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Review

The Effect of Olive Cake, With or Without Enzymes Supplementation, on Growth Performance, Carcass Characteristics, Lymphoid Organs and Lipid Metabolism of Broiler Chickens

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ABSTRACT

An experiment was carried out to investigate the effect of using olive cake (OC) in broiler diets, when it is supplemented with multi-enzymes or phytase enzyme. The OC was included in isocaloric, isonitorgneous diets at 5 and 10% levels and fed to broilers from 1–28 days of age. Experimental diets were fed with or without either of the two enzymes: galzym or phytase. This resulted in 3 OC levels (0, 5, 10%) × 3 enzyme supplementations (no enzyme, galzym enzyme, phytase enzyme). This included nine treatments, and each treatment was replicated eight times with seven broiler chickens each. Feed intake, feed conversion ratio, body weight gain, survival rate, dressing, inner and immune organ´s weights (compared to live body weight); and blood lipids constituents were investigated. According to the findings in this study, it could be concluded that OC is a valuable ingredient and might be included in the broiler diet up to 10% without galzym or phytase enzyme addition. Also, further studies should investigate the possibility of using higher ratios of it or mixed with another by-product in poultry diets; as a very cheap by-product. Moreover, these studies can be associated with suitable additives at different concentrations that might help to increase the utilization of olive cake or at least to keep performance equal to the control. On the other hand, it is worthwhile to follow the positive effect of phytase enzyme on cholesterol and very low density lipoprotein (VLDL) concentrations, which may relate it´s use with chicken´s health.

INTRODUCTION

Agricultural by-products are useful feed resources for feeding livestock including domestic animals (Attia *et al*., 2003; Al-Harthi *et al*., 2009; 2011). These resources can be used as livestock feed supplies and save traditional feed sources, such as grains, for human consumption, and also decrease waste management cost (Molina-Alcaide & Yáñez-Ruiz, 2008). Recently, there has been an increase in the cultivation of olive crop for olive oil production that is used for human food and health benefits (Amici *et al*., 1991; Al-Harthi and Attia, 2016a;b). Olive cake, olive leaves and olive pulp are the by-products of olive oil extraction; however, olive pulp with or without seeds are dominant (Amici *et al*., 1991; Al-Harthi & Attia, 2016 a;b).

The utilization of olive cake (OC) in chicken diets is a useful way for recycling these waste products (Sadeghi *et al*., 2009). These wastes are a rich source of residual oil 6.8% and can be utilized as complementary energy. In addition, unsaturated fatty acids (oleic acid, linoleic acid, linolenic acid) of olive pulp can affect the fatty acids profile in animal tissue (Molina-Alcaide & Yáñez-Ruiz, 2008).

Cell wall contents of olive cake are non-starch polysaccharides (NSPs), that contain xyloglucan and xylan-xyloglucan complexes (Coimbra *et al*., 1995), and glucuronoxylans with a xylose/glucose ratio of 7:1 (Domingues, 2002), and that have anti-nutritive influence on monogastric animals, e.g. poultry and pigs (Coimbra *et al.,* 1995). However, it is well known that NSPs are not digestible by intestinal enzymes of chickens (Petersen *et al.,* 1999).

During the last two decades, the use of commercial enzymes to promote nutrient utilization by poultry has been accepted; this is due to the potential of multi-enzymes in improving the performance of poultry that are fed corn-soybean meal containing agro-industrial by-products products (Cowieson, 2010). The positive effect of multi-enzymes has been attributed in literature, to increasing nutrient digestibility, improving nutrient utilization, decreasing nutrient waste and limiting anti-nutritional factors (Attia *et al*., 2003) and improving gut ecology (Cowieson, 2010; Attia *et al*., 2014b).

The use of OC in broiler chicken´s nutrition was recommended at about 5–10% (Abo Omar, 2005; Rabayaa *et al.,* 2001; El Hachemi *et al.,* 2007; Al-Harthi & Attia, 2016a;b). These authors concluded that using olive cake (the raw material after olive oil extraction) did not negatively affect organ and gastrointestinal tract weights, carcass cuts, carcass fractions and dressing percentage; however, feeding 10% OC resulted in the heaviest average live body weight. Also, a positive effect of using enzymes on feed intake, feed conversion ratio and the utilization of energy and protein in broilers was evident (Hajati, 2010; Attia *et al*., 2014a).

