Impact of dietary supplemental bile salts on growth performance, carcass, immunity and antioxidant parameters and bacteriology of broiler chicks

Salah H. Mohamed, Adel I. Attia, Fayiz M. Reda and Ismail E. Ismail

Department of Poultry, Faculty of Agriculture, Zagazig University, Zagazig, Egypt

ABSTRACT
The aim of the present study was to evaluate the effect of bile salts on the growth performance, carcass estimates, immunity and antioxidant measurements, some blood biochemical parameters, intestine enzyme activities and microbiology of broiler chickens. One hundred twenty 1 day old of Ross 308 broiler chicks were randomly assigned to 4 treatments with 5 replicates of 6 chicks each for 42 days. The first group was the basal diet only and served as control. The second, third and fourth groups were fed the basal diet plus 0.5, 1 and 1.5 ml bile salts/kg diet, respectively. Body weight and daily body weight gain were linearly \( p < .01 \) increased with increasing bile salt levels compared to control. Addition of bile salts to broiler diets significantly \( p < .01 \) decreased feed intake and improved feed conversion than control. The addition of 1.0 ml bile salts to broiler chicks’ diets increased plasma proteins concentrations, total antioxidant capacity and superoxide dismutase compared with the control group. The cholesterol and triglyceride levels, liver enzymes and intestinal enzymes (amylase, protease and lipase) activities were significantly \( p < .05 \) or \( .01 \) increased in bile salt groups. While, malondialdehyde was significantly \( p < .01 \) decreased in all treated groups compared to control. Total bacteria, total fungi, \Escherichia coli\, and \Salmonella\ were decreased in broiler chicks fed diet supplemented with bile salts. It could be concluded that bile salts especially levels (0.5 and 1.0 ml/kg diet) have a positive impact on growth performance and carcass traits, blood biochemical measurements, intestinal enzymes activities, digestibility of nutrients and microorganism’s content of broiler chicks.

HIGHLIGHTS
- Addition bile salt to broiler diets improved growth rate and feed conversion.
- Addition bile salt to broiler diets improved nutrients digestion.
- The addition of 1.0 ml bile salts in broiler diets increased total antioxidant capacity and superoxide dismutase.
- Total fungi, \Escherichia coli\ and \Salmonella\ were decreased in chicks fed diet supplemented with bile salts.

Introduction
The diversity of oils and fats must be taken into account when evaluating their nutritive value (Mohamed et al. 2019; Alagawany, Abdel-Latif et al. 2020; Alagawany, El-Hindawy et al. 2020; Reda, El-Kholy et al. 2020; Reda, Alagawany et al. 2020). Quantity and quality of dietary fats play an important role in the formation of micelle in the intestine. The secretion of gall bladder acts as a natural emulsification (Elnesr et al. 2020). The low digestibility of fat in young broiler chicks may be due to the reduced amount of bile salts or the process of recycling inactive bile salts (Leeson and Summers 2005).

Whereas, secretion of bile salts with small amount in young chicks is considered, along with a decrease in the activity of pancreatic lipase during the first 3 weeks of age. Therefore, several efforts have been made to enhance digestion of fat in poultry. Bile salts as an exogenous source play an important role in improving digestion and absorption of fatty acids.

Bile acids are composed of cholesterol and complemented with glycine or taurine during the liver pericentral hepatocytes, concentrated and stored in the gallbladder, and secreted into the duodenum where, playing an important role in improving the digestion of fat thorough bile salts dissolvable fats and due to absorption increasing (Abudabos 2014). In poultry...
nutrition, the worth of the several fat origins builds on adipose acids tenor, power contents, digestible coefficient and assimilation. There are many factors that affect the digestion of fats such as dietary fat sources and level of bile secretion (Reshetnyak 2013). Azman et al. (2005) reported that broiler performance was improved with improving dietary metabolisable energy (ME) when nutrition containing mishmash of palmitic and oleic acids supplemented with cholic acid. Viscosity plays an important role in hypothesis reabsorption of bile salts with no mediation the activity of microflora (Azman et al. 2005). The previous studies on bile salts and their effects on growth, antioxidant and immune measurements, digestive enzymes microbiological aspects in the caeca of broiler chickens are scarce. Thus, the present study was designed to investigate the effect of bile salts at levels of 0.5, 1.0 and 1.5 mL/kg diet, on performance, carcass traits, blood parameters, antioxidant and immune measurements, digestive enzymes, digestibility of nutrients and caecum microbiology of broiler chickens.

Material and methods

Design and diets

One hundred twenty 1 day old of Ross 308 broiler chicks were randomly assigned to 4 treatments with 5 replicates of 6 chicks each for 42 days. The first group was the basal diet only and served as control. The second, third and fourth groups were fed the basal diet plus 0.5, 1 and 1.5 mL bile salts/kg diet, respectively. The basal diet or the other diets contained the requirements suggested by the NRC (1994). The basal diet of different phase of experimental period is presented in Table 1. Bile salts were purchased from ICC Industrial Comercio Exportacao e Import Lemma, Sao Paulo Brazil (purchased from Total Vet Company for vet medicine, Qalyubia Governorate, Egypt). Bile salts were gently mixed with a portion of the basal diet then, mixed with all the experimental diets. The composition of bile salts is 70% bile salts, 46.9% cholic acid, 17.8% desoxycholic and 4.06% chenodeoxycholic. Chicks were individually weighted at 1, 14, 28 and 42 days of age. Feed intake was determined weekly until marketing age on a replicate basis. Consequently, feed conversion (g feed/g gain) and body weight gain were estimated during each experimental period (1–14, 15–28, 29–42 and 1–42 days).

