Impact of phytase on improving the utilisation of pelleted broiler diets containing olive by-products

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A feeding trial was run to assess the effect of olive cake (OC) in pelleted diets at 0, 10 and 15% with or without 500 FTU/kg diet of a bacterial E. coli phytase (BECP) for broiler chickens during early growth phase period (7–28 days of age). The experimental setup included six treatments as 3 OC levels × 2 supplementations (no enzyme and 500 FTU of BECP). Each treatment was replicated eight times, having five male chickens each. Phytase supplementation significantly increased growth rate and improved production index regardless of the level of OC. Inclusion of OC in broiler diets at 10 and 15% did not significantly affect growth rate, European production index and economic efficiency irrespective of BECP supplementation. In addition, phytase supplementation significantly increased growth rate, European production index and economic efficiency, irrespective of OC level. Plasma cholesterol and triglycerides were decreased considerably due to phytase supplementation to 10 and 15% OC diets. In addition, phytase supplementation significantly increased plasma inorganic phosphorus of broiler fed different levels of OC, and decreased plasma triglycerides and cholesterol of 10 and 15% OC diets. In conclusion, broiler chickens during early growth stage can be fed pelleted diet contained up to 15% OC with or with BECP supplementation, without adverse effects on growth performance, carcass characteristics, plasma lipid profiles, while phytase supplementation to 10% OC diets yield the highest return over cost and economic efficiency.

HIGHLIGHTS

1. Inclusion of olive cake in broiler diets at 10 and 15% did not significantly affect growth rate, European production index and economic efficiency irrespective of phytase supplementation.
2. Phytase supplementation significantly improved growth, European production index, and economic efficiency, regardless of OC level.
3. Phytase supplementation also markedly increased plasma inorganic phosphorus of broiler fed different levels of OC, and economic efficiency of broilers fed 10% OC, regardless of olive cake level.
4. Plasma cholesterol and triglycerides were significantly decreased due to phytase supplementation to 10 and 15% olive cake diets.
5. Phytase supplementation to 10% olive cake diets yields the highest return over cost and economic efficiency.

Introduction

Globally, feeding cost and the price of poultry products have recently increased due to increasing competition between human and animals for the same feed resources. Agriculture by-products are valuable feedstuffs that can be used in animal nutrition (Al-Harthi et al. 2018a, 2018b). In animal nutrition feed processing and feed additives as well have been used for improving the feeding values of feeds particularly of agricultural by-products (Dozier et al. 2010; Zangeneh and Torki 2011; Al-Harthi et al. 2019). For example, different processing methods were used to improve feed quality and overcome the anti-nutritional factors (Bedford...
et al. 1991; Bedford 2000). Infeed manufacture, pelleting is used to enhance agriculture by-products utilisation due to several beneficial effects (Attia et al. 2014; Al-Harthi et al. 2018b).

Recycling OC as a feed resource in livestock feeding is a possible tool to overcome the shortage in animal feed resources (Luciano et al. 2013). Inclusion of OC in mash feed between 5–10% did not cause adverse influence on production of egg of layers and meat of broilers (Abo Omar 2000, 2005; El hachemi et al. 2007; Al-Harthi and Attia 2015, 2016), and up to 15% in pelleted feed for broilers (Al-Harthi et al. 2019).

Nowadays, phytase is utilised extensively to improve animal feeds utilisation and economic of livestock farming due to many potential, such as overcome anti-nutritional factors such as phytic acid, improve digestion, and gut ecology and thus enhancing the use of nutrients for meat and egg production, reduce environmental pollution and increase profits due to nutrient equivalency value (Dailin et al. 2018; Attia et al. 2020). Phytase mainly improved phytate phosphorus utilisation, particularly of high phytic acids diets and enhanced the use of protein/amino acids, energy, phosphorous calcium and several trace minerals (Choct 2006; Dailin et al. 2018).