On the other hand, it is well known that phytase enzyme is not excreted by the poultry digestive tract, and that about 65% of the phosphorus element in plants is found in the phytic acid compound as suggested by National Research Council (NRC, 1994). This phytic acid acts as an anti-nutritional component and, in turn, reduces the utilization of feed (Ceylan *et al.,* 2012). Therefore, many researchers have used phytase enzyme in poultry diets, and some of them have concluded that this enzyme increases the utilization of phytic acid in poultry diets (Tiwari *et al.*,2010).

This study aims to investigate the effect of various concentrations of olive cake with or without supplementation of multi-enzymes or phytase enzyme on broiler performance, carcass characteristics, inner and immune organs, and blood lipids constituents.

MATERIALS AND METHODS

Chicks, experimental design and diets

This research was carried out in the Hada Al-Sham Research Station, College of Meteorology, Environment and Arid Land Agriculture, King Abdulaziz University, Jeddah, Saudi Arabia. Olive cake, the residual material after oil extraction by screw press, including pulp and stones, was transported from Al-Jouf area to the Hada Al-Sham Research Station. Then, it was distributed on the floor of a big room (10×5 m), which has four big fans, two on each side, to pull warm air from the outside area (environment temperature was about 45 °C) and to push it outside again as a ventilation cycle. Olive cake was continuously stirred until completely dried. Then, it was crushed to dry powder and sieved in order to reach the appropriate size of ~0.5 mm for feeding, and then stored in bags until used in diet formulation. The chemical composition of sundried olive cake (AOAC, 1990) showed, 5.2% CP, 0.79% Ca, 0.90% total phosphorus of which about 0.35 was considered available based on assumption by NRC (1994), 0.03% methionine, 0.15% methionine and cysteine, 0.03% lysine and 6.7 MJ/kg ME. Experimental diets were formulated to be, iso-caloric and iso-nitrogenous diets, which met the nutrient requirements suggested by the NRC (1994). The OC was included at 0, 5 or 10%, and each experimental diet was fed alone or supplemented with galzyme enzyme (multi-enzyme) or phytase enzyme (GE or PE, respectively).

Galzym is a product of Tex Biosciences (P) Ltd., India. Galzym is a multi-enzyme containing cellulase 100.000.000 U/kg, xylanase 1.500.000 U/kg, lipase 10.000 U/kg, amylase 125.000 U/kg, protease 15.000 U/kg, pectinase 30.000 U/kg, arabinase 7.000 U/kg, Phytase 200.000 U/kg, α-galactosidase 10.000 U/kg, and β-glucosidase 10.000 U/kg. The recommended dose by the producer is 0.5 g/kg diet. The phytase enzyme product is Phyzyme XP (Danisco Animal Nutrition, Marlborough, UK), an *Escherichia coli*. derived phytase. The recommended dose by the producer is 0.5-1 g/kg diet.

The aim of using these two ratios (5 and 10%) and no higher is attributed to the high content of fibre in olive cake (14.12%, data not shown), and also to the weak digestive tract of broilers, especially at the early stage of 1 to 28 days of age (Noy & Uni, 2010). Another reason is that this study is the first one to investigate this by-product in Saudi Arabia, where no previous data is available for such ratios.

Thus, a total of 504, 1 day old Ross-308 male broiler chickens, were randomly distributed among nine dietary treatments of three levels of OC (0, 5, 10%) × 3 enzyme additions (0, galzym 0.5 g/kg diet, phytase 1g/kg diet), each treatment consisting of eight replicates of seven chicks per replicate, thus, the experimental chickens are 56 per treatment. The groups were fed experimental diets from 1 to 28 days of age (Table 1).

Table 1 – Calculated and determined compositions of the diets used in this study.