Blood measurements

At the end of trial period, 10 birds from each group were weighed and slaughtered to obtain blood samples. Blood samples were collected into tube with EDTA

Table 1. Composition and calculated analysis of the experimental basal diets.

| Ingredients | Basal diet (as DM) |
|-------------|--------------------|
|             | Starter (1–14 days) | Grower (14–28 days) | Finisher (28–42 days) |
| Corn        | 57.00              | 61.40              | 65.74              |
| Soybean meal (48%) | 35              | 30              | 25              |
| Corn gluten meal (62%) | 2.5              | 2.5              | 2.5              |
| Limestone  | 1.89              | 1.96              | 1.96              |
| Di-calcium phosphate | 1.9              | 1.8              | 1.6              |
| Sodium chloride | 0.25              | 0.25              | 0.25              |
| DL-Met     | 0.20              | 0.15              | 0.13              |
| L-Lys_HCl  | 0.15              | 0.13              | 0.01              |
| Oil        | 0.8               | 1.5               | 2.5               |
| Sodium bicarbonate | 0.01             | 0.01              | 0.01              |
| Broiler vitamin-mineral Premixa | 0.3               | 0.3               | 0.3               |
| Total      | 100               | 100               | 100               |
| Calculated analysis |         |                  |                  |
| DM%        | 87.5              | 86.73             | 85.92             |
| Crude protein (CP %)| 23.2              | 21.0              | 19.0              |
| Metabolic energy (k cal/kg) | 2950              | 3020              | 3125              |
| Ether extract (EE %)| 3.44              | 4.25              | 5.35              |
| Crude fibre (CF %)| 2.42              | 2.36              | 2.30              |
| Ash %      | 2.76              | 2.55              | 2.34              |
| Ca %       | 1.20              | 1.18              | 1.12              |
| Available phosphorus | 0.50              | 0.47              | 0.43              |
| Lys %      | 1.39              | 1.22              | 1.00              |
| Met %      | 0.60              | 0.53              | 0.48              |
| Met + cys %| 0.97              | 0.87              | 0.79              |

*Broiler vitamin premix provided the following per 1.5 kg: vitamin A, 12,000,000 IU; vitamin D3, 3,000,000 IU; vitamin E, 40,000 mg; vitamin K, 3000 mg; pantothenic acid, 12,000 mg; B1 2000 mg, B2 6000 mg; B6 5000 mg, B12 20 mg, niacin, 45,000 mg; biotin, 75 mg and folic acid 2000 mg.

Mineral premix provided the following per 1.5 kg: I 1000 mg; Mn, 100,000 mg; Cu 10,000 mg; Zn 60,000 mg, Se 200 mg; Fe 30,000 mg; choline 260,000 mg, Cobalt 100 mg.

*bCalculated according to NRC (1994).
to determine some blood constituents, and then non-
coagulated blood used for estimating (WBCs) White
blood cells count \((x10^3/mm^3)\), lymphocyte (%),
Monocytes (%) and Heterocytes (%) (Stoskopf
et al. 1983).

Plasma was obtained by centrifugation of the
whole blood at 3000 \(x\) for 20 min and stored at
\(-20^\circ\text{C}\) until analysis. Total protein (g/dL) was esti-
mated by the Biuret method according to Armstrong
and Carr (1964). The concentration of albumin (g/dL)
was estimated calorimetrically according to Wise
(1965). Globulin concentration (g/dL) was calculated.
Creatinine (mg/dL) and urea (mg/dL) levels were
determined spectrophotometrically using commercial
kits from Biodiagnostic Company (Giza, Egypt).

Digestibility coefficient

At the end of the study, 10 birds were used to deter-
mine the apparent digestion coefficients of each diet-
ary nutrient and to calculate the nutritive values of
the experimental diets. Birds were individually settle in
metallic cage and fed their respective trail diets. The
preliminary period continued for 3 days and the col-
collection period extended to 5 days in which excreta were
quantitatively collected every 24 h and daily feed intake
was recorded. The excreta were clean out from feathers
and samples diet then weighed and dried in oven
at \(70^\circ\text{C}\) for 36 h. Finally, grind and placed in screw
top glasses till analysis. The direct analysis of diets and
excreta was executed according to AOAC (2006).

Microbiology traits

The authors collected the caecal fresh contents and
subjected to a stream of CO2 in bottles and trans-
ported to the lab. The count of total bacteria was
determined via plate count agar at \(30^\circ\text{C}\) for 2 days. Salmonella and \(E.\ coli\) bacteria were determined on
Eosin Methylene Blue agar plates and on XLD agar
plates after incubation at \(37^\circ\text{C}\) for 1 day, respectively
(Alagawany et al. 2018).

Statistical analysis

Data were statically analysed by using the GLM pro-
cedure of SAS (package program, SPSS 2008).

Orthogonal polynomial contrasts (linear and quadratic)
were used to test the significance of the different levels of dietary bile salts.

Results and discussion

Growth performance

**Live body weight and body weight gain**
At 28 and 42 days old, body weight (BW) was linearly increased with the bile salt supplementation (Table 2). However, daily body weight gain (DBWG) through 14–28, 28–42 and 1–42 days old was linearly improved in chick groups fed diet plus bile salt when compared with control. On the other hand, the results of average BW at 14 days of age and DBWG during 1–14 days old were not significantly affected due to bile salts supplementation (Table 2). It could be noticed that the highest values of BW and DBWG were recorded for birds having 1.5 ml bile salts.

These results agreed with the observation obtained by Alzawqari et al. (2016) and Lai et al. (2018) who indicated that BWG improving significantly in broiler chicks fed diet plus bile acid matched with the non-treated group. However, Rezaeipour et al. (2016) indicated that bile salts preparation failed to take out any increased significantly in broiler chick’s performance (LBW and BWG). The improvement in live body weight and body gain due to bile salts supplementation in broiler diets may be attributed to higher digestion coefficients of nutrients. Brzózska et al. (2013) indicated broiler growth enhanced when birds fed diet containing 0.3–0.9% organic acids. Improving digestion and absorption of lipid due to bile salts improves the bioavailability of fatty vitamins (A, D, E and K) (Stamp and Jenkins 2008).

