Yeast, Lactose, and Organic Acids Mixture Improved Growth and Immune Status of Japanese quails

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Short title: Organic production of Japanese quail

Abstract: The present study aimed to investigate the effect of incorporating some organic feed additives, lactose (G2), yeast (G3), lactose+yeast (G4), benzoic+citric acid (G5), lactose+benzoic+citric acid (G6) and diet prepared without additives (G1), on performances and immune status of one-week-old growing Japanese quails in 35 days growth performance trial. Chicks were randomly divided into 6 groups (30 birds each; mean weight 30.5g), each was subdivided into 3 replicates (10 chicks each). Body weight (BW), body weight gain (BWG), feed intake, feed conversion ratio and feed efficiency were determined weekly. At the end of the growing trial, 36 birds (2birds/replicate) were slaughtered for determination of carcass traits, and the economic efficiency. Blood samples were collected for measuring some serum biochemical parameters and immunoglobulin M (IgM). The results showed that the BW increased significantly in G2 and G6 compared to control (G1). The BWG was significantly higher in all treatments compared to G1. Feed conversion ratio was improved in G2, G3 and G6. There were significant increases of circulating natural agglutinins and mean serum levels of IgM in G4, G5 and G6, while other treatments showed increased natural agglutinins or IgM levels. Biochemical parameters showed significant decrease in cholesterol levels in G4, G5 and G6, and significant increase in high density lipoprotein levels in G3, G4 and G5. G6 and G2 showed higher relative economic efficiency compared to G1.
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Introduction

Researchers worldwide has started the investigation for suitable replace to antibiotic growth boosters in poultry production(Mahdi et al., 2015). Now nutriceutical components are being used as alternative for antibiotics. They include probiotics, prebiotics and organic acids. Models describing the effects of yeast on poultry performance are currently based on the ability of yeast strains to stimulate the growth and activities of gastrointestinal bacteria, but this stimulatory characteristic may not be common to all strains of yeast(Stanley et al., 2004; Zhang et al., 2005). Many results suggested that, *Saccharomyces cerevisiae* (SC) could act as a growth promoter; because of it is natural improvement of digestibility and absorption of nutrients and controlling infections by enteric pathogens(Cruickshank, 2002; Miazzo et al., 2005; Onifade et al., 1999).

Prebiotics are feed additives, mainly in the form of specific types of carbohydrates that are indigestible to the host while serving as substrates to select beneficial bacteria and altering the gut microflora(Roberfroid, 2001; Roto et al., 2015). In the case of poultry species, lactose hypothetically fits within the prebiotic concept, because birds cannot digest it, and it is therefore available to microflora in the hindgut(Denbow, 2000).

Both single acids and/or blends of several acids are candidate replacements of antibiotics, their addition in drinking water or feed has been documented to improve the performance of birds. Citric acid was demonstrated to achieve similar performance results in broilers as the antibiotic growth promoter avilamycin, moreover; recently, benzoic acid has been identified as an efficient feed additive to improve growth performance, nutrient digestibility, and nitrogen balance(Chowdhury et al., 2009; Stringfellow et al., 2009; Weber et al., 2012). Articles with effect of novel combinations of organic acids, lactose and yeast on growing Japanese quails are limited. The present study aimed to investigate the effect of incorporation of lactose, yeast and organic acids mixture in diets of growing Japanese quails on performance, carcass quality, some serum biochemical parameters, immune status and economic efficiency of each additive.

Materials And Methods

I-Growth Performance Trial

Experimental birds, housing and management

A total of 180 unsexed one-week-old healthy Japanese quail chicks were used in 35 day growth performance trial. Chicks were randomly divided into six groups of similar mean weight (30.5g). Each treatment group contained 30 birds which were subdivided into three replicates of 10 chicks. The experimental groups were G1 control group that had only the basal diet. G2 had the basal diet supplemented by 1 g lactose/kg diet. G3 had the basal diet supplemented by 3 g yeast/ kg diet. G4 had the basal diet supplemented by 1 g lactose plus 3 g yeast/ kg diet. G5 had the basal diet supplemented by 2g benzoic acid plus 5g citric acid / kg diet. G6 had the basal diet supplemented by 1g lactose plus 2g benzoic acid plus 5g citric acid / kg. Chicks were housed in wire battery cages of 86L×50W×25H cm which equally partitioned into 3 pens (29x50x25cm) According to(Hassan et al., 2003). The chicks were allowed *adlibitum* access to feed and water. Birds were provided 24 hours of lighting. The diets were freshly prepared (every week) and offered regularly 3 times at 6 am, 2 and 10 pm daily.

Feed additives

1- Lactose monohydrate A.R $C_{12}H_{22}O_{11}.H_2O$, WINLAB. M.W 360.31, CAS NO.; 63-42-3 Lot No L 33155/12. UK.

2- Instant dried yeast (*Saccharomyces cerevisiae*), PANTHER, $8x10^9$ c.f.u. Turkey.

3- Benzoic Acid A.R $C_7H_6O_2$, WINLAB. M.W 122.12, Assay 99.5%, LOT No. B33155/15. UK.

4- Citric Acid Monohydrate, $C_6H_8.H_2O$, SIGMA. FW 210.1, Lot 125H0061. Austria.