	Olive cake concentration,%		
Ingredients, g/kg	0	5	10
Maize	550.5	477.5	404.5
Soybean meal, 48% CP	373.0	380	387.0
Dried olive cake	0.0	50.0	100
Dical phosphate	19.0	18.5	18.5
Limestone	12.5	11.65	11.0
Sodium chloride, %	3.0	3.0	3.0
Vitamin+mineral premix[1]	1.0	1.0	1.0
DL-methionine	2.5	2.6	2.8
L-lysine	2.0	2.0	2.2
Soybean meal oil	36.5	53.75	70.0
Total	1000	1000	1000
Calculated[2]and determined[3]analysis (g/kg)			
ME, kcal/kg[2]	3086	3091	3088
Dry matter [3]	899	901	900
CP[3]	227.1	225.7	226.2
Lysine[2]	1.30	1.30	1.32
Methionine[2]	5.8	5.8	5.9
Methionine + Cystine[2]	9.2	9.2	9.3
Ether extract[3]	46.7	67.4	83.2
Linoleic acid[2]	38.4	49.3	59.3
Crude fibre[3]	38.2	44.2	50.2
Ash[3]	119.3	116.9	112.5
Ca[2]	10.1	9.99	10.0
Available phosphorus[2]	4.96	4.93	4.98

[1]Vit+Min mix. provides per kilogram of the diet: vit. A 1.700 IU, vit. E 13 IU, vit. K1 mg, vit. D3 250 ICU, riboflavin 4 mg, pantothenicacid 12 mg, niacin 40 mg, choline 1.500 mg, vit. B12 0.02 mg, vit. Pyridoxine 4 mg, thiamin 2 mg, folacin 1 mg, biotin 0.2 mg. Trace minerals (mg/kg of diet): Mn 70, Io 0.4, Zn 50, Fe 90, Cu 10, and Se 0.2 mg. [2]Calculated values. [3]Analysed values.

*Calcium and phosphorus were adjusted according to phytase equivalent when phytase was supplemented, and the diet was supplemented with sand to compensate the difference in dicalcium phosphate.

Husbandry of chickens

All the procedures were approved by the department committee that recommends animal rights, welfare and minimal stress, that is subjected to, the Government Law No. 9 in 24-8-2010, about the regulations of research ethics on living creatures. The chicks were housed in floor pens (1×1 m) under similar managerial and hygienic conditions, furnished with wood shavings as padding material. Water and mash diets were offered *ad libitum* over the experimental period (1–28 d). The brooding temperature was 33, 29, 26 and 23 °C during the first, second, third and fourth weeks of age, with 45% RH. The chickens were illuminated with a 23:1 light/dark cycle.

Collection of data

Body weight and feed intake were recorded weekly and presented for the whole period (1–28 days), then the feed conversion ratio was calculated. Also, the survival rate was recorded during the entire period. At the end of the experiment (28 days), eight chickens from each treatment were randomly taken and sacrificed to determine carcass characteristics and inner organs ratios to live body weight. In addition, lymphoid organs including spleen and Fabricius bursa were removed, weighed, and finally, the ratio to live weight was calculated.

In addition to that, eight blood samples from each treatment were collected to determine blood lipids constituents, where 5 ml was collected from the brachial vein into heparinized tubes. Plasma was collected after blood centrifugation at 1500xg for 20 min. The blood plasma lipids profile was determined using commercial diagnostic kits (Diamond Diagnostics company, USA) as cited by Al-Harthi *et al*., (2009) and Attia *et al*., (2014a).

Statistical analysis

Data were analyzed using the GLM procedure (SAS, 2003) using two-way factorial analyses according to the 2-factors randomized complete block design of the experiment (3×3). The replicate was the experimental unit. Before analysis, all percentages were subjected to logarithmic transformation (log10 x + 1) to normalize data distribution. Differences between means were tested using Student-Newman-Keuls test at $p \leq 0.05$ (SAS, 2003). Interactions were only presented and, in case of significance, discussed.

RESULTS

Productive performance

Table 2 shows the effects of OC level and different enzymes addition on body weight gain (BWG), final body weight and survival rate of broilers raised between 1 and 28 days of age. Neither the increasing OC level nor enzymes addition had an effect on the previous observations.

The effect of OC level and different enzymes addition on feed intake (FI) and FCR is shown in Table 3. There was a significant interaction in feed intake, which revealed that using 5% OC + GE (GE, galzyme enzyme) led to a significant increase in feed intake

Table 2 – The effect of olive cake level, galzym and phytase addition on the body weight gain, final body weight and survival rate of broiler chickens raised during the period of 1–28 d of age.