The use of phytase to improve animal diets containing olive cake is limited in the literature (Al-Harthi 2017). Recent evidence indicates that protease and phytase supplementation influences the microbiota composition in the last part of the small intestine, and to a lesser extent, in digestibility of prececal amino acids (Alagawany et al. 2018). However, the deviations in the composition of microbiota and digestibility of AA in prececal were not connected together, showing a lack of a causal relationship (Borda-Molina et al. 2019). It is found that broilers exhibited a higher inositol P₆ degradation and hydrolysis of lower inositol phosphorus compared with turkeys; however, the results are affected by the source of maize fed (Ingelmann et al. 2019). Hence, this study targets to investigate the impact of phytase on enhancing the use of pelleted feeds containing different concentrations of olive cake on performance, carcass traits, red blood cell characteristics, lipid metabolites and plasma calcium, phosphorus, and alkaline phosphatase of broiler chickens during 7–28 days of age.

Materials and methods

Birds, experimental design and diets

The trial was conducted at Hada Al-Sham Agriculture Research Station belongs to Faculty of Meteorology, Environment and Arid Land Agriculture, King Abdulaziz University, Jeddah, KSA. The source and preparation of OC were as cited by Al-Harthi et al. (2019). The OC was included at 10 and 15% levels in starter-grower diets during 7–28 days of age (Table 1). Diets were fed with or without commercial bacterial phytase. This resulted in 3 OC levels (0, 10, and 15% in the starter-grower feeds) × 2 phytase supplementations (no phytase and bacterial E. coli phytase supplemented at 0.1% to provide a 500 FTU/kg diet). Thus, a total of 240, seven days old Ross 308 male broiler chicks were distributed randomly among six feeding regimens with eight replicates of five chicks each. Diets were pelleted (2–2.5 mm pellet diameter) under the temperature of 80 °C, moisture 17% in the pellets and pressure between (1.5–2.0) bar/kg.

Table 1. Composition and analysis of the experimental diets containing different levels of olive cake fed during 7–28 days of age needs revision.

| Ingredients, g/kg as fed | Olive cake, g/kg | 7–28 days of age |
|--------------------------|------------------|------------------|
|                          |                  | 0                | 100              | 150              |
| Maize                    | 616              | 467              | 391              |
| Soybean meal, 44% CP     | 310              | 324              | 335              |
| Dried olive cake         | 00               | 100              | 150              |
| Di-calcium phosphate     | 16.0             | 17.7             | 17.4             |
| Limestone                | 11.0             | 8.5              | 8.0              |
| Sodium chloride, %       | 3.0              | 3.0              | 3.0              |
| Vitamin + mineral premix  | 3.0              | 3.0              | 3.0              |
| DL-methionine            | 2.7              | 2.5              | 2.6              |
| L-lysine HCL             | 2.3              | 2.3              | 2.0              |
| Soybean meal oil         | 36.0             | 72.0             | 88.0             |
| ME, MJ/kg               |                  |                  |                  |
| Dry matter               |                  |                  |                  |
| CP                       |                  |                  |                  |
| Lysine                   |                  |                  |                  |
| Methionine               |                  |                  |                  |
| Methionine + Cystine     |                  |                  |                  |
| Ether extract            |                  |                  |                  |
| Linoleic acid            |                  |                  |                  |
| Crude fibre              |                  |                  |                  |
| Ash                      |                  |                  |                  |
| Ca, %                   |                  |                  |                  |
| Available phosphorus     |                  |                  |                  |
| Feed price/ ton feed, SR |                  |                  |                  |

This diet was fed also during 1–6 days of age and served as the control diets during 7–28 days of age.

bChemical composition of dried olive pulp showed 5.2% CP, 0.55% Ca, 0.80% total phosphorus of which 0.27 was considered available based on assumption by NRC (1994), 0.009% methionine, 0.0110% methionine and cystine and 0.03% lysine and 6.7 MJ/kg ME.

cCalculated analysis.
dDetermined analysis.

SR: Saudi Riyals; CP: crude protein.
pelleting after diluted 10 times with pelleted feed and mixed well at the top of the feeds to assure homogeneity of distribution. Then, feeds were dried to less than 10% moisture was dried in a stirrer oven. The experimental diets were isocaloric and isonitrogenous and met the nutrient requirements of broilers, according to the National Research Council (NRC 1994). The chemical analyses of feeds were according to AOAC (2007).