Basal experimental diet

Diet for growth performance trial was formulated to meet the nutritional requirements as suggested by the(NRC., 1994), as shown in the table (1).

Studied Parameters for Growth Performance trial

Individual body weight, body weight gain, feed consumption and feed conversion ration were determined weekly. Protein efficiency ratio (PER) and energy efficiency ratio (EER) were calculated according to (Ashayerizadeh et al., 2011; McDonald et al., 2002) respectively. Economic Efficiency (EE) was calculated by the following equations:

$$\textbf{EE} = \frac{\text{Net revenue}}{\text{Total production cost}} \times 100$$

Net revenue= selling price – production cost

Total production cost was calculated including price of one week quail chicks, feed and veterinary care. Also cost of feed (LE)/kg gain was calculated according to (El-Dein et al., 2000).

Carcass traits

At the end of the growing trial when birds were 42 days old, 36 birds (2 birds/ replicate) were randomly slaughtered to determine carcass characteristics. Their blood samples were collected during slaughtering. Feathers were plucked, and head and feet (shank) were cut. The carcass and edible visceral organs were taken; cleaned and weighed (heart, liver, gizzard), and expressed as a percentage of live body weight(Esen et al., 2006). Weights of thymus and bursa were also recorded.

Table 1. Composition and calculated chemical analysis of the experimental basal diet.

Ingredients	**%**
Ground yellow corn	57.83
Soya bean meal (45%)*	32.94
Fish meal (morocco 60.5%)*	3.5
Corn gluten (62%)*	3.48
Di-calcium Phosphate (22%Ca&19%P)	0.33
Limestone (38% Ca)	1.16
DL – Methionine (purity 98%)	0.09
Lysine (purity 98%)	0.07
Iodized sodium chloride	0.3
Mineral& Vitamins premix**	0.3
Calculated composition	
Crude protein (%)	24.0
ME (kcal per kg)	2900.0
Calorie/protein ratio(C/P)	120.83
Calcium (%)	0.8
Phosphorus (%)	0.3

* Determined according to AOAC, 1995.

**Vit. A 12 mIU, vit. D_3 2 mIU, vit. E 1000mg, vit. k_3 1000mg, vit. B_1 1000mg, vit. B_2 5000mg, vit. B_6 1500mg, vit. B_{12} 10mg, biotin 50mg, pantothinic acid 10000mg, nicotinic acid 30000mg, folic acid 1000mg, manganese 60000mg, zinc 50000mg, iron 30000mg, copper 4000mg, iodine 300mg, selenium 100mg, cobalt 100mg, carrier($CaCO_3$) to 3kg. (Golden premix- Selim Pharm Elasher, Egypt).

Measurement of pH of different parts of small intestine

To determine the gut pH of birds, 10g of gut content were aseptically collected from duodenum, jejunum and ileum in 90 ml sterilized physiological saline (90:10 dilution) then pH values were measured(Al-Natour and Alshawabkeh, 2005).

II-Immunological studies

Lymphoid organ weight index

Four indices were defined to evaluate the relative sizes of the lymphoid organs. The bursa fabricius (BF) index, thymus (T) index, BF/T index, and spleen index were calculated as ratio of BF/ body weight, T/body weight, BF/T weight, and spleen/ body weight, respectively. (Organ weight/live body weight) X 1000 (Al-Sultan, 2003).

Heterophils /Lymphocytes ratio (H/L ratio)

Blood smears were made on duplicate glass slides. The H/L ratio was calculated for each bird(Gross and Siegel, 1983).

Evaluation of natural agglutinin response and Serum level of Immunoglobulin M (IgM)

Antibodies were measured with a haemagglutination test (Giambrone et al., 1978). Immunoglobulin M (IgM) serum levels were measured using diagnostic kits by an automatic biochemical analyzer (Roche Diagnostics Elecsys® 2010, Hitachi High-Technologies Corporation, Japan).

Serum biochemical parameters and antioxidant status

Colorimetric determination of serum total protein was measured according to(Cannon et al., 1974). Also, albumin was determined and globulin was calculated according to(Sturkie, 1986). The Alb/Glo ratio was also calculated using automatic biochemical analyzer (Selectra junior, The Netherlands). The serum concentrations of total triglyceride, cholesterol, high density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL), cholesterol/ HDL and HDL/ LDL ratio in serum samples were analyzed by an automatic biochemical analyzer (Selectra junior, The Netherlands), following the instructions of the corresponding reagent kit. Very low-density lipoprotein (VLDL) cholesterol was calculated from triglycerides divided by the factor 5 (Panda et al., 2006). The LDL cholesterol was calculated by using the formula: LDL cholesterol = Total cholesterol – HDL cholesterol - VLDL cholesterol. Alanine transaminase (ALT) and aspartate transaminase (AST) were measured using commercial kits. Antioxidant status was determined by estimation the serum levels of catalase and superoxide dismutase enzymes using commercial kits of Bio-diagnostic.

III. Statistical analysis

Data were analyzed by one-way ANOVA. Differences among means were tested at the 5% probability level using Duncan Multiple Range test. All the statistical analyses were done using SPSS 16 (Coakes et al., 2009).

