Use of *Aspergillus japonicas* culture filtrate as a feed additive in quail breeder’s nutrition

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**ABSTRACT**

This study was carried out to study the beneficial role of organic acids and other beneficial compounds produced by *Aspergillus japonicas* and their effects on the egg production, egg quality, fertility, and hatchability as well as blood metabolites of quail breeders. A number of 150 mature 8-week of age Japanese quails (100 females and 50 males) were used and divided into 5 groups. The 1st, 2nd, 3rd, 4th, and 5th groups were fed basal diet with 0 (control), 1, 2, 3, and 4 ml *Aspergillus japonicas* culture filtrate/kg diet, respectively. The use of *Aspergillus japonicas* filtrate improved egg number, egg mass and feed conversion ratio when compared to control. The use of *Aspergillus japonicas* filtrate quadratically improved feed conversion ratio when compared to control at all ages. Quail breeders fed 2 and 3 ml *Aspergillus japonicas* filtrate-treated diets consumed less feed than the other diets (0, 1, or 4 ml) during 8–12 week-old and the overall period. Supplementation of *Aspergillus japonicas* filtrate to quail diets improved fertility percentage during 12–16 and 8–16 week of age, the best level was 2 ml/kg of filtrate. Addition of *Aspergillus japonicas* filtrate to quail diets reduced the shell percentage and shell thickness, but increased the Haugh unit. The activity of SOD and TAC was higher in *Aspergillus japonicas* filtrate groups than the control. In conclusion, supplemental *Aspergillus japonicas* filtrate could improve productive and reproductive performance, lipid profile, immunity, and antioxidant indices.

**Introduction**

Natural and synthetic feed additives have been effectively used in livestock for increasing productivity and improving the public health and wellbeing (Hajiaghapour and Rezaeipour 2018; Alagawany et al. 2019a, 2019b, 2020d, 2020b; Nabi et al. 2020). Recently, in several countries, especially in the developed countries, there have been increasing desires for designer and organic poultry products, may be due to their ability to decline the problems of several ailments and improve the consumer health (Alagawany et al. 2018a, 2018b; Abdel-Latif et al. 2020; Alagawany et al. 2020a, 2020c). To provide industrial organic acids from species fungi aiming to adjust the fermentation, many factors must be analysed (carbon source, culture components, fermentation time, temperature and pH). In poultry nutrition, the application of agro-industrial wastes like bran, may contribute decreasing the production costs of organic acids and enzymes, generating a cheap final product (Alagawany et al. 2018c; Mohamed et al. 2019). Organic acids and other components of some fungal species are effectively used in industries including poultry industry due to their health benefits for biotechnological applications (Coban and Demirci 2017; Abd El-Hack et al. 2020; Ismail et al. 2020; Reda et al. 2020). *Aspergillus japonicus* is an excellent organic acid and enzyme producer in a culture medium using agricultural wastes and