Observations Treatments	Initial body weight, g	Body weight gain 1-28 d of age, g	Final body weight 28 d of age, g	Survival rate 1–28 d of age,%
Interaction among olive cake (OC%) and enzymes supplementation				
Control	45	1070	1115	95.1
Control with Galzym, 0.5 g /kg	47	1048	1095	97.8
Control with Phytase, 1 g/kg	44	1073	1117	95.6
5 OC	45	1105	1150	95.6
5 OC with Galzym, 0.5 g /kg	45	1143	1188	100.0
5 OC with Phytase, 1 g/kg	46	1067	1113	97.8
10 OC	48	1088	1136	95.6
10 OC with Galzym, 0.5 g /kg	46	1131	1177	97.8
10 OC with Phytase, 1 g/kg	47	1090	1137	100.0
SEM	1.48	21.18	21.99	1.59
Interaction	0.854	0.339	0.373	0.397

OC= olive cake, SEM = standard error of mean.

compared with the counterpart group on 10% OC. No differences were noted elsewhere among parallel groups, as a result of elevating the OC level. Also, no significant effect was recorded as a result of applying different enzymes inside each OC level.

The effect of the OC level revealed that FCR was significantly improved for the group fed on 10% OC + GE compared to the other respective groups (GE, but with 0 or 5% OC). Within the OC level, the FCR for the chickens on an OC-free and no enzyme addition diet was better than the FCR for the chickens on the same diet but given GE. Also, it was significantly better with group fed 10% OC + GE than for those on the same OC level but given PE (PE, phytase enzyme). However, no other significant effects were obtained due to increasing OC level or enzymes addition among counterpart groups.

Table 3 – The effect of olive cake level, galzym and phytase addition on feed intake and feed conversion ratio of broiler chickens raised during the period of 1–28 d of age.

Observations Treatments	Feed intake 1–28 d of age, g	Feed conversion ratio 1–28 d of age
Interaction among olive cake (OC%) and enzymes supplementation		
Control	1514^{b}	1.42^{bcd}
Control with Galzym, 0.5 g /kg	1607^{ab}	1.53^{a}
Control with Phytase, 1 g/kg	1547^{b}	1.44^{abc}
5 OC	1580^{ab}	1.43^{bc}
5 OC with Galzym, 0.5 g /kg	1663^{a}	1.46^{abc}
5 OC with Phytase, 1 g/kg	1593^{ab}	1.49^{ab}
10 OC	1512^{b}	1.39^{cd}
10 OC with Galzym, 0.5 g /kg	1499^{b}	1.33^{d}
10 OC with Phytase, 1 g/kg	1578^{ab}	1.45^{abc}
SEM	29.93	0.028
Interaction	0.002	0.025

$^{a-d}$ means within a column not sharing similar superscripts are significantly different; OC= olive cake, SEM = standard error of mean.

Dressing percentage and inner body organs ratios

Data in Table 4 indicate the effect of the OC level and enzymes types on the dressing percentage and inner body organs ratios (abdominal fat, liver and heart) to live body weight. Neither increasing OC level nor enzymes addition had an effect on the previous observations. Similar results were found with the pancreas, gizzard, intestinal and cecum ratios to live body weight (Table 5).

Immune organs ratios

Table 6 shows the data of immune organs ratios (spleen and bursa) to live body weight. Neither increasing OC level nor enzymes addition had an effect on the previous observations.

Blood lipids constituents

Data in Table 7 displays the influence of OC level and enzymes addition on blood lipids constituents (triglycerides, cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL), HDL/LDL ratio and VLDL). However, there was a significant effect on triglycerides, cholesterol and VLDL.

Not considering to enzymes addition, triglycerides were significantly higher with chickens on 5 or 10% OC diets compared with those on OC-free diets. However, no significant differences were noted within the OC level.

Cholesterol was lower with chickens on the control diet and no enzyme supplemented, compared with the counterparts, but on 10% OC diet. Within GE groups, cholesterol was lower for those on an OC-free

Table 4 – The effect of olive cake level, galzym and phytase addition on dressing and inner organs ratios to live body weight of broiler chickens raised during the period of 1–28 d of age.