Results

Results showed improved performance due to dietary feed additives used as seen in (Table 2)

Body weight increased significantly in G2 and G6 compared to control (G1). The body weight gain was significantly higher in all treatments compared to G1. Feed conversion ratio was improved in G2, G3 and G6. Also (Table 3) showed a significant increase of circulating natural agglutinins and mean serum levels of IgM in G4, G5 and G6, while other treatments showed increased natural agglutinins or IgM levels. Biochemical parameters (Table 4) showed significant decrease in cholesterol levels in G4, G5 and G6, and significant increase in high density lipoprotein levels in G3, G4 and G5. G6 and G2 showed higher relative economic efficiency compared to G1 (Figure 1).

Table 2. Effect of different feed additives on growth performance of Japanese quail.

Parameters	G1	G2	G3	G4	G5	G6
Initial wt.(g) One week	30.3±1.2	29.3±0.88	31.3±1.66	31.66±1.45	29.6±0.33	30.6±0.88
Final Wt.(g)	255.53±3.97^{c}	269.6±1.45^{b}	263.7±4.57^{bc}	259.7±2.11^{bc}	266.4±6.14^{bc}	283.7±3.28^{a}
Weight gain (g)	225.2±4.38^{c}	240.3±1.85^{ab}	232.3±5.18^{bc}	228.1±3.05^{bc}	236.8±6.1^{bc}	253.1±4.0^{a}
Feed consumption	695.0±6.9^{c}	703.0±6.6^{bc}	694.4±4.15^{c}	713.0±2.92^{b}	729.0±1.93^{a}	730.8±1.48^{a}
Feed conversion ratio	3.08±0.08^{ab}	2.92±0.02^{ab}	2.99±0.06^{ab}	3.12±0.04^{a}	3.08±0.07^{ab}	2.89±0.05^{b}
PER	1.35±0.03^{bc}	1.42±0.01^{ab}	1.39±0.02^{abc}	1.33±0.01^{c}	1.35±0.03^{bc}	1.44±0.02^{a}
EER	11.18±0.3^{bc}	11.79±0.1^{ab}	11.5±0.2^{abc}	11.0±0.15^{c}	11.2±0.28^{abc}	11.9±0.2^{a}
Dressed carcass %	68.66±1.06	70.52±1.08	70.26±0.88	70.72±0.98	70.4±0.6	68.1±1.3
Giblets %	4.36±0.1	4.63±0.2	4.23±0.1	4.6±0.3	4.38±0.16	4.8±0.28
Edible parts%	73.8±1.2	75.0±0.8	73.8±1.0	74.8±0.9	74.3±0.6	71.8±1.3
Liver %	2.02±0.05^{b}	2.38±0.08^{ab}	2.02±0.1^{b}	2.05±0.1^{b}	2.18±0.1^{b}	2.75±0.2^{a}
Gizzard %	1.4±0.1	1.58±0.1	1.5±0.1	1.46±0.04	1.54±0.11	1.46±0.04
Heart %	0.77±0.03	0.84±0.05	0.83±0.02	0.84±0.02	0.79±0.03	0.86±0.03
pH duodenum	7.0±0.05^{a}	6.83±0.06^{a}	6.53±0.12^{b}	6.83±0.03^{a}	6.9±0.1^{a}	6.7±0.03^{ab}
pH jejunum	6.83±0.03	6.83±0.12	6.6±0.11	6.83±0.03	6.9±0.05	6.7±0.05
pH ileum	6.93±0.17	7.03±0.03	6.7±0.05	6.9±0.1	6.9±0.12	6.8±0.05

Values are means ± standard error (SE)

Means within the same row with different superscripts are significantly different (P<0.05)

G1= control; G2= 0.1% lactose; G3= 0.3% yeast; G4= 0.1% lactose +0.3% yeast; G5= 0.5% citric acid+ 0.2% benzoic acid; G6= 0.5% citric acid+ 0.2% benzoic acid +0.1% lactose.

Table 3. Effect of different feed additives on immune status of Japanese quail.

Parameters	G1	G2	G3	G4	G5	G6
Bursa index	0.94±0.11	1.8±0.44	1.7±0.33	1.29±0.21	1.14±0.3	1.1±0.04
Spleen index	1.02±0.09	1.08±0.17	1.17±0.19	1.06±0.17	1.07±0.18	1.76±0.47
Thymus index	1.15 ± 0.08^{d}	4.59 ± 0.34^{a}	3.39 ± 0.47^{abc}	2.33 ± 0.53^{cd}	2.9 ± 0.7^{bc}	4.02 ± 0.6^{ab}
(BF)/T	0.70±0.17	0.6±0.10	0.59±0.12	0.77±0.26	0.42±0.1	0.34±0.05
Natural agglutinin	4.34 ± 0.33^{c}	5.35 ± 0.33^{bc}	5.35 ± 5.35^{bc}	5.68 ± 0.33^{b}	6.35 ± 0.3^{ab}	7.02 ± 0.5^{a}
IgM (mg/dl)	3.00 ± 0.14^{b}	3.6 ± 0.49^{b}	3.8 ± 0.23^{b}	5.4 ± 0.29^{a}	4.76 ± 0.35^{a}	5.36 ± 0.24^{a}
Total. L.C /ul	46270±896.9	128700±5811.9	47470±437.1	66670±5925.5	75330±37706	57330±5456.9
Heterphil	14023 ± 88.1^{b}	32547 ± 2032.3^{a}	15037 ± 660.6^{ab}	20987 ± 1207.2^{ab}	22600 ± 1131.9^{ab}	13880 ± 926.1^{ab}
Lymphocyte	31141 ± 311.7^{b}	83120 ± 3240.6^{a}	30201 ± 834.8^{b}	38040 ± 3628.4^{b}	44813 ± 22417.7^{b}	39800 ± 4051.3^{b}
H/L ratio	0.45±0.01	0.39±0.02	0.50±0.03	0.56±0.03	0.34±0.17	0.35±0.01

