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Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorijps@gmail.com

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Effect of Canola and Olive Oils on Productive, Immunological and Some Biochemical Parameters of Broiler Chickens Fed Iso Caloric and High Caloric Diets

A.S. Ahmed[1], S.M. El-Bahr[2] and A.A. Al-Azraqi[2]
[1]Department of Animal and Fish Production, College of Agriculture and Food Science, King Faisal University, Al-Ahsa, Saudi Arabia
[2]Department of Physiology, Biochemistry and Pharmacology, College of Veterinary Medicine and Animal Resources, King Faisal University, Al-Ahsa, Saudi Arabia

Abstract: One day old broiler chicks (n=240) were used to assess the effect of canola and/or olive oil on performance, immunological and biochemical parameters of broiler chickens fed iso caloric and high caloric diets under a hot climate condition. Birds were divided into 2 groups each of 120 birds based on the level of diet energy as iso caloric and high caloric diet groups. Each group were divided equally into four diet treatments in three replicates, control diet free of examined oils, diet containing 2% canola oil, diet containing 2% olive oil and diet containing 1% canola oil + 1% olive oil. Birds housed in wire cage system in an open house system. Experimental diets started at 15 days old. Weight gain , food conversion, hemoglobin, hematocrite and immune response were determined. Liver and kidney functions were evaluated by assessing the activities of Liver enzymes namely alanine transaminase (ALT) and aspartate transaminase (AST), In addition to kidney function biomarkers (Creatinine, Uric acids and blood urea nitrogen, BUN). Inclusion of canola and olive–canola oils combination in broilers ration increased body weight gain, improved feed conversion and keel length. Both olive and canola oils decreased AST in birds fed iso caloric ration, both oils increased the activity of this enzyme in serum of birds fed high caloric diet. In contrast to canola oil, olive oil did not affect the activity of ALT in birds fed iso and high caloric diets whereas oils combination were not recommended whenever iso caloric diet was available. Moreover, renal function was improved in birds fed ration mixed with olive oil or both oils combination. The examined doses of olive oil was effective than canola oil as feed additives to broilers at the levels of peripheral blood and immune response tested against sheep red blood cells.

Key words: broiler, canola oil, olive oil, production, immune response, biochemical indicators

INTRODUCTION

Using the term fat and oil, refer to triglycerides of several profiles of fatty acids. The fats and oils are esters of glycerol and fatty acids ; fats are solid whereas oils are liquid at room temperature. Lipids constitute the main energy reserve of animals and it has the highest caloric value among all nutrients (Baiao and Lara, 2005). The value of oils is based partially on their quality, in terms of their energy value and factors as essential fatty acid content, oxidative stability and palatability (Nobakht and Mehmannavaz, 2012).

From broiler industry prospective, improve body weight and feed efficiency of the birds without disruption of their biochemical status is essential therefore, feed intake in relation to biochemistry of the birds is an important factor in ration formulation. For instance Regulating dietary energy by supplementing fat is believed to be one of the most effective ways to adjust feed intake of broiler chicks (Bryant *et al*., 2005). In addition, the fatty acid composition of poultry muscle is an important quality parameter especially with respect to potentially affecting human health from poultry meat consumption (Rahimi *et al*., 2011). Oils that predominantly contain polyunsaturated fatty acids (PUFAs) have been used previously through supplementation of diets with lipids from oilseeds (Salamatdoustnobar *et al*., 2011 ; Ahmed *et al*., 2009; Ramesh and Gita, 2004; Bean and Lesson, 2002). Canola oil has been reported as very rich source of monounsaturated oleic acid , it contains considerable amounts of linoleic (LA) and alpha-linoleic (ALA) acids, the precursors of omega-6 and omega-3 fatty acids and is poor of saturated fatty acid (Antongiovanni *et al*., 2009). Many of the healthful effects of olive oil are usually attributed to olive oil's high oleic acid content (Visioil and Galli, 2000). Olive oil is considered a source of oil rich in monounsaturated fatty acids (MUFA) (Crespo and Esteve-Garcia, 2001). Birds fed an olive oil-supplemented diet showed better production performance compared with control diet-fed birds, whether under thermo neutral conditions or when