**Highlights**

- Use of *Aspergillus filtrate* in quail diets improved egg production and egg mass.
- Use of *Aspergillus japonicas* filtrate to quail diets improved fertility percentage.
- Use of *Aspergillus filtrate* in quail diets improved immunity and antioxidant indices.
- Dietary addition of *Aspergillus filtrate* improved lipid profile.
inexpensive components (Maller et al. 2014). Dietary acids are divided into organic and inorganic acids, but the organic part is effectively and widely used in poultry feeds (Salah et al. 2019). There are several organic acid applications in poultry industry such as the short-chain organic acids (formic, acetic, butyric and propionic acids, in addition to others like lactic, fumaric, malic, tartaric and citric acids (Dibner and Buttin 2002; Pearlin et al. 2020). These feed additives gained considerable attention as excellent alternatives to growth and production enhancer antibiotics owing to its beneficial impact on pathogens; they lower the pH in the gastrointestinal tract, thus, enhancing the absorption of nutrients in livestock (Kil et al. 2011; Hazrati et al. 2020). The use of organic acids in poultry diets improved the performance, production, egg mass, feed efficiency, and resistance against several ailments (Islam 2012; Fouladi et al. 2018). Additionally, several studies showed that organic acids including formic acids and propionic have antimicrobial impact against pathogenic bacteria like Coliforms, Salmonella spp., and Escherichia coli in the intestine of poultry (Ruhnke et al. 2015; Gowda and Shivakumar 2019). Furthermore, Chowdhury et al. (2009) observed an improvement in the immunological indices of chickens fed citric acid at 0.5%. Abdel-Fattah et al. (2008) observed a similar improvement in the immune response of birds due to organic acids supplementation. It is hypothesised that the use of Aspergillus japonicas culture filtrate in the diets is expected to have beneficial impacts on quail breeders. Thus, the present study was planned to investigate the influence of dietary addition of Aspergillus japonicas culture filtrate (oxalic acid, citric acid, lactic acid, ascorbic acid, maleic acid, formic acid, and salicylic acid) on the egg production, egg quality, fertility and hatchability, liver and kidney functions, lipid profile, antioxidant, and immunity parameters of quail breeders.

**Materials and methods**

**Experimental design and animal husbandry**

A number of 150 mature 8-week of age Japanese quails (100 females and 50 males) were used. The birds were allotted to 5 experimental groups with 5 replications of 9 birds (2 male and 4 females). The duration of our study was 2 months (8–16 weeks). The treatments were the following: (1) basal diet without any supplements (control), (2) basal diet + 1 ml A. japonicas filtrate/kg diet; (3) basal diet + 2 ml A. japonicas culture filtrate/kg diet; (4) basal diet + 3 ml A. japonicas filtrate/kg diet; (5) basal diet + 4 ml A. japonicas filtrate/kg diet. The basal ration (Table 1) was formulated to meet the requirements of quail breeders (NRC 1994). Quails were reared in conventional type cage (50 × 30 × 50 cm³; 1500 cm² of floor space) with water and feed provided *ad libitum*. Quails were exposed to 17 h light:7 h dark cycle during the trail. Aspergillus japonicas (accession no. MN960315). Culture filtrate contained oxalic acid, citric acid, lactic acid, ascorbic acid, maleic acid, formic acid, and salicylic acid by GCMASS.

### Table 1. Ingredients and nutrient contents of basal diet of Japanese quail.

| Ingredient                         | Quantity (g/kg as-fed basis) |
|-----------------------------------|------------------------------|
| **Yellow corn**                   | 602                          |
| Soybean meal (44%)               | 250                          |
| soybean oil                       | 15.0                         |
| Corn gluten meal, 60%            | 57.0                         |
| Di-calcium phosphate             | 13.0                         |
| Limestone                        | 55.0                         |
| NaCl                              | 3.00                         |
| l-Lysine                          | 2.00                         |
| Premix*                          | 3.00                         |
| Composition (g/kg)               |                              |
| Protein                           | 199                          |
| Metabolizable energy (kcal/kg)    | 2918                         |
| Calcium                           | 25.0                         |
| Available phosphorus              | 3.50                         |
| Methionine                        | 3.50                         |
| Lysine                            | 10.9                         |
| Methionine + cystine              | 7.10                         |

*Provides per kg of diet: Vitamin A, 12,000 I.U; Vitamin D3, 5000 I.U; Vitamin E, 130.0 mg; Vitamin K3, 3.605 mg; Vitamin B1 (thiamin), 3.0 mg; Vitamin B2 (riboflavin), 8.0 mg; Vitamin B6, 4.950 mg; Vitamin B12, 17.0 mg; niacin, 60.0 mg; D-biotin, 200.0 mg; calcium D-pantothenate, 18.333 mg; folic acid, 2.083 mg; manganese, 100.0 mg; iron, 80.0 mg; zinc, 80.0 mg; copper, 8.0 mg; iodine, 2.0 mg; cobalt, 500.0 mg; and selenium, 150.0 mg.

