J. Basic Sci. Appl. Res., 1(4)1-10, 2016

ISSN 2356-8879
Journal of Basic Sciences and Applied Research
www.textroad.com

Physiological Responses and Semen Characteristics of Male Chickens Fed Olive Cake under South Sinai Conditions

Amal M. Hassan[1]; Morsy[1*], A.S.; Emam[2], K. R. S. and Abd El-Galil[3], K.

[1]Animal and Poultry Physiological Department, Desert Research Center, Egypt
[2]Animal Production Department, Fac. of Agric., Beni Suef Univ., Egypt
[3]Animal and Poultry Nutrition Department, Desert Research Center, Egypt

ABSTRACT

The present study was conducted to investigate the effects of diets inclusion of olive cake (OC) and enzyme supplementation on semen quality and blood constituents of Mamora cocks under South Sinai conditions. The olive cake was included at 0, 8 and 16 %. The diets were also supplemented with 0 and 0.05 % Allzyme SSF. A total number of 48 cocks were randomly distributed among the 6 treatments (8 cocks / treatment). Semen quality characteristics showed significant decrease in the cocks fed 16 % OC than that of the control diet (0.0 % OC). On the other hand, ion hydrogen (pH) was increased (P<0.05) in the cocks fed 8 and 16 % OC as compared with the cocks fed 0 % OC. Red blood cells (RBC's) count and hemoglobin concentration (Hb) were significantly decreased in the cocks fed 16 % of olive cake (OC) than that of the control diet. Albumin (Alb), albumin/globulin ratio (A/G ratio) and cholesterol values were significantly increased in the cocks fed 8 and 16 % of OC than that of the control diet. In contrary, cocks fed 16 % OC showed decrease (P<0.05) globulin (Glo) as compared with the cocks fed 8 and 0 % OC. Semen quality characteristics were significantly improved in the cocks fed Allzyme SSF (0.05 %) as compared to control group. RBC's, Hb TP, Alb and Glo values were increased (P<0.05) in the cocks fed Allzyme SSF (0.05 %) as compared to the control group. In opposite, cholesterol and aspartic transaminase (AST) concentrations were significantly decreased in the cocks fed Allzyme SSF compared to control group. Results showed that supplementation of Allzyme SSF in the diets of cocks fed 16 % OC significantly improved semen quality characteristics and increased count of RBC's.
It could be concluded that, the addition of 8% OC with or without Allzyme SSF supplementation to the diets of cocks might be the avenue to reduce the gap of feed stuff. Moreover, supplementing the 8 and 16 % OC diet with 0.05% Allzyme SSF may improve cock's performance.
KEY WORDS: Cocks, olive cake, semen quality, blood constituents

INTRODUCTION

Feeding agricultural by-products to livestock advantages by less dependency of livestock on grains that can be consumed by humans and the reduction of costs related to waste management.

Olive cake is the remainder of olive cake (the raw material resulting from extraction of olive oil) after the removal of the seed fractions. It can be achieved by sieving the dry olive cake (OC) to separate most of the seeds. About 0.3 of cell wall fraction will be removed by sieving [1- 2]. Olive cake could be successively and safely included up to 12 % in laying hens diets without adversely affecting productive performance and blood constituents under South Sinai conditions [3].

Olive cake is considered as a good source of calcium, copper and cobalt but poor in phosphorus, magnesium and sodium and with fair levels of manganese and zinc [4]. [5] reported that olive cake tended to have low levels of lysine, methionine and histidine. Indeed, the olive mill waste could be of particular interest in chickens for its level of residual oil (6.8%), this can constitute a complementary energy source and for its particular composition of unsaturated fatty acids (62.4% of oleic acid, 18.2% of linoleic acid, 1.1% of linoleic acid and 2.7% of palmitoleic acid) which could influence the accumulation of fatty acid in the various body compartments during the animal's life and as such could have a certain impact on the positive animals performance and meat quality [6].

The utilization of olive by-products as animal feed is a good way of recycling these waste products. However, presence of xyloglucan (non-starch polysaccharides) on olive cake cell walls which has anti-nutritive effects on poultry [7 – 8]. Hence, there is a need to formulate Optimized rations for different animal uses to avoid metabolic disorders caused by the unbalanced rations of energy and protein and to reduce the tasty factors which might limit feed intake and then the animal performance that leads to low profitability such as high percentage of raw fiber (27–41%), tannin and phenol [9].

* **Corresponding Author:** Amal M. Hassan Morsy, Animal and Poultry Physiological Department, Desert Research Center, Egypt. *Email: alisaber_drc@yahoo.com

Concerns associated with olive cake as a feedstuff for livestock are the amounts of total-extractable tannins (approximately 10 percent) and total-extractable polyphenols (approximately 14 percent) because these compounds might reduce feed intake and/or animal performance. Moreover, their nutritive elements are masked with the occurrence of anti – nutritional factors such as tannin which lead to significant physiological and biochemical alterations in the body of livestock animals [10].

The poultry feed industry has greatly increased its use of exogenous enzymes. Adding the appropriate exogenous enzymes to the feed can improve utilization of nutrients from the feed, thereby decreasing feed cost, improving bird performance, and decreasing the environmental impact of manure application to land [11]. Allzyme SSF is a natural complex that improves profitability through maximizing nutrient release. It is produced by solid-state fermentation using a carefully selected strain of *Aspergillus niger* that allows flexibility in feed formulation through the inclusion of by-products and alternative raw materials. Allzyme SSF which is characterized by a 21% CP and 2.6 % Fat and 7 types of enzymes (proteases, amylases, xilanases, beta-glucanases, pectinases, celluloses and phitases). Diets containing Allzyme SSF can be reformulated through reduction its energy value by 75 kcal and Ca and P content by 0,1 % reducing its cost with production performances at a high level [12].

Numerous trials have been conducted to investigate the use of Allzyme SSF in poultry diets [12, 13, 14, 15, 16, 17].

