Effect of *Aspergillus japonicas* culture filtrate on performance, carcase yield, digestive enzymes, intestinal microbiota and blood constituents of quail

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**ABSTRACT**

This study was carried out to examine the beneficial role of organic acids and other beneficial compounds produced by *Aspergillus japonicas* and their effects on performance, carcase yield, digestive enzymes, intestinal microbiota and blood constituents of quail. 240 quails (7 days old) were allotted to 5 groups consisting of 48 quail chicks (4 replicates of 12 chicks). The first, second, third, fourth and fifth groups were fed basal diet with 0 (control), 1, 2, 3, and 4 mL *A. japonicas* culture filtrate/kg diet, respectively. The addition of *A. japonicas* culture filtrate up to 3 mL/kg feed increased body weight at 3 and 5 weeks-old and body weight gain from 1-3, 3-5 and 1-5 weeks, the best level was 3 mL/kg feed. Quails fed *A. japonicas* filtrate diets recorded the best feed conversion ratio in comparison with the control. Dietary addition of *A. japonicas* filtrate tended to decline triglyceride, LDL, and VLDL and increased HDL values in the plasma. Use of *A. japonicas* filtrate in diets up to 2 mL/kg improved the immune indices. The levels of SOD and TAC, GSH and CAT as well as digestive enzymes were improved due to *A. japonicas* filtrate supplementation compared to control. Plasma levels of MDA were decreased (*p* = .0001) in the quails fed *Aspergillus* filtrate (1 or 2 mL/kg). Quails fed rations enriched with filtrate exhibited lower colonisation of *Escherichia coli*, coliform and Salmonella. In conclusion, supplemental *A. japonicas* culture filtrate could improve the performance, digestive enzymes, immunity, antioxidant indices and mitigate intestinal pathogens.

**HIGHLIGHTS**

- Use of *Aspergillus* filtrate improved in quail diets performance and digestive enzymes.
- Use of *Aspergillus* filtrate in quail diets improved immunity and antioxidant indices.
- Dietary addition of *Aspergillus* filtrate mitigated intestinal pathogens.

**Introduction**

Feed additives and supplements have been effectively used in animal and poultry for improving the public health, performance and wellbeing (Alagawany et al. 2019; Elwan et al. 2019; Elnesr et al. 2020; Hazrati et al. 2020). Recently, in several countries, there have been increasing desires for organic products/foods, may be returned to their efficacy to decrease the disorders of several diseases and improve the public health of the consumer (Alagawany et al. 2018; Khaleghipour et al. 2019; Reda et al. 2020a). Dietary acids are divided into organic and inorganic acids, but organic acids are widely used in poultry rations (Salah et al. 2019). The poultry industry commonly uses the short-chain organic acids like acetic, formic, propionic, and butyric acids, in addition to other organic acids like malic, lactic, fumaric, citric acids and tartaric (Dibner and Buttin 2002). Organic acids gained considerable attention as effective alternatives to growth promoter antibiotics owing to its impact on pathogens; it lowers the pH in the gut, thus enhancing the absorption of nutrients in poultry (Kil et al. 2011). Subsequently, organic acids improve the growth rate, feed efficiency, and resistance against several diseases (Ricke 2003; Islam 2012). Furthermore, in broilers, formic acid was supplemented in the feed and water with the aim of improving performance indices and intestinal microbiota (Ruhnke et al. 2015; Gowda and Shivakumar 2019). Also, Chowdhury et al. (2009) found that an improvement in the immunological parameters of broilers fed 0.5% citric acid; Abdel-Fattah et al. (2008) observed a similar improve in the immune function of chickens due to organic acids supplementation. It is hypothesised that the use of these acids in
the diets is expected to have beneficial impacts on Japanese quail. Therefore, the present study was planned in order 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 salisylic acid) on the growth, feed utilisation, carcasses, blood indices, caecal microbiota, antioxidant, digestive enzyme and immunity of the broiler quails.

**Materials and methods**

This experiment was designed at Poultry Department and conducted at Poultry Research Farm, Faculty of Agriculture, Zagazig University, Egypt. All procedures were carried out according to the EACC (Local Experimental Animal Care Committee).

**Quails, design and diets**

A total of 240 Japanese quails chicks (7 days old, and average weight 29.95 ± 0.10 g) were haphazardly allotted to 5 experimental groups, with 4 replications of 12 birds.

