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RESEARCH ARTICLE

Effects of Yeast Addition to the Diet of Japanese Quails on Growth Performance, Selected Serum Parameters and Intestinal Morphology as well as Pathogens Reduction

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A B S T R A C T

The objectives of the current study were to investigate the impact of dietary yeast (*Saccharomyces cerevisiae*) supplementation on growth performance, some serum parameters, intestinal morphology and pathogens reduction in Japanese quails. In total, 300 d-old Japanese quails were randomly allocated to 5 dietary groups (6 replicates of 10 Japanese quails per pen). At beginning of the experiment (d 14), Japanese quails fed a basal diet without contain any yeast while, the other treatments provided basal diet plus 0.5, 1.5, 2.5 and 3.5% yeast on feed basis. The results showed that dietary supplementation of yeast, particularly at a level of inclusion of 3.5% on feed basis, resulted in highest BW for Japanese quails ($P<0.05$). Japanese quails fed diets supplemented with yeast reduced ($P<0.05$) cholesterol and triglycerides concentrations in serum compared to control. Dietary yeast supplementation at 3.5% reduced ($P<0.05$) interleukin 1 β (IL-1 β), IL-6 and TNF-α levels in serum compared to those fed dietary yeast at level of 0.5% or fed non supplemented diets. Feeding yeast at level of 3.5% led to increase in duodenal villus height significantly compared to other treatments. Japanese quails fed yeast supplemented diets at 2.5 or 3.5% reduced *E. coli* ($P<0.05$) and *C. perfringens* CFU counts in excreta than those fed non supplemented diet. It is concluded that dietary yeast supplementation of Japanese quails improved growth rate as a result of an increase in villus height and a reduce in the counts of *E. coli* and *C. perfringens*.

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INTRODUCTION

Poultry industry is one of the biggest growing parts worldwide of the animal production economy with expected to reach about 4% higher in 2020 to a record 103.5 million tons (USDA, 2019). Japanese quails are recently attracted attention in the poultry sector for being economically viable (Bolacali and Irak, 2017). The development of intensive poultry industry has made the role of feed additives in poultry diets more and more important. The proper use of feed additives can increase feed utilization, improve production and promote health. Years ago, antibiotics were used at sub-therapeutic levels in diets to enhance poultry performance (Chattopadhyay, 2014). However, it has caused residues in feed and environment and hence a bacterial resistant in animals and human. Thus, medical alarms motivated on the complete removal of the antibiotics from animal feed (Ronquillo and Hernandez, 2017). Thus, there is a need to find substances capable of replacing antibiotic growth promoters in the diets. The aim of these alternatives is to enhance performance while protecting environment and animal health (Mehdi *et al*., 2018). Thus, probiotics like yeast *S. cerevisiae* have been investigated as a feed additive for improve animal performance and health (Ogbuewu *et al*., 2018). Probiotics are live microorganisms that improve animals' health by competing with undesirable microorganisms, improve intestinal microbial balance and absorption of nutrients (Al-Khalaifah, 2018). *Saccharomyces cerevisiae* (also known as 'baker's yeast') is considered as one of the most yeast species that are added to dietary formulations in poultry diets (Duarte *et al*., 2012). *Saccharomyces cerevisiae* contain substantial levels of digestible proteins, vitamins, magnesium, zinc and its wall has many characteristics such as polysaccharides α-D-mannan, chitin and β-D-glucan (Elghandour *et al*., 2019)

which play an important role in microbial balance in intestine towards beneficial organisms. Furthermore, proliferation of tissues in intestine and lymphocytes with a rapid cell turnover depend mostly on dietary nucleotides where de novo synthesis of nucleotides cannot meet their demand (Alizadeh *et al*., 2016).

In most studies, no reliable results were obtained by supplementation diets with yeast. Beneficiary effects on animal health and performance (Bolacali and Irak, 2017) as well as no effects with using yeast have been found. The variations in the results might be due to using of distinguishable species and levels of yeast in addition to differences in diet composition, species of animals and/or age. Therefore, the present study was conducted to investigate the potential effects of using yeasts in the diet of Japanese quails as a feed additive on growth performance, serum parameters, intestinal morphology and counts of *E. coli* and *C. perfringens* in excreta.