The Phyzyme XP, was a product of Danisco Animal Nutrition, Marlborough, UK. It is an E. coli, derived phytase. The dose of phytase addition was 1 g/kg diet to provide 500 FTU/kg diet. In the phytase supplemented non-phytate phosphorus and calcium contents of the feeds were adjusted according to phytase equivalent value of phytase as previously suggested (Attia et al. 2016; Dailin et al. 2018; Dersjant-Li et al. 2019). The aim of using these two rates (10 and 15% OC in the starter and grower diets during 7–28 days of age) is to increase these ratios compared to the previous rates (5 and 10%) obtained by the research group (Al-Harthi and Attia 2016; Al-Harthi 2017) as 10% was found to be safe for feeding broiler chickens up to 28 days of age. It is worth noting that, in chickens, the digestive tract is under development up to 28 days of age (Attia et al. 2012; Al-Harthi et al. 2019). In addition, the fibre content of OC was 14.12%.

**Husbandry and housing of chickens**

All the procedures were approved by the committee of the Department of Arid Land Agriculture under proposal number G 198-155-1440H, that recommends animal rights, welfare and minimal stress and did not cause any harm or suffering to animals according to the Royal Decree number M59 in 14/9/1431H.

Chicks were housed under similar rearing conditions in floor pens (1 x 1 m). The padding material was wood shavings. Pelleted feed and water were available all time (5–30 d). The temperature during the 1st, 2nd, 3rd and 4th week of age was 32, 30, 28 and 26°C, respectively. The relative humidity ranged from 43–52% RH during the same period. During 1–5 days of age, broilers were fed the control diet-fed during 7–28 days of age. The flight schedule was a 23:1 light/dark phase. During 1–6 days of age, broilers were fed starter diets containing 221 g/kg crude protein and 13 MJ/kg diet, 10 g/kg calcium and 5 g/kg available phosphorus.

**Data collection**

Growth performance, including initial body weight, final body weight, body weight gain (BWG), feed consumption and feed conversion ratio (FCR), were recorded during 7–28 days of age on replicates basis. Mortality was recorded daily, and the survival percentage was recorded during 7–28 days of age. At the end of the experiment (28 days of age), 5 ml of blood samples as 8 samples per treatment representing all treatment replicate were collected from the brachial vein in tubes containing heparin as an anti-Coagulant agent. Plasma was collected after blood centrifugation at 1,500xg for 20 min.

The determinations of the red blood cell (RBCs) characteristics, including RBC count and haemoglobin (Hgb), were measured according to Hepler (1966). However, Haematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC), were calculated to the following equations:

\[
\text{Hct} = \text{Hgb} \times 3 \\
\text{MCV} = \text{Hct} \div \text{RBC} \times 10 \\
\text{MCH} = \text{Hg} \div \text{RBC} \times 10 \\
\text{MCHC} = \text{Hg} \div \text{Hct} \times 100
\]

The determinations of calcium and phosphorus, and alkaline phosphatase, total lipids, triglyceride (TG), total cholesterol (T-C) and HDL-cholesterol (HDL-C), were carried out by colorimetric methods using commercial medical digenetic kits (Diamond Diagnostics Company, USA). However, LDL-cholesterol (LDL-C) and VLDL-cholesterol (VLDL-C) were calculated according to the following equation (Friedewald et al. 1972):

\[
\text{LDL-C} = \text{total cholesterol} - (\text{HDL-C} + \text{VLDL-C} + \text{TG/5})
\]

Where TG/5 is an estimate for VLDL-cholesterol.

Risk of cholesterol = total cholesterol/LDL

Economic efficiency was carried out using output (selling price of the chickens –total cost (cost of feeding + cost of husbandry + cost of day old chicks) divided the total cost and multiple by 100. The European production index was estimated, according to Marcu et al. (2013).

**Statistical analysis**

Two-way analysis of variance (3 x 2) was applied using the GLM procedure of SAS® (SAS 2006) for data analysis. The replicate was considered as experiment unit. Percentages data were transformed to arcsine to normalise data distribution. Differences among treatment means were examined according to the Student-Newman-Keuls test at \( p < .05 \). It should be mentioned that \( p \) values for \( .10 < p < .05 \) are considered a trend.
Table 2. The effect of olive cake concentration and/or phytase supplementation\(^1\) on growth during 7–28 days of broiler chicks fed a pelleted diet.