Values are means ± standard error (SE)

Means within the same row with different superscripts are significantly different (P<0.05)

Table 4. Effect of different feed additives on serum biochemical parameters and antioxidant status of Japanese quail.

文章名 Parameters	G1	G2	G3	G4	G5	G6
Cholesterol mg/dl	222 ± 16^{a}	206.6 ± 8.3^{ab}	226.3 ± 13.3^{a}	160.3 ± 7.2^{c}	169.3 ± 4.2^{c}	188.6 ± 5.2^{bc}
HDL mg/dl	36 ± 3^{c}	42.3 ± 1.8^{bc}	43 ± 1^{b}	48 ± 2.1^{ab}	49.6 ± 1.2^{a}	41.5 ± 0.5^{bc}
Tri glyceride mg/dl	170 ± 32^{a}	149.6 ± 4.2^{ab}	164.6 ± 12.8^{ab}	93.3 ± 7.8^{c}	143.3 ± 17.4^{abc}	111 ± 1.7^{bc}
LDL mg/dl	152 ± 20.3^{a}	134.4 ± 7.5^{a}	150.4 ± 13.9^{a}	93.6 ± 7.6^{b}	91 ± 6.6^{b}	125.1 ± 5.3^{ab}
VLDL mg/dl	34 ± 6.4^{a}	29.9 ± 0.8^{ab}	32.9 ± 2.5^{ab}	18.6 ± 1.6^{c}	28.6 ± 3.4^{abc}	22.2 ± 0.3^{bc}
T. protein (g/dl)	5.36±0.14	5.8±0.15	5.6±0.2	5.4±0.17	5.5±0.12	5.5±0.14
Albumin (g/dl)	1.25 ± 0.02^{bc}	1.31 ± 0.05^{abc}	1.39 ± 0.01^{a}	1.28 ± 0.04^{abc}	1.36 ± 0.04^{ab}	1.19 ± 0.02^{c}
Globulin (g/dl)	4.1±0.16	4.49±0.1	4.2±0.2	4.18±0.17	4.17±0.16	4.33±0.17
ALT ul	7.0 ± 0.5^{b}	9.6 ± 0.6^{ab}	12.0 ± 1.0^{a}	9.3 ± 0.8^{ab}	12.3 ± 0.8^{a}	10.0 ± 1.5^{ab}
AST ul	107.6 ± 2.4^{b}	119.3 ± 2.7^{b}	118.0 ± 4^{b}	123.3 ± 3.9^{b}	159.3 ± 19.4^{a}	181.6 ± 4.1^{a}
CA (U / ml)	196.2 ± 5.1^{b}	210.3 ± 6.1^{b}	204.1 ± 2.9^{b}	206.3 ± 5.1^{b}	205.1 ± 2.8^{b}	234.0 ± 5.8^{a}
SOD (U/ml)	406.25 ± 43.3^{b}	456.25 ± 25^{ab}	481.25 ± 25^{ab}	431.25 ± 25^{ab}	481.25 ± 50^{ab}	556.25 ± 66.1^{a}

Values are means ± standard error (SE)

Means within the same row with different superscripts are significantly different (P<0.05)

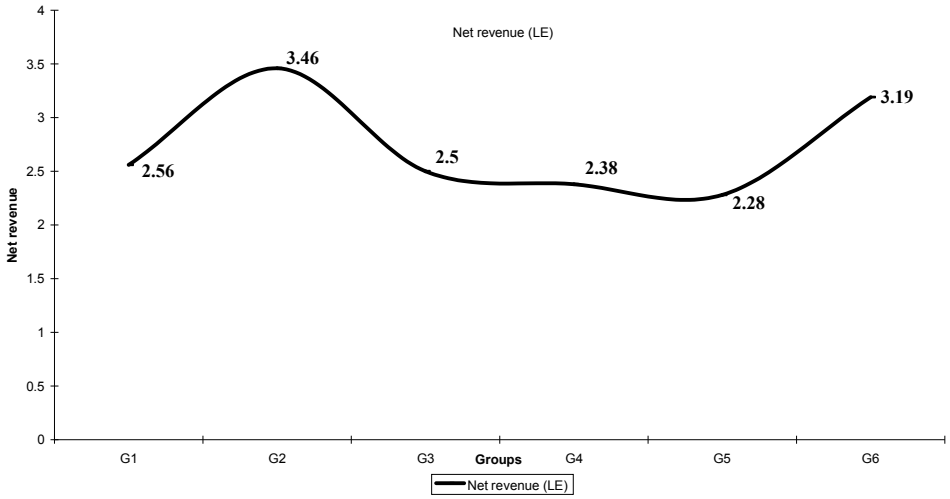

Figure 1. Net revenue of the experimental groups.