Corresponding Author: A.S. Ahmed, Animal Production Department, College of Agriculture, Cairo University, Egypt
Co-Author: A.M. El Bahr, Department of Biochemistry, Faculty of Veterinary Medicine, Alexandria, Egypt

exposed to heat stress (Mujahid *et al*., 2009). Essential fatty acids provided through vegetable oils besides supplying energy they improve the absorption of fat-soluble vitamins, and increase the efficiency of utilization of the consumed energy (Fouladi *et al*., 2008). Previous studies concluded that canola oil supplement improved body weight, and feed conversion in broilers (Shahryar *et al*., 2011; Bryant *et al*., 2005). It has been described that, the administration of high caloric diet caused the development of dyslipidaemia, abdominal obesity (Innis, 2007), fatty liver disease (Altunkaynak, 2005), hepatomegaly (Oldenburg and Pijl, 2001), splenomegaly (Altunkaynak *et al*., 2007a) and renal diseases (Altunkaynak *et al*., 2007b). In addition, the hipolipidemic and hypolcholesterolemic effects of polyunsaturated and monounsaturated fatty acids containing oils were fully documented (Spadaro *et al*., 2008). Furthermore, dietary oils in chickens have demonstrated anti-inflammatory and immunostimulent effects (Korver and Klasing, 1997; Korver *et al*., 1998). In addition the variation in the balance level of nutrients can have a substantial impact on metabolism and immunity (Kidd, 2004). In a recent study Salamatdoustnobar *et al*. (2011) using canola oil as fatty acid source they mentioned that increase of diet n-3 fatty acids have direct effects in the tibia bone mineralization in broilers and significantly increased calcium content in the experimental treatments, but has not effect on the phosphorus. As well known, changes in ration formulation could be a stress factor which may affect physiological and biochemical status of the birds. Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were used as the biochemical indicators for liver function; the increase of these enzyme activities indicates the liver damage (Han *et al*., 2008). In addition, biomarkers of renal functions include Blood urea nitrogen (BUN), Creatinine and uric acid are reliable indicators for renal function, the elevated level of creatinine is an indicative of kidney dysfunction. In broilers, uric acid (UA) is produced as the main end product of N metabolism (Donsbough *et al*., 2010). The present study was conducted to evaluate the effect of canola and olive oils and their combination on performance, immunological and biochemical parameters of broiler chicken fed normal and high caloric diets, trying to assess safe and effective ration formulation parallel to best broiler performance. This has been done considering the suspected effect of this supplement on hemoglobin concentration, shank and keel length. The present study aimed also to estimate liver and kidney functions in all examined birds to clarify whether high caloric diets disturb the liver and kidney performance or not. The aims of the study were extended to evaluate whether these oils (canola and olive) improve the function of these organs whenever affected or not under a hot arid environment.

MATERIALS AND METHODS

Experimental design: Two hundred and forty Day old broiler chicks (ROSS 308) were obtained from a commercial hatchery for the current study and reared at Research station, King Faisal University, Saudi Arabia. All chicks were randomly divided into 2 main dietary treatments iso caloric and high caloric diets each of 120 chicks. Each group were divided into four diet treatments formulated as following: control diet (T1), diet containing 2% canola oil (T2), diet containing 2% olive oil (T3), and diet containing 1% canola oil + 1% olive oil (T4). All diets formulated to meet or exceed the nutrient requirements for poultry of the National research council, (NRC) 1994, feed formulation for all diets are shown in Table 1. Each treatment included 30 chicks in 3 replicates. All chicks were housed in a wire cage system for broilers 10 birds/cage. Cages were placed in an open house with ventilation fans. The regular vaccination program at the experimental station applied to all birds. Birds were exposed to 22 h of light a day during the experimental time. For the first 2 weeks all birds fed iso caloric, control diet, then they exposed to the experimental diets. Feed and water were provided *ad libitum*. All birds under the current study were treated according to the ethical and animal right roles of the scientific research committee of King Faisal University, Saudi Arabia .

Data collection and analysis: At one day old, all chicks were weighted for each cage and a preliminary statistical analysis were done to ensure that there were no differences in the initial body weight. Starting from 21d old and after 1 week of dietary treatment provided, food consumption for each replicate were measured every week to establish each bird mean daily food intake. Body weights were measured individually to the nearest 10 g on a weekly basis starting from 21d of age. Data of weekly body weight and feed consumption were used to calculate weekly feed conversion rate and weight gain. Shank length and keel length were recorded weekly for each bird starting from 21d of age. Shank length was determined by measuring the length of the tibiotarsus (from the top of the hock joint to the footpad), and keel length was determined by measuring the distance from the hypocleidoclavical joint to the caudal end of the sternum. Blood samples were collected from all bird at 42d old into plain and heparinized vacutainers, Hematocrite (HTC) percent were measured using microhematocrit technique, and Hemoglobin (Hb) values were determines through cyanomethemoglobin method using commercial kit (Ref. No. 042A, United Diagnostics Industry, Dammam, KSA). Commercial diagnostic kits (United Diagnostic Industry, UDI, Dammam, Saudi Arabia) were used for determination of ALT (EP07-500), AST (EP15-500), BUN (EP20-420), Uric acid (EP61-620) and Creatinine (EP33K-660) on ELIPSE full automated chemistry

analyzer (Rome, Italy). Concentration of the biochemical constituents was calculated according to the manufacture instruction.