**Data collection**

Feed intake (FI) was recorded weekly, while feed conversion ratio (FCR, g feed/g egg) was computed as the amount of FI divided by the egg mass. Egg weight and egg number were recorded every day to calculate the egg yield or mass. Egg quality indices were measured using 3 eggs per replicate at the end of each month. Yolk, albumen, and shell percentages; thick of shell; USSW (unit surface shell weight), ESI (shape index of egg), and Haugh unit) were measured (Romanoff and Romanoff 1949).

**Fertility and hatchability**

At 12 and 16 weeks of age, 50 fertile eggs from each treatment were incubated. After hatching, hatched chicks were counted and non-hatched eggs were checked and broken to compute the fertility and hatchability percentages (Alagawany and Attia 2015).
Blood parameters

At 16 weeks of age, after slaughter, blood samples were randomly collected from five quail breeders per treatment into heparinised tubes. Blood samples were centrifuged (G force rate = 2146.56 × g) for 900 s. Plasma total protein (g/dL), globulin (g/dL), albumin (g/dL), aspartate transaminase (AST; IU/L), alanine transaminase (ALT; IU/L), lactate dehydrogenase (LDH, IU/L), creatinine (mg/dL), urea (mg/dL), total cholesterol (TC; mg/dL), triglyceride (TG; mg/dL), high-density lipoprotein (HDL) cholesterol (mg/dL), and low-density lipoprotein (LDL) cholesterol (mg/dL) levels were determined spectrophotometrically using commercial kits from Biodiagnostic Company (Giza, Egypt). The level of superoxide dismutase (SOD), reduced glutathione (GSH), catalase (CAT), total antioxidant capacity (TAC), and malondialdehyde (MDA) were determined in plasma using commercial kits and a spectrophotometer (Shimadzu, Tokyo, Japan). The level of immunoglobulin G (IgG; mg/dL), A (IgA; mg/dL), and M (IgM; mg/dL) and lysozyme (mg/dL) also were measured using commercial kits.

Statistics

All of the statistical methods were carried out using the SAS (2001). All data (productive and reproductive performance, egg quality, liver and kidney functions, immunity, antioxidant indices, and lipid profile) were analysed with one-way ANOVA. Orthogonal polynomial contrasts (linear and quadratic) were used to test the significance of the different levels of dietary Aspergillus japonicas filtrate.

Results and discussion

Productive performance

Based on the results in Table 2, in general, supplemental A. japonicas filtrate in the diets of quail breeders led to significant differences in productive performance. Apart from the period from 12 to 16 weeks of age, the use of A. japonicas filtrate in quail breeders quadratically improved egg number and mass when compared to control. Also, use of Aspergillus japonicas filtrate quadratically improved feed conversion ratio when compared to control at all ages. On the same trend, the best values of egg weight were recorded with 3 or 4 ml/kg feed, which is in agreement with Fouladi et al. (2018) who stated that the rations containing acetic acid or lactic acid or butyric acid considerably increased feed conversion ratio, egg weight, egg mass, and egg production (p < .01). On the same context, the use of organic acid in quail diets improved egg weight, weight of first egg and age of laying 50% egg laying intensity when compared to control (Alrahawi 2019).

Quail breeders fed 2 and 3 ml A. japonicas filtrate-treated diets consumed less feed than the other diets (0, 1, or 4 ml) during 8–12 week-old (quadratic, p = .0001) and the overall period (quadratic, p = .0020). The reduction of feed consumption in the groups of 2 and 3 ml filtrate can be returned to the strong taste of the content of organic acids in A. japonicas filtrate.
which would have lowered the palatability of the diets, thereby lowered feed intake. During 8–12 and 8–16 week of age, the quail breeders fed diets enriched with *A. japonicas* filtrate achieved the best feed conversion ratio when compared to control. The 1 ml *A. japonicas* filtrate-treated quails were the best feed conversion ratio, the improvement in the conversion of feed in our study could be attributed to better nutrient absorption and utilisation resulting in enhanced productive performance in the birds fed organic acid-diets such as lactic, butyric, and fumaric acids (Adil et al. 2011).