**Feed intake (FI) and feed conversion ratio (FCR)**
Results in Table 2 show that FI was linearly reduced \((p < .0261)\) through 1–28 and 1–42 days with increasing levels of bile salts. On the other hand, during 1–42 days of age, FI was quadratically \((p < .01)\) decreased by the levels of dietary bile salts supplementation (Table 2). FCR was linearly improved

---

**Table 2. Growth performance of broiler chicks as affected by different levels of bile salts.**

| Items                  | Bile salt levels, mL/kg diet | SEM   | Linear  | Quadratic |
|------------------------|-----------------------------|-------|---------|-----------|
| Live body weight (g)   |                             |       |         |           |
| 1                      | 41.50                       | 0.330 | 0.8133  | 0.6000    |
| 14                     | 332.20                      | 6.090 | 0.1230  | 0.1477    |
| 28                     | 908.00                      | 9.815 | 0.0005  | 0.0282    |
| 42                     | 1736.67                     | 10.475| <.0001  | 0.3357    |
| Daily body weight gain (g/day) |                  |       |         |           |
| 1–14                   | 20.76                       | 2.265 | 0.440   | 0.1544    |
| 14–28                  | 41.13                       | 4.471 | 0.553   | 0.0939    |
| 28–42                  | 59.19                       | 6.900 | 0.618   | <.0001    |
| Daily feed intake (g/day) |                            |       |         |           |
| 1–14                   | 33.80                       | 3.256 | 3.138   | 0.563     |
| 14–28                  | 90.71                       | 8.721 | 8.072   | 1.340     |
| 28–42                  | 146.33                      | 13.08 | 14.01   | 1.473     |
| Feed conversion ratio (g feed/g gain) |         |       |         |           |
| 1–14                   | 1.63                        | 1.44  | 1.45    | 0.033     |
| 1–42                   | 90.28                       | 8.08  | 8.64    | 0.780     |

Means within the same row with different common superscripts differ significantly.

---

**Table 3. Digestion coefficient and nutritive values affected by different levels of bile salts.**

| Items                  | Bile salt levels, mL/kg diet | SEM   | Linear  | Quadratic |
|------------------------|-----------------------------|-------|---------|-----------|
| DM                     | 71.73                       | 0.775 | 0.0009  | 0.1260    |
| OM                     | 74.39                       | 0.573 | 0.001   | 0.1945    |
| CP                     | 70.65                       | 0.463 | <.0001  | 0.0046    |
| EE                     | 61.31                       | 0.543 | 0.0522  | <.0001    |
| CF                     | 38.99                       | 0.853 | 0.0522  | <.0001    |
| NFE                    | 69.60                       | 0.658 | <.0001  | 0.5126    |
| TDN                    | 60.25                       | 0.518 | <.0001  | 0.0578    |
| ME                     | 2530.60                     | 2.180 | <.0001  | 0.0588    |

Means within the same row with different common superscripts differ significantly.

DM: Dry matter; OM: organic matter; CP: curd protein; EE: ether extract; CF: curd fibre; NFE: nitrogen-free extract; TDN: total digestible nutrient; ME: metabolic energy.
throughout overall period of the trail by levels of dietary bile salts supplementation. The improvement in FCR was associated with gradually increasing bile salts from 0.5 to 1.5 ml/kg diet. It could be noticed that chickens fed diet plus 1.5 ml bile salts/kg diet recorded the best value of FCR: while, chicks fed the control diet recorded the poorest value. The reduction in feed intake and improvement in feed conversion may be related to the fatty acid content in the bile salts (Xie et al. 2013).

Our results agree with Alzawqari et al. (2016) who stated that feeding broiler on diet supplemented with bile salts improved feed conversion from 21 to 42 days of age. The chenodeoxycholic acid and cholic acid predominate in broiler biliary acids, it appears that chenodeoxycholic acid can decrease feed intake of broilers (Lai et al. 2018). Also, our findings are partially agree with El-Katcha et al. (2019) who found that laying hens fed on diet supplemented with 400 g/ton of dried bile acids with biotin increased significantly \( p < 0.05 \) daily FI and improved FCR compared to the control group. On the contrary, our results disagreed with those obtained by Maisonnier et al. (2003) and Karimi et al. (2011) who stated that utilise bile salts in broiler feeds did not progress feed efficiency rate. Lai et al. (2018) found that average feed consumption did not affected by dietary inclusion bile acids. Also, Parsaie et al. (2007) showed an increase significantly in feed consumption of broiler chicks fed a diet plus bile acids.

\( (p < 0.01) \) throughout overall period of the trail by levels of dietary bile salts supplementation. The improvement in FCR was associated with gradually increasing bile salts from 0.5 to 1.5 ml/kg diet. It could be noticed that chickens feed diet plus 1.5 ml bile salts/kg diet recorded the best value of FCR: while, chicks fed the control diet recorded the poorest value. The reduction in feed intake and improvement in feed conversion may be related to the fatty acid content in the bile salts (Xie et al. 2013).