Therefore, this study aimed to investigate the effects of diets inclusion of olive cake and enzyme supplementation on semen quality, physiological responses and blood constituents of Mamora cocks.

MATERIALS AND METHODS

The present experiment was carried out at South Sinai Experimental Research Station (Ras Sudr) which belongs to the Desert Research Center. The experiment started in June to September 2012.

Olive cake was collected from local olive processing factory during summer 2012 at Ras-Suder city then transported to experimental research station (Ras-Suder city). Crude olive cake: The residue of the first extraction of oil from the whole olive by pressure. The chemical analysis of the tested by product was as follows: 91.5 % DM, 7.02 % CP, 40.27 % CF, 10.07 % EE, 13.63 % Ash, 29.01 % NFE and 86.37 % OM.

A total number of 48 Mamora cocks (22 weeks old and body weight of 2322.5 ± 30.5 g) were used until 34 week of age. The olive cake was added at levels of 0, 8 and 16 %. The diets were also supplemented with 0 and 0.05 % Allzyme SSF. This resulted in a factorial experimental design of 3 (olive cake %)× 2 (Allzyme SSF %), or 6 treatments. Then 48 cocks were randomly distributed into the 6 treatments (8 cocks/ treatment). The experimental diets were formulated in granular form according to NRC [18] in Table (1).

Table (1). Composition and calculated analysis of the experimental diets

Ingredients (%)	0 %	8 %	16 %
Olive cake meal	0.00	8.00	16.00
Yellow corn	62.10	58.50	53.00
Soybean meal (44 % CP)	14.65	18.20	16.50
Corn gluten meal (60 % CP)	5.00	3.30	4.60
Wheat bran	8.25	2.10	0.00
Limestone ground	2.20	2.20	2.10
Dicalcium phosphate	1.70	1.70	1.70
Vit. and min. premix*	0.30	0.30	0.30
Salt	0.20	0.10	0.20
DI-methionine	0.28	0.28	0.29
Sand	5.6	5.6	5.6
Total	**100**	**100**	**100**
Calculated values**			
Crude protein	16.11	16.04	16.01
Crude fiber	3.32	3.06	5.09
Ether extract	3.29	2.91	3.11
Ash	2.30	2.65	3.11
Metabolizable energy (kcal/kg)	2700	2700	2700
Calcium (%)	1.25	1.24	1.25
Available phosphorus (%)	0.46	0.43	0.48
Methionine (%)	0.60	0.60	0.60
Lysine (%)	0.71	0.78	0.72
Methionine+ Cyc (%)	0.87	0.88	0.81
Cystine	0.28	0.27	0.26

* Each 2.5 kg Vitamins and minerals premix contains (per ton of feed), Vit. A 10000000 IU, Vit. D_3 2000000 IU, Vit.E I0g, Vit.K_3 1000 mg, Vit. B_1 1000 mg, Vit. B_2 5000mg, Vit. B_6 1.5g, Vit. B_{12} 10 mg, Pantothenic acid 10g, Niacin 30g, Folic acid 1g, Biotin 50 mg, Iron 30g, Manganese 70g, Choline chlorite 10g, Iodine 300 mg, COCper 4g, Zinc 50g and Selenium 100 mg.

** According to NRC (1994).

All groups were reared under month's condition (average maximum indoor ambient temperature, relative humidity and temperature-humidity index were 36.7 ºC, 25.5 % and 31.4, respectively).

Experimental cocks were housed in wire cages of triple deck batteries. Birds were exposed to natural day- light and provided with artificial light to increase the day light length until reaching 14 h. at 18 weeks of age. Then, the day light length period was increased 30 minutes every other week until fixed at 16 h. daily from 30 weeks of age to the end of experiment (34 weeks). Birds were kept under the same managerial and hygienic conditions. Birds were examined against diseases and treated with antibiotics and vaccines to keep them healthy.

Blood samples were randomly withdrawn from the wing vein into tube containing EDTA to examine immediately red blood cells counted in blood under the microscope by means of hemocytometer and hemoglobin concentration according to [19]. Hematocrite (%) estimated using microhematocrit tubes by win robe methods. Serum was collected by using centrifugation for 15 minutes at 3000 rpm and it stored at –20 ˚C until analysis. Blood metabolites (total protein, albumin, cholesterol, triglycerides, alanine transaminase, aspartic transaminase and total antioxidant capacity) were determined calorimetrically by using commercial kits. Globulin was calculated by the difference between total protein and albumin.

Concentrations of triiodothyronine and testosterone hormones were determined by ELISA method using commercial kits.

Semen was collected from cocks using the massages method. The ejaculate volume was determined to the nearest 0.01 ml using tuberculin syringe. Sperm concentration was determined by using Thomes–Zeis haemocytometer [20]. Total sperm output was calculated by multiplying ejaculate volume and spermatozoa concentration. Percentage of live and abnormal sperms were determined after staining with Eosine and nigrosine [21], then calculated as a percentage out of randomly chosen 100 sperm counted. Percentage of motile sperm was estimated a phase-contrast microscope according to [22]. Total number of motile sperm (TMS) calculated by multiplying percentage of motile sperm and total sperm output. Semen quality factor (SQF), calculated according to the following pattern was used: SQF= (sperm concentration× ejaculate volume× live spermatozoa)/ 100. Hydrogen ion concentration (pH) of semen was determined immediately after collection using pH paper.

Statistical analysis was carried out using General Linear Model (GLM) procedures by SAS [23] using two-way analysis of variance according to this model: $Y_{ijk} = \mu + T_i + Z_j + TZ_{ij} + e_{ijk}$

Where: Y_{ijk} = observations value of the kth animal, μ = overall mean, T_i = effect of ith olive cake (i: 1-3), Z_j= effect of jth enzyme (j: 1-2), TZ_{ij}= the interaction between olive cake and enzyme, e_{ijk} = experimental error.

Significant differences among treatment means were tested using Duncan multiple range test [24].