In broiler chickens, consistent with the present data, Paul et al. (2007) observed a decrease in the consumption of feed of broilers fed 3 g/kg of organic acids; but, this level improved the performance indices. According to Chamba et al. (2014) greater feed conversion ratio were observed when birds fed 700 mg/kg of organic acids. The enhancement in the performance traits may be due to the addition of organic acid, because these feed additives increase the ability of the intestinal wall to absorb nutrients (proteins, carbohydrates, and minerals) (Nair and Kollanoor 2019).

**Reproductive (fertility and hatchability)**

The impact of *A. japonicas* filtrate on reproductive performance is illustrated in Table 3. Supplemental *A. japonicas* filtrate to the diets of quail breeders quadratically improved the fertility percentage during 12–16 and 8–16 week of age ($p = .0031$ and .0050, respectively), the best level was 2 ml/kg of filtrate. No significant effect ($p > .05$) of dietary *A. japonicas* product on the fertility and hatchability (8–12 week of age; Table 3). Addition of *A. japonicas* filtrate up to 2 ml/kg to quail diets quadratically improved the hatchability percentage by 19.49% when compared to control ($p = .0089$); but, the high levels of filtrate (4 ml/kg diet) were similar to the control groups during the production phase (12–16 week of age). Our results are confirmed by those obtained by researchers (Zanaty et al. 2001; Garcia et al. 2005; Mohamed and Bahnas 2009). This improvement in fertility percentage might be due to diets containing acetic acid and lactic acid (Yakout et al. 2004; Garcia et al. 2005; Mohamed and Bahnas 2009). Anyway, several studies have shown a strong correlation between sexual maturity and live body weight (Chan and Decker 1994). The results of the present study also reported that rations containing 1 and 2 ml of organic acids and other compounds produced by *A. japonicas* had the most impact on the increase in total serum protein and globulin, respectively. So, it can be concluded that the improvement in serum levels of these proteins, increased the protein concentrations secreted to the eggs of Japanese quail by the reproductive system resulting in high egg weight that could have high fertility impact (Garcia et al. 2005; Mohamed and Bahnas 2009).

**Egg quality**

The impact of *A. japonicas* filtrate on egg quality is illustrated in Table 4. No polynomial effect ($p > .05$) of dietary *A. japonicas* filtrate on the parameters of egg quality except yolk, shell, shell thickness, Haugh unit, and USSW. The addition of *A. japonicas* filtrate to quail diets reduced the shell percentage and shell thickness when compared to control (quadratic, $p < .0001$ or .0120, respectively); but increased the yolk percentage and Haugh unit, the best values (33.80 and 84.38) were observed by quails fed diet enriched with 1 and 2 ml/kg diet, respectively. Also, the value of USSW was linearly improved ($p = .0391$) with dietary supplementation of *A. japonicas* filtrate. These results partially agreed with Fouladi et al. (2018) who found that the rations diets contained acetic and butyric acids affected ($p < .01$) some egg quality parameters like eggshell weight, eggshell thickness and shell surface. Also, the use of organic acid (lactic acid 2.5 mg/kg diet) in quail diets improved ($p < .05$) yolk weight, albumen weight, height of yolk, height of albumen,

**Table 3.** Reproductive performance of laying Japanese quail as affected by *Aspergillus japonicas* filtrate.

| Items          | *A. japonicas* filtrate (ml/kg diet) | p Value                  |
|----------------|-------------------------------------|--------------------------|
|                | 0        | 1        | 2        | 3        | 4        | SEM         | Linear | Quadratic |
| Fertility %    |          |          |          |          |          |             |        |           |
| 8–12 week      | 81.35    | 87.25    | 92.68    | 83.20    | 82.01    | 3.705       | .8286  | .0726     |
| 12–16 week     | 78.33    | 88.73    | 93.33    | 86.19    | 81.27    | 2.800       | .7269  | .0031     |
| Hatchability % |          |          |          |          |          |             |        |           |
| 8–12 week      | 79.84    | 87.99    | 93.00    | 84.70    | 81.64    | 2.563       | .9723  | .0050     |
| 12–16 week     | 76.11    | 88.73    | 90.95    | 81.43    | 76.67    | 2.209       | .3986  | .0002     |