MATERIALS AND METHODS

Birds and diets: Three hundred Japanese quails 1-d old were divided into 5 different treatments according to completely randomized design, each containing 6 replicate pens of 10 Japanese quails. Each replicate is considered as an experimental unit. The birds were kept in wire floor pens (60×60×50 cm) for 3 weeks. At the beginning of the experimental period (d 14), the body weight (BW) of the Japanese quails was ~70 g. The Japanese quails in the control treatments were fed a basal diet with no yeast. While, the other treatments were fed with the basal diet plus 0.5, 1.5, 2.5 and 3.5% yeast (Leiber-Aromor XR, LS, Leiber® GmbH, Germany). The additive Leiber-Aromor XR® is a preparation of *S. cerevisiae*. The level of yeast in this product is about ≤100 CFU/g and it contains about 7% for nucleotides. A standard basal diet was formulated in accordance with the NRC (1994) requirements for poultry. The ingredients of the basal diet and its chemical composition are presented in Table (1).

Growth performance: Feed and water intakes were measured daily. Japanese quails were wing tagged and the individual BW was weekly recorded. The feed conversion ratio (FCR) and body weight gain (BWG) were estimated individually.

Carcass characteristics and blood sampling: At the end of the experiment (d 35), 30 Japanese quails per treatment (5 from each replicate) were weighed and slaughtered. Visceral and lymphoid organs were also weighed and recorded. About 10 g of the contents from duodenum, jejunum and ileum were collected in 90 mL physiological saline for pH value measurement. Blood samples were collected from the same Japanese quails used for carcass traits and were centrifuged at 3000 rpm for 10 min, then stored for later analysis at -20°C.

Biochemical analysis: Colorimetric determination of serum contents of total protein, albumin, cholesterol, triglycerides, alanine transaminase (ALT) and aspartate aminotransferase (AST) were measured in an auto analyzer (ADVIA 1800 Chemistry System). Determination of catalase (CAT) activity and superoxide dismutase (SOD) were determined by enzymatic colorimetric method. Determination of Interleukin 1 β (IL-1 β); IL-6 and TNF-α levels in serum were measured.

Microbiological examination: Standard plate count methodology was used for *E. coli* and *C. perfringens* colony forming units (CFU) counts in excreta of Japanese quails. A plastic sheet was used in each replicate for 30 min to collect excreta. Then excreta samples of each replicate were collected and mixed. Briefly, one g of the sample was aseptically weighted and decimally diluted with 9 mL Phosphate Buffer Saline (PBS, Oxoid UK) till dilution between 10^5 and 10^8. Thereafter, 0.1 mL of each dilution was surface spread in duplicate onto Eosin Methylene Blue agar (EMB, Oxoid, UK) for detection of *E. coli*. Tryptose Sulfite Cycloserine agar (TSC, perfringens agar base supplement with perfringens selective supplement Oxoid, UK) was used for enumeration of *C. Perfringens*.

Histopathological findings: At the end of the experiment, 5 Japanese quails were randomly selected from each replicate/treatment. Duodenum specimens were collected and fixed in 10% neutral buffered formalin solution for 24 h, then embedded in paraffin and sectioned at 4 µm. The following parameters were measured: (i) villous height (VH), (ii) depth of crypt (CD) and (iii) ratios of VH/CD.

Statistical analysis: All data obtained from the experiment were carried out by software SPSS program package version 17, Use one-way ANOVA variance analysis. Results for each group are expressed as Mean±SEM. Differences between means were tested for significance by using Duncan's Range test. Differences at the level of ($P<0.05$) were considered statistically significant.

RESULTS

First of all, it has to be mentioned that all animals enrolled got to the end of the experimental trial and were healthy.