RESULTS AND DISSCUSION

1. Semen quality

The obtained results revealed that providing olive cake (OC) in cocks diets at level of 8% decreased (P<0.05) ejaculate volume (EV), total motile sperm (TMS), semen quality factor (SQF) and increased (P<0.05) hydrogen ion (pH). While there were any significant differences as a result of adding olive cake in cocks' diets on sperm concentration (SC), live spermatozoa (LS), dead spermatozoa (DS), sperm abnormalities (SA) and the concentration of testosterone hormone (T_2) (Table 1). Moreover, increasing the percentage of OC to 16% in cocks' diet decreased significantly most of the semen quality parameters; EV, TSO, SM, TMS and SQF. However, SC, DS, SA and T_2 did not change significantly (Table 1).

The significant reduction in semen characteristics as a result of increasing the OC percentage up to 16% might be attributed to inhibition in the metabolism of dietary protein. [25] reported a reduction in semen volume of boars when fed with low protein dietary level. Although the dietary protein levels given to the birds in this study were considered adequate, the combined effect of anti-nutritional factors such as tannin and polyphenols in OC may cause a substantial part of the ingested protein unavailable for normal semen production and reduced efficiency of spermatogenesis processes and/or indirectly via hypothalami-pituitary-testis-axis, which controls the reproductive efficiency [26]. [27] and [28] reported that a possible interference with protein metabolism, enzyme activities and hormonal regulation in the cocks by the high tannin and polyphenols contents in the OC.

The high tannin content in the OC might led to adverse effects on the testis and a consequent reduction in the semen characteristics of cockerels [29, 30, 31, and 32].

Regarding the enzyme effect, the obtained results demonstrated that providing Allzyme SSF (0.05%) improved EV, SC, TSO, SM, TMS, LS and SQF significantly by 33.3, 33.0, 77.4, 31.1, 27.3 and 132.6 %, respectively. On the other hand, Allzyme SSF supplementation decreased (P<0.05) DS and SA by 27.3 and 22.6 %, respectively. The improvement recoded in semen quality as a result of

adding Allzyme SSF to the OC diets might be owing to that appropriate exogenous enzymes improve utilization of nutrients from the feed, thereby improving cock performance [33]. Allzyme SSF which is characterized by a 21.0 % CP, 2.6 %, Fat and 7 types of enzymes (proteases, amylases, xilanases, beta-glucanases, pectinases, cellulases, phitases) which may increasing the digestibility of phytate-bound phosphorus, proteins, triglycerides, xylans, hemicellulose, cellulose and starch in diets. [11] indicated that diets containing the enzymatic complex had a marked effect on the spermatogenesis process. However, exogenous fibrolytic enzymes involved in the feed have been shown to improve fiber digestion by cellulolytic ruminal bacteria, microbial growth and production of microbial protein [34].

On the other hand, enzyme providing elevated insignificantly blood testosterone concentration as compared to the non-supplemented ones (Table 1). The beneficial effect of the exogenous enzyme addition might be due to a stimulatory effect of nutrients made available to the animal on testicular steroidogenesis, as improved nutrition enhances testicular functions, stimulating testosterone synthesis [35].

Concerning the interaction between OC and enzyme supplementation, results showed that there were significant differences in EV, TSO, TMS and SQF, while there were no significant for SC, DS, SA, pH and T_2. Supplementation of Allzyme SSF in the diets of cocks fed 16 % OC significantly increased EV, TSO, SM, TMS and SQF comparing with control group (0% OC). These results revealed that the importance role of Allzyme SSF to decrease the deleterious effect of inclusion OC on semen quality.

These actions may be improved the nutrition status of cockerels taking the Allzyme SSF by improved intestinal mucosal development and enhances nutrient digestibility, which in turn promote nourishment of the serotonin cells and seminal fluid that nurse the germ cells. [36] reported that there is a strong correlation between animal nutrition and spermatogenesis processes, sperm maturation and male reproductive system development. Hence, enzyme addition may encourage the availability of macronutrients and micronutrients caused by the action of proteases, amylases and cellulose's essential for the synthesis of diverse components of the spermatozoa. Also, this improved in nutrient availability with the enzyme supplementation may enhance the endocrine activity of gonads, which created a supportive environment for spermatogenesis [11].

Table (1). Effect of feeding different levels of olive cake and enzyme supplementation on semen characteristics and testosterone level (T_2) of Mamora cocks