Means in the same raw with no superscript letters after them or with a common superscript letter following them are not significantly different ($p < .05$). SEM: standard error mean.
egg length, shape index, and yolk diameter when compared to control (Alrahawi 2019). The enhancement in egg quality indices may be returned to increase the secretion of albumin from magnum so that the albumin weight and egg weight were increased (Alrahawi et al. 2019).

### Blood parameters

#### Liver and kidney functions

As shown in Table 5, apart from plasma albumin, A/G ratio, AST and creatinine, there were significant influences on plasma total protein, globulin, ALT, LDH, and urea with the A. japonicas filtrate ($p < .05$). The quail breeders received A. japonicas filtrate had significantly higher total protein (linear, $p = .0081$) and globulin (linear, $p = .0220$). ALT ($p < .0001$), LDH ($p = .0002$), and urea ($p = .0041$ or .0017) levels were decreased with A. japonicas filtrate suplementations. Liver function data of the present study partially agreed with the results of Ahmad et al. (2018) who found that ALT and AST insignificantly affected by addition of organic acid ($p > .05$; 40 g citric/kg diet). There was no polynomial effect of dietary supplementation of organic acid mixture (5% citric acid, 70% propionic acid, and 25% soft acid) on the liver functions (ALT, AST, total protein, and albumin) of hens ($p > .05$) (Kaya et al. 2014).

Abdel-Fattah et al. (2008) stated that liver enzymes (AST and ALT), and total protein and its fractions were not adversely influenced by citric, acetic, and lactic acids at 1.5% and 3.0% in poultry rations.

### Lipid profile

As shown in Table 6, there were polynomial influences on plasma lipid parameters with the A. japonicas filtrate. The use of A. japonicas filtrate in quail breeder’s diets tended to decline total cholesterol, triglyceride, LDL, and VLDL in plasma. But, there was no significant ($p = .1024$ or .2019) difference due to A. japonicas filtrate regarding HDL-cholesterol. Our results were supported by the data obtained by Kamal and Ragaa (2014) who found that the lowest values of lipid profile indices including total lipids, cholesterol, and LDL-cholesterol were observed by birds received diets enriched with organic acids. Furthermore, inclusion of organic acids in rations of birds lowered serum cholesterol, LDL, and total lipid (Youssef et al. 2017; Naveenkumar et al. 2018). On the same context, dietary inclusion of citric acid in the diets of quail reduced ($p < .001$) cholesterol, LDL-cholesterol, and VLDL-

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**Table 4.** Internal and external egg quality of laying Japanese quail as affected by A. japonicas filtrate.

| Items                  | Aspergillus japonicas filtrate (ml/kg diet) | SEM | Linear | Quadratic |
|------------------------|--------------------------------------------|-----|--------|----------|
|                        | 01234 Linear Quadratic                      |     |        |          |
| Albumin %              | 53.94 54.00 53.75 54.85 54.40              | 0.735 | .5539  | .9675    |
| Yolk %                 | 31.50 33.8 33.30 32.12 31.85                | 0.648 | .4471  | .0025    |
| Shell %                | 15.80 12.25 12.94                           | 0.559 | .6213  | <.0001   |
| Shell thickness        | 0.24 0.19 0.20                              | 0.007 | .1817  | .0120    |
| Egg shape index        | 77.59 79.82 80.68 77.99 76.65               | 1.208 | .3927  | .0544    |
| Yolk index             | 44.76 50.03 50.23                           | 1.490 | .1845  | .1165    |
| Haugh unit             | 80.88 83.18 84.38                           | 0.744 | .6228  | .0061    |
| USSW                   | 46.99 47.53 47.53                           | 0.310 | .0391  | .6638    |

Means in the same raw with no superscript letters after them or with a common superscript letter following them are not significantly different ($p < .05$). SEM: standard error mean; USSW: unit surface shell weight (mg/cm²).