Discussion

I. Growth Performance Trial

Final body weight and body weight gain were significantly improved in G2 and G6 with a trend of improved gain in all other treatments compared to the control (Table 2). G6 (citric and benzoic acids combination with lactose) resulted in improved daily live body weight (LBW) and weight gain (WG) when compared to control one. These in accordance with (Boling et al., 2000); (Snow et al., 2004); and (Ghosh et al., 2007) who observed that, organic acid combination, or OAC with prebiotic in growing Japanese quail diet significantly improved the performance in terms of BWG. (Hedayati et al., 2013) showed that dietary acidifier increased the growth performance of broiler chicken; they concluded that it is an option for maintaining or improving broiler growth and efficiency. (Abdel-Fattah et al., 2008) mentioned that, significantly improved LBW, BWG and FCR may be due to enhancement in the thyroid gland activity also, the attained non-significant reduction in pH values of different GI-tract segments have a beneficial effect in the inhibition of intestinal bacteria competing with the host for available nutrients and a reduction of possibly toxic bacterial metabolites, e.g. ammonia and amines, thus improving weight gain of the host animals. Furthermore, the growth inhibition of potential pathogen bacteria, e.g. *E. coli* and *Salmonella*, in the feed and in the GI-tract benefited animal health (Berchieri and Barrow, 1996; Hedayati et al., 2013; Iba and Berchieri Jr, 1995; Thompson and Hinton, 1997). (Kirchgessner and Roth, 1988) observed that, dietary acidification increased gastric proteolysis and protein and amino acid digestibility. Organic acids also serve as substrates in intermediary metabolism. Moreover, the improvement in body weight gain of birds fed citric acid may be due to the utilization of minerals that positively affecting the growth (El-Hakim et al., 2009), such as phosphorus and trace minerals (Boling et al., 2000).

The improved performance with dietary G2(0.1% lactose) was in accordance with (Torres-Rodriguez et al., 2007; Van Der Wielen et al., 2002). Lactose effect on the intestinal tract of poultry is characterized by a lowering of pH and a change of intestinal flora to an acidophilic type(Bailey, 1993; Gibson et al., 2004). This stimulates the synthesis of B-vitamins by the intestinal bacteria. Lactose significantly improved the CF apparent digestibility and ME when compared to that in the control groups of broilers, pigeon and turkey (Barnhart et al., 1999; Buteikis et al., 2008; Sales and Janssens, 2003). Dietary lactose decreased *lactobacillus*, *clostridium* and *proteus* species and increased bifidobacteria in the chickens ceca (Morishita et al., 1982; Van Der Wielen et al., 2002) which play an important role in the ecology of intestinal tract and are increasingly employed in the production of functional foods (Biavati et al., 2000) explained that, the positive results of prebiotic on body weight and weight gain may be attributed to that additive may help to maintain the microflora balance of the intestinal tract of chicken resulting in a more efficient use of nutrients from feed, more intensive processes of protein metabolism and subsequently in better health.

The numerically improved LBW and BWG effect of G3 (0.3% yeast) when compared to control one, was in accordance with (Fuller, 2012; Kabir et al., 2004; Mountzouris et al., 2007; Patterson and Burkholder, 2003). Probiotics, such as yeast, have the ability to stimulate digestion and aid in maintaining microbial equilibrium in the gut. Live yeast, such as *Saccharomyces cerivisae*, contains numerous enzymes that could be released into the intestine and aid existing enzymes within the digestive tracts. Also, yeast contains vitamins and other nutrients that may produce beneficial production responses(Kornegay et al., 1995). The critical role of yeast culture in the metabolic functions was suggested by (Stockland, 1993) as a strong stimulating action on the activity of certain important bacteria, which are actively involved with the digestive processes, protein synthesis and nutrient absorption in the GI-tract. (Santin et al., 2001) reported a significant increase in weight gain when birds fed 0.2% cellular wall from *Saccharomyces cervisiae* (Mannanoligosaccharide).

Feed consumption was significantly improved in G4, G5 and G6 compared to control; while other dietary treatments were not significantly different compared to the control (Table 2). G5 (0.5% citric acid plus 0.2% benzoic acid combination) and G6 (OAC with 0.1% lactose) significantly increased the feed intake. Similar results were reported by (Atapattu and Nelligaswatta, 2005; Boling et al., 2000) who studied the inclusion of citric acid with low energy diets and speculated that, the increase of feed intake may be due to the dilution of energy in the diet plus inclusion of citric acid. However, diets of the present experiment were isocaloric. Even if it is assumed that citric acid provides some energy, it should lead to a reduction of feed intake in citric acid supplemented diet. Inclusion of citric acid reduced the pH levels of the feeds and digesta of the crop and gizzard. Appetite control nerve endings are located in the crop of the birds. Therefore, it can be assumed that citric acid itself and/or low pH environment created by it, stimulated the feed intake(Atapattu and Nelligaswatta, 2005). Also a study by (Lokhande et al., 2005) on comparative evaluation of prebiotic, probiotic and acidifier in broiler diets concluded that, birds received different growth promoters recorded marginally better