Observations Treatments	Dressing, %	Abdominal fat,%	Liver, %	Heart, %
Interaction among olive cake (OC%) and enzymes supplementation				
Control	68.06	0.356	2.20	0.618
Control with Galzym, 0.5 g /kg	69.10	0.584	2.27	0.677
Control with Phytase, 1 g/kg	67.97	0.224	2.26	0.721
5 OC	70.28	0.542	2.11	0.765
5 OC with Galzym, 0.5 g /kg	68.86	0.696	2.45	0.753
5 OC with Phytase, 1 g/kg	70.45	0.912	2.30	0.712
10 OC	69.88	0.498	2.11	0.700
10 OC with Galzym, 0.5 g /kg	67.70	0.458	2.20	0.759
10 OC with Phytase, 1 g/kg	68.58	0.432	2.29	0.626
SEM	0.675	0.094	0.059	0.006
Interaction	0.312	0.103	0.291	0.417

OC= olive cake, SEM = standard error of mean.

Table 5 – The effect of olive cake level, galzym and phytase addition on inner organs ratio of broiler chickens raised during the period of 1–28 d of age.

Observations Treatments	Pancreas, %	Gizzard, %	Intestinal, %	Cecum, %
Interaction among olive cake (OC%) and enzymes supplementation				
Control	0.300	1.77	4.24	0.572
Control with Galzym, 0.5 g /kg	0.300	1.69	3.96	0.508
Control with Phytase, 1 g/kg	0.306	1.64	4.34	0.588
5 OC	0.314	1.79	4.20	0.498
5 OC with Galzym, 0.5 g /kg	0.328	1.96	4.02	0.560
5 OC with Phytase, 1 g/kg	0.312	1.58	4.01	0.602
10 OC	0.320	2.11	3.82	0.648
10 OC with Galzym, 0.5 g /kg	0.311	2.17	4.06	0.484
10 OC with Phytase, 1 g/kg	0.307	2.06	3.70	0.63
SEM	0.069	0.088	0.209	0.041
Interaction	0.113	0.565	0.696	0.323

OC= olive cake, SEM = standard error of mean.

Table 6 – The effect of olive cake level, galzym and phytase addition on immune organs ratio of broiler chickens raised during the period of 1–28 d of age.

Observations Treatments	Spleen, %	Bursa, %
Interaction among olive cake (OC%) and enzymes supplementation		
Control	0.098	0.203
Control with Galzym, 0.5 g /kg	0.100	0.243
Control with Phytase, 1 g/kg	0.290	0.294
5 OC	0.099	0.174
5 OC with Galzym, 0.5 g /kg	0.092	0.175
5 OC with Phytase, 1 g/kg	0.118	0.228
10 OC	0.096	0.249
10 OC with Galzym, 0.5 g /kg	0.100	0.279
10 OC with Phytase, 1 g/kg	0.138	0.244
SEM	0.038	0.023
Interaction	0.278	0.379

OC= olive cake, SEM = standard error of mean.

diet compared with its counterpart groups, and no differences were noted among PE groups.

Within the OC level, the cholesterol of chickens on 5% OC + GE was higher compared to those on the same diet and no enzyme added, which in turn, was greater than the PE group, while at 10% OC, it was lower for the group fed on PE than the other groups.

Chickens on the control diet (OC-free diet and no enzyme supplemented) had lower VLDL when compared with groups on a similar diet but with 5 and 10% OC included. A similar trend was noted with those groups fed GE, and no differences were detected for groups fed PE.

VLDL at 5% within the OC level was greater with those fed GE compared to PE, and at 10% OC, it was lower with those on PE than other groups.

DISCUSSION

The results indicate that including OC up to 10% without enzyme addition in broiler diets did not adversely affect broiler performance (BWG, final BW, survival rate, FCR; Tables 2 and 3). This was confirmed by dressing, inner and immune organs ratios, which

Table 7 – The effect of olive cake level, galzym and phytase addition on blood lipids constituents of broiler chickens raised during the period of 1–28 d of age.