**Table 5.** Liver and kidney functions of laying Japanese quail as affected by A. japonicas filtrate.

| Itemsa               | Aspergillus japonicas filtrate (ml/kg diet) | SEM | Linear | Quadratic |
|----------------------|--------------------------------------------|-----|--------|----------|
|                      | 01234 Linear Quadratic                      |     |        |          |
| TP (g/dL)            | 3.68 3.95 4.39 4.08 3.85                   | 0.085 | .0081  | .1975    |
| ALB (g/dL)           | 2.30 2.33 2.23 2.37 2.32                   | 0.041 | .3081  | .5585    |
| GLOB (g/dL)          | 1.38 1.62 2.16 1.71 1.53                   | 0.114 | .2200  | .4013    |
| A/G (%)              | 1.70 1.45 1.04 1.42 1.65                   | 0.139 | .0892  | .0547    |
| AST (IU/L)           | 239 185 231 183 222                       | 1.664 | .0097  | .0635    |
| ALT (IU/L)           | 20.42 6.70 10.79 10.91 14.87               | 1.259 | .1295  | <.0001   |
| LDH (IU/L)           | 321 242 301 152 210                       | 1.471 | .0002  | .3415    |
| Creatinine (mg/dL)   | 0.59 0.57 0.51 0.47 0.52                   | 0.037 | .0647  | .3174    |
| Urea (mg/dL)         | 2.96 1.48 1.54 1.41 1.74                   | 0.204 | .0041  | .0017    |

Means in the same raw with no superscript letters after them or with a common superscript letter following them are not significantly different ($p < .05$). SEM: standard error mean

aTP: total protein; Alb: albumin; GLOB: globulin; A/G: albumin/globulin ratio; AST: aspartate aminotransferase; ALT: alanine aminotransferase and LDH: lactate dehydrogenase.
cholesterol (Ahmad et al. 2018). Also, Fouladi et al. (2018) found that the groups containing acetic and butyric acids decreased the contents of triglyceride in quail serum during the production phase ($p < .05$).

Immunity
Apart from IgA and IgM levels, inclusion of *A. japonicas* filtrate in diets of quail breeders linearly ($p = .0001$) and quadratically ($p = .0018$) improved the immune parameters (IgG and lysozyme, respectively) (Table 7). These results confirm that organic acids play an important role in improving the immunity (Dibner and Buttin 2002). Also, our results are similar with the data obtained by Chowdhury et al. (2009) who found that supplementation of citric acid at 0.5% in broiler rations enhanced the immunity. Dietary inclusion of organic acids positively affects the immune responses (Dibner and Buttin 2002; Abdel-Fattah et al. 2008). In line, there was a linear increase in the content of IgG of broiler due to the use of organic acid mixture (Nguyen et al. 2018). The enhancement in immunological indices can be supported by the results of Yang et al. (2018) who observed an improvement in the spleen size in birds that received 0.30 g/kg of organic acids plus thymol. On the other hand, the level of IgA of ileal mucosa was higher in the diets of organic acids (Liu et al. 2017).

Antioxidant parameters
In Table 7, there were polynomial differences among the treatments regarding the antioxidant indices ($p < .05$) except the level of reduced glutathione ($p = .2259$ or .3128). The activity of SOD ($p = .0128$ or .0102), CAT ($p = .0152$) and TAC ($p < .0001$) was higher in *A. japonicas* filtrate groups than the control. Plasma MDA was linearly decreased ($p = .0027$) in the quail fed *A. japonicas* filtrate when compared to the un-supplemented group. Our results regarding the antioxidant indices agreed with the outputs of Ahmad et al. (2018) who found that the antioxidant enzyme of GPX was higher in acidified group. Contrarily, no significant alterations were observed in TAC for birds fed organic acid rations than control (Abudabos et al. 2017).