**Digestion coefficients and nutritive values**

Chicks received diets contained 0.5, 1.0 and 1.5 ml bile salts/kg diet had higher and linearly \( p < 0.0009 \) or \( p < 0.0001 \) improved digestion coefficient for OM and DM than the control (Table 3). The digestion coefficient of NFE, TDN and ME was improved linearly \( (p < 0.00001) \) with increasing bile salts level. The digestion coefficient of EE and CF was quadratically increased in chicks given 0.5, 1.0 and 1.5 ml bile salts compared with the control. The digestion coefficient of CP was linearly and quadratically improved with 0.5, 1.0 and 1.5 ml bile salts. The amelioration in fat assimilation ability by extension of bile salts might be towards deficient gall salts excretion by the animal or regeneration process catabolism of gall salts by illumes bacteria (Adrizal and Yayota 2002; Maisonnier et al. 2003). Other investigators suggested another characteristic of dietary gall salts in improving the digestion of appeased fatty acids characterised long bonds (Gomez and Poplin 1976). Mostly, the enhancement of digestion coefficients by adding bile salts to the feed placed in order to non-fat nutrients turn off minimal preserved by fat droplets and after that there no need to add extra digestion enzymes. Ndelekwe et al. (2019) observed that digestibility of protein, fibre, and ether extract were significantly improved by addition of organic acids than control. Birds feeding on bile salts due to decrease intestines pH consequence the environment unsuitable for Salmonella and Kelly Basil which need pH about 7 and decrease

### Table 4. Relative weights of carcass traits as affected by different level of bile salts.

| Items       | Bile salt levels, mL/kg diet | SEM   | Linear     | Quadratic |
|-------------|------------------------------|-------|------------|-----------|
| Carcase %   | 63.43 63.25 67.44 65.69     | 0.728 | 0.0119     | 0.2938    |
| Dressing %  | 69.98 70.66 74.84 72.26     | 0.761 | 0.0160     | 0.0771    |
| Giblets %   | 6.55 7.41 7.39 6.57         | 0.132 | 0.8477     | 0.0006    |
| Liver %     | 2.56 3.58 3.27 2.76         | 0.138 | 0.7126     | 0.0019    |
| Heart %     | 0.52 0.56 0.55 0.50         | 0.028 | 0.6436     | 0.2005    |
| Gizzard %   | 3.47 3.27 3.58 3.31         | 0.100 | 0.7517     | 0.7842    |

Means within the same row with different common superscripts differ significantly.

### Table 5. Total protein, its fractions and lipid profile parameters as affected by different levels of bile salts.

| Items                  | Bile salt levels, mL/kg diet | SEM   | Linear     | Quadratic |
|------------------------|------------------------------|-------|------------|-----------|
| Total Protein (g/dL)   | 3.37 4.12 4.24 4.02         | 0.130 | 0.0995     | 0.0534    |
| Alb (g/dL)             | 1.03 1.69 1.72 1.57         | 0.218 | 0.8540     | 0.1513    |
| Glob (g/dL)            | 2.34 2.43 2.53 2.45         | 0.124 | 0.0436     | 0.0011    |
| A/G (%)                | 0.44 0.70 0.71 0.64         | 0.290 | 0.3540     | 0.0367    |
| Total Cholesterol (mg/dL) | 79.84 100.52 86.57 90.16   | 2.979 | 0.0214     | 0.2311    |
| Triglycerides (g/dL)   | 56.05 83.70 60.33 63.37     | 3.859 | 0.2047     | 0.0211    |
| HDL (mg/dL)            | 48.75 62.49 51.67 57.10     | 4.071 | 0.8372     | 0.6707    |
| LDL (mg/dL)            | 19.89 21.29 19.04 20.39     | 1.991 | 0.0215     | 0.0660    |

Means within the same row with different common superscripts differ significantly.

Alb: albumin; Glob: globulin; HDL: high-density lipoprotein; LDL: low-density lipoprotein.
the damage of intestinal wall, and due to renewing intestinal epithelial cells decreases and increase villi length (Sarangi et al. 2016). The present results harmonise with Kussaibati et al. (1982) who observed that fat absorption enhanced in broiler chicks fed on diets plus bile salts. The improvement in FCR may be due to organic acids whereas, the acidic anions have been found to promote the cation absorption of minerals such as calcium, phosphorus, magnesium and zinc (Edwards and Baker, 1999; Alagawany et al. 2018; Abdel-Latif et al. 2020; Pearlin et al. 2020). Alzawqari et al. (2016) found that fat digestibility increased in broilers fed diet supplemented with bile salts. [314x74]p \textless .05) serum concentrations of triglycerides were higher in groups fed diet plus various levels of bile salts than the control group, also LDL values were linearly (p \textless .05) increased with bile salts addition than control. While, total cholesterol (TC) and high density lipoprotein (HDL) concentrations were insignificantly increased in all experimental groups than control birds (Table 5). Similarly, Hegsted et al. (1960) showed that the serum cholesterol content of young chickens increased when fed diet containing 0.8% of cholesterol plus cholic acid (which a serious ingredient in the gall acids). Our results may be due to increase the digestion and absorption of lipids; whereas, bile acids can improve the absorption of dietary lipids which are not stored in abdominal and the ability to transport cholesterol from peripheral tissues to the liver was unaffected by supplemental bile acids fat Lai et al. (2018). Alzawqari et al. (2010) demonstrated that birds received diet inclusion 0.5% DBA had significantly higher serum triglycerides, cholesterol, LDL and HDL concentrations at 21 and 42 days old as compared 0.0%. However, Lai et al. (2018) reported that broilers at 42-day old fed diet plus bile acids unaffected (p \textless .05) serum concentrations of triglyceride, HDL and LDL, the synthesis of adipose tissue and

### Carcase traits

The effect of experimental additives on carcase traits is presented in Table 4. It was observed the relative weights of carcase and dressing were linearly (p \textless .05) increased while, giblets and liver percentages were quadratically (p \textless .01) enhanced by adding bile salts levels in broiler chicken’s diets. The highest values of the aforementioned relative weights were recorded with 1.0 and 0.5 ml bile salts comparison with the control, respectively. The results in Table 4 showed tested bile salts levels did not affect heart and gizzard weight. The addition of bile acids to broiler diet may decrease body fat mass and increase the percentage of the carcase (Lai et al. 2018), these to broiler diet may decrease body fat mass and increase avert heart and gizzard weight. The addition of bile acids (Lai et al.2018), these to broiler diet may decrease body fat mass and increase