Table 1: Ingredients and calculated composition of basal diet (as fed)

Item (%)	
Yellow corn	571
Soybean meal	300
Corn gluten	20
Wheat bran	73
Corn oil	5
Limestone	15
Dicalcium phosphate	7
Minerals and vitamins mixture*	3
NaCl	3
DL-Methionine	1
Lysine	2
Chemical analysis	
ME (kcal/kg) **	2902
Crude protein (g/kg)	240
Lysine (g/kg)	15
Methionine (g/kg)	5.5
Ca (g/kg)	11
P (g/kg)	7

*Supplies per kg diet: Vitamin A, 16,500 IU; vitamin D3, 750 IU;vitamin E, 12 IU; vitamin K, 2 mg; vitamin B1, 1.2 mg; vitamin B2 10mg; vitamin B6, 2.4 mg; vitamin B12, 12 µg; niacin, 18 mg; pantothenic acid, 12 mg; Mn, 190 mg as manganese sulfate; Zn, 72 mg as zinc oxid; Fe, 380 mg as ferrous sulfate; copper, 13 mg as copper sulfate; iodine, 0.4 mg as potassium iodide. ** Calculated according to NRC (1994) for poultry.

Growth performance: The outcomes of dietary yeast addition on feed intake and growth rate in Japanese quails are presented in Table (2). Final BW, and BWG were affected (P<0.05) by dietary treatments. During the experimental period (14 d to 35 d), Japanese quails fed diets containing 1.5, 2.5 and 3.5% of yeast had higher (P<0.05) BWG than those fed control diets. At end of experimental period the FCR was improved significantly by adding dietary yeast at contents of 2.5 and 3.5%. No significant differences were observed in final BW or FCR between birds fed 0.5% supplemented yeast and those fed non supplemented diets.

Carcass traits: No differences in the relative weights of liver, gizzard and heart were observed among treatments (Table 3). By contrast, Japanese quails fed diets supplemented by yeast at different levels had decreased (P<0.05) relative abdominal fat weight than those fed control diet. Furthermore, carcass yield was (P<0.05) higher for groups fed supplemented yeast at level of 3.5% compared to groups fed only 0.5% supplemented yeast or non-supplemented groups. Effect of dietary yeast supplementation on weight of some lymphoid organs is presented in Table 4. Thymus weight was affected between groups fed supplemented diets by yeast and those fed the control diets significantly. However, there were no differences between the treatments in weights of spleen and bursa of Fabricius. pH values of duodenal, jejunal and ileal digesta have not been affected by dietary yeast supplementation in the present study (Table 4).

Serum parameters: Japanese quails fed different doses (1.5-3.5%) of dietary yeast had increased (P<0.05) in serum protein and albumin concentrations in comparison to those fed non supplemented diets (Table 5). In contrast, Japanese quails fed diets supplemented with yeast have shown decreased serum concentrations of cholesterol and triglycerides compared to those fed non supplemented diets. Furthermore, the activities of ALT and AST in serum were not affected by dietary yeast supplementation (Table 5). Dietary yeast supplementation increased (P<0.05) SOD and CAT contents in serum comparison to those fed non supplemented diets (Table 6). While, IL-1 β, IL-6 and TNF-α levels were decreased (P<0.05) with using the greatest inclusion rate of dietary yeast at 3.5% compared to control groups (Table 6).

Intestinal morphology: No histopathological lesions of duodenum were observed in all treatments. Feeding yeast at level of 3.5% showed an increase (P<0.05) in villus height of duodenum (1188 μm) compared to those fed only 0.5% supplemented yeast or non-supplemented (950 and 683 μm, respectively). Also, villus height/crypt depth ratio was significantly higher for groups fed 3.5% supplemented yeasts compared to those fed non supplemented diets (22.1 vs. 13.9 μm). However, no differences were noted in crypt depth of duodenum in Japanese quails fed different dietary treatments (data not shown).