The duration of this study was 4 weeks (1–5 weeks). The treatments were: 1) Basal diet without any additives (control), 2) Basal diet + 1 mL *A. japonicas* culture filtrate/kg diet; 3) Basal diet + 2 mL *A. japonicas* culture filtrate/kg diet; 4) Basal diet + 3 mL *A. japonicas* culture filtrate/kg diet; 5) Basal diet + 4 mL *A. japonicas* culture filtrate/kg diet. The basal ration (Table 1) was formulated to meet quail requirements (NRC 1994). Quails were reared in conventional type cage (50 × 30 × 50 cm3; 1,500 cm2 of floor space). Feed and water were provided *ad libitum*. The lighting program was 23 h light with 1 hour dark period through the first 3 days, after this period, quails were subjected to 16 h light with 8 h dark until 5 weekss of age.

*Aspergillus japonicas* (Accession number: MN960315). Culture filtrate contained oxalic acid, citric acid, lactic acid, ascorbic acid, maleic acid, formic acid, and salisylic acid by Gas chromatography–mass spectrometry (GCMASS). *Aspergillus japonicas* culture filtrate was obtained from Microbiology Department, Faculty of agriculture, Zagazig University, Zagazig, Egypt.

**Growth and carcase measurements**

At 1, 3 and 5 week of age, quail weights were recorded to compute the body weight and gain. Values of feed intake and conversion were calculated during the experiment. At weeks 5, five birds per treatment were haphazardly taken and manually slaughtered for carcase examination (Reda et al. 2020b).

**Microbiological analysis**

At weeks 5, after slaughter, we collected the caecal fresh contents and subjected to a stream of CO2 in bottles and transported to the lab. The number of total bacteria was determined via plate count agar at 30°C for two days. Coliform bacteria were incubated at 37°C for one day on Violet Red Bile Agar. Salmonella and *E. coli* were determined on Eosin Methylene Blue agar plates and on XLD agar plates after incubation at 37°C for one day, respectively. Also, lactobacilli number was determined according to Xia et al. (2004).

**Blood parameters**

Samples of blood were collected from the slaughtered quails into heparinised tubes and centrifuged (G force rate = 2 146.56 × g) for fifteen minutes, and plasma were kept at −20°C until making analysis. We used the centrifuge (Janetzki, T32c, Wall-hausen, Germany) to separate the plasma. All blood biochemical parameters were determined as lipid profile (TC: total cholesterol, TG: triglycerides, HDL: high-density lipoprotein, LDL: low-density lipoprotein and VLDL: very low

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

| Ingredient                  | g/kg as-fed basis |
|-----------------------------|-------------------|
| Maize 8.5%                  | 518.0             |
| Soybean meal 44%            | 367.0             |
| Maize gluten meal 62%       | 52.1              |
| Soybean oil                 | 29.0              |
| Limestone                   | 7.0               |
| Di-calcium phosphate        | 16.5              |
| Salt                        | 3.0               |
| Premix<sup>a</sup>          | 3.0               |
| L-Lysine                    | 1.3               |
| Di-Methionine               | 1.1               |
| Choline chloride            | 2.0               |
| Total                       | 1000              |

<sup>a</sup>Provides per kg of diet: Vitamin A, 12,000 IU; Vitamin D3, 5000 IU; 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.

<sup>b</sup>According to NRC (1994).
density lipoprotein), liver and kidney (TP: total protein; ALB: albumin, GLOB: globulin, A/G: albumin/globulin ratio, AST: aspartate aminotransferase, ALT: alanine aminotransferase, LDH: lactate dehydrogenase, urea and creatinine), functions, and immunity parameters (IgG, IgM and IgA: immunoglobulin G, M and A) by kits from Spectrum Company (Cairo, Egypt). Also, the antioxidant parameters (SOD: superoxide dismutase, MDA: malondialdehyde, TAC: total antioxidant capacity, CAT: catalase and GSH: reduced glutathione) of plasma were measured using a spectrophotometer (Shimadzu, Japan).

Digestive enzymes assay

The activities of α-amylase, lipase and protease were measured in the ileal content of quails at 5 weeks-old (5 quails/group). The ileum from Meckel’s diverticulum to 2 cm above the ileocecal junction was dissected, and the ileal content was collected in screw-capped sterile specimen vials for analysis. Ileal content (1 g) was put on ice, and fifteen mL of physiological saline (9 g of sodium chloride/L) was added and centrifuged at 2000 g for ten minute at 4°C (Najafi et al. 2005, 2006).