Conclusions
From these results, it could be proposed that supplements with *A. japonicas* filtrate (oxalic acid, citric acid, lactic acid, ascorbic acid, maleic acid, formic acid, and salicylic acid) could improve productive and

### Table 6. Lipid profile of laying Japanese quail as affected by *A. japonicas* filtrate.

| Items | 0 | 1 | 2 | 3 | 4 | SEM | Linear | Quadratic |
|-------|---|---|---|---|---|-----|--------|----------|
| TC (mg/dL) | 400 | 244 | 284 | 285 | 267 | 11.95 | .0001 | .0004 |
| TG (mg/dL) | 1258 | 917 | 932 | 820 | 924 | 32.57 | <.0001 | <.0001 |
| HDL (mg/dL) | 13.68 | 23.80 | 26.99 | 19.20 | 23.35 | 2.646 | .0111 | .0008 |
| LDL (mg/dL) | 135.21 | 37.54 | 70.90 | 108.90 | 59.13 | 5.557 | .0001 | .0001 |
| VLDL (mg/dL) | 251 | 183 | 186 | 164 | 184 | 6.515 | <.0001 | <.0001 |

Means in the same raw with no superscript letters after them or with a common superscript letter following them are not significantly different ($p < .05$). SEM: standard error mean.

### Table 7. Antioxidant and immunological indices of laying Japanese quail as affected by *Aspergillus japonicas* filtrate.

| Items | 0 | 1 | 2 | 3 | 4 | SEM | Linear | Quadratic |
|-------|---|---|---|---|---|-----|--------|----------|
| IgM (mg/dL) | 0.32 | 0.52 | 0.35 | 0.33 | 0.49 | 0.030 | .1326 | .6091 |
| IgG (mg/dL) | 0.42 | 0.52 | 0.55 | 0.77 | 0.65 | 0.034 | .0001 | .0778 |
| IgA (mg/dL) | 0.32 | 0.32 | 0.38 | 0.40 | 0.32 | 0.047 | .6500 | .2986 |
| Lysozyme (mg/dL) | 0.11 | 0.22 | 0.20 | 0.21 | 0.14 | 0.019 | .4438 | .0018 |
| SOD (U/mL) | 0.10 | 0.23 | 0.20 | 0.18 | 0.21 | 0.016 | .0128 | .0102 |
| MDA (nmol/mL) | 0.08 | 0.29 | 0.23 | 0.42 | 0.23 | 0.027 | .0027 | .0512 |
| TAC | 0.10 | 0.26 | 0.24 | 0.18 | 0.19 | 0.013 | .0526 | <.0001 |
| CAT (ng/mL) | 0.10 | 0.14 | 0.19 | 0.18 | 0.21 | 0.027 | .0152 | .4747 |
| GSH (ng/mL) | 0.13 | 0.14 | 0.22 | 0.16 | 0.18 | 0.031 | .2259 | .3128 |

Means in the same raw with no superscript letters after them or with a common superscript letter following them are not significantly different ($p < .05$). SEM: standard error mean.

| IgG, IgM and IgA: immunoglobulin G, M and A; SOD: superoxide dismutase; MDA: malondialdehyde; TAC: total antioxidant capacity; CAT: catalase; GSH: reduced glutathione. |
reproductive performance, lipid profile, immunity, and antioxidant indices of quail breeders.

Ethical approval

Animal care and maintenance were performed in accordance with the guidelines of the Egyptian Research Ethics Committee and the guidelines specified in the Guide for the Care and Use of Laboratory Animals (2011).

Disclosure statement

No potential conflict of interest was reported by the author(s).

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