Table 2: Growth performance and FCR of Japanese quails fed diets with different levels of yeast

Parameter	Dietary supplementation of yeast (%)				
	0	0.5	1.5	2.5	3.5
Total feed intake g/bird	436.7	443.5	463.5	462.0	482.5
Final BW (g)	153^{d}±15.7	156^{d}±13.7	176^{c}±17.8	185^{b}±18.9	197^{a}±14.4
BWG (g)	141^{d}±9.72	146^{d}±8.58	160^{c}±13.0	170^{b}±12.1	180^{a}±11.7
FCR	3.09^{a}±0.92	3.04^{a}±0.61	2.89^{b}±0.79	2.71^{c}±0.84	2.68^{c}±0.55

[a,b]Means in the same row with different superscripts are significantly different (P<0.05).

Table 3: Effects of yeast supplementation on weight (g) of visceral organs and dressed carcass (% of live weight)

Organ	Dietary supplementation of yeast (%)				
	0	0.5	1.5	2.5	3.5
Liver	2.25±0.44	2.31±0.28	2.34±0.18	2.37±0.25	2.42±0.28
Gizzard	3.42±0.27	3.48±0.86	3.54±0.80	3.59±0.29	3.61±0.59
Heart	0.77 ±0.09	0.78 ±0.06	0.82±0.22	0.82±0.16	0.83±0.10
Abdominal fat	1.21^{a}±1.16	0.90^{b}±0.06	0.82^{b}±0.15	0.64^{bc}±0.08	0.58^{c}±0.82
Dressed carcass	64.9^{c}±2.43	68.7^{b}±6.26	71.7^{ab}±13.4	73.1^{ab}±5.82	76.5^{a}±2.54

[a,b]Means in the same row with different superscripts are significantly different (P<0.05).

Table 4: Impacts of dietary supplementation with yeast on weight (g) of lymphoid organs and pH values in intestinal digesta

Organ (g)	Dietary supplementation of yeast (%)				
	0	0.5	1.5	2.5	3.5
Thymus	0.27^{e}±0.11	0.39^{d}±0.22	0.43^{c}±0.08	0.49^{b}±0.23	0.54^{a}±0.26
Spleen	0.09±0.05	0.10±0.07	0.12±0.07	0.14±0.02	0.17±0.03
Bursa of Fabricius	0.15±0.05	0.12±0.03	0.14 ±0.01	0.17±0.06	0.19±0.05
pH					
Duodenum	6.11±0.10	6.17±0.08	6.14±0.11	6.20±0.24	6.24±0.23
Jejunum	6.28±0.06	6.21±0.09	6.22±0.30	6.17±0.19	6.21±0.12
Ileum	6.55±0.13	6.41±0.24	6.48±0.33	6.38±0.19	6.52±0.26

[a,b]Means in the same row with different superscripts are significantly different (P<0.05).

Table 5: Influences of graded levels of yeast in diet on some serum parameters and activity of some liver enzymes

Parameter	Dietary supplementation of yeast (%)				
	0	0.5	1.5	2.5	3.5
Total protein (g/dL)	2.0^{c}±11.7	2.2^{c}±8.2	4.0^{b}±6.4	4.9^{a}±9.4	5.3^{a}±11.6
Albumin (g/dL)	1.3^{c}±6.7	1.8^{c}±8.3	2.6^{b}±7.4	2.9^{b}±4.6	3.6^{a}±6.2
Cholesterol (mg/dL)	184^{a}±19.3	165^{b}±16.6	152^{c}±14.1	130^{d}±18.2	110^{e}±15.7
Triglycerides (mg/dL)	99^{a}±15.2	65^{b}±12.4	62^{b}±10.7	60^{b}±14.5	42^{c}±11.8
ALT (U/L)	68±11.2	69±15.4	70±13.8	70±10.8	71±10.1
AST (U/L)	239±19.5	240±17.3	241±16.7	241±15.8	243±18.4

[a,b]Means in the same row with different superscripts are significantly different (P<0.05).