Statistics

All of the statistical methods were carried out using the SAS (SAS 2001). All data (performance, carcase, blood chemistry, immunity, antioxidant, digestive enzyme and caecal microbes) were analysed with one-way ANOVA and the statistical model is as follows:

\[ Y_{ij} = \mu + t_i + e_{ij} \]

where \( Y_{ij} \) is an observation, \( \mu \) is the overall mean, \( t_i \) is the treatment effect, and \( e_{ij} \) is experimental random error. Tukey’s test was used for comparison among significant means \( (p < .05) \).

Results and discussion

Growth parameters

Based on the results in Table 2, apart from feed intake during 3-5 weeks, supplemental A. japonicas filtrate in the quail diet led to a significant difference in growth parameters. The addition of this filtrate up to 3 mL/kg feed increased \( (p < .05) \) body weight at 3 and 5 weeks-old and body weight gain from 1–3, 3–5 and 1–5 weeks when compared to the control, the best level was 3 mL/kg feed, which is in agreement with García et al. (2007) who found that the inclusion of up to 10,000 ppm of organic acid enhanced the growth rate of chickens. The use of the acetic acid (30 g/kg diet) in broilers improved rate (Rehman et al. 2016). The substantial role of organic acids in increasing the growth parameters may be due to improve digestibility of dry matter and other nutrients the ileum (Hernández et al. 2006; Pearlin et al. 2020).

Birds fed 2, 3 and 4 mL A. japonicas filtrate-treated rations consumed less feed than the control or 0.1 mL filtrate during 1-3 and 1-5 weeks-old \( (p < .05) \). Quails that received 0 or 1 mL A. japonicas filtrate diets consumed more feed than those received the other levels. Low feed consumption can be attributed to the strong taste of these acids which would have reduced the palatability of the rations, consequently, reducing feed consumption (García et al. 2007). During 1-3, 3-5 and 1-5 weeks of age, the quails fed organic acid diets recorded the best feed conversion ratio. The 3 mL organic acid-treated birds were the best FCR; the

Table 2. Growth performance of growing Japanese quail as affected by dietary treatments.

| Item                        | Aspergillus japonicas culture filtrate (mL/kg diet) | SEM | p Value |
|-----------------------------|---------------------------------------------------|-----|---------|
|                             | 0        | 1       | 2       | 3        | 4       |
| Body weight (g)             |          |         |         |          |         |
| 1 weeks                     | 29.93    | 30.02   | 29.88   | 29.90    | 30.02   | 0.097  | 0.8072 |
| 3 weeks                     | 97.13    | 108.56a | 106.85a | 108.35a  | 96.10b  | 1.296  | <.0001 |
| 5 weeks                     | 186.50b  | 200.25b | 197.68b | 206.11a  | 190.72c | 1.652  | <.0001 |
| Body weight gain (g/day)    |          |         |         |          |         |
| 1–3 weeks                   | 4.80b    | 5.61a   | 5.50a   | 5.60a    | 4.72b   | 0.088  | <.0001 |
| 3–5 weeks                   | 6.39b    | 6.55b   | 6.49b   | 6.98a    | 6.76ab  | 0.101  | 0.0248 |
| 1–5 weeks                   | 5.59b    | 6.08b   | 5.99b   | 6.29a    | 5.74c   | 0.059  | <.0001 |
| Feed intake (g/day)         |          |         |         |          |         |
| 1–3 weeks                   | 15.07ab  | 15.50a  | 12.94c  | 13.66bc  | 13.67bc | 0.421  | 0.0278 |
| 3–5 weeks                   | 21.56    | 20.50b  | 20.37   | 19.87    | 20.63   | 0.458  | 0.2828 |
| 1–5 weeks                   | 18.31b   | 18.00ab | 16.66c  | 16.77c   | 17.15bc | 0.317  | 0.0224 |
| Feed conversion ratio (g feed/g gain) |          |         |         |          |         |
| 1–3 weeks                   | 3.14b    | 2.76b   | 2.36c   | 2.44c    | 2.90ab  | 0.092  | 0.0016 |
| 3–5 weeks                   | 3.37b    | 3.13b   | 3.14b   | 2.85c    | 3.05bc  | 0.060  | 0.0030 |
| 1–5 weeks                   | 3.27b    | 2.96bc  | 2.78cd  | 2.67d    | 2.99b   | 0.057  | 0.0003 |

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.
improvement in the feed utilisation in the present study could be possibly attributed to better nutrient absorption and utilisation resulting in improved weight gain in the chickens fed diet supplemented with organic acids such as butyric, lactic and fumaric acids (Adil et al. 2011).