Items	Enzyme	Olive cake (%)			
	(%)	0	8	16	Overall mean
EV (ml)	0	$0.44^{ab}\pm0.03$	$0.36^{bc}\pm0.03$	$0.10^{e}\pm0.03$	$0.30^{B}\pm0.02$
	0.05	$0.38^{b}\pm0.03$	$0.30^{d}\pm0.03$	$0.51^{a}\pm0.03$	$0.40^{A}\pm0.02$
	Overall mean	$0.41^{A}\pm0.02$	$0.33^{B}\pm0.02$	$0.30^{B}\pm0.02$	
SC ($\times10^6$ml)	0	543.3±79.3	390.0±79.3	493.3±79.3	$475.5^{B}\pm45.7$
	0.05	570.0±79.3	700.0±79.3	627.5±68.6	$632.5^{A}\pm43.8$
	Overall mean	556.6±56.0	545.0±56.0	560.4±52.4	
TSO ($\times10^6$)	0	$239.0^{b}\pm34.2$	$140.4^{bc}\pm34.2$	$49.3^{c}\pm34.2$	$142.6^{B}\pm19.7$
	0.05	$216.6^{b}\pm34.2$	$210.0^{b}\pm34.2$	$320.0^{a}\pm29.6$	$253.0^{A}\pm18.9$
	Overall mean	$228.2^{A}\pm24.1$	$179.8^{AB}\pm24.1$	$168.1^{B}\pm22.6$	
SM (%)	0	$93.3^{ab}\pm5.8$	$73.3^{c}\pm5.8$	$53.3^{d}\pm5.8$	$73.3^{B}\pm3.3$
	0.05	$96.6^{a}\pm5.8$	$96.6^{a}\pm5.8$	$95.0^{a}\pm5.0$	$96.1^{A}\pm3.2$
	Overall mean	$95.0^{A}\pm4.1$	$85.0^{AB}\pm4.1$	$77.1^{B}\pm3.8$	
TMS ($\times10^6$)	0	$222.9^{b}\pm33.6$	$102.9^{d}\pm33.6$	$26.2^{e}\pm33.6$	$104.5^{B}\pm19.4$
	0.05	$209.2^{b}\pm33.6$	$202.8^{bc}\pm33.6$	$304.0^{a}\pm29.1$	$243.1^{A}\pm18.5$
	Overall mean	$216.7^{A}\pm23.7$	$152.8^{B}\pm23.7$	$129.6^{B}\pm22.2$	
SQF	0	$167.3^{b}\pm25.0$	$101.9^{bc}\pm25.0$	$38.2^{d}\pm25.0$	$104.5^{B}\pm14.5$
	0.05	$177.6^{b}\pm25.0$	$166.5^{b}\pm25.0$	$257.9^{a}\pm21.6$	$203.9^{A}\pm13.8$
	Overall mean	$173.4^{A}\pm17.7$	$136.6^{B}\pm17.7$	$132.7^{B}\pm16.5$	
LS (%)	0	70.0±3.0	72.6±3.0	77.5±2.6	$73.3^{B}\pm1.6$
	0.05	82.0±3.0	79.3±3.0	80.6±3.0	$80.6^{A}\pm1.7$
	Overall mean	76.0±2.1	76.0±2.1	79.0±2.0	
DS (%)	0	30.0±3.0	27.4±3.0	22.5±2.6	$26.7^{A}\pm1.6$
	0.05	18.0±3.0	20.7±3.0	19.4±3.0	$19.4^{B}\pm1.7$
	Overall mean	24.0±2.1	24.0±2.1	21.0±2.0	
SA (%)	0	10.6±0.83	8.0±0.83	10.5±0.83	$9.7^{A}\pm0.46$
	0.05	7.3±0.83	7.3±0.83	8.0±0.72	$7.5^{B}\pm0.48$
	Overall mean	9.0±0.59	7.6±0.59	9.2±0.55	
pH	0	7.96±0.03	8.00±0.03	8.11±0.03	8.02±0.02
	0.05	7.93±0.03	8.10±0.03	8.10±0.03	8.04±0.02
	Overall mean	$7.95^{B}\pm0.02$	$8.05^{A}\pm0.02$	$8.10^{A}\pm0.02$	
T_2 (ng/ml)	0	2.5±1.4	2.1±1.3	3.7±1.3	2.8±0.77
	0.05	3.0±1.4	3.2±1.3	5.3±1.3	4.0±0.77
	Overall mean	3.0±1.0	2.7±0.91	4.5±0.91	

EV = ejaculate volume; SC = sperm concentration; TSO = total sperm output; SM = sperm motility; TMS= total motile sperm; LS= live spermatozoa; SQF= semen quality factor; DS= dead spermatozoa; SA = sperm abnormalities; pH = hydrogen ion.

A, B Means of olive cake with different superscripts are significant differences (P<0.05), also means of enzyme supplementation with different superscripts are significant differences (P<0.05).

a,b,c,d,e Means of different experimental sub-groups with different superscripts are significant differences (P<0.05).

2. Hematological parameters

Concerning the olive cake effect, red blood cells (RBC's) was significantly decreased in the cocks fed 8 and 16 % of olive cake (OC) by 14.8 and 33.3 %, respectively, than that of the control diet (Table 2). However, cocks fed 16 % OC had lower (P<0.05) RBC's by 21.7 % as compared with the cocks fed 8 % OC. On the other hand, cocks fed 16 % OC showed decreased (P<0.05) hemoglobin concentration (Hb) by 14.1 and 16.7 % when compared to the cocks fed 8 and 0 % OC, respectively. In contrary, hematocrite % (Ht) was increased (P<0.05) in the cocks fed 16 % OC by 7.4 % as compared to the cocks fed 8 % OC and insignificantly increased by 4.3 % as compared to the cocks fed 0 % OC. These results were in agreement with the results of [37], [38] and [3] where they concluded that the lower RBC count and Hb level for hens fed 16 % OC might be attributed to the more presence of anti-nutritional factors in OC particularly phenols and condensed tannins, that have been reported to have an antinutritional action.

Table (2). Effect of feeding different levels of olive cake (OC) and enzyme supplementation on hematological parameters of Mamora cocks

Items	Enzyme	Olive cake (%)			
	(%)	0	8	16	Overall mean
RBC ($\times 10^6$)	0	$5.4^{ab}\pm0.11$	$4.1^{d}\pm0.11$	$3.3^{f}\pm0.11$	$4.2^{B}\pm0.06$
	0.05	$5.5^{a}\pm0.11$	$5.2^{bc}\pm0.11$	$3.9^{de}\pm0.11$	$4.8^{A}\pm0.06$
	Overall mean	$5.4^{A}\pm0.07$	$4.6^{B}\pm0.07$	$3.6^{C}\pm0.07$	
Hb (g/dl)	0	15.7±1.0	14.7±1.0	11.9±1.0	$14.1^{B}\pm0.59$
	0.05	16.6±1.0	16.5±1.0	14.8±1.0	$16.0^{A}\pm0.59$
	Overall mean	$16.1^{A}\pm0.72$	$15.6^{A}\pm0.72$	$13.4^{B}\pm0.72$	
Ht (%)	0	46.0±0.98	45.6±0.98	48.1±0.98	46.5±0.56
	0.05	45.6±0.98	43.3±0.98	47.5±0.98	45.5±0.56
	Overall mean	$45.8^{AB}\pm0.69$	$44.5^{B}\pm0.69$	$47.8^{A}\pm0.69$	

RBC's = red blood cells; Hb = hemoglobin; Ht = hematocrite %.
A, B, C Means of olive cake with different superscripts are significant differences (P<0.05), also means of enzyme supplementation with different superscripts are significant differences (P<0.05).
a,b,c,d,e,f Means of different experimental sub-groups with different superscripts are significant differences (P<0.05).