Table 6: Effects of yeast addition to diets on SOD, CAT activities and interleukin levels in serum

Parameter	Dietary supplementation of yeast (%)				
	0	0.5	1.5	2.5	3.5
SOD (U/mL)	302^{e}±14.5	313^{d}±13.8	346^{c}±17.4	400^{b}±16.8	419^{a}±18.1
CAT (U/mL)	3.60^{d}±9.34	5.20^{c}±4.78	5.50^{c}±8.12	7.30^{b}±7.65	9.80^{a}±5.71
IL-1 β (pg/mL)	68.4^{a}±11.4	62.0^{b}±10.1	57.3^{b}±11.8	52.2^{bc}±13.2	50.3^{c}±12.6
IL-6 (pg/mL)	52.8^{a}±12.7	50.2^{a}±13.4	42.7^{b}±16.7	41.7^{b}±15.3	28.5^{c}±14.2
TNF-α (pg/mL)	55.0^{a}±10.2	49.0^{b}±9.77	45.0^{c}±11.4	40.0^{d}±15.8	31.0^{e}±13.2

[a,b]Means in the same row with different superscripts are significantly different (P<0.05).

Table 7: *E. coli* and *C. perfringens* counts (log_{10} CFU) in excreta of Japanese quails fed different treatments

Pathogen	Dietary supplementation of yeast (%)				
	0	0.5	1.5	2.5	3.5
E. coli	3.83^{a}±2.02	3.41^{a}±2.26	3.06^{ab}±2.15	2.73^{b}±2.24	1.77^{c}±2.17
C. perfringens	4.13^{a}±3.34	3.75^{a}±4.78	3.12^{ab}±5.12	2.67^{b}±4.65	1.22^{c}±2.51

[a,b]Means in the same row with different superscripts are significantly different (P<0.05).

Pathogens: Dietary supplementation can change intestinal microflora markedly and thus diet has an effect on excreta. The effects of yeast addition in diets on *E.coli* and *C. perfringens* CFU counts are presented in Table (7). Colony forming units counts of *E. coli* and *C. perfringens* were reduced (P<0.05) in excreta by dietary yeast supplementation particularly at levels of 2.5% and 3.5%.

DISCUSSION

To the best of our knowledge the effect of high levels (3.5%) of yeast in diets of Japanese quails has not been previously reported. Dietary yeast supplementation improves BWG, FCR, intestinal morphological structure, reduces enteric pathogens counts. According to Borda-Molina *et al*. (2018) yeast has many desirable effects regarding nutrients digestibility, pathogens inhibition and gut immune system interaction.

In the present study dietary yeast addition at greater concentrations (3.5%) could be used in Japanese quails to improve growth performance. Smith *et al*. (2014) showed that the weight gains markedly influenced by increased digestive and absorption area. Moreover, it has been found that the broilers fed yeast for 5 weeks have greater BWG than those of the control (Zhang *et al*., 2005). Also, Ashok *et al*. (2016) observed that dietary supplementation of yeast at contents of 5% and 10% to the basal diet improved BWG. Different authors have shown that the improvement in growth with yeast addition could be due to: (i) promotion of digestive enzyme activity and hence improvement in feed digestibility (ii) increase the absorption and utilization of nutrients via improve gut morphological structure (Pourabedin *et al*., 2014). Nevertheless, the variety in yeast products could lead to differences in animal response.

There is a controversial debate regarding the effect of dietary yeast supplementation on carcass traits. The data in this present study is in agreement with previous reports that supplemented dietary yeast had no effect on weights of gizzard, spleen and bursa of Fabricius (Morales-López *et al*., 2009). By differentiate; the relative weight of abdominal fat was significantly lower in groups fed diets with yeast than those fed non supplemented diets. According to Yalçın *et al*. (2013) it can be that the additional energy was not being stored by the birds fed yeast supplemented diets whereas, was being utilized to up-regulate the immune system. The pH values of duodenal, jejunal and ileal digesta in the present study were not influenced by yeast addition to the diets. Similarly, some researchers found that addition of yeast to the diets did not affect or increase the pH value of the ileal digesta (Markovic *et al*., 2009). In contrast to our findings, some researchers noted that by yeast addition to the diets the pH values of jejunal and ileal digesta were decreased (Yalçın *et al*., 2013).