| Treatments                        | Initial body weight at 7 days of age, g | Final body weight at 28 days of age, g | Growth during 7–28 days of age, g | Feed intake during 7–28 days of age, g | Feed conversion efficiency during 7–28 days of age, % | Survival rate during 7–28 days of age, % | European Broiler Index during 7–28 days of age\(^{2}\) |
|----------------------------------|----------------------------------------|----------------------------------------|-----------------------------------|----------------------------------------|-----------------------------------------|----------------------------------------|---------------------------------------------|
| Control diet                     | 145                                    | 1111                                   | 966                               | 1372                                   | 1.42                                    | 89.0                                    | 288                                         |
| Control diet + phytase           | 153                                    | 1129                                   | 976                               | 1347                                   | 1.38                                    | 95.6                                    | 322                                         |
| 10% Olive cake                   | 149                                    | 1131                                   | 982                               | 1434                                   | 1.46                                    | 95.6                                    | 306                                         |
| 10% Olive cake + phytase         | 151                                    | 1128                                   | 977                               | 1417                                   | 1.45                                    | 95.6                                    | 307                                         |
| 15% Olive cake                   | 154                                    | 1119                                   | 965                               | 1399                                   | 1.45                                    | 100                                     | 317                                         |
| 15% Olive cake + phytase         | 149                                    | 1148                                   | 999                               | 1389                                   | 1.39                                    | 97.8                                    | 335                                         |

RMSE: root-mean-square error, The European Broiler Index was calculated according to the following equation (Marcu et al. 2013): European Broiler Index = \[\text{survival} \times \text{growth/chick/day} \times (\text{FCR} \times 10).\]

Results and discussion

Growth rate and vitality

The results presented in Table 2 showed the lack of the significant effect of the OC and/or BECP on 5 days of age body weight. The rate of growth during the experimental periods (7–28 days of age) and final body weight at days 28 of age was not significantly influenced by the OC concentrations and/or BECP supplementation. The current results show the possibility of including OC in the pelleted diet for broiler chickens up to 15% without adverse effect on growth during 7–28 days of age. Likewise, OC can be used from 7.5 to 15% in broiler chickens’ feed (Rabayaa 2000; Abo Omar 2000; Al-Shanti 2003; El hachemi 2007; Al-Harthi et al. 2019). However, 20% OC in the broilers’ feed during 28–49 days of age reduced BWG, but 10% OC did not (Al-Harthi 2017). The reduction, therefore, can be attributed to high fibre contents and anti-nutritional factors (Al-Harthi 2017).

Addition of BECP significantly improved the growth performance of broilers by 1.3%, regardless of OC level (Table 2). The results indicated that phytase improved growth of broiler fed pelleted diets suggests that phytase enhanced phosphorus availability and other essential nutrients (energy, amino acids, and Ca) and yields additional effects over pelleting. The pelleting process improved nutritional values of OC diets, including the availability of minerals due to heat, pressure and humidity treatments (Behnke 1998; Al-Harthi 2017). In addition, the pelleting process eliminates the anti-nutritional factors, enhances in the utilisation of dietary components by reducing the microbial load of feed, improving digestibility and increasing intestinal health by reducing the harmful microbes (Attia et al. 2012, 2014). Pelleting also improves feed homogeneity and reduces the separation of feed components, thereby reducing feed waste (Behnke 1998) and energy lost in feed consumption (Amerah et al. 2007; Dozier et al. 2010). The absence of response of OC diets to BECP supplementation found herein can be attributed to the enhancing effects of pelleting on digestion and utilisation and ecology of gut (Amerah et al. 2007; Al-Harthi 2017).

In agreement with the present results, addition of phytase to the pelleted feed for broilers improved the rates of growth and FCR during 1–20 days of age (Attia et al. 2003; Attia et al. 2012) and also during the period from 21 to 37 days of age (El-Ghamry et al. 2005). However, multi-enzymes addition to pelleted diets for chickens containing rice bran and whole corn improved the growth performance and feed utilisation, showing an additive effect of enzymes cocktail over pelleting, however overlapping in the mode of action may exist between enzymes and pelleting (El-Serwy et al. 2008, 2009). In addition, multienzymes or phytase addition to broiler diets containing different levels of OC up to 10% did not affect growth rates (Sateri et al. 2017; Al-Harthi 2017). Thus, OC at 10% could be fed to broilers without enzyme contained β-glucanase, phytase and hemicellulase (Sateri et al. 2017).