At 28d of age after 2 weeks of dietary treatment exposure, all chickens were injected intramuscularly with 0.5 ml of 15% Sheep red blood cells (SRBC) to evaluate their humoral immunity . Blood samples were obtained from each bird at 3, 7, and 10 days post immunization . After centrifugation of blood samples, sera were harvested and stored frozen at -20ºC till the time of the analysis . The SRBC antibodies were assayed by micro agglutination method (Nelson *et al*., 1995).

Statistical analysis: The data from this study were subjected to a two-way analysis of variance (ANOVA) for the effect energy level in diets and treatment and their interactions. Means were separated by use of Duncan's multiple range tests. Data were analyzed using the general linear model procedure of SAS software (SAS, 2000). Statistical significance was considered as $P \leq 0.05$ throughout the paper.

RESULTS

Figure 1 demonstrates the weekly body weight and Fig. 2 illustrates weight gain of the treatment groups in high caloric and iso caloric diets. The means of body weight within each week showed a significant ($P<0.05$) magnitude of body weight for the high caloric diets supported with oils compared to iso caloric diets. This increase in body weight for high caloric diets reduced at the six week of age. Regarding the iso caloric diets, all treatment showed early significant high body weight compared to control treatment at 3 weeks of age. However, this increase in body weight disappeared gradually with progress in age to reach insignificant values at 6 weeks of age. For birds fed high caloric diets an early significant increase in body weight of birds fed diets containing canola oil and mixed canola- olive oils compared to control group, this trend disappeared at the following weeks. While, birds fed olive oil treatment recorded the lower body weight ($P<0.05$) compared to the other two oil treatments at week four.

Dietary treatments did not produce any obvious effect on the weight gain values within or between the two different caloric levels of diets at the 3rd and 6th weeks of age except the early lowest weight gain of iso caloric diet control birds (Fig. 2). Birds fed iso caloric diet containing mixed oil recorded a high weight gain compared to control birds ($P<0.05$). Figure 3 illustrates the changes in feed conversion rate in birds under two caloric levels in response to different treatments. There were no significant effects of caloric level or treatment on feed conversion rate at 3rd and 6th weeks of age. Both control treatments birds recorded the best significant feed conversion rate compared to birds treated with canola or olive oil in under iso caloric diet treatments at 4th week of age. However, birds fed iso caloric diet provided with olive oil or mixed oils recorded better feed conversion rate than the control treatment of high caloric diet birds.

Dietary treatments did not affect the bird's shank length (Fig. 4). However, using single oil, canola or olive, in iso caloric diets increased significantly the keel length of the birds compared to the control group. While, the results declared that mixed oil in high caloric diet produced the significant ($P<0.05$) longest keel length of birds.

As shown in Fig. 5, no changes have been observed in hematocrit percent due to diet or treatments. While, hemoglobin concentration of birds fed high caloric diets, except the mixed oil treatment, recorded the highest significant ($P<0.05$) heamoglobin concentration among all treatments and diets.

Dietary treatments affected the immune response to SRBC at 3, 7, 10 days post exposure (Table 2 and 3). Birds received iso caloric diets with olive oil and mixed oils showed high significant ($P<0.05$) response to SRBC at 7 days post exposure compared to control treatment. While, birds received high caloric diets with mixed oils recorded high significant response compared to control treatment at 3, 7, 10 days post exposure. However, there were no differences in response between birds received olive oil or canola oil in both iso caloric and high caloric diets.

The activities of AST were reduced significantly ($P<0.05$) in birds fed isocaloric diet supplemented with either canola or olive oil but not the combination compare to control. This effect was more pronounced ($P<0.05$) in birds fed isocaloric diet mixed with olive oil than that fed the same diet mixed with canola oil. In the contrary, addition of canola and/or olive oil to high caloric diet induced significant ($P<0.05$) increase in activities of AST in serum of birds fed such diets compare to control. ALT activities were reduced significantly ($P<0.05$) in birds fed isocaloric diet supplemented with olive oil 2% compare to control treatment. However, the activities of the same enzymes were increased significantly ($P<0.05$) in birds fed high caloric diet supplemented with canola oil 2% or combination of both oil (1% for each). The data summarized in Table 5 indicated that, ALT activities were increased significantly ($P<0.05$) in birds fed high caloric diet mixed with canola oil 2% whereas addition of olive oil or combination did not affect the activities of these enzymes compare to the control treatment. The data shown in Table 4 and 5indicated that, the creatinine and blood urea nitrogen values were not affected in serum of all birds in both groups (iso and high caloric diets). However, uric acid values were reduced significantly ($P<0.05$) in birds fed either isocaloric or high caloric diets supplemented with olive oil 2% or combination compare to control treatment. However, the values of the same parameter in birds fed iso or high caloric diet supplemented with canola oil 2% were comparable to the control treatment.