feed conversion ratio than the control. The results of this study were in accordance with (Denli et al., 2003; Ghosh et al., 2007; Torres-Rodriguez et al., 2007; Van Der Wielen et al., 2002) who observed best FCR with supplementation of diets of quail and chickens with OAC and OAC with prebiotic. The numerical improvement of FCR observed in G2 (lactose 0.1%) was consisted with (Guclu, 2003; Pelicano et al., 2004; Torres-Rodriguez et al., 2007; Waldroup et al., 2003; Xu et al., 2003) who reported that, addition of prebiotic to quail and broiler chickens diets at a level of 0.75 and 1g/kg enhances feed conversion ratios. Moreover, (Kirkpinar et al., 2004; Tomasik and Tomasik, 2003) stated that, prebiotics belong to a group of indigestible dietary carbohydrates improved feed conversion. A non-significant response due to G3 (yeast) was not in agreement with (Madkour et al., 2008) who reported that, using probiotic and prebiotic in broiler diets significantly improved both feed consumption and feed conversion ratio from 0-42 days of age. This may be attributed to the difference in species used as well as this study began with quails at 7 days old.

A significant improvement in both protein efficiency ratio (PER) and energy efficiency ratio (EER) were seen in G6 (OAC with lactose). G2 (lactose) and G3 (yeast) were numerically improved while G4 and G5 were not affected in by additives compared to the control (Table 2). This improvement was in agreement with (Falaki et al., 2010; Nayebpor et al., 2007; Thitaram et al., 2005; Zulkifli et al., 2000). Also (Ashayerizadeh et al., 2011) who revealed that, all the growth promoting additive treatments had better protein intake, protein efficiency ratio (PER) and energy efficiency ratio (EER) than control birds. However, these results disagree with that of (Boling et al., 2000) who concluded that, the highest value of PER and EER was shown by broilers under synbiotic treatment.

There was a trend of increased dressed carcass %, edible parts% and giblets% of quail chicks at in groups G2, G4 and G5 (Table 2). The assumption of non-improved dressed weight of carcass while improved growth rate especially in G6 (OAC with lactose), may be due to that, dietary citric acid decrease intestinal pH and increase calcium availability (Snow et al., 2004) that reflected on meat as well as bone and feathering in males and early egg production in female. Significant improvement was detected in relative weight of liver in G6 and numerically in G2 compared to the control one (Table 2). These results disagree with those of (Denli et al., 2003) who stated that dietary organic acids had no effect on the carcass yield and liver weight of broiler chickens at 42 d old. On the other hand, (Abdel-Azeem et al., 2000) declared that, addition of citric acid to the diet was associated with higher dressing and lower liver percentages, he suggested that, the lack of significance in the relative liver weights between the acidified and control chicks may be ascribed to the more storage of glycogen and lower lipid repletion induced by dietary organic acid. This supposition may emphasize the hypothesis of (Fushimi et al., 2001) who stated that dietary acidification might stimulate glycogenesis by increasing the influx of glucose 6-phosphate (G-6-P) into the glycogen synthesis pathway through the inhibition of glycolysis due to an increase in citrate concentration. Results of G3 are in accordance with (Abdel-Azeem et al., 2000) he indicated that, carcass traits and internal organs were not affected due to addition of yeast culture at lg/Kg in quail diet. Contradiction to (Mahajan et al., 1999) who observed a significant improvement in giblets, hot dressed weight, cold dressed weight and dressing percentage were significantly higher for -yeast supported-probiotic (Lacto-Sacc) fed broilers

Only G3 showed a significant reduction in the pH of duodenum compared to the control. Other intestinal segments (jejunum and ileum) did not show any alteration in pH compared to the control (Table 2). (Atapattu and Nelligaswatta, 2005) suggested that, citric acid doesn't alter pH of the GI tract after the gizzard. It seems that unprotected form of OA used in feed can be neutralized easily by bile and pancreatic secretions(Gheisari and Kholeghipour, 2006). Also (Chaveerach et al., 2002) demonstrated the magnitude of antimicrobial effect of organic acids varies from one acid to another and it depends on concentration and pH. (Hernández et al., 2006) reported no effect on intestinal pH with the use of a product containing combination of propionic acid and formic acid. They referred this insignificant effect to the strong buffering action of the GI-tract in poultry.

II-Effect on immunity

There is a non-significant increase in relative weight of bursa and spleen comparable to control. All dietary treatments except G4 significantly increased the relative weight of thymus. The positive effect of dietary additives on immune status was confirmed by BF/T ratio, which was narrower than the control one in dietary treatment of G6, G5, G3 and G2 respectively (Table 3). (Katanbaf et al., 1989) reported that the increase in the relative organs weight is considered as an indication of the immunological advance. Our results agree with (Rahmani and Speer, 2005; Tollba, 2010) who reported that, citric acid and lactose supplementation did significantly improve relative weight of thymus and bursa that reflect higher immunity. (Mohamed and Bahnas, 2009)observed that, Malic acid (MA) supplementation resulted in significantly increased edible giblets and lymphoid

organs (thymus gland). (Ao et al., 2004) revealed that the immune status of the birds was supported by increased relative weight of bursa due to the use of prebiotic in broilers diets.