### Protein and lipids fractions

The effect of bile salts on total protein and its fractions are presented in Table 5. Globulin concentrations and A/G % were quadratically (p \textless .01 and 0.05) enhanced with bile salts trail. Total protein and albumin concentrations were numerically increased without statically significant by bile salts addition. Our finding agreed with those obtained by Natsir et al. (2017) who reported that organic acids had better effects on serum total protein and albumin compared with the control in broiler drinking acidifying water. The triglycerides (TG) values were quadratically (p \textless .0211) higher in groups fed diet plus various levels of bile salts than the control group, also LDL values were linearly (p \textless .05) increased with bile salts addition than control. While, total cholesterol (TC) and high density lipoprotein (HDL) concentrations were insignificantly increased in all experimental groups than control birds (Table 5). Similarly, Hegsted et al. (1960) showed that the serum cholesterol content of young chickens increased when fed diet containing 0.8% of cholesterol plus cholic acid (which a serious ingredient in the gall acids). Our results may be due to increase the digestion and absorption of lipids; whereas, bile acids can improve the absorption of dietary lipids which are not stored in abdominal and the ability to transport cholesterol from peripheral tissues to the liver was unaffected by supplemental bile acids fat Lai et al. (2018). Alzawqari et al. (2010) demonstrated that birds received diet inclusion 0.5% DBA had significantly higher serum triglycerides, cholesterol, LDL and HDL concentrations at 21 and 42 days old as compared 0.0%. However, Lai et al. (2018) reported that broilers at 42-day old fed diet plus bile acids unaffected (p \textless .05) serum concentrations of triglyceride, HDL and LDL, the synthesis of adipose tissue and

#### Table 6. Liver and kidney functions as affected by different levels of bile salts.

| Items                     | Bile salts levels, mL/kg diet | p value |
|---------------------------|-------------------------------|---------|
|                           | 0    | 0.5  | 1.0  | 1.5  | SEM   | Linear | Quadratic |
| Liver function            |      |      |      |      |       |        |           |
| ALT (u/L)                 | 4.86 | 9.69 | 17.79| 6.46 | 1.230 | 0.0556 | 0.0002    |
| AST (u/L)                 | 181.10 | 201.85 | 291.01 | 225.66 | 110.88 | 0.0043 | 0.0094    |
| Total bilirubin (mg/dL)   | 0.55 | 0.66 | 0.67 | 0.67 | 0.048 | 0.1550 | 0.3011    |
| Direct bilirubin (mg/dL)  | 0.12 | 0.12 | 0.13 | 0.14 | 0.015 | 0.2624 | 0.7546    |
| Kidney function           |      |      |      |      |       |        |           |
| Creatinine (mg/dL)        | 0.65 | 0.77 | 0.76 | 0.56 | 0.066 | 0.3640 | 0.0325    |
| Uric acid (mg/dL)         | 5.56 | 5.71 | 5.24 | 4.66 | 0.495 | 0.2605 | 0.5519    |

Means within the same row with different common superscripts differ significantly.

ALT: alanine–aminotransferase; AST: aspartate–aminotransferase.

Youssef et al. (2017) who reported that percentages of carcase yield did not affected significantly of broilers chickens fed diet treated by organic acids.
fat deposition in birds is dependent on available serum triglycerides. The most fatty acids are synthesized in the liver and transported via LDL or chylomicrons for storage in adipose tissue as TG (Hermier 1997). In contrast, LDL elevates the uptake of cholesterol from peripheral tissues and expedites the transport of cholesterol to the liver for catabolism (Miller and Miller 1975). Contradicting results were obtained by Edwards (1962) who illustrated that laying hen fed diet plus cholic acid or lithocholic acid at grades of 0.05 or 0.025% decreased numerically cholesterol concentrations. El-Katcha et al. (2019) concluded that serum total cholesterol, TG and LDL concentrations insignificantly reduced with addition (400 g/ton) of dried bile acids with vitamin B12 or biotin to laying hen diet compared with control.

Liver and kidney functions

Results in Table 6 indicated that liver enzymes (ALT and AST) were increased significantly in bile salts groups than the control group. Whereas, AST values were linearly and quadratically increased ($p < .0043$ and 0.0094), respectively, while ALT and creatinine concentrations were increased quadratically only in chicks treated with bile salts compared to the control. Dietary supplementation of bile salts did not affect total bilirubin, direct bilirubin and uric acids of broiler chicks in comparison with control. Liver as a master organ in poultry metabolism is sensible to nutritional adjustment and actions of ALT and AST in serum are generally looked as a serious index for perception the liver health. It is purified that when liver doing healthy, liver enzymes concentrations in serum will be minimize in broiler chickens (Corduk et al. 2007). Passive diffusion and active transport are principle pathways for absorption of nearly 95% of bile acids from the ileum (Hofmann and Hagey 2008). Diets contain high fat could modify the concerning enzyme picture in liver tissue and serum (Ishii et al., 2010). Our finding showed a significant increase of LDL values which elevate the uptake of cholesterol from peripheral tissues and easily the transport of cholesterol to the liver for catabolism (Miller and Miller, 1975). This mechanism may be makes fatty liver and effect on liver function especially with bile salts or fat treatments that due to increased liver enzymes concentrations. The results cleared that broiler fed diets contained bile salts increased concentricity of ALT and AST as compared to control birds. On the other hand, El-Katcha et al. (2019) stated that serum ALT and AST activities insignificantly increased in laying hens fed on diet plus 400 g/ton of dried bile acids with vitamin B12 or biotin compared with control birds. Our results were contrast with Rezaei pour et al. (2016) who found that concentrations of liver enzymes including ALT and AST were not affected by dietary bovine bile salts.
Relative lymphoid organs, some immune measurements and oxidative parameters

The relative weight of spleen and thymus gland was quadratically (P = 0.0162 and 0.0439) increased in birds fed diet supplemented with bile salts (Table 7). The highest value of relative spleen weight was recorded with birds fed diet supplemented by 0.5 and 1.0 ml/kg diet, while use of 1.0 ml in broiler chick’s diet enhanced the thymus relative weight compared with the untreated birds. But, tested bile salts levels insignificantly effect on bursa relative weight, WBCs, lymphocytes, monocytes and heterocytes percentages when compared to control birds (Table 7). Our results agreed with the findings obtained by Alzawqari et al. (2016) who indicated that bursa and spleen at 42 days of did not affected broiler fed diets plus bile acid, the stimulatory effect of the bile salts and acids on evolution and growth of the lymphoid organs may be perfect of improving immunity. The immune system plays important role in maintain the bird’s health (Yang et al. 2018).