Table 1: Feed ingredients and calculated composition of the experimental diets

	Iso caloric diet				High caloric diet			
Feed Ingredients	T1[1]	T2	T3	T4	T1	T2	T3	T4
Yellow Corn	50.51	50.31	50.21	50.21	53.41	53.21	53.11	53.11
Soybean Meal 44%	40.00	40.00	40.00	40.00	28.60	28.60	28.60	28.60
Fish Meal	-	-	-	-	6.50	6.50	6.50	6.50
L. Stone	1.20	1.20	1.20	1.20	1.20	1.20	1.20	1.20
Premix[2]	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Dl methionine	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
DCP	1.39	1.39	1.39	1.39	1.39	1.39	1.39	1.39
Salt	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Corn Oil	6.00	4.20	4.30	4.30	8.00	6.20	6.30	6.30
Antioxidant	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Canola Oil	-	2.00	-	1.00	-	2.00	-	1.00
Olive Oil	-	-	2.00	1.00	-	-	2.00	1.00
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Calculated composition								
Crude Protein	21.89	21.88	21.87	21.87	21.82	21.81	21.80	21.8
Me, Kcal/kg	3160.00	3157.00	3159.00	3161.00	3402.00	3399.00	3401.00	3403.00
Calcium (%)	0.91	0.91	0.91	0.91	1.02	1.02	1.02	1.02
Phosphorus (%)	0.40	0.40	0.40	0.40	0.48	0.48	0.48	0.48
Me/cp	144.35	144.28	144.44	144.53	155.91	155.84	156.00	156.10

[1]T1, T2, T3 and T4 are diets containing 0% canola and/or olive oil (control), 2% canola oil, 2%olive oil and 1%canola oil+ 1% olive oil, respectively

[2]The multi vitamin-minerals premix provide the following per kilogram of diet: vitamin A, 4800 IU (retinal acetate); cholecalciferol, 880 IU; vitamin E, 10 mg (dl-"-tocopheryl acetate); vitamin K (menadione sodium bisulfate),1.2 mg; thiamin, 0.8 mg; riboflavin, 2.4 mg; pantothenic acid, 12 mg; niacin, 3 mg; vitamin B12, 0.006 mg; biotin, 0.04 mg; pyridoxine, 0.8 mg; choline chloride 100 mg; anti oxidant 4 mg; 2 Mineral premix provided the following per kilogram of feed: manganese, 40 mg; zinc, 24 mg; iron, 16 mg; copper, 2 mg; iodine, 0.4 mg; selenium, 0.08 mg; Ca, 280 mg and choline chloride 100 mg

Table 2: Effect of Iso caloric diet containing canola and/or olive oils treatment on broiler Primary antibody response to SRBC at 3, 7 and 10 days post exposure

Days post	Treatment			
exposure	T1[1]	T2	T3	T4
3	1.37±0.27^{B}	1.37±0.28^{B}	2.00±0.26^{AB}	2.11±0.27^{AB}
7	6.00±0.26^{C}	6.44±0.27^{BC}	7.00±0.26^{AB}	7.10± 0.26^{AB}
10	2.66±0.25^{AB}	2.66±0.26^{AB}	3.00±0.25^{AB}	3.12±0.25^{AB}

a,b,c values within a measuring day with different superscript differ significantly (p = 0.05).

[1]T1, T2, T3 and T4 are diets containing 0% canola and/or olive oil (control), 2% canola oil, 2% olive oil and 1%canola oil + 1% olive oil respectively

Table 3: Effect of High caloric diet containing canola and/or olive oils treatment on broiler Primary antibody response to SRBC at 3, 7 and 10 days post exposure

Days post	Treatment			
exposure	T1[1]	T2	T3	T4
3	1.37±0.27^{B}	2.00±0.28^{AB}	2.00±0.27^{AB}	2.33±0.27^{A}
7	6.33±0.25^{BC}	7.00±0.26^{AB}	7.00±0.25^{AB}	7.33±0.27^{A}
10	2.44±0.26^{B}	3.00±0.25^{AB}	3.00±0.26^{AB}	3.34±0.25^{A}

a,b,c values within a measuring day with different superscript differ significantly (p = 0.05).