Regarding the enzyme effect, RBC's and Hb values were increased (P<0.05) in the cocks fed Allzyme SSF (0.05 %) by 14.2 and 13.4 %, respectively, as compared to the cocks fed 0% Allzyme SSF (control group). On the other hand, no significant differences were observed between the cocks fed Allzyme SSF (0.05 %) and control group in Ht %.

Concerning the interaction between OC and enzyme supplementation, the results showed that the interaction effect was significant in RBC's, while there were no significant interaction for Hb and Ht. Adding Allzyme SSF (0.05 %) in the diets of cocks fed 8 and 16% OC increased significantly the RBC's by 26.8 and 18.1 %, respectively, when comparing with the cocks without adding Allzyme SSF. This improvement in hematological parameters might be attributed to exogenous enzymes used to improve digestibility of a wide range of feed components such as fibre, phytate, protein, etc. Fibre-degrading enzymes break down specially non-starch polysaccharides (NSP), which are large polymers, to smaller polymers to alleviate their anti-nutritive activities [39]. This reflected in better flock performance and improved bird health [40 and [41].

3. Biochemical parameters

Regarding the olive cake effect, albumin (Alb) and albumin/globulin ratio (A/G ratio) values were significantly increased in the cocks fed 8% OC (by 23.8 and 33.3%, respectively) and 16% of OC (by 33.3 and 73.3%, respectively) than that of the control diet (Table 3). In contrary, cocks fed 16 % OC showed a decrease (P<0.05) in globulin (Glo) levels by 19.2 and 25.0 % as compared with the cocks fed 8 and 0 % OC, respectively.

Cholesterol (Cho) levels were significantly increased in the cocks fed 8% OC by 35.9% and by 28.3% in the cocks fed 16% of OC than that of the control diet (Table 3). However, blood cholesterol and total lipids increased significantly for growing lamb fed 25% OC. These results don't agree with the results of [42], [43], [44] and [3], they reported that a low dietary fiber concentration or enzyme supplementation of the diet may increase plasma cholesterol levels [45] and [46].

On the other hand, no significant differences were observed among treatments in total protein (TP), total antioxidant capacity (TAC) and triglycerides (TG) concentrations. These results agreed with [47], [48] and [49].

Table (3). Effect of feeding different levels of olive cake and enzyme supplementation on total protein and its fractions and total antioxidant capacity of Mamora cocks

Items	Enzyme (%)	Olive cake (%) 0	8	16	Overall mean
TP (g/dl)	0	4.7±0.22	4.7±0.22	4.5±0.22	4.6^{B}±0.13
	0.05	5.1±0.22	5.8±0.22	5.3±0.22	5.4^{A}±0.13
Overall mean		4.9±0.16	5.2±0.16	4.9±0.16	
Alb (g/dl)	0	2.2^{de}±0.09	2.3^{d}±0.09	2.7^{bc}±0.09	2.4^{B}±0.05
	0.05	2.0^{e}±0.09	3.0^{a}±0.09	2.8^{ab}±0.09	2.6^{A}±0.05
Overall mean		2.1^{B}±0.06	2.6^{A}±0.06	2.8^{A}±0.06	
Glo (g/dl)	0	2.5±0.25	2.4±0.25	1.8±0.25	2.2^{B}±0.14
	0.05	3.1±0.25	2.8±0.25	2.5±0.25	2.8^{A}±0.14
Overall mean		2.8^{A}±0.17	2.6^{A}±0.17	2.1^{B}±0.17	
A/G ratio	0	0.88±0.15	0.95±0.15	1.5±0.15	1.1±0.09
	0.05	0.64±0.15	1.0±0.15	1.1±0.15	0.92±0.09
Overall mean		0.75^{B}±0.11	1.0^{A}±0.11	1.3^{A}±0.11	
TAC(mM/L)	0	0.49±0.08	0.44±0.08	0.43±0.08	0.46±0.06
	0.05	0.54±0.08	0.59±0.08	0.67±0.08	0.60±0.06
Overall mean		0.51±0.06	0.51±0.06	0.55±0.06	
Cho (mg/dl)	0	106.7±8.7	143.2±8.7	142.6±8.7	130.8^{A}±5.0
	0.05	86.5±8.7	119.4±8.7	105.4±8.7	103.8^{B}±5.0
Overall mean		96.6^{B}±6.2	131.3^{A}±6.2	124.0^{A}±6.2	
TG (mg/dl)	0	291.8±48.2	291.5±48.2	303.2±48.2	295.5±27.8
	0.05	280.7±48.2	263.1±48.2	281.4±48.2	275.1±27.8
Overall mean		286.3±34.1	277.4±34.1	292.3±34.1	

TP = total protein; Alb = albumin; Glo = globulin; A/G ratio = albumin / globulin ratio; TAC = total antioxidant capacity; Cho = cholesterol; TG = triglycerides.
A, B Means of olive cake with different superscripts are significant differences (P<0.05), also means of enzyme supplementation with different superscripts are significant differences (P<0.05).
a,b,c,d,e Means of different experimental sub-groups with different superscripts are significant differences (P<0.05).

About the enzyme effect, TP, Alb and Glo values increased (P<0.05) in the cocks fed Allzyme SSF (0.05%) by 17.3, 8.3 and 27.2%, respectively, as compared to the cocks fed 0% Allzyme SSF (control group). In opposite, Cho concentrations were significantly decreased in the cocks fed Allzyme SSF by 20.6% as compared to control group. On the other hand, no significant differences were observed between the cocks fed Allzyme SSF (0.05%) and control group in A/G ratio, TAC and TG values. Nutritional factors (diet quantity and composition) also affect intermediary metabolism, resulting in the changes of plasma metabolite levels in poultry [50] and [51]. Exogenous enzymes used in the diets to improving nutrient digestibility.