Data in Table 7 showed that Gama immunoglobulin (IgG) was linearly and quadratically enhanced in broiler groups fed diet enriched with bile salts compared to control birds. While, white blood cells and their fractions insignificantly affected by bile salts supplementation. Bile salts which used in the present work enriched with organic acids that may be improved immune system as supported by Devi et al. (2016) found that WBC, Gama immunoglobulin level and lymphocyte % were improved in lactating sow treated with protected organic acid. Lee et al. (2017) found that trypsin and chymotrypsin activities of intestinal tract were higher of broiler chickens fed diet enriched with organic acids. Increase activity of digestive enzymes improving nutrients digestibility. Ndelekwute et al. (2018) reported that digestibility of protein, fibre, and ether extract were significantly improved in broiler by addition organic acids to drinking water. Addition of bile salts to broiler diet increased dietary oils improved digestive enzymes activities. Jang et al. (2007) indicated that the higher activation of pancreatic trypsin, α-amylase and intestinal maltase may be due to oil supplementation. Our results harmonised with those found by Lai et al. (2018) who observed that the activity of duodenum lipase and lipoprotein lipase on 42 days old significantly increased by adding 60 and 80 mg bile acids/kg diet. Also, bile salts play an important role in digestion and absorption of lipid in small intestine and enhanced ME (Stamp and Jenkins 2008). Lipase activity in intestinal can be an indicator of utilisation of lipids in animals whereas, lipase plays a critical role in lipid metabolism. The insoluble fat molecules are broken into microscopic micelles to higher their solubility and hydrolysed by lipase to convert the triacylglycerols to monoacylglycerols, diacylglycerols, fatty acids, and glycerol by catalysing the hydrolysis of triglycerides (Lai et al. 2018).

Digestive enzymes

Amylase enzyme activity was linearly and quadratically enhanced in groups treated with dietary bile salts, however, lipase activity only quadratically (p < .0077) increased in treated groups. The activities of protease enzymes was linearly (p < .0225) decreased in bile salts groups than the control group (Table 8). Liu et al. (2017) found that trypsin and chymotrypsin activities of intestinal tract were higher of broiler chickens fed diet enriched with organic acids. Increase activity of digestive enzymes improving nutrients digestibility. The results presented in Table 7 show improvement of MDA in chicks fed diet plus bile salts, SOD values were enhanced linearly and quadratically while, TAC values were increased quadratically (p < .0006) only in broilers fed diet inclusion different levels of bile salts compared with control birds. Our results were supported by Awaad et al. (2018) who stated that use of organic acids in broilers drinking water improved antioxidant parameters.

Bacteriology

Total bacteria was linearly and quadratically reduced in broiler chicks fed bile salts diets as compared to the untreated group; while, total fungi, E. coli and Salmonella were only linearly (p < .01) decreased in broiler groups fed diet enriched with bile salts compared with the untreated group (Table 9). Bile salts can use as an antimicrobial substance, whereas
microorganisms count was significantly decreased in broiler chickens fed bile salt diets in our experiment. Kocsar et al. (1969) stated that bile acids had a substantial function in the protection technique of the microorganism versus bacterial endotoxins. The aggregation of bile salts in the intestine related to reduce the pH intestine which inappropriate of salmonella and another microorganisms (Sarangi et al. 2016). Hassan et al. (2010) reported that broiler chicks treated by a combination of organic acids or salts decreased intestinal E. coli and Salmonella spp compared with control. Furthermore, the ability of bile acids decreased the ability of endotoxin absorption (Sheen-chen et al. 2002).

Conclusions
From our results, it could be concluded that bile salts especially levels (0.5 and 1.0 ml/kg diet) have a positive impact on growth performance and carcase estimates, blood biochemical measurements, intestinal enzymes activities, digestibility of nutrients and microorganism’s content of broiler chicks.

Animal welfare statement
This work was carried out at Department of Poultry, Faculty of Agriculture, Zagazig University, Egypt. Animal care and maintenance were performed in accordance with guidelines of Egyptian Research Ethics Committee. This study was approved by the institutional committee.

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).