The rate of vitality was numerically increased due to phytase addition to the control. This may be due to improving gut ecology as a result of enhancing beneficial microorganisms (Attia et al. 2012). In addition, OC diets markedly increase vitality compared to OC-free feeds. This increase could be attributed to the unsaturated fatty acids profile of OC mainly monounsaturated fatty acids and to the antioxidants such as polyphenols and vitamin E, which improve immunity and disease cure (Dal Bosco et al. 2012; Al-Harthi and Attia 2015, 2016; Al-Harthi 2017). In this context, the use of the OC at a rate of 35% in lamb feed increased...
the concentration of vitamin E in the muscles and improved the oxidative stability of sheep meat (Luciano et al. 2013). In addition, OC at 20% in mash feeds for broilers during 28–49 days of age did not affect the survival rate of chickens (Al-Harthi 2017). The olive cake contains antimicrobial substances that protect chickens from pathogens and improve vitality (Al-Mughrabi et al. 2001). There was no significant impact of the interaction between OC and BECP on the survival rate of broilers during 7–28 days of age. The current results indicate OC can be fed at 15% in the feeds when fed in pelleted form.

**Feed intake, FCR and European production efficiency index**

The results reported in Table 2 shows the rate of feed intake, FCR and European production index during the experimental period. The results indicate that all the feed intake were unaffected by the concentration of OC and/or phytase supplementation during 7–28 days of age. On the other hand, broilers on olive cake diets significantly impaired FCR by about 3% compared to OC free diets regardless of phytase supplementation. The low feed utilisation of broilers on 10 and 15% OC diets without phytase supplementation may be due to the fact that the gut of young chickens is under development up to 28 days of age (Attia et al. 2012; Al-Harthi et al. 2019). On the other hand, Al-Shanti (2003) and El hachemi (2007) reported that OC could be included in broilers’ feed up to 15% without detrimental effect on feed intake and FCR. Similar results were also found by (Rabayaa 2000; Abo Omar 2000; Al-Harthi 2017). Also, a broiler diet in mash form containing 20% OC during days 28–49 of age did not affect FCR (Al-Harthi and Attia 2016).

Supplementation with BECP improved European production index by 5.8% compared to unsupplemented control irrespective of OC level without affecting feed intake and FCR. The effect of BECP on production index reflected the improvement in growth rate. The results indicated that the best European production index was from broilers fed OC at 10% and supplemented with phytase during 7–28 days of age. Thus, phytase supplementation to broilers’ diets containing up to 15% OC may be beneficial from a production index point of view (Al-Harthi 2017). It is worth mentioning that the response to the addition of enzymes are affected by many factors, including differences in feed form, age of chickens and the type and dose of enzymes and the presences of target substrates (Choc 2006; Khan et al. 2013; Lee et al. 2017).

**Carcass traits and inner organs**

The results in Table 3 displayed the effects of OC and/or BECP on carcase traits and internal organs of 28-day-old broilers. Inclusion of OC and BECP did not significantly affect weight and percentage of carcase, dressing, heart, pancreas, and caecum. However, the addition of OC to broiler diets at 10 and 15% similarly decreased abdominal fat and liver percentage compared to the control group, regardless of BECP supplementation, but increased gizzard and intestinal proportional. The decrease in the abdominal fat and liver percentage may be due to PUFA fat acids content of OC diets (Dal Bosco et al. 2012; Luciano et al. 2013). Whereas, the reduction in the gizzard and intestinal percentage could be due to the increase in crude fibre in OC diets (Al-Harthi 2017). Likewise, the inclusion of OC in broilers feeds tended to increase gizzard percentage, and phytase decreased it in broilers fed 15% OC (Sateri et al. 2017). In addition, Al-Harthi (2017) and Al-Harthi et al. (2019) reported similar results using multienzymes and phytase addition to broiler diets. In general, the lack of significant influences of OC and BECP indicates that OC up to 15% during 7–28 days of age did not affect muscle growth and inner body organs of broilers (Abo Omar 2005; Al-Harthi 2017; Al-Harthi et al. 2019). Also, BECP