With regard to the interaction between OC and enzyme supplementation, the results showed that the interaction was only significant in Alb concentration. Adding Allzyme SSF (0.05%) in the diets of cocks fed 8% OC increased significantly Alb concentration by 30.4% when comparing to the cocks without adding Allzyme SSF. No adverse effect on some biochemical metabolites may indicate that cocks were in a good health as result of inclusion of wastes supplemented by olive cake cocks.

4. Liver and kidney functions

Regarding the olive cake effect, no significant differences were observed among treatments in ALT, AST and urea concentrations (Table 4). The present results of plasma ALT, AST and urea values for the different experimental treatments are within the normal values for poultry and in agreement with those obtained by [3]. [52] reported that on the basis of the hematological variables and the catalytic activities of serum enzymes, it can be concluded that dehydrated olive cake, when added to the feed at a level of 10% or 20%, exerted no harmful effect on the hematopoietic system or on the catalytic activity of serum enzymes. In other words, it was not detrimental to the health of rabbits during the 56-day fattening period.

Concerning the enzyme effect, AST value was lower (P<0.05) in the cocks fed Allzyme SSF (0.05%) by 13.2% as compared to the cocks fed 0% Allzyme SSF (control group). Moreover, values of ALT and urea were insignificantly decreased in the cocks fed Allzyme SSF by 15.1 and 8.6%, respectively as compared to control group. Blood plasma transaminase enzymes activity (ALT and AST) are the most important indicators of liver cells activity where increasing the concentration of these enzymes indicated that the tissue activity are destroyed [3].

Table (4). Effect of feeding different levels of olive cake and enzyme supplementation on liver and kidney function of Mamora cocks

Items	Enzyme (%)	Olive cake (%) 0	8	16	Overall mean
ALT (I.U./L)	0	25.4±5.5	27.5±5.5	27.9±5.5	27.0±3.2
	0.05	25.7±6.2	20.2±5.5	22.6±5.5	22.9±3.3
Overall mean		25.6±4.1	23.9±3.9	25.3±3.9	
AST (I.U./L)	0	47.4±3.9	49.5±3.9	52.0±3.9	49.7^{A}±2.2
	0.05	45.7±3.9	39.8±3.9	44.0±3.9	43.1^{B}±2.2
Overall mean		46.6±2.7	44.6±2.7	48.0±2.7	
Urea (mg/dl)	0	37.9±2.9	38.8±2.9	44.9±2.9	40.5±1.7
	0.05	39.9±3.2	35.2±2.9	35.4±2.9	37.0±1.7
Overall mean		38.9±2.1	37.0±2.0	40.2±2.0	

Cho = cholesterol; TG = triglycerides; ALT = alanine transaminase; AST = aspartic transaminase.
A, B Means of enzyme supplementation with different superscripts are significant differences (P<0.05).

With regard to the interaction between OC and enzyme supplementation, the results showed that the interaction was insignificant in ALT, AST and urea concentrations. Although, supplementation of Allzyme SSF (0.05%) in the diets of cocks fed 8 and 16% OC decrease insignificantly the values of ALT, AST and urea when comparing to the cocks without adding Allzyme SSF.

5. Triiodothyronine hormone (T_3)

Regarding the olive cake effect and enzyme effect, no significant differences were observed among treatments in T_3 hormone (Table 5). However, value of T_3 was insignificantly increased in the cocks fed Allzyme SSF by 17.3% as compared to control group. The results indicated that the interaction between OC and enzyme supplementation on T_3 hormone was insignificant. This insignificant increase may suggest that enzyme addition directly or indirectly promoted an enhanced activity of deiodinase in liver and kidney tissues, promoting the transformation of T4 into T3 [53] and [54].

Table (5). Effect of feeding different levels of olive cake (OC) and enzyme supplementation on hormonal assay of Mamora cocks

Items	Enzyme (%)	Olive cake (%) 0	8	16	Overall mean
T_3 (ng/ml)	0	2.1±0.43	2.4±0.43	2.3±0.43	2.3±0.25
	0.05	2.3±0.43	3.4±0.43	2.5±0.43	2.7±0.25
Overall mean		2.2±0.30	2.9±0.30	2.4±0.30	

T_3 = triiodothyronine hormone.

CONCLUSION

It could be concluded that, the addition of 8% OC with or without Allzyme SSF supplementation to the diets of cocks might be the avenue to reduce the gap of feed stuff. Moreover, supplementing the 8 and 16 % OC diet with 0.05% Allzyme SSF may improve cock's performance.

Acknowledgements

This research is part of a project of "Utilization of some agricultural by-products and desert plants as non-conventional feedstuffs in poultry diets" which is financially supported by Ministry of International Cooperation, Egypt.

REFERENCES

1. Abo Omar, J. (2000). Effect of different levels of olive pulp on the digestibility of broiler chicks. Bethlehem. Univ. J., 12: 34-40.
2. Abd El-Galil, K. (2001). Utilization of olive pulp meal in feeding growing rabbits. J. Agric. Sci., Mansoura Univ., 26: 727-736.
3. Abd El-Galil, K.; Morsy, A.S.; Emam, K.R.S. and Amal M. Hassan (2015). Physiological responses and productive Performance of laying hens fed olive pulp under South Sinai conditions. International Conference of Agriculture and Environment for Sustainable Development; National Research Center; 25-27 May, 2015; 281-281 (Abstract).
4. Harb, M. (1986). Use of olive cake pulp for fattening Awassi lambs. Dirasat. 13: 37-55.

5. Rabayaa, E.; Abo Omar, J.M and Othman, R.A. (2001). Utilization of Olive Pulp in Broiler Rations. An-Najah Univ. J. Res., 15: 133-144.

6. El-Hachemi, A.; El-Mecherfi, K.E.; Benzineb, K. and Saidi, D. (2007). Supplementation of olive mill wastes in broiler chicken feeding. Afr. J. Biotechnol., 6: 1848-1853.