[1]T1, T2, T3 and T4 are diets containing 0% canola and/or olive oil (control), 2% canola oil, 2%olive oil and 1%canola oil+ 1% olive oil respectively

DISCUSSION

Level of energy in diet regardless the oil content in the present study resulted in increased body weight although, this increase has decline with age which suspected to be affected with the normal decline in growth curve of the broilers. An increase in body weight showed off at early ages of birds fed high caloric diet provided with canola or mixed oil compared to control birds, while birds fed diet provided with olive oil recorded low body weight at 28 days of age. These results may be explained through the highest level of essential fatty acids, unsaturated fatty acids and malabsorption of fatty acids in canola oil which can play a major role in reduces the rate of feed passage through the digestive system, which allows a better absorption of all nutrients present in the diet (Rahimi *et al*., 2011). While, lower level of unsaturated fatty acids in olive oil may not have the same effect on body weight. Our results are partially agreed with Baiao and Lara (2005); Taylor (2000) they reported an increase of body weight of broilers fed diets provided with canola oil.

Birds feed conversion rate in general did not improved by adding canola oil or olive oil in the high caloric diets, this suggested to be due to the presence of the same amount of energy of all high caloric diets, which provided the same rate of metabolizable energy to protein in diets (Wongsuthavas *et al*., 2007). While the observed improvement in feed conversion rate at the 5th week of age of birds fed iso caloric diet containing olive oil or mixed oils, suspected to be due to the effect of olive oil in decrease heat stress effect through production of lower levels of mitochonderial reactive oxygen species (ROS) which reduce the heat stress effect and allow better performance (Mujahid *et al*., 2009). This effect of olive oil is probably synergized with the relatively lower body weight of iso caloric feed birds compared to birds fed high caloric feed under current experimental heat conditions.

Table 4: Effect of Iso caloric diet containing canola and/or olive oils treatment on broiler blood profile at 42 days of age

	Treatment			
	T1[1]	T2	T3	T4
AST (U L^{-1})	237.1±4.7200^{D}	208.0±4.720^{E}	171.1±4.7200^{G}	292.2±4.7200^{A}
ALT (U L^{-1})	8.86±0.300^{D}	10.83±0.30^{A}	6.43±0.300^{CD}	10.93±0.300^{A}
Creatinine (mg dL^{-1})	0.493±0.05^{AB}	0.363±0.05^{B}	0.515±0.05^{AB}	0.525±0.05^{AB}
Uric acid (mg dL^{-1})	3.68±0.230^{A}	3.82±0.230^{A}	2.13±0.240^{BC}	2.25±0.240^{BC}
BUN (mg dL^{-1})	8.53±1.830^{AB}	9.16±1.830^{AB}	10.93±1.830^{AB}	8.16±1.830^{AB}

^{a,b,c}Values within a parameter with different superscript differ significantly ($P \leq 0.05$).
[1]T1, T2, T3 and T4 are diets containing 0% canola and/or olive oil (control), 2% canola oil, 2%olive oil and 1%canola oil+ 1% olive oil, respectively

Table 5: Effect of High caloric diet containing canola and/or olive oils treatment on broiler blood profile at 42 days of age

	Treatment			
	T1[1]	T2	T3	T4
AST (U L^{-1})	190.0±4.7200^{F}	265.4±4.7200^{BC}	253.2±4.7200^{C}	274.8±4.7200^{B}
ALT (U L^{-1})	6.10±0.300^{D}	9.20±0.300^{B}	7.33±0.300^{C}	6.83±0.300^{CD}
Creatinine (mg dL^{-1})	0.617±0.05^{A}	0.542±0.05^{A}	0.619±0.05^{A}	0.593±0.05^{A}
Uric acid (mg dL^{-1})	2.24±0.230^{BC}	2.85±0.240^{B}	1.76±0.230^{C}	1.90±0.230^{C}
BUN (mg dL^{-1})	9.66±1.830^{AB}	6.23±1.830^{B}	10.83±1.830^{AB}	12.50±1.830^{A}

^{a,b,c}Values within a parameter with different superscript differ significantly ($P \leq 0.05$). T1, T2, T3 and T4 are diets containing 0% canola and/or olive oil (control), 2% canola oil, 2%olive oil and 1%canola oil+ 1% olive oil, respectively

Our results indicated positive effect of oil source on skeletal bone, where keel length of birds fed canola oil or olive oil in an iso caloric diet showed higher keel length than control birds. While, birds fed mixed oils in the high caloric formula recorded the highest keel length. The role of lipids and polyunsaturated fatty acids in bone metabolism and skeletal healt is well documented (Watkins *et al*., 2001). The differences in the keel length in the current research under different oil sources in diet may be due to the type and amount of polyunsaturated fatty acids (PUFA) which influence bone formation and osteoblastic cell functions in culture (Watkins *et al*., 2003). Probable explanation is due to increase of calcium percent affected with levels of canola oil in broiler diets as mentioned most recently (Salamatdoustnobar *et al*., 2011). The response of keel length but not the shank length suggested being under genetic effect of the broiler breed. Treatments did not affect the hematoctite percent of different birds groups. Hemoglobin concentration did not show a trend as affected with dietary treatment, generally hemoglobin concentration was higher for birds fed high caloric diets, which may be due to higher metabolic activity due to high energy provided.