| Treatments          | dressing, % | Abdominal fat, % | liver, % | Heart, % | Pancreas, % | Gizzard, % | Intestine, % | Caecum, % |
|---------------------|-------------|------------------|---------|----------|-------------|------------|--------------|-----------|
| Control diet        | 68.3        | 0.716            | 2.57    | 0.792    | 0.320       | 2.02       | 4.41         | 0.502     |
| Control diet + phytase | 68.9        | 0.711            | 2.23    | 0.734    | 0.294       | 2.10       | 4.03         | 0.574     |
| 10% Olive cake      | 70.0        | 0.674            | 2.16    | 0.731    | 0.296       | 2.42       | 4.70         | 0.540     |
| 10% Olive cake + phytase | 67.9        | 0.640            | 2.13    | 0.690    | 0.326       | 2.47       | 4.67         | 0.532     |
| 15% Olive cake      | 68.3        | 0.676            | 2.22    | 0.726    | 0.320       | 2.55       | 4.58         | 0.562     |
| 15% Olive cake + phytase | 67.9        | 0.708            | 2.29    | 0.699    | 0.310       | 2.21       | 4.39         | 0.552     |

**RMSE**: root-mean-square error.
supplementation to diets containing different levels of OC did not affect the characteristics of carcase and inner body organs.

**Red blood-cell properties**

The results presented in Table 4 showed the effect of OC and/or BECP addition on red-blood-cell characteristics that can reflect the general health status of chicken. The results indicate that the attributes of RBCs, such as the Hgb, PCV, MCH and MCHV, were not significantly affected by OC and/or BECP. However, RBCs was markedly decreased due to feeding 10 and 15% OC diets compared to OC free diets, but MCV increased. In addition, MCV has significantly increased also due to BECP supplementation. These results suggested that up to OC 15% in broiler diets during 7–28 days of age had no adverse effect on indices of the health status. These results are consistent with those published by Al-Harthi and Attia (2016) and Al-Harthi et al. (2019). They indicated that broilers on up to 15% OC diets supplemented with a mixture of different enzymes during 28–49 days of age exhibited no detrimental changes in the RBCs characteristics. Similar results were observed by Dal Bosco et al. (2012) who indicated that 20% OC in rabbits’ feed improved the immunity of rabbits and the defence system against oxidising damage due to polyphenols and vitamin E contents (Luciano et al. 2013; Branciari et al. 2017).

**Lipid metabolites**

The results reported in Table 5 show the influence of OC and/or BECP on blood plasma protein biochemistry. Different concentration of BECP did not significantly affect total plasma lipids, LDL, VLDL and risk of cholesterol. In general agreement, with the present results, Attia et al. (2020) observed that enzymes did not negatively affect the biochemical constituents of blood. On the other hand, plasma triglycerides, and cholesterol were significantly decreased due to phytase supplementation to the 10 and 15% OC diets. Similarly, Al-Harthi (2017) found that phytase supplementation affects lipid metabolism of broilers.

Lipid profile such as plasma total lipids, cholesterol, HDL, LDL, HDL/LDL, VLDL and risk of cholesterol were significantly affected by OC diets. The resulted showed

**Table 4.** The effect of olive cake concentration and/or phytase supplementation on haematological characteristics at 28 days of age of broiler chicks fed a pelleted diet.

| Treatments                  | RBCs, 10^6/μl | Hct, % | Hgb, g/dl | MCV, fl/cel | MCH, pg/cell | MCHC, % |
|-----------------------------|---------------|--------|-----------|-------------|--------------|---------|
| Control diet                | 2.60          | 31.8   | 10.6      | 122         | 40.8         | 33.1    |
| Control diet + phytase      | 2.46          | 33.0   | 11.0      | 134         | 44.7         | 34.1    |
| 10% Olive cake              | 2.41          | 31.8   | 10.6      | 132         | 44.0         | 33.7    |
| 10% Olive cake + phytase    | 2.34          | 32.4   | 10.8      | 139         | 46.2         | 33.5    |
| 15% Olive cake              | 2.45          | 32.4   | 10.8      | 132         | 44.1         | 33.8    |
| 15% Olive cake + phytase    | 2.49          | 34.2   | 11.4      | 137         | 45.8         | 33.4    |

**Statistical analyses**

RMSE: 0.457, 4.69, 1.20, 22.53, 6.71, 4.63; Interaction: 0.154, 0.373, 0.725, 0.518, 0.017, 0.167; Olive cake: 0.005, 0.010, 0.011, 0.016, 0.011, 0.012; Phytase: 0.118, 0.007, 0.172, 0.010, 0.076, 0.192.