ORCID
Fayiz M. Reda http://orcid.org/0000-0002-2014-3418

References
Abdel-Latif EA, Ibrahim ZA, Reda FM, Alagawany M. 2020. Effect of Aspergillus japonicas culture filtrate on performance, carcass yield, digestive enzymes, intestinal microbiota and blood constituents of quail. Ital J Anim Sci. 19(1):1057–1064.
Abudabos AM. 2014. Effect of fat source, energy level and enzyme supplementation and their interactions on broiler performance. SA J Sci. 44(3):280–287.
Adrizal SO, Yayota M. 2002. Dietary energy source and supplements in broiler diets containing defatted rice bran. J Appl Poult Res. 11:410–417.
Alagawany M, Abd El-Hack M, Al-Sagheer A, Naiel M, Saadeldin I, Swelum A. 2018. Dietary cold pressed watercress and coconut oil mixture enhances growth performance, intestinal microbiota, antioxidant status, and immunity of growing rabbits. Animals. 8(11):212.
Alagawany M, Abd El-Hack ME, Laudadio V, Tufarelli V. 2014. Effect of low-protein diets with crystalline amino acid supplementation on egg production, blood parameters and nitrogen balance in laying Japanese quails. Avian Biol Res. 7(4):235–243.
Alagawany M, Abdel-Latif EA, Ibrahim ZA, Reda FM. 2020. Use of Aspergillus japonicas culture filtrate as a feed additive in quail breeder’s nutrition. Ital J Anim Sci. 19 (1): 1291–1298.
Alagawany M, El-Hindawy MM, Mohamed LA, Bilal RM, Soomro J. 2020. The use of cold pressed oils as eco-friendly alternatives for antibiotics in high and low-CP diets of laying Japanese quail. Anim Biotechnol.DOI: 10.1080/10495398.2020.1837846.
Alagawany M, El-Nesr SS, Farag MR. 2018. The role of exogenous enzymes in promoting growth and improving nutrient digestibility in poultry. Ir J Vet Res. 19(3):157–164.
Alagawany M, Nasr M, Al-Abdullatif A, Alhotan RA, Azzam MMM, Reda FM. 2020. Impact of dietary cold-pressed chia oil on growth, blood chemistry, hematology, and antioxidant and immunity status of growing Japanese quail. Ital J Anim Sci. 19:896–904.
Allain CC, Poon LS, Chan CS, Richmond WS, Fu PC. 1974. Enzymatic determination of total serum cholesterol. Clin Chem. 20(4):470–475.
Alzawqari M, Kermanshahi H, Nassirimoghaddam H. 2010. The effect of glycine and desiccated ox bile supplementation on performance, fat digestibility, blood chemistry and ileal digesta viscosity of broiler chickens. Glob Vet. 5: 187–194.
Alzawqari MH, Al-Baadani HH, Alhidiary IB, Al-Owaimer AN, Abudabos AM. 2016. Effect of taurine and bile acid supplementation and their interaction on performance, serum components, ileal viscosity and carcass characteristics of broiler chickens. SA J Sci. 46(4):448–457.
AOAC. 2006. Official methods of analysis. 18th ed. Washington (DC), USA: Association of Official Analytical Chemists.
Armstrong WD, Carr CW. 1964. Physiological chemistry laboratory direction. 3rd ed. Minneapolis, MN: Burses Publishing Co.
Awaad MHH, Elmenawey MA, Bashandy MM, Mohamed FF, Salem HM, Morsy EA, Gossens T. 2018. Heat stress impedance by acidifiers in broiler chickens. Acta Sci Med Sci. 2: 84–93.
Azman MA, Ibrahim H, Birben N. 2005. Effects of various dietary fat sources on performance and body fatty acid composition of broiler chickens. Turk J Vet Anim Sci. 29: 811–819.
Bianchi AT, Moonen-Leusen HW, Van der Heijden PJ, Bokhour BA. 1995. The use of a double antibody sandwich
ELISA and monoclonal antibodies for the assessment of porcine IgM, IgG and IgA concentrations. Vet Immunol Immunopathol. 44(3–4):309–317.

Brzózska F, Sliwiński B, Michałik-Rutkowska O. 2013. Effect of dietary acidifier on growth, mortality, post-slaughter parameters and meat composition of broiler chickens. Ann Anim Sci. 13(1):85–96.

Corduk M, Ceylan N, Ilidiz F. 2007. Effect of dietary energy density and L-carnitine supplementation on growth performance, carcass traits and blood parameters of broiler chickens. S Afr J Anim Sci. 37:65–73.

Devi SM, Lee KY, Kim IH. 2016. Analysis of the effect of dietary protected organic acid blend on laying sows and their piglets. R Bras Zootec. 45(2):39–47.

Edwards HM, Baker DDH. 1999. Effect of dietary citric acid on zinc bioavailability from soy products using an egg white diet with zinc sulfate heptahydrate as the standard. Poult Sci. 78:576–580.

Edwards, H.M. (1962). Observations on feeding cholic acid to bovine bile powder and enzyme treatment on performance, carcass characteristics, serum lipid metabolites and intestinal enzyme activities of broiler chickens. Poult Sci. 97(1):196–202.

Lee IK, Bae S, Gu MJ, You SJ, Kim G, Park SM, Jeung WH, Ko KH, Cho KJ, Kang JS, et al. 2017. H9N2-specific IgG and CD4 + CD25 + T cells in broilers fed a diet supplemented with organic acids. Poult Sci. 96(5):1063–1070.

Leeson S, Summers JD. 2005. Commercial poultry nutrition. 3rd ed. Nottingham (UK): Nottingham University Press.

Liu Y, Yang X, Xin H, Chen S, Yang C, Duan Y, Yang X. 2017. Effects of a protected inclusion of organic acids and essential oils as antibiotic growth promoter alternative on growth performance, intestinal morphology and gut microbiota in broilers. Anim Sci J. 88(9):1414–1424.

Lynn KR, Clevette-Radford NA. 1984. Purification and characterization of hevin, a serum protease from Hevea braziliensis. Biochem J. 23:963–964.

Maisonnier S, Gomez J, Bree A, Berri C, Baeza E, Carre B. 2003. Effects of microflora status, dietary bile salts and guar gum on lipid digestibility, intestinal bile salts, and histomorphology in broiler chickens. Poult Sci. 82(5):805–814.

Mihara M, Uohiyama M. 1978. Determination of malondialdehyde precursors in tissues by thiobarbituric acid test. Anal Biochem. 86:271–278.

Miller GJ, Miller NE. 1975. Plasma-high-density-lipoprotein concentration and development of ischaemic heart-disease. Lancet. 1(7897):16–19.