In the present study an obvious alteration in immune response to SRBC antigen have been recognized. It is noticed that birds provided with feed containing olive oil or mixture olive/canola oil recorded the highest antibody response especially at 7 days post exposure. It is suspected that under hot environmental conditions the olive oil play an important role to alleviate heat stress on birds, thereby allow better performance of birds. While the observed enhancement in immune response of birds fed diet containing mixed canola/olive oil suggested to be affected by the heat alleviation through olive oil supplement which allowed a suitable physiological condition for the effect of the high PUFA content in the canola oil to enhance the immune response. On the other hand, support birds with one of the two oils in feed formula in the high caloric diet suspected to be not enough to modulate the immune response, which may be due to the low proportion of olive oil to tackle heat stress of relatively high body weight birds, therefore no differences in immune response have been reported between birds provided with single oil supplement in high caloric feed formula with the control birds. The effect of olive oil supplement in heat alleviation have been reported by Mujahid *et al*. (2009). They reported that olive oil supplement in broiler feed down regulate the oxidative damage in birds under heat stress conditions, and altered respiratory chain activity in skeletal muscle mitochondria. Canola oil effect on the immune response has been suggested to be due to its adequate mixture of essential fatty acids, and unsaturated fatty acids. Yang *et al*. (2000) reported that canola oil has been recognized as adequate mixture of essential fatty acids, unsaturated fatty acids such a linolenic acid (C18:3) that can improve broiler performance, also linolenic acids can be converted to longer chain omega-3 fatty acids. The action of n-6 PUFA as a pro-inflammatory factor and the action of n-3 PUFA as an anti-inflammatory factor is well documented (Calder, 2001). Dietary lipid source may affect immunocompetence via affecting membrane fatty acid composition and therefore fluidity, flexibility and function and/or affecting the inflammatory process and other cell-signaling pathways (Trushenski and Lochmann, 2009). The performance and immunological data can conclude

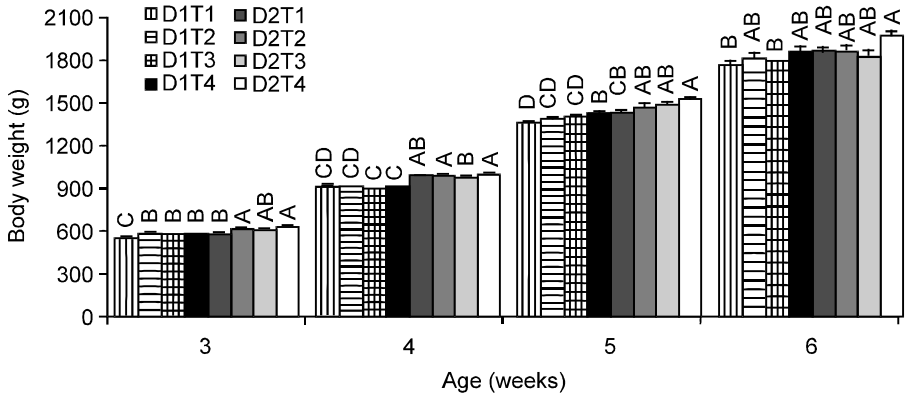

Fig. 1: Effect of High caloric and iso caloric diets containing canola and/or olive oils treatment on broiler body weight
D1, and D2 are iso caloric and high caloric diets respectively
T1, T2, T3, and T4 are diets containing 0% canola and/or olive oil (control), 2% canola oil, 2%olive oil, and 1% canola oil+ 1% olive oil respectively. Columns within a week with different superscript differ significantly ($P \leq 0.05$).