RMSE: root-mean-square error. RBC: red blood cells; Hct: haematocrit; Hgb: Haemoglobin; MCV: mean corpuscular volume; MCH: mean corpuscular haemoglobin; MCHC: mean corpuscular haemoglobin.

**Table 5.** Effect of different olive cake concentrations and/or phytase supplementation on plasma lipid metabolites at 28 days of the age of broiler chicks fed a pelleted diet.

| Treatments                  | Total lipids, mg/dl | Triglyceride, mg/dl | Total cholesterol, mg/dl | HDL, mg/dl | LDL, mg/dl | HDL/LDL ratio | VLDL, mg/dl | Risk of cholesterol |
|-----------------------------|---------------------|---------------------|--------------------------|------------|------------|---------------|--------------|-------------------|
| Control diet                | 512                 | 166b                | 199c                     | 67.0       | 98.8       | 0.678         | 33.2         | 2.01              |
| Control diet + phytase      | 513                 | 167b                | 198c                     | 79.0       | 85.6       | 0.923         | 33.4         | 2.31              |
| 10% Olive cake              | 525                 | 174b                | 211a                     | 77.5       | 98.7       | 0.785         | 34.8         | 2.14              |
| 10% Olive cake + phytase    | 517                 | 169b                | 205a                     | 81.6       | 88.4       | 0.923         | 35.0         | 2.32              |
| 15% Olive cake              | 577                 | 175a                | 212a                     | 79.7       | 97.3       | 0.819         | 35.0         | 2.18              |
| 15% Olive cake + phytase    | 560                 | 166b                | 202bc                    | 77.1       | 90.5       | 0.852         | 34.4         | 2.23              |

**Statistical analyses**

RMSE: 102, 3.23, 4.64, 10.42, 12.55, 0.133, 5.41, 0.302; Interaction: 0.171, 0.001, 0.001, 0.315, 0.259, 0.142, 0.162, 0.259; Olive cake: 0.007, 0.052, 0.006, 0.001, 0.001, 0.001, 0.002, 0.324; Phytase: 0.028, 0.354, 0.877, 0.886, 0.616, 0.762, 0.755, 0.186.

Mean values within a column not sharing similar superscripts are significantly different (p < .05). RMSE: root-mean-square error. HDL: high density lipoprotein; LDL: low density lipoprotein; VLDL: very low density lipoprotein; Risk of cholesterol = total cholesterol/LDL-cholesterol.
a beneficial increase in HDL and HDL/LDL ratio, reflecting the increase in PUFA content of OC diet (Luciano et al. 2013; Sateri et al. 2017).

**Calcium, phosphorus and alkaline phosphatase**

The results presented in Table 6 display the influence of OC and/or BECP on plasma calcium, phosphorus, and calcium/phosphorous ratio and alkaline phosphatase. Inclusion OC in broiler diets resulted in a stepwise increase in the plasma phosphorus concentrations, suggesting that OC is a suitable source of available phosphorus for poultry. However, OC as an independent variable significantly decreased plasma Ca, Ca/P ratio and alkaline phosphatase compared to OC free-diets. The decrease in plasma Ca in OC diets have been due to increased fat content of the experimental feeds and thus, the formation of Ca-soaps (Attia et al. 2003).

There was a significant interaction between OC and BECP on plasma calcium, calcium/phosphorus ratio and alkaline phosphatase. It was found that plasma phosphorus was significantly increased due to phytase supplementation to diets with different OC levels. These results indicate that the positive effect of BECP on phytate phosphorus utilisation, maybe due to liberating phosphorus from the phytic acid molecule of main components of the experimental ingredients such as maize, soybean meal and OC. These results are consistent with those reported in previous studies (Chocä 2006; Attia et al. 2012; Attia et al. 2016; Ingelmann et al. 2019).