Observations Treatments	Triglyceride, mg/dl	Cholesterol, mg/dl	HDL, mg/dl	LDL, mg/dl	HDL/LDL ratio	VLDL, mg/dl
Interaction among olive cake (OC%) and enzymes supplementation						
Control	165[b]	197[c]	36.38	83.55	0.406	76.75[c]
Control with Galzym, 0.5 g /kg	167[b]	196[cd]	34.63	83.88	0.413	77.13[c]
Control with Phytase, 1 g/kg	166[b]	196[cd]	32.50	82.50	0.395	80.63[bc]
5 OC	177[a]	199[bc]	34.50	78.75	0.438	85.63[ab]
5 OC with Galzym, 0.5 g /kg	174[a]	203[a]	34.25	81.50	0.421	87.50[a]
5 OC with Phytase, 1 g/kg	176[a]	193[de]	35.25	77.38	0.456	80.63[bc]
10 OC	174[a]	202[ab]	35.50	81.25	0.437	84.75[ab]
10 OC with Galzym, 0.5 g /kg	175[a]	202[ab]	35.50	82.00	0.433	84.88[ab]
10 OC with Phytase, 1 g/kg	176[a]	196[cd]	37.13	79.75	0.466	79.13[c]
SEM	0.893	1.33	0.767	1.04	0.011	1.98
Interaction	0.003	0.001	0.126	0.659	0.587	0.0387

[a-e] means within a column not sharing similar superscripts are significantly different; OC= olive cake, SEM = standard error of mean.HDL = high density lipoprotein, LDL = low density lipoprotein, VLDL = very low density lipoprotein.

were not significantly different among groups fed 0, 5 and 10% OC (Tables 4, 5 and 6). This indicates that, iso-caloric and iso-nitrogenous diets, containing up to 10% OC¸ provide adequate nutrients for growth performance, carcass characteristics, and inner, and lymphoid organs.

However, feed intake was significantly increased with the group fed 5% OC + GE compared to the counterpart group, on 10% OC. This could be explained by the higher fibre content in the later diet compared with 5% OC diet (50 versus 44 g/kg, respectively; Table 1). It is well known that the poultry´s digestive tract lacks the enzymes that can deal with fibre (Petersen *et al.*, 1999). This will lead to the slow movement of feed through the digestive tract, which in turn increases the time required for offering space to new meal, and finally leads to less feed intake (Savory & Hodgkiss, 1984).

This also might elucidate that the concentration of GE 0.5 g/kg, which contains mixed enzymes as: cellulase, xylanase, pectinase, arabinase, α-galactosidase and β-glucosidase that are responsible for non-starch polysaccharides digestion, was sufficient for 5% level but not for 10% OC.

Furthermore, this confirms, to some extent, the positive role of GE as a multi-enzyme for improving digestion for all different ingredients found in the diet (carbohydrates, proteins, lipids), where feed intake was also higher for the group fed on 5% OC and supplemented with GE¸ than the control diet (OC-free diet without enzyme supplementation).

Other possibilities for increasing feed intake with 5% OC + GE diet than the control diet (OC-free diet without enzyme supplementation) are firstly, the flavour, rich and valuable nutrients that compose OC, such as, polyphenols, essential amino acids, essential fatty acids and important elements (data not shown), as well as the difference in ingredient´s composition such as soybean meal oil (e.g. 37 and 54 g/kg in OC-free diet and 5% OC + GE diet, respectively), which is an important factor. An increase in energy intake in sheep fed high-polyphenolic Lotus, and this was attributed to the role of polyphenolic in decreasing condensed tannin content by the binding with the latter (Barry & Duncan, 1984).

Secondly, the effect of energy utilisation on feed intake is another suggestion (Leeson et *al*., 1996), since this might help broilers on the control diet (OC-free diet without enzyme supplementation) to get the energy required by less feed intake in grams, as fibre is lower with this group compared with the group on 5% OC + GE, as mentioned above.

The positive effect of PE on feed intake was also noted. In general, it helped to increase feed intake to some extent, and could keep the amount intake between no enzyme supplementation and those with GE diets, particularly, at 0 and 5% OC levels, and it´s effect on FI was better than GE at 10% OC; however, these differences were not significant.