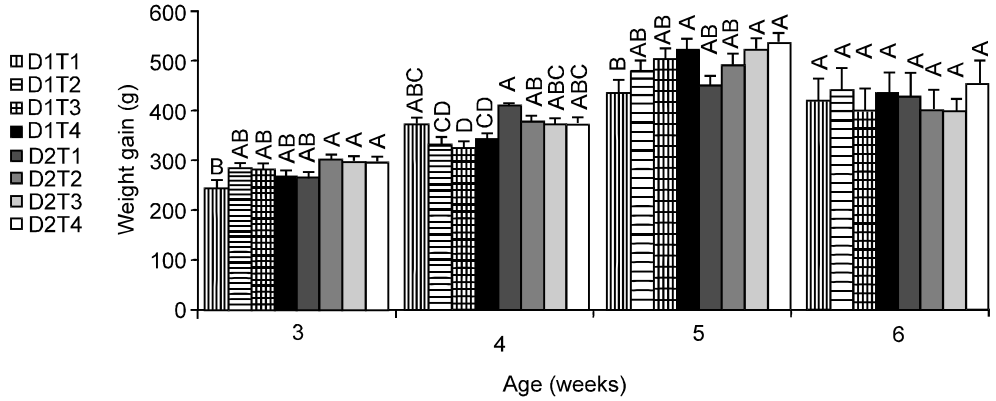

Fig. 2: Effect of High caloric and iso caloric diets containing canola and/or olive oils treatment on broiler weight gain
D1, and D2 are iso caloric and high caloric diets respectively
T1, T2, T3, and T4 are diets containing 0% canola and/or olive oil (control), 2% canola oil, 2% olive oil, and 1% canola oil+1% olive oil respectively. Columns within a week with different superscript differ significantly ($P \leq 0.05$).

that, high caloric diets as expected increased body weight, and weight gain compared to iso caloric diet in some measuring points. While mixed Canola/olive oil in the current study suggested having an obvious effect on the humoral immune response in the current hot experimental environment through heat alleviation mechanism supported by olive oil content and high PUFA content in canola oil which support immune response.

Biochemical markers play an important role in accurate diagnosis and also for assessing risk and adopting therapy that improves clinical outcome. In the assessment of liver dysfunction, the determination of enzyme levels such as AST and ALT is largely used. Elevated levels of these serum enzymes are indicative of loss of functional integrity of cell membrane in liver (Drotman and Lawhan, 1978). The significant ($P<0.05$) decrease of AST activity in birds fed either olive or canola oils and not the combination compare to control indicated two major outcomes, first is that the combination of canola and olive oils at the examined concentrations (1% for each) is not recommended to be added to the broiler diets. Secondly a dose of 2% was more effective for olive oil than canola oil in broilers isocaloric diets. Parallel to our findings, Nakbi *et al.* (2010) demonstrated that olive oil reduced the elevated ALT and AST in rats intoxicated with 2,4-dichlorophenoxy acetic acid. In addition, Farag *et al.* (2010) reported that non fried and fried canola oil induced worst alteration in rat liver due to the presence of erucic acid. The significant ($P<0.05$) increase in activities of AST in serum of birds fed high caloric diet supplemented with canola and/or olive oil compare to control might indicated that, the addition of these oils to high caloric diet is not recommended in birds reared under hot climate and investigation of the effect of dietary supplementation of lower concentration of olive oil and canola oils to high caloric diets could be verified in future

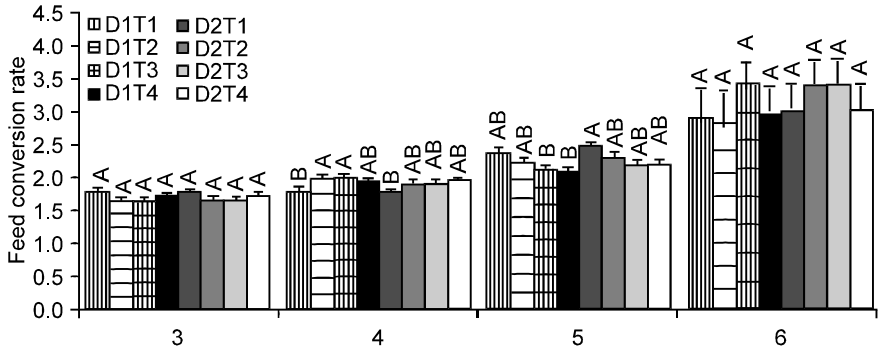

Fig. 3: Effect of High caloric and iso caloric diets containing canola and/or olive oils treatment on broiler Feed conversion rate

D1, and D2 are iso caloric and high caloric diets respectively

T1, T2, T3, and T4 are diets containing 0% canola and/or olive oil (control), 2% canola oil, 2% olive oil, and 1%canola oil+ 1% olive oil respectively. Columns within a week with different superscript differ significantly ($P \leq 0.05$).