**Economic efficiency**

The results presented in Table 7 show the influence of OC and/or BECP on economic efficiency traits. Most of the financial parameters were not significantly affected by OC and the interaction between OC and phytase supplementation. However, the total revenue, net return and economic efficiency were increased considerably due to phytase supplementation compared to the unsupplemented control, irrespective of OC level. The results indicate a considerable increase in economic efficiency account to 31.8%. These improvements were maximised when phytase was added to 10% OC diet and minimised when added to 15% OC diet. These results suggested that phytase addition to pelleted diets for broilers containing 10% OC was beneficial.

**Conclusions**

From the present findings, OC represents a valuable ingredient for inclusion in broilers’ pelleted diet up to 15% during 7–28 days of age with phytase addition.

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**Table 6.** The effect of olive cake concentration and/or phytase supplementation on plasma calcium, phosphorus, calcium/phosphorous ratio and alkaline phosphatase at 28 days of the age of broiler chicks fed a pelleted diet.

| Treatments                  | Calcium, mg/dl | Phosphorus mg/dl | Calcium/phosphorus ratio | Alkaline phosphatase, U/L |
|-----------------------------|----------------|------------------|--------------------------|---------------------------|
| Control diet                | 8.40           | 3.70             | 2.27                     | 128                       |
| Control diet + phytase      | 8.55           | 3.88             | 2.20                     | 135                       |
| 10% Olive cake              | 8.18           | 4.02             | 2.03                     | 120                       |
| 10% Olive cake + phytase    | 7.98           | 4.10             | 1.95                     | 133                       |
| 15% Olive cake              | 8.48           | 4.08             | 2.08                     | 118                       |
| 15% Olive cake + phytase    | 8.28           | 4.28             | 1.93                     | 115                       |

**Statistical analyses**

| RMSE       | 0.781 | 0.127 | 0.096 | 9.51 |
| Interaction| 0.181 | 0.001 | 0.075 | 0.299|
| Olive cake | 0.003 | 0.003 | 0.001 | 0.017|
| Phytase    | 0.340 | 0.875 | 0.329 | 0.150|

Mean values within a column not sharing similar superscripts are significantly different ($p < .05$). RMSE: root-mean-square error; U/L: Unit per liter.

**Table 7.** Effect of different olive cake concentration and/or phytase supplementations on economic evaluation of broiler chicks fed a pelleted diet.

| Treatments                  | Feeding cost, SR | Total revenue, SR/chick | Total cost, SR/chick | Net Return, SR/chick | Economic efficiency, % |
|-----------------------------|------------------|-------------------------|----------------------|----------------------|------------------------|
| Control diet                | 2.11             | 6.31                    | 4.85                 | 1.46                 | 30.1$^{ab}$            |
| Control diet + phytase      | 2.22             | 6.86                    | 4.97                 | 1.89                 | 38.3$^{a}$            |
| 10% Olive cake              | 2.15             | 6.22                    | 4.90                 | 1.32                 | 26.8$^{ab}$           |
| 10% Olive cake + phytase    | 2.27             | 7.20                    | 5.02                 | 2.18                 | 43.5$^{a}$            |
| 15% Olive cake              | 2.23             | 6.41                    | 4.97                 | 1.44                 | 29.0$^{b}$            |
| 15% Olive cake + phytase    | 2.33             | 6.67                    | 5.08                 | 1.59                 | 31.3$^{b}$            |

**Statistical analyses**

| RMSE       | 0.237 | 0.459 | 0.237 | 0.361 | 72.3 |
| Interaction| 0.996 | 0.167 | 0.996 | 0.068 | 0.045|
| Olive cake | 0.512 | 0.642 | 0.513 | 0.279 | 0.225|
| Phytase    | 0.163 | 0.001 | 0.163 | 0.001 | 0.001|

Mean values within a column not sharing similar superscripts are significantly different ($p < .05$). RMSE: root-mean-square error; SR: Saudi Riyals.
supplementation. However, the best production index and economic efficiency was found in broilers fed 10% OC and supplemented with 500 FTU of E.coli phytase during 7–28 days of age. In addition, phytase supplementation significantly decreased triglycerides, and cholesterol of diets containing 10 and 15% OC and increased plasma phosphorus concentration, suggesting a positive impact of phytase on biochemical constituents of broilers.

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The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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