7. Gil-Serrano, A. and Tejero-Mateo, P. (1988). A xyloglucan from olive pulp, Carbohydr. Res., 181: 278-281. doi:10.1016/0008-6215(88)84048-5.

8. Coimbra, M.A.; Rigby, N.M.; Selvendran, R.R. and Waldron, K.W. (1995). Investigation of the occurrence of xylan-xyloglucan complexes in the cell walls of olive pulp (*Olea eurOCaea*). Carbohydr. Polymers, 27(4): 277-284.

9. Abo Omar, J.M. (2005). Carcass composition and visceral organ mass of broiler chicks fed different levels of olive pulp. Gaza Univ. J., 13: 75-84.

10. D'Mello, J.P.P (1989) Toxic amino acids. In: Anti-nutritional factors, potentially toxic substances in plants. Warwick Association of Applied Biology. Pp 29-50.

11. Gado, H.; Mellado, M.; Salem, A.Z.M.; Zaragoza, A. and Seleem, T.S.T. (2015). Semen characteristics, sexual hormones and libido of Hy-Plus rabbit bucks influenced by a dietary multi-enzyme additive. World Rabbit Sci., 23: 111-120.

12. Ramesh, K.R. and Devegowda, G. (2005). Effect of enzyme (Allzyme SSF TM) on performance, intestinal viscosity and toe ash of broiler chickens fed corn-soybean meal based diets. IPSACON.

13. Njeru, H.K. (2013). Effects of enzyme complex (ALLZYME SSF) and yeast metabolites (DIAMOND V XPC) on performance, immune responses, gastrointestinal morphology and intestinal microbiota in broiler chicken (*Gallus domesticus*). M. Sci., Faculty of Veterinary Medicine, University of Nairobi.

14. Barros A.C.C, F.N. Takata, S.B.P. Lima, B.H.S. Moura and J.E. Neto (2007). Effect of Allzyme SSF and Bio- Mos on the intestinal morphology of broilers. The New Energy Crisis: Food, Feed, or Fuel? Proceedings of Alltech's 23 rd Annual Symposium May 20-23, 2007 Lexington, Kentucky, USA, 2007, p.32.

15. Pierce J.L., T. Ao, B.L. Shafer, A.J. Pescatore, A.H. Cantor and M.J. Ford (2007). Effect of Allzyme SSF on growth performance of broilers receiving diets containing high amounts of distillers dried grains with solubles. The New Energy Crisis: Food, Feed, or Fuel? Proceedings of Alltech's 23rd Annual Symposium May 20-23, 2007 Lexington, Kentucky, USA, 2007, p.5.

16. Ao, T.; Pierce, L.; Hoskins, B.; Paul, M.; Pescatore, A.J.; Cantor, A.H.; Ford, M.J. and King, W.D. (2011). Allzyme® SSF increased AMEn of the corn-soy diet and improved performance of broilers. Science and Technology in the Feed Industry, 27th International Symposium, May 22-25.

17. Clave, H.; Geliot, P. and Spring, P. (2007). Effect of Allzyme SSF on performance and health of semi-intensive broiler chicks. The New Energy Crisis; Food, Feed, or Fuel? Proceedings of Alltech's 23rd Annual Symposium May 20-23, 2007 Lexington, Kentucky, USA, p. 33.

18. National Research Council (1994). Nutrient Requirements of Poultry. 9th edition. National Academic Press. Washington, D.C.

19. Jaime, S. (2000). Text book of Avian Medicine. Chapter 3, Edited by Judith C. H. Harcourt publishers limited, London.

20. Kalamah, M. A., El-Nadi, M. M., Goher, L. M. and Soliman, M. M. (2000). Some factors affecting fertility and hatchability using artificial insemination in Norfa chickens. 3rd All Africa Conference on Animal Agric. and 11th Conference of the Egyptian Society of Animal Production, Alex. Egypt, 6-9 November, 597-605.

21. Blom, E. (1950). A 1-min live-dead sperm stain by means of eosin-nigrosin. J. Fertil. Steril., 1: 176-177.

22. Melrose, D.R. and Laing J.A. (1970). The characteristics of normal semen. Chap. 4-Fertility and infertility in the domestic animals. Ed. By J.A. Laing Bailliere Tindall and Gassell, London.

23. SAS Institute (2004). SAS User's Guide: Statistics. Release 9.1. SAS Institute Inc., Cary, NC.

24. Duncan, D.B. (1955). Multiple range and multiple F- test, Biometrics, 11:42.

25. Louis, G.F, Lewis, A.J., Weldon, W.C, Miller, P.S, Kittak, R.J and Stroup, W.W (1994). The effect of protein intake on boar libido, semen characteristics and plasma hormone concentrations. Journal of Animal Science, 72 (8): 2038 – 2050.

26. Salem, M.H, Zahraa A.E, Abd-Allah, G.A, Hassan, G.A and Shaker, N (1988). Effects of toxic compounds on semen quality of rabbits. . Journal of Environmental Science and Health B30 (4) 513-534.

27. Jaffe, W.G. (1980) Hemagglutinins. In: Toxic constituents of plant foodstuffs. Liener, I.E (eds). New York. Pp 69-101.

28. Putsztai, A. (1989) Biological effects of dietary lectins. In: Recent Advances of Research in antinutritional factors in legume seeds. Pudoc, Wageningen, pp 17-29.

29. Ahmed A.E., Smithar R. and Ellis M., (1991). Activities of enzymes of the pancreas and the lumen and mucosa of the small intestine in growing broiler cockerels fed on tannin containing diets. British Journal of Nutrition, 65, 189-197.

30. Horrigorne, T., Kumar, R.I. and Okamoto, K. (1988). Effects of condensed tannins prepared from leaves of fodder plants on digestive enzymes in vitro and in the intestine of rats. British journal of Nutrition 60, 275-285.

31. Yousef, M.I., Salem, M.H; Ibrahim, H.Z, Helmi, S; Seehey, M.A and Bertheussan, K(1995). Toxic effects of carbofuran and glyphosate on semen characteristics in rabbits. Journal of Environmental Science and Health B30 (4) 513-534.