FCR, seemingly, was affected by different factors, such as, feed intake and diet composition. The best FCR was recorded with chickens fed on 10% OC + GE (1.33), where a lower amount of feed intake was recorded with this group (1499 g), too. This could be attributed to the negative effect of fibre in OC, where it was 50 g/kg. As mentioned above, this fibre leads to a decrease or slowing of the motion of digesta that, in turn, will provide a greater chance for different enzymes to digest feed, or lead to greater absorption or synergistic action; and finally, more utilization of feed will occur (Zurpizal *et al*., 1993).

However, the FCR with the control group (OC-free diet without enzyme supplementation) was 1.42, which was not significantly different from 10 OC + GE group; this could be explained by the diet composition, where fibre is low (38 g/kg) with this group, which leads to good utilization of feed (Attia *et al*., 2014a;b). It is worth noting that these improvements in FCR among different treatments finally resulted in similar weight gain (Table 1).

However, by looking to the complete picture of feed intake, FCR and weight gain, it could be concluded that the inclusion of OC up to 10% in an isocaloric and isoproteic diet did not negatively affect the performance of broilers, and this can be achieved without any enzyme addition. This conclusion was confirmed by similar weight gain between broilers fed 10% OC diet and the control diet (based on maize and soybean), both without enzyme supplementation, and by the lack of a negative effect of using this ratio of OC on survival rate, carcass characteristic (dressing percentage), inner and immune organs ratios to live body weight.

No effects of multi-enzyme and phytase enzyme addition on carcass characteristics of poultry were previously reported (Attia *et al*., 2014a).

Triglyceride values were significantly increased in all 5% OC and all 10% OC diets compared with all OC-free diets. Also, the OC level and different enzymes supplementation affected cholesterol values significantly, where this component, within no enzyme addition diets, was increased at 10% OC compared with OC-free diet; a similar trend was noted with GE groups at 5 and 10% OC. However, this effect was not noted with PE groups, which had similar cholesterol values. Similar findings, for cholesterol, were noted with VLDL.

By looking at the previous results, and generally, it could be concluded that there is an increase in triglycerides with 5 and 10% OC groups, cholesterol and VLDL also increased as OC level increased, except the PE groups. This might be attributed to the increase in soybean meal oil in the diet as OC level increases (37 g/kg in OC-free diet, 54 g/kg in 5% OC diet and 70 g/kg in 10% OC diet; Table 1) and, in turn, an increase in intake of saturated fatty acids also occurs. Soybean oil contains saturated fatty acids (SFA) such as palmitic, stearic, arachidic and behenic (Martin *et al*., 2008), and the ratio of the SFA amount to 18.26% of its oil (Chowdhury *et al*., 2007). Also, saturated fatty acids in OC (data not shown) cannot be ignored, which possibly played a synergistic role. Thus, the content of fatty acids in the diet could play an important role in modifying plasma lipid fractions.

However, it is worth mentioning that phytase enzyme led to cholesterol and VLDL concentrations to remain low in all OC levels. As it is known and was previously mentioned, phytic acid compound is the target of phytase enzyme. Phytic acid (known as inositol hexakisphosphate (IP6)) is a complex compound, which correlates with proteins and metal elements such as Ca, Zn, Mn, Fe, Na and Cu (Coulibaly *et al*., 2011; Sebastian *et al*., 1998; Vohra *et al*., 1965).

When phytase enzyme acts on phytic acid, the availability and the utilization of these nutrients for the chicken´s increases (Sebastain *et al*., 1998; Ceylan *et al*., 2012; Cowieson, 2010). Recently, it was reported that using organic elements such as Zn, Mn, Cu and Se in broiler breeders reduced plasma triglycerides, cholesterol, LDL and VLDL (Qiujuan *et al*., 2012), and it was explained by the role of organic minerals in lipid metabolism by regulating lipoprotein synthesis. Moreover, it was shown that using phytase enzyme in laying hen´s diets resulted in less egg yolk cholesterol and better egg shell (Zyla *et al*., 2012); this was attributed to many factors such as phosphorus availability, Ca/P balance and the role of iron element in fatty acids absorption.

Hence, it could be the explanation for the reduction in plasma cholesterol and VLDL; however, this was not the case with triglycerides concentration, which was high at 5 and 10% OC. This effect of phytase enzyme could be a good subject for further studies, which, possibly, relate its function with chicken´s health.

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