A significant increase in IgM levels in Japanese quail serum was observed in G4, G5 and G6 while; it was numerically increased in G2 and G3. Also a significant increase in circulating natural unspecified agglutinins was observed in dietary treatments in G4, G5 and G6 while the increase was numerically observed in G2 and G3 (**Table 3).** These results revealed the positive effect of used dietary feed additives on humeral immune status of Japanese quail. This is in agreement with findings of (Gheisari and Kholeghipour, 2006) who stated that, dietary inclusion of live yeast (particularly in powder form) could be an effective stimulator of humeral immune responses in broiler chickens. However, more studies are required to show the effect of organic acids and lactose on these natural agglutinins. The prebiotic-mediated immunological changes may in part be due to direct interaction between prebiotics and gut immune cells as well as due to an indirect action of prebiotics via preferential colonization of beneficial microbes and microbial products that interact with immune cells (Janardhana et al., 2009). (Kabir et al., 2004) reported significantly higher antibody production in dietary yeast fed broilers as compared to control ones. They also demonstrated that, the differences in the weight of spleen and bursa of broilers could be attributed to different level of antibody production in response to probiotics. (Haghighi et al., 2005) reported that, administration of probiotics enhances serum and intestinal natural antibodies to several foreign antigens in chickens. Antibody responses have been used as measures of the humoral immune status of bird (Davis and Sell, 1989; Sklan et al., 1994). This parameter was significantly increased in the mannanoligosacchaide (MOS) group in the trials of (Shashidhara and Devegowda, 2003), who suggested that, prebiotic MOS may also be influencing systemic immunity. This effect on antibody titers might have been due to influence of the MOS on immune system; improved intestinal absorption of some nutrients, such as Zn, Cu, Se; or both effects. Much of the nature of mechanism accountable for immunomodulation associated with the MOS remains to be delineated. One the other hand, (Cotter et al., 2000; Raju and Devegowda, 2000) reported no such improvement in the antibody titers against IBDV and Newcastle disease virus in broilers fed MOS.

We measured a numerical increase in total leucocytic counts (TLC) in all groups compared to control one. Heterophils revealed a significant increase in G2 while numerically increased in G3, G4 and G5. Lymphocytes (L) significantly increased in G2 while, numerically increased in G4, G5 and G6. Heterophil: lymphocyte (H/L) ratio showed a numerical decrease in G2, G5 and G6 while numerically increased in G3 and G4 compared to the control (Table 3).The positive effects of G2 (lactose), G5 (OAC) and G6 (OAC with lactose) could have potential benefits for growing quail performance, as reflected on reduction of H/L ratio and enhancement of physiological resources. This result is contrary to (Houshmand et al., 2012) who reported that, supplementation with a prebiotic had no significant effect on performance, immunity, and stress indicators (blood glucose, cholesterol, corticosterone, and heterophil: lymphocyte ratio). However, (Maassen et al., 2000) stated that, beneficial bacteria (probiotic) had stimulated healthy gut structure and systemic immune system. The yeast can stimulate immune system of chicks' body as it affects white blood cells (WBCs) (Paryad and Mahmoudi, 2008) observed that, all yeast-fed chicks in comparison to control diet, had a higher WBCs count as observed in the present study and lower H/L ratio by the higher populations of lymphocytes than control diet. The higher lymphocyte populations may be indicative of higher activity of humeral immune responses in chicks fed yeast supplemented diets (Gheisari and Kholeghipour, 2006).

***Blood biochemical** parameters*

Results of cholesterol reflect the hypocholestrolemic proprieties of experimental additives except G3 (yeast) where, a significant decrease was observed in G4, G5 and G6 and numerical decrease due to addition of G2 (lactose). Results of triglycerides revealed a significant decrease in G4 and g6 while the decrease was numerically in G2, G3 and G5 (Table 3). The results in G5 and G6 are in agreement with findings of (El-Kerdawy, 1996) and (Abdo and Zeinb, 2004) who reported that blood total lipids and cholesterol decreased significantly by dietary acidifiers. Also (Mahdi et al., 2015) showed the same blood lipid profile in poultry by consumption of probiotic and acidifier in the feed. However, (El-Afifi et al., 2001)observed no significant effect on blood lipid profile in broiler chicks fed on citric acid. The beneficial role of organic acids in reducing the blood lipid profile may be interpreted through their influence in decreasing the microbial intracellular pH. Thus, inhibits the action of important microbial enzymes and forces the bacterial cell to use energy to release the acid protons, leading to an intracellular accumulation of acid anions (Young and Foegeding, 1993) Also, (Abdel-Fattah et al., 2008) observed hyperthyroidism associated with dietary organic acidification could also explain the observed reduction in serum lipid profile. (Sturkie, 1986) reported that hyperthyroidism (T_3 elevation) induced energy