Consistent with our results, in broiler chickens, Paul et al. (2007) reported a decrease in the feed consumption of chicks fed 3 g/kg of organic acid (ammonium salt of formic acid); however, this addition improved the growth parameters. These improvements can be supported by the outputs of Abdel-Fattah et al. (2008) who found that growth parameters were improved with the addition of 1.5 and 3% acetic or citric acids in the diet, respectively. According to Chamba et al. (2014) greater weight gain and feed conversion ratio were achieved in the growth and finishing stage of chickens fed 700 ppm of organic acids. The improvements in performance parameters may be due to the organic acid addition, because they increase ability of the intestinal wall to absorb nutrients by enhancing the structure and function of the villi and the digestive enzymes and secretions that lead to improved absorption of nutrients including proteins, carbohydrates, and minerals (Nair and Kollanoor 2019).

**Carcass measurements**

No significant impact of dietary organic acid and other compounds produced by A. japonicas on the carcase, liver, gizzard, heart, giblets and dressing (p > .05; Table 3). Our results are confirmed by those obtained by Brzóska et al. (2013) who found that the weights of liver, gizzard, carcase, abdominal fat, breast muscles and leg were not influenced by acidifier at 3, 6 and 9 g/kg ration. On the same line, carcase traits (heart, breast, gizzard, thigh and liver) of broilers were not influenced by Nufocidil (1 mL/L drinking water) as an organic acid supplement (Heidari et al. 2018). On the same line, the edible parts of broilers were not influenced by lactic acid supplementation. Organic acid effect is confirmed by the data of some studies which stated that the organic acid did not affect carcase yield and dressing parameters of broilers (Kopecký et al. 2012; Ghasemi et al. 2014; Pearlin et al. 2020).

**Blood parameters**

As shown in Table 4, there were no significant influences on plasma TP, ALB, GLOB, and creatinine with the A. japonicas filtrate (p > .05). But, there were statistical differences in other parameters of liver and kidney (A/G ratio, AST, ALT, LDH and urea). The quails received A. japonicas culture filtrate (0.1–0.4 mL) had significantly lower AST (p < .0001), ALT (p = .0011), and LDH (p = .0038). Apart from 0.3 mL A. japonicas filtrate, A/G ratio was decreased with A. japonicas filtrate supplemen-tations (p = .0014). In comparison with control, plasma urea level was significantly decreased (p = .0109) in quails fed diets containing A. japonicas filtrate (2 mL/kg). Ahmad et al. (2018) found that liver enzymes activity (ALT and AST) insignificantly (p > .05) affected by organic acid supplementation (40 g citric/kg diet).

As shown in Table 5, dietary addition of A. japonicas filtrate tended to decline plasma triglyceride (p = .0123), LDL (p = .0040), and VLDL (p = .0123) and increased HDL (p = .0070). However, there was no significant (p > .1011) difference among treatments regarding the total cholesterol level of the plasma. The lowest concentrations of serum total lipids, cholesterol and LDL were achieved for birds received organic acids when compared to control (Kamal and Ragaa 2014) confirmed the current results of plasma lipid indices. Moreover, use of organic acids in broiler diets reduced serum cholesterol, LDL and total lipid (Youssef et al. 2017; Naveenkumar et al. 2018). When compared to untreated group, dietary addition of organic acid (citric acid) in quail diets reduced cholesterol, LDL and VLDL (p < .001) (Ahmad et al. 2018). Abdel-Fattah et al. (2008) stated that the noted lower feed intake throughout the growth period and

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**Table 3.** Carcase traits and relative organs of growing Japanese quail as affected by dietary treatments.

