772	0.003

The means within the same column with at least one common letter, do not have significant difference (P>0.05).  
SEM: standard error of the means.

Nevertheless, MOS supplementation to the diets did not have significant effects on FI and FCR. In addition, [Das et al. \(2012\)](#) reported that adding MOS and organic acid to the diet of Japanese quail caused longer villi and improved growth performance.

In other poultry species, it has been reported that supplementation of EHY improved flock uniformity. Supplementation of EHY in broiler diets effectively enhanced bird performance by significantly improving BWG, lowering FCR, and improving bird uniformity at slaughter under anticoccidial control program practices ([Mathis et al. 2012](#)).

In addition, [Gómez et al. \(2012\)](#) evaluated EHY in broilers under optimal conditions. The EHY-fed broilers showed the lowest FI and FCR and had both more energy and greater ileal digestibility of dry matter. Furthermore, [Huff et al. \(2007\)](#) showed that yeast extract supplementation significantly improved both the BWG and the FCR of challenged poult.

In line with these results, [Toghyani et al. \(2012\)](#) reported that broilers receiving diets supplemented with another yeast product (YP) exhibited the highest BWG and lowest FCR relative to other treatments.

In a study by [Salianeh et al. \(2011\)](#), broiler chickens were fed either a corn-soybean meal-based unsupplemented diet (control) or one supplemented with either a YP or a probiotic fed. The BWG and FCR were improved simply by supplementation of the prebiotic. In a recent study, diets supplemented with yeast cell wall products resulted in improved BWG with a decrease in FCR in starter broilers under experimentally-induced challenge conditions ([Fowler et al. 2015](#)). [Sadeghi et al. \(2013\)](#) reported that prebiotic supplementation improved the immune responses and health of chicks infected with pathogens. [Khaksar et al. \(2014\)](#) reported no significant effect on Chukar partridge BWG and cumulative FCR from a diet of 0.18% prebiotic *Aspergillus* meal supplementation. However, prebiotic supplementation decreased FI of the birds. Nevertheless, some previous studies concluded that prebiotics do not exert strong growth-promoting effects in an optimal setting ([Baurhoo et al. 2009](#); [Alizadeh et al. 2016a](#)). Recently, prebiotics have been supplemented to broilers under stress with some considerable findings ([Sohail et al. 2011](#); [Seifi et al. 2017](#)). In line with current research, [Sohail et al. \(2013\)](#) reported MOS supplement could reduce some detrimental effects of heat stress in broilers. Interestingly, transport-stress dramatically increased oxidative burst and that increase was significantly modulated in yeast extract-fed turkeys ([Huff et al. 2010](#)).

### Internal organs

No pronounced effect of prebiotics was found in the internal organs of quail under normal production conditions herein. In contrast to the current results, [Bonos et al. \(2010\)](#) reported that dietary supplementation of MOS increased body and carcass weight of quail, whereas the relative weight of the liver and ether extract content of the breast meat was decreased. Also, [Adb-Allah and Abdel-Raheem \(2012\)](#) revealed that quail fed diets containing medium MOS level (3 g/kg feed) recorded significant improvements in BWG as well as increased the dressing and edible giblets percentages, while the offal's and carcass abdominal fat percentages were significantly decreased compared with other treatment groups. Crude protein and moisture values of quail's meat were higher in medium MOS supplemented birds diet than in other groups, while fat and ash values were lower. Furthermore, [Vahdatpour et al. \(2011\)](#) reported that the relative weight of heart decreased in the males fed prebiotics compared to the control group. In line with our results, [Çakir et al. \(2008\)](#) revealed no beneficial effect of a dietary supplement containing MOS on proportional organ weight in Japanese quail. They proposed that the beneficial effects of such supplements could be more evident in other circumstances such as stress. For instance, [Sandikci et al. \(2004\)](#) found that jejunal and ileal goblet cell number was

increased in yeast-bacitracin zinc treated quail exposed to heat stress.