Mohamed LA, El-Hindawy MM, Alagawany M, Salah AS, El-Sayed SA. 2019. Effect of low- or high-CP diet with cold-pressed oil supplementation on growth, immunity and antioxidant indices of growing quail. J Anim Physiol Anim Nutr. 103(5):1380–1387.

Myers GL, Cooper GR, Henderson LO, Hassemmer DJ, Kimberly M. 1994. Standardization of lipid and lipoprotein Measurements. In: N Rifai, GR Warnick, editors. Laboratory measurement of lipid and lipoproteins and apolipoproteins. Washington, DC, USA: AACC Press; p. 177–205.

Natsir MH, Hartuttik SJofjan O, Widodo E, Widyawasthi ES. 2017. Use of acidifiers and herb-acidifier combinations with encapsulated and non-encapsulated intestinal microflora, intestinal histological and serum characteristics in broiler. AIP Conference Proceedings 1844, 020012.

Ndelekwute EK, Assam ED, Assam EM. 2018. Apparent nutrient digestibility, gut pH and digesta viscosity of broiler chickens fed acidified water. MOJ Anat Physiol. 5(4):250–253.
Ndelekwute EK, Unah UL, Udoh UH. 2019. Effect of dietary organic acids on nutrient digestibility, faecal moisture, digesta pH and viscosity of broiler chickens. MOJ Anat Physiol. 6(2):40–43.

Nishikimi M, Roa NA, Yogi K. 1972. Biochemistry. Bioph Res Common. 46:849–854.

NRC. 1994. Nutrient requirements of poultry, 9th rev. ed. Washington (DC): National Academy Press.

Parsae S, Shariatmadari F, Zamiri MJ, Khajeh K. 2007. Influence of wheat-based diets supplemented with xylanase, bile acid and antibiotics on performance, digestive tract measurements and gut morphology of broilers compared with a maize-based diet. Brit Poult Sci. 48(5):594–600.

Pearlin BV, Muthuvel S, Govidasamy P, Villavan M, Alagawany M, Farag MR, Dhama K, Marappan G. 2020. Role of acidifiers in livestock nutrition and health: a review. J Anim Physiol Anim Nutr. 104(2):558–569.

Poorghasemi M, Chamani M, Mirhosseini SZ, Sadeghi AA, Seidavi A. 2018. Effect of probiotic and different sources of fat on performance, carcass characteristics, intestinal morphology and Ghrelin Gene Expression on broiler chickens. Kafkas Univ Vet Fak Derg. 24(2):169–178.

Reda FM, Alagawany M, Mahmoud HK, Mahgoub SA, Elnesr SS. 2020. Use of red pepper oil in quail diets and its effect on performance, carcass measurements, intestinal microbiota, antioxidant indices, immunity and blood constituents. Animal. 14(5):1025–1033.

Reda FM, El-Kholy MS, Abd El-Hack ME, Taha AE, Othman SI, Allam AA, Alagawany M. 2020. Does the use of different oil sources in quail diets impact their productive and reproductive performance, egg quality and blood constituents? Poult Sci. 99:3511–3518.

Reitman S, Frankel SA. 1957. Colorimetric method for determination of serum glutamicoxaloacetic and glutamic pyruvic transaminases. Ann J Clin Pathol. 26:1–13.

Reshetnyak VI. 2013. Physiological and molecular biochemical mechanisms of bile formation. WJG. 19(42):7341–7360.

Rezaeipour V, Alinejad H, Asadzadeh S. 2016. Growth performance, carcass characteristics and blood metabolites of broiler chickens fed diets formulated on total or digestible amino acids basis with bovine bile salts powder and soybean oil. J Cent Eur Agric. 17(2):272–284.

Roberts SA, Xin H, Kerr BJ, Russell JR, Bregendahl K. 2007. Effects of dietary fiber and reduced crude protein on nitrogen balance and egg production in laying hens. Poult Sci. 86(8):1716–1725.

Sarangi NR, Babu LK, Kumar A, Pradhan CR, Pati PK, Mishra JP. 2016. Effect of dietary supplementation of prebiotic, probiotic, and symbiotic on growth performance and carcass characteristics of broiler chickens. Vet World. 9(3):313–319.

Sheen-Chen SM, Chen HS, Ho HT, Chen WJ, Sheen CC, Eng HL. 2002. Effect of bile acid replacement on endotoxin-induced tumor necrosis factor-alpha production in obstructive jaundice. World J Surg. 26(4):448–450.

Somogyi M. 1960. Modification of two methods for the assay of amylase. Clin Chem. 6(1):23–27.

SPSS. 2008. Statistical package for the social sciences, Release. Chicago, IL: SPSS INC; p. 16.

Stamp D, Jenkins G. 2008. An overview of bile-acid synthesis, chemistry and function. In: G Jenkins, L. Hardie, editors. Bile acids: toxicology and bioactivity. Cambridge (UK): Royal Society of Chemistry; p. 1–13.

Stoskopf MK, Neely E, Mangold B. 1983. Avian hematology in clinical practice. Mod Vet Pract. 64:629–632.

Tietz NW, Fiereck EA. 1966. A specific method for serum lipase determination. Clin Chem Acta. 13(3):352–355.

Titus HW. 1960. The scientific feeding of chickens. 5th ed. Norwell, MA: Danville Interstate.

Wise WA. 1965. Determination of serum albumen. King Wasmr. 43:273.

Xie P, Wang Y, Wang C, Yuan C, Zhou X. 2013. Effect of different fat sources in parental diets on growth performance, villus morphology, digestive enzymes and colorectal microbiota in pigeon squabs. Arch Anim Nutr. 67(2):147–160.

Yang X, Xin H, Yang C, Yang X. 2018. Impact of essential oils and organic acids on the growth performance, digestive functions and immunity of broiler chickens. Anim Nutr. 4(4):388–393.