| Item          | 0      | 1      | 2      | 3      | 4      | SEM   | p Value |
|---------------|--------|--------|--------|--------|--------|-------|---------|
| Carcase (%)   | 67.37  | 73.07  | 70.36  | 71.11  | 72.25  | 3.169 | 0.7840  |
| Liver (%)     | 3.25   | 3.28   | 2.97   | 3.70   | 3.43   | 0.421 | 0.8463  |
| Gizzard (%)   | 2.28   | 2.46   | 2.86   | 2.71   | 2.48   | 0.374 | 0.8948  |
| Heart (%)     | 1.46   | 0.93   | 0.80   | 1.03   | 1.05   | 0.186 | 0.4498  |
| Giblets (%)   | 7.00   | 6.68   | 6.63   | 7.43   | 6.96   | 0.288 | 0.4125  |
| Dressing (%)  | 74.37  | 79.74  | 76.99  | 78.54  | 79.22  | 3.105 | 0.7927  |

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.
consequently lower the intake of fat that resulted in depletion of fat may also help in lowering lipid content in blood.

Use of *Aspergillus japonicas* filtrate in quail diets up to 2 mL/kg improved the immune indices (IgG, IgM, IgA and lysozyme) compared to the control and the other levels (3 or 4 mL/kg) (Table 5). These results are similar with the outputs obtained by Chowdhury et al. (2009) who stated that use of 0.5% citric acid in broiler diets improved the immunological status. According to Dibner and Buttin (2002) and Pearlin et al. (2020) the inclusion of organic acids in the poultry diet positively affects the immunological indices of poultry. Abdel-Fattah et al. (2008) showed a similar improvement in the immune functions of broiler chickens due to organic acids. In the same line, there was linearly improvement in IgG content associated with the use of the blend of organic acids in the broiler rations (Nguyen et al. 2018). The improvements in immunity parameters can be illustrated by the findings of Yang et al. (2018) who found an increase in the size of spleen in birds that received 0.30 g/kg of organic acids and thymol essential oil through the grower or the finisher phases. Furthermore, concentration of IgA of ileal mucosa was higher in the organic acids (0.30 g/kg diet) treatment (Liu et al. 2017).

In Table 5, there were significant differences due to *Aspergillus japonicas* filtrate regarding the antioxidant indices ($p < .05$). The SOD and TAC of *Aspergillus japonicas* filtrate (1, 2 and 3 mL/kg diet) supplement groups were higher ($p < .0001$ and 0.0030, respectively) than the control and 4 mL/kg diet. The GSH ($p = .0004$) and CAT ($p = .0095$) activities were improved in the quails fed diets enriched with 0.1 or 0.2 mL/kg of *Aspergillus japonicas* filtrate when compared to the other groups. Plasma levels of MDA were decreased ($p = .0001$) in the animals fed *A. japonicas* filtrate (1 or 2 mL/kg). Antioxidant indices data of the present study agreed with the

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**Table 4.** Liver and kidney function of growing Japanese quail as affected by dietary treatments.

| Itema | 0    | 1    | 2    | 3    | 4    | SEM  | $p$ value |
|-------|------|------|------|------|------|------|-----------|
| TP (g/dL) | 2.98 | 3.10 | 3.22 | 2.76 | 2.68 | 0.113 | 0.0665    |
| ALB (g/dL) | 1.42 | 1.39 | 1.41 | 1.31 | 1.21 | 0.061 | 0.2255    |
| GLOB (g/dL) | 1.56 | 1.71 | 1.81 | 1.45 | 1.47 | 0.076 | 0.0987    |
| A/G (%) | 0.91a | 0.81b | 0.77b | 0.90a | 0.82b | 0.015 | 0.0014    |
| AST (IU/L) | 177.61a | 137.70b | 157.15b | 103.89d | 109.15b | 3.938 | <.0001    |
| ALT (IU/L) | 10.97a | 6.99b | 8.47b | 9.63b | 9.21b | 0.422 | 0.0011    |
| LDH (IU/L) | 200.50a | 198.30b | 189.50b | 165.00c | 131.50c | 10.069 | 0.0038    |
| Creatinine (mg/dL) | 0.29 | 0.32 | 0.23 | 0.32 | 0.29 | 0.018 | 0.0069    |
| Urea (mg/dL) | 7.29a | 7.18a | 7.59b | 6.62a | 6.74b | 0.229 | 0.0109    |

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; LDH: lactate dehydrogenase.