Because of their utmost importance, prebiotics is presently thought to be a forerunner amongst several nutraceuticals for application towards routine maintenance of health as well as for ecological treatment of disorders of the gastrointestinal tract ([Samanta et al. 2013](#)). For example, [Gómez et al. \(2012\)](#) reported that the thickness of the villus was greater in broilers fed EHY plus live *Bacillus subtilis* compared to the control group. The area of the villus was greater in the control group, and in broilers fed EHY combined with *Bacillus subtilis*. In addition, EHY-fed broilers showed greater breast yield, nitrogen retention, and ash digestibility. The results of [Gomez and Angeles \(2011\)](#) showed that EHY-fed broilers trended toward greater FI and BWG, improved FCR, and better yield of carcass, breast, and legs. The EHY-fed broilers also had greater dry matter and ash intake and retention. Moreover, it has been shown that ileum villus height, surface area, lamina propria thickness, crypt depth, and goblet cell density were enhanced by yeast extract fed on days 7 and 21. Duodenum villus height, surface area, and goblet cell density were higher for the prebiotic groups on day 7; however, intestinal morphology of the duodenum was not found to be different in either control or treated birds on day 21 ([De los Santos et al. 2007](#)).

Different action mechanisms of prebiotics have been suggested up to now. For instance, 1-3/1-6  $\beta$ -glucan as a component of the yeast cell wall can bind to macrophage-specific complement receptor 3 (CR3) in glycoproteins of the epithelial surface. This triggers a cascading reaction that activates macrophage to phagocytosis and eventually proliferates lymphocytes ([Swiatkiewicz et al. 2014](#)). Also, yeast cell wall preparations can directly block *Salmonella* and *E. coli* by attaching MOS to bacterial mannose-binding lectins or indirectly confronting the *Clostridia* population ([Dawson, 2001](#); [Tian et al. 2016](#)). Furthermore, yeast cell extract is an important source of nucleotides from yeast RNA, which are involved in many biological processes such as being precursors of nucleic acids. In addition, yeast extract containing biopeptides and nucleotides has beneficial effects on nitrogen metabolism, intestinal development, and overall growth. Although the action mechanism of nucleotides is not yet completely understood; however, is it probably connected to the fact that fast proliferating tissues may preferentially utilize nucleotides and nucleobases derived from yeast extract ([Swiatkiewicz et al. 2014](#)). Moreover, the immunomodulatory effects of nucleotides on different types of immune response have been confirmed in numerous *in vitro* and *in vivo* model experiments on different animals ([Carver and Walker, 1995](#); [Alizadeh et al. 2017](#)).

**Table 5** Evaluation of a liquid prebiotic on relative weight of liver, heart, gizzard, proventriculus, and spleen as well as relative length of small intestine in quails at 42 d of age

Treatments	Liver <sup>1</sup>	Heart <sup>1</sup>	Gizzard <sup>1</sup>	Proventriculus <sup>1</sup>	Spleen <sup>1</sup>	Small intestine <sup>2</sup>
Control	2.29	0.90	1.62	0.35	0.05	25.30
Liquid prebiotic	2.11	0.77	1.71	0.34	0.06	25.42
SEM	0.135	0.049	0.095	0.032	0.007	1.598
P-value	0.375	0.051	0.523	0.932	0.592	0.957

<sup>1</sup> Relative weight of organ to live body weight as percentage.

<sup>2</sup> Relative length of intestine to live body weight as percentage (cm/g).

SEM: standard error of the means.

**Table 6** Evaluation of a liquid prebiotic on relative weight of breast, thighs, and carcass of quails at 42 d of age

Treatments	Breast <sup>1</sup>	Thighs <sup>1</sup>	Carcass <sup>2</sup>
Control	25.95	16.00	59.35
Liquid prebiotic	24.82	15.79	59.88
SEM	1.22	0.566	0.859
P-value	0.543	0.801	0.678

<sup>1</sup> Relative weight of organ to live body weight as percentage.

<sup>2</sup> Carcass percentage is sum of breast, thighs, wings, neck and back without skin relative to live body weight.

SEM: standard error of the means.

All in all, Swiatkiewicz *et al.* (2014) summarized the data from poultry experiments and noted in their review article that production results from yeast derivatives depend on type, source, and usage level of the product. Furthermore, rearing conditions and the health status of birds are determining factors for outcomes.

## CONCLUSION

In conclusion, the crosstalk among all components of this LP and gut microbiome might exert modulatory effects for gut health and digestion process. Consequently, the addition of this LP had some helpful impacts on the productive traits of quail. Nevertheless, further research is needed to specify the molecular aspects of this additive on quail and other poultry species.

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