metabolism and decreased fat deposition. Hence, the concentration of avian blood lipids is influenced by the physical and nutritional status of the bird. The non-significant, addition of lactose as a prebiotic numerically decreased the serum cholesterol and triglyceride levels were in agreement with (Siegel et al., 1995; Tarasewicz et al., 1998) who revealed a sharp decrease of cholesterol in blood as an effect of oligosaccharide-enriched Japanese quail diet. Prebiotic sugars remain longer in the large intestine, thus they enhance the excretion of bile acids caused by volatile fatty acids production. As a result of this process the level of cholesterol in blood decreases. Furthermore, volatile fatty acids and products of their metabolism also decrease the production of endogenous cholesterol (Hidaka et al., 1991; Terada et al., 1994). The mechanism involved in the overall hypo-cholesterolaemic effect of MOS supplementation is not fully documented. However, MOS (yeast cell wall component) as a prebiotic is considered a substrate for lactic acid producing bacteria such as *Clostridium* spp. and *Bifidobacterium bifidum (Van Loo, 2004)*. It is reported that some *Lactobacillus spp.* are able to incorporate cholesterol into the cellular membrane of the organism, so cholesterol assimilation by *Lactobacillus* in turn decreases cholesterol absorption in the system(Kannan et al., 2005). Lactose group results coincided with (Taherpour et al., 2009) who stated that, by the addition of prebiotics, probiotics and organic acids the serum total cholesterol and serum LDL concentrations were significantly reduced by dietary treatments compared by control group; but HDL was not significantly increased.

It has been pointed out that dietary yeast 0.3% significantly increased HDL with no effect on LDL and VLDL (Table 3) that is in accordance with (Gheisari and Kholeghipour, 2006) who observed that, diets containing 0.2 % powdery *S.C.* increased serum HDL concentration in broiler chicks, while with increasing level of granular *S.C.* in experimental diet, serum LDL and cholesterol concentration increased. On the other hand, chickens fed diet containing 0.1% *S.C.* in powder form decreased serum cholesterol concentration compared to control group.The results of the present study contradicts with the results of other researchers (Onifade, 1997; Onifade et al., 1999) who stated that, the addition of innocuous microorganisms including yeast to diet of rabbit and broiler chickens decreased serum cholesterol and triglycerides.

Data presented in **Table (3)** showed that, dietary feed additives exhibited numerical increase in serum concentration of total protein especially in G2, G3, G5 and G6 compared to the non-supplemented control and yeast G3. This could be due to the achieved numerically increased serum concentration of globulin levels especially in G2 and G6 that agreed with (Abdel-Fattah et al., 2008). Globulin level has been used as indicator of immune responses and source of antibody production. Table 3 showed non-significant increase levels of globulin. This is in agreement with (Ali et al., 2008; El-Hakim et al., 2009) who reported that, no difference was observed in the total protein, albumen or globulin in the plasma of broilers fed diet supplemented with citric acid, lactic acid and their combinations. On the other hand (Rahmani and Speer, 2005) observed that, higher percentage of gamma globulin in broilers given organic acids than the control ones.

An ALT activity, which is specific mirror of liver functions, was significantly increased in G3 and G5 compared to the control. Also data revealed numerical increase in ALT levels in G2, G4 and G6. AST levels showed a significant increase due to dietary supplementation of OAC, G5 and OAC plus lactose, G6 (Table 3). This in accordance with (Grassmann and Klasna, 1986) who reported that, dietary addition of 3% citric acid (a higher level than the present study) significantly increased the activities of both AST and ALT enzymes. In-contrary, (Abdel-Azeem et al., 2000; Abdel-Fattah et al., 2008; El-Kerdawy, 1996) they demonstrated that, levels of AST was reduced in growing rabbits fed supplemental citric acid, although ALT was not significantly affected. This disagreement may be due to differences in levels of acid supplementation and species.

There was a trend of enhanced serum superoxide dismutase (SOD) and catalase (CA) activity due to all dietary supplementations when compared to the control (Table 3). This effect was significant in G6 (OAC with lactose). SOD minimizes oxidative stress in quail chicks by inhibiting the oxygen free radical production and scavenging the superoxide ions(Öztürk - Ürek et al., 2001). In the cell catalase reacts with generated hydrogen peroxide to form water and molecular oxygen thereby protecting the cells against hydrogen peroxide toxicity and lipid peroxidation (Yamaguchy, 1991). Result of the present study may be due to synergistic effect of OAC with lactose on scavenging free radicals and hydrogen peroxides. (Holovská et al., 2003; Wang et al., 1998) observed age-related increase in plasma antioxidant enzyme levels by antioxidants supplementation in broilers. These supplementations might have enhanced enzyme levels to scavenge ROS and free radicals which are produced more during rapid growth period (from 1st to 6th week). We concluded that the feed additives in this experiment can combat oxidative stress caused by rapid growth rate in quails, by effectively enhancing the SOD, and catalase activities in the body.

Economical evaluation

Japanese quail has attained economic importance as an agricultural species producing meat and eggs that are enjoyed for their unique flavor. Results of economic efficiency (E.E.) for quail chicks fed experimental diets during the growth period are summarized in **Figure (1)**. There are considerable cost saving with using the inclusion of G2 (0.1% lactose) and G6 (organic acids mixture with lactose) as compared to the control group. This improvement could be due to improving weight gain and feed conversion.

Conclusion

In conclusion the present study showed that, 0.3% yeast, 0.1% lactose and OAC or OAC with lactose improved the growth performance, immune response and reduced cost of production and relative economic efficiency especially due to 0.1% lactose and OAC with lactose.

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