32. Adetayo A.; Olubunmi F.; Ademola O. and Adewale E. (2016). Effects of Some Toxic Plant Seeds on Semen and Sperm Quality of Adult Cockerels. Journal of Biology, Agriculture and Healthcare, Vol.6, No.3, 120-126.

33. Mihai B.; Aurel Ş.; Liviu P. and Liviu C. (2010). The Effects of Enzymatic Complex Allzyme SSF and Organic Selenium on some Growth and Consumption Indices of Broiler Turkey. Benţea M. et. al./Scientific Papers : Animal Science and Biotechnologies, 2010, 43 (1). 9-14.

34. Gado H.M., Salem A.Z.M., Robinson P.H., Hassan, M. (2009). Influence of exogenous enzymes on nutrient digestibility, extent of ruminal fermentation as well as milk production and composition in dairy cows. Anim. Feed Sci. Technol., 154: 36-46. doi:10.1016/j.anifeedsci.2009.07.006.

35. Attia Y.A., Kamel K.I. (2012). Semen quality, testosterone, seminal plasma biochemical and antioxidant profiles of rabbit bucks fed diets supplemented with different concentrations of soybean lecithin. Animal, 6: 824-833. doi:10.1017/S1751731111002229.

36. Cheah Y., Yang W. (2011). Functions of essential nutrition for high quality spermatogenesis. Adv. Biosci. Biotechnol., 2: 182-197. doi:10.4236/abb.2011. 24029.

37. Robins, C. and Brooker, J.D. (2005). The effect of *Acacia aneura* feeding on abomasal and intestinal structure and function in sheep. Anim. Feed Sci. Technol. 121, 205-215.

38. Rubanza, C.D.K.; Shem, M.N.; Otsyina, E.R. and Fujihara, T. (2005). Performance of Zebu 005): Performance of Zebu steers grazing on western Tanzania native forages supplemented with *Leucaena leucocephala* leaf meal. Agrof. Syst. 65, 165-174.

39. Choct, M and G. Annison (1992).The inhibition of nutrient digestion by wheat pentosans. *Br. J. Anim. Nutr., 67: 123-132.*

40. Saleh, F., M. Yamamoto, M. Tahir, A. Ohtsuka and K. Hayash (2005)**.** A new natural feed additive for broiler chickens. *Poult. Sci. Asso. Annual Meeting. Edmonton, Canada, pp: 36-54.*

41. Cowieson, A. J. and V. Ravindran (2008). Effect of exogenous enzymes in maize-based diets varying in nutrient density for young broilers: Growth performance and digestibility of energy, minerals and amino acids. *British Poultry Science, vol. 49, 37-44.*

42. El-Husseiny, O. M.; Hanafy, M.A.; Radwan, M.A.H. and Azouz, H.M.M. (1997). Evaluation of traditional and untraditional protein sources in rabbit diets. *Egyptian Journal of Animal Prod*uction, 34: 57-66.

43. Laila. D. Abd El-Samee and Samia M. Hashish (2007). Olive cake in laying hen diets for modification of yolk lipids. PDF created with pdfFactory Pro trial version www.pdffactory.com.

44. Taneja, S.K. and Rakha, A. (2005). Influence of low cholesterol eggs enriched with vitamin-E and omega-3 fatty acid on blood lipid profile of wister rats. *Indian Journal of Experimental Biology.,* 43: 601-605.

45. Mancini, M. and M. Parillo, 1991 Lipid intake and therosclerosis. Ann. Nutr. Metab., 35: 103-108. DOI: 10.1159/000177689.

46. Sutton, C.D., W.M. Muir and G.E.J. Mitchell (1985). The effect of dietary cholesterol, energy intake and oxygen consumption on cholesterol metabolism in the chick. Poult. Sci., 64: 502-509. PMID: 3991424

47. El-Kerdawy, D.M.A. (1997). Olive pulp as a new energy source for growing rabbits. Egyptian J. Rabbits Sci., 7: 1-12.

48. Mousa, M.R.M. (2000). Effect of feeding olive pulp on productive performance of Awassi ewes and their lambs under subtropical conditions. J. Agric. Sci. Mansoura Univ., 25: 4967-4975.

49. Mehrez, A.Z. and Mousa, M.R.M. (2011). Growth Performance of Rabbits Fed Olive Pulp in North Sinai. *Asian Journal of Animal Sciences, 5: 317-329.*

50. Buyse, J., K. Jassens, S. van der Geyten, P. van As, E. Decuypere and V.M. Darras (2002). Pre-and postprandial changes in plasma hormone and metabolite levels and hepatic deiodinase activities in meal-fed broiler chickens. Br. J. Nutr., 88: 641- 653. DOI: 10.1079/BJN2002741

51. Swennen, Q., G.P.J. Janssens, S. Millet., G. Vansant, E. Decuypere and J. Buyse (2005). Effect of substitution between fat and protein on feed intake and its regulatory mechanisms in broiler chickens: endocrine functioning and intermediary metabolism. Poult. Sci., 84: 1051-1057. PMID: 16050122.

52. Rupic,V.; V. Bozikov; R. Bozac; S. Muzic; N. Vranesic and M. Đikic (1999). Effect of feeding olive by-products on certain blood parameters and serum enzyme activities of fattening rabbits. Acta Veterinaria Hungarica 47 (1), pp. 65.75.

53. Collin, A., R.D. Malheiros, V.M.B. Moraes, P.V.A. Veerle and M. Darras et al. (2003). Effects of dietary macronutrient content on energy metabolism and uncoupling protein mRNA expression in broiler chickens. Br. J. Nutr., 90: 261-269. PMID: 12908886.

54. Hajati, H. (2010). Effects of enzyme supplementation on performance, carcass characteristics, carcass composition and some blood parameters of broiler chicken. American Journal of Animal and Veterinary Sciences 5 (3): 221-227.