**Table 5.** Lipid profile, antioxidant and immunological indices of growing Japanese quail as affected by dietary treatments.

| Item           | Aspergillus japonicas culture filtrate (mL/kg diet) | 0      | 1      | 2      | 3      | 4      | SEM  | $p$ value |
|----------------|----------------------------------------------------|--------|--------|--------|--------|--------|------|-----------|
| Lipid profile  |                                                    |        |        |        |        |        |      |           |
| TC (mg/dL)     | 205.07                                            | 187.75 | 194.25 | 198.24 | 200.50 | 3.731 | 0.1011 |
| TG (mg/dL)     | 254.39                                            | 211.50b| 188.35a| 215.00c| 231.00b| 8.822 | 0.0123 |
| HDL (mg/dL)    | 47.96b                                            | 57.25a | 60.19b | 62.32a | 57.55b | 1.698 | 0.0070 |
| LDL (mg/dL)    | 106.24a                                           | 88.20c | 96.39b | 92.93a | 96.75b | 2.242 | 0.0040 |
| VLDL (mg/dL)   | 50.87a                                            | 42.30bc| 37.67a | 43.00bc| 46.20bc| 1.764 | 0.0123 |
| Immunity and antioxidants |                                                        |        |        |        |        |        |      |           |
| IgM (mg/dL)    | 0.48b                                             | 1.05a  | 0.99a  | 0.51b  | 0.49b  | 0.051 | 0.0001 |
| IgG (mg/dL)    | 0.81a                                             | 1.26c  | 1.02c  | 0.90c  | 0.83c  | 0.042 | 0.0022 |
| IgA (mg/dL)    | 0.59c                                             | 1.13b  | 0.86b  | 0.55c  | 0.55c  | 0.056 | 0.0003 |
| Lysozyme (IU/mL) | 0.11b                              | 0.21a  | 0.20c  | 0.15c  | 0.11b  | 0.012 | 0.0012 |
| SOD (IU/ml)    | 0.11b                                             | 0.24a  | 0.23a  | 0.20a  | 0.12b  | 0.010 | <.0001 |
| MDA (nmol/mL)  | 0.40b                                             | 0.24b  | 0.26b  | 0.37a  | 0.38a  | 0.014 | 0.0001 |
| TAC (ng/mL)    | 0.10b                                             | 0.20b  | 0.21a  | 0.22a  | 0.11b  | 0.018 | 0.0030 |
| CAT (ng/ mL)   | 0.11b                                             | 0.23b  | 0.23b  | 0.13b  | 0.15b  | 0.021 | 0.0095 |
| GSH (ng/ mL)   | 0.12b                                             | 0.25a  | 0.24a  | 0.14b  | 0.12c  | 0.014 | 0.0004 |

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.

TC: total cholesterol; TG: triglycerides; HDL: high-density lipoprotein; LDL: low-density lipoprotein; VLDL: very low density lipoprotein; IgG, IgM and IgA: immunoglobulin G, M, and A; SOD: superoxide dismutase; MDA: malondialdehyde; TAC: total antioxidant capacity; CAT: catalase; GSH: reduced glutathione.
results of Ahmad et al. (2018) who demonstrated that GPX activity was higher in organic acid group \( (p < .05) \). On the contrary, no significant change was observed in TAC for chickens fed acidified rations than control at slaughter (Abudabos et al. 2017).

**Digestive enzyme**

Use of *A. japonicas* filtrate in growing quail diets significantly affects digestive enzymes (Table 6). The inclusion of this filtrate in quail diets up to 3 mL/kg improved digestive enzymes activities including protease \( (p < .0001) \), amylase \( (p = .0017) \) and lipase \( (p = .0028) \) in comparison with the control and the other treatment levels. The improvements in digestive enzymes can be supported by the findings of Dittoe et al. (2018) who stated organic acids can improve the digestive physiology of poultry, the nutrients digestibility and the proteolytic enzyme activity; enhance the pancreatic secretions; and balance the intestinal microbiota of poultry. Furthermore, activities of endogenous digestive enzyme in intestinal tract were higher in the organic acids at 0.30 g/kg (Liu et al. 2017). Also, use of fumaric acid, sorbic acid and thymol mixture can be effectively used in broiler chicken, Nair and Kollanoor (2019) found that organic acids decrease the number of Salmonella and bacteria of the family Enterobacteriaceae. Furthermore, several other studies showed that propionic and formic acids have a microbial effect against *Coliforms*, *Salmonella* spp. and *E. coli* in the gastrointestinal tract of bird (Ruhnke et al. 2015; Gowda and Shivakumar 2019). Regassa and Nyachoti (2018) stated that dietary addition of butyric acid can reduce the infection of *Salmonella Enteritidis* in the gut of birds. Nair and Kollanoor (2019) also reported that organic acids in the rations decrease the vertical transmission of pathogens such as *Salmonella* in broilers. Chickens fed organic acid diets showed a linear decrease in the count of *E. coli* and increase in the count of Lactobacillus (Nguyen et al. 2018).