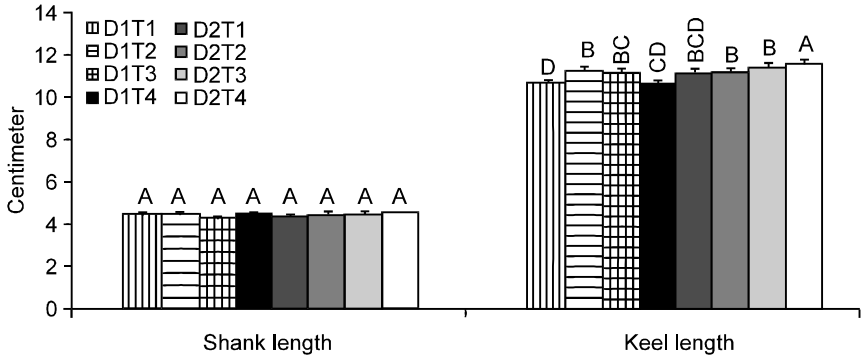

Fig. 4: Effect of High caloric and iso caloric diets containing canola and/or olive oils treatment on broiler Shank and Keel length

D1, and D2 are iso caloric and high caloric diets respectively

T1, T2, T3, and T4 are diets containing 0% canola and/or olive oil (control), 2% canola oil, 2% olive oil, and 1% canola oil + 1% olive oil respectively. Columns within a week with different superscript differ significantly ($P \leq 0.05$).

studies taken in consideration that, canola oil is expensive and using higher concentrations could be avoided in broilers industry. The arbitrary differences in blood biochemical parameters as indicators for liver and kidney function suspected to be affected mainly by heat stress conditions and its interaction with experimental treatments than the experimental treatments alone. Kinetic investigation over broiler life time and under different environmental conditions is needed for accurate explanation of those changes. The significant ($P<0.05$) decrease of ALT activities in birds fed isocaloric diet supplemented with olive oil 2% with significant ($P<0.05$) increase in the activities of the same enzymes in birds fed high caloric diet supplemented with canola oil 2% or combination of both oil (1% for each) when all compare to control treatment confirmed and evidenced the earlier findings that reported the beneficial effect of olive oil at the examined concentration over canola oil or combination in broilers reared under hot climate . This findings might be attributed to the well-known effect of olive oil as antioxidants and subsequently reduced the heat stress on examined birds (Mujahid *et al*., 2009). The increased activities of ALT in birds fed high caloric diet mixed with canola oil 2% gives final conclusion that, the examined dose can be used effectively and safely for olive oil as feed additives in broilers reared in hot climate however, this dose is not recommended for canola or combination of both oils. Biomarkers of renal functions include BUN, creatinine and uric acid. The elevated level of creatinine is an indicative of kidney dysfunction. In broilers, uric acid (UA), and not urea, is produced as the main end product of nitrogen metabolism (Donsbough *et al*., 2010). The elevation of uric acid occurs following significant disease in the kidneys (Fudge, 1997). The significant ($P<0.05$) reduction of uric acid values in birds fed either isocaloric or high caloric diets supplemented with olive oil 2% or combination compare to control treatment indicated that,

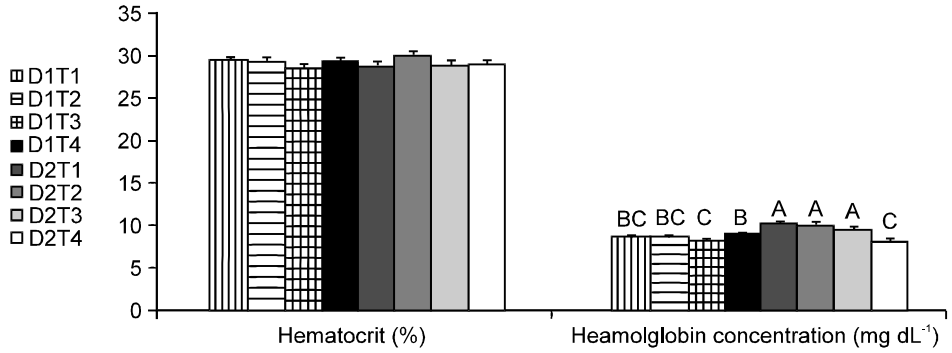

Fig. 5: Hematocrit percent and hemoglobin concentration at 42 days of age
D1, and D2 are iso caloric and high caloric diets respectively
T1, T2, T3, and T4 are diets containing 0% canola and/or olive oil (control), 1% canola oil, 1% olive oil, and 1% canola oil + 1% olive oil respectively. Columns within a week with different superscript differ significantly ($P \leq 0.05$).

the examined dose of olive oil can be used effectively as feed additives in broilers reared in hot climate than canola oil and their combination. The present study can concluded that, the examined doses of olive oil was effective than canola oil and combination as feed additives to broilers at peripheral blood level in spite of immunostimulent effect of oil combination when tested against sheep red blood cells. Future studies are needed to specify the optimum concentration of both oils or its combination as feed additives for broilers.

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