It is well known that diagnosis of some diseases and dysfunctions can be detected by measuring some serum parameters. The values of serum protein, cholesterol and triglycerides were affected by yeast supplementation but the activities of ALT and AST were not affected. Similar to the present study, Tomaszewska *et al*. (2018) observed that feeding yeast supplementation led to significantly lower serum cholesterol content in comparison to feeding non supplemented yeast. Furthermore, in agreement with our results it has been observed that levels of serum ALT and AST in laying hens were not influenced by yeast supplementation (Yalçın *et al*., 2014).

Main anti-oxidant enzymes that constitute the first line of anti-oxidant enzymatic defenses include SOD and CAT (Aluwong *et al*., 2013). The activities of SOD and CAT in the present study were increased significantly by addition of yeast to the diets. A similar study reported that the mechanisms of oxidative defense could be stimulated by mannan-oligosaccharides and β-glucans which are component of *S. cerevisiae* (Ognik and Krauze, 2012). This suggests that dietary yeast can protect the gut rather than just removing undesirable bacteria.

The pro-inflammatory cytokines such as IL-1 β, IL-6 and TNF-α play a critical role in both the innate and the adaptive immune response (Jacob and Pescatore, 2017). Nevertheless, it is well known that overproduction of those pro-inflammatory cytokines during the inflammatory response is often energetically costly (Sanz *et al*., 2007). Our results showed that supplemented yeast at level of 3.5% could significantly inhibit the elevation of serum IL-1β, IL-6 and TNF-α compared to non supplemented groups or those supplemented only at level of 0.5%. To our knowledge, the present study demonstrates for the first time that yeast supplementation could decrease the concentrations of serum IL-1β, and TNF-α in Japanese quails. This could suggest that on the absence of an immune challenge or infectious pathogens, Japanese quails supplemented with yeast may not exhibit inflammatory responses as also was stated by Alizadeh *et al*. (2016).

The gut microbiota is one of the main defense components against enteric pathogens. Thus, any disturbance of the gut microbiota plays a major role in the

development of gut disorders. Precise mode of yeast action to reduce intestinal *E. coli* and *C. perfringens* have not been clearly understood, but yeast has increased the production of cytokines by macrophages and intestinal IgA that binds to antigens (Gao *et al*., 2008). Moreover, it is well known that yeast contain mannan-oligosaccharide and fructo-oligosaccharides, the two most commonly oligosaccharides have a role in reducing undesirable microorganisms in gut of poultry by moving them away through the intestine without colonization (Iji *et al*., 2001). Additionally, Lactobacilli can ferment fructo-oligosaccharides, which can help to reduce the growth of pathogenic bacteria such as *C. perfringens* (Hofacre *et al*., 2005). According to Suarez and Guevara (2018) the yeast has variable mechanisms including stimulation of the animal's immune system, attachment and removal of pathogens. However, some researchers reported that the addition of yeast had no significant effect on *E. coli* CFU count in the intestinal digesta (Ghosh *et al*., 2012).

The villi length and crypt depth of intestine are of special interest as they considered the major effect on nutrient absorption occurring in the small intestine. It is well known that intestinal villi increase surface area for nutrient absorption, while greater crypt depth can be related to the turnover of epithelial cells (Sims *et al*., 2004). Similarly to our findings, Tomaszewska *et al*. (2018) found that dietary yeast supplementation increased villus height in comparison to control treatment. Gao *et al*. (2008) reported that using 2.5 g/kg and 7.5 g/kg of yeast addition was associated with the highest villi in duodenum on d 21 and d 42, respectively. Also, Pourabedin *et al*. (2014) found that yeast addition improved the villus height in duodenum of broilers significantly.

Conclusions: Dietary yeast supplementation improved growth rate, FCR and increased the absorption of nutrients as a result of increased villus height in Japanese quails. Finally, greater concentrations of yeast addition were effective in reducing *E. coli* and *C. perfringens* CFU counts in excreta.

Authors contribution: Amr Abd El-Wahab designed, conducted the experiments, supervised, interpreted the data and wrote the manuscript. Rania E. Mahmoud conducted the experiments and wrote the manuscript. Basma Marghani and Hossam Gadallah performed analyses and drafted the manuscript. All authors read and approved the final manuscript.

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