**Caecal microbiota**

In comparison with the control group, birds fed rations enriched with *A. japonicas* filtrate exhibited lower colonisation of *E. coli* \( (p < .0001) \), coliform \( (p = .0002) \) and Salmonella \( (p = .0099) \) (Table 6). Regarding the total bacterial count and lactobacillus, the trend is not clear, because the highest values were recorded with 3 mL *A. japonicas* filtrate, but the 4 mL recorded the lowest total bacterial count and lactobacillus. Feed additives including organic acids have been added effectively in poultry feed to lower the existence of pathogenic bacteria, especially *Salmonella* spp. and mycotoxins produced by fungi; organic acids have the ability to lower pathogens in the intestines of poultry (Dittoe et al. 2018; Al-Hashedi et al. 2019; Ayoub et al. 2019; Reda et al. 2020b). The results of the present study were in agreement with Attia et al. (2018) who stated that organic acids inhibit the activity of pathogenic bacteria and improve the beneficial bacteria in the gut. Further, in broiler chicken, Nair and Kollanoor (2019) found that organic acids decrease the number of Salmonella and bacteria of the family Enterobacteriaceae. Furthermore, several other studies showed that propionic and formic acids have a microbial effect against *Coliforms*, *Salmonella* spp. and *E. coli* in the gastrointestinal tract of bird (Ruhnke et al. 2015; Gowda and Shivakumar 2019). Regassa and Nyachoti (2018) stated that dietary addition of butyric acid can reduce the infection of *Salmonella Enteritidis* in the gut of birds. Nair and Kollanoor (2019) also reported that organic acids in the rations decrease the vertical transmission of pathogens such as *Salmonella* in broilers. Chickens fed organic acid diets showed a linear decrease in the count of *E. coli* and increase in the count of Lactobacillus (Nguyen et al. 2018).

**Conclusions**

From these results, it could be proposed that supplements with *A. japonicas* culture filtrate (oxalic acid, citric acid, lactic acid, ascorbic acid, maleic acid, formic acid, and salsylac acid) could be used in the near future to improve the growth and health status of birds. Also, supplemental *A. japonicas* culture filtrate up to 3 mL/kg diet could improve the growth, digestive enzymes, immunity, antioxidant indices and mitigate intestinal pathogens.

### Table 6. Digestive enzymes and Caecal bacterial count of growing Japanese quail as affected by dietary treatments.

| Item                                      | Aspergillus japonicas culture filtrate (mL/kg diet) | SEM | p Value |
|-------------------------------------------|--------------------------------------------------|-----|---------|
| Digestive enzymes (unit)                  |                                                  |     |         |
| Protease                                  | 0.27c                                            | 0.055| <.0001  |
| Amylase                                   | 4.63d                                            | 1.495| 0.0017  |
| Lipase                                    | 1.84b                                            | 0.923| 0.0028  |
| Caecal bacterial count (Log CFU/g)        |                                                  |     |         |
| Total bacterial count                     | 9.08b                                            | 0.010| <.0001  |
| Lactobacillus                             | 7.16d                                            | 0.029| <.0001  |
| Coliform                                  | 4.76c                                            | 0.086| 0.0002  |
| *E. coli*                                 | 4.42a                                            | 0.050| <.0001  |
| Salmonella                                | 3.46a                                            | 0.117| 0.0099  |

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.
Animal welfare statement
The authors confirm that they have followed EU standards for the protection of animals used for scientific purposes.

Ethical Approval
This experiment was designed at Poultry Department and conducted at Poultry Research Farm, Faculty of Agriculture, Zagazig University, Egypt. All procedures were carried out according to the EACC (Local Experimental Animal Care Committee).

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

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