

Performance, Carcass Characteristics and Blood Fat Metabolites of Broilers Fed Oil Supplemented Diets

Mohammad EL-QUB¹ and Jamal Abo OMAR^{2,*}

¹*Palestine Poultry Company, Tulkarem, Palestine*

²*Department of Animal Production, Faculty of Agriculture, An-Najah National University, Nablus, Palestine*

(*Corresponding author's e-mail: aboomar57@najah.edu)

Received: 27 November 2014, Revised: 5 January 2015, Accepted: 6 February 2015

Abstract

The objective of this study was to compare the effects of olive oil sediment (OOS) with soybean oil soap stock (SOY) traditionally used in poultry rations, on broilers growth performance, dressing proportions, carcass cut, blood lipid metabolites and meat quality (i.e. water holding capacity, WHC; colony forming unit, CFU). A total of 416 day-old Cobb-500 chicks were used in this experiment. Birds were divided into 2 experimental treatments of 208 birds in each. Each treatment was composed of 8 replicates with 26 birds in each. Oil supplements were added (day 22) to the finishing diets at a level of 30 g/kg diet. Chicks fed the OOS had a better ($p < 0.05$) feed conversion ratio (FCR) compared to those of the SOY fed birds. Similar effect of OSS on the dressing proportions was observed. Carcasses of broilers fed the OOS had higher ($p < 0.05$) WHC compared to that of SOY fed broilers. The OOS resulted in more than 100 % improvement in WHC compared to the traditionally used SOY. Carcasses of SOY fed broilers had more ($p < 0.05$) CFU count compared to that of birds fed OOS diets. Levels of all tested blood metabolites at day 28 and 35 compared to the baseline levels (day 21), prior to oil supplementation, were significantly affected by the type of oil.

Keywords: Broilers, performance, carcass, blood fat metabolites

Introduction

The energy density in diets can be improved by the addition of fat. The increased concentration of energy has certain advantages in improving feed conversion ratio (FCR) by reducing feed intake (FI) [1,2]. Increasing palatability of rations is the main objective of the poultry industry in order to improve body weight (BW) and feed efficiency of the birds. Harms *et al.* [3] showed that increasing dietary energy or fat supplementation decreased FI and improved FCR of broiler chicks which is important in decreasing the broilers marketing age. The effects of fats and vegetable oils need to be examined not only for production characteristics such as growth rate and feed efficiency, but also for meat quality and blood parameters relative to human health [4]. The positive effects of replacing dietary carbohydrates with lipid sources, especially under warm environmental temperatures, are mainly ascribed to a reduction in metabolic heat produced during digestion [5,6].

Several studies suggest that in both birds and mammals, dietary polyunsaturated fatty acids (PUFA) inhibit lipid synthesis and increase fatty acid oxidation and induce thermogenesis [7]. These effects could explain why PUFA reduce abdominal fat. Zollitsch *et al.* [8] reported that vegetable oil resulted in higher metabolizable energy through decreasing fecal energy. Fat utilization in chickens is age dependent [9]. At ages lower than 3 weeks, utilization of fat is low because of the limited activity of lipase and bile salts [9-11]. The OOS, is the precipitated fraction of olive oil in oil storage facilities. The estimated amount of OOS by our unpublished work is 2 % of total stored olive oil. There is no information on the advantages of feeding this type of oil supplement in broilers diets and its impact on carcass and blood fat metabolites.

The objective of this study was to compare the effects of olive oil sediment (OOS) with soybean oil soap stock (SOY) which is traditionally used in poultry rations, on broilers growth performance, dressing percentage, carcass cut, blood lipid profile and meat quality (i.e. WHC, CFU).

Material and methods

Animals and experimental design

Four hundred and sixteen one-day-old broiler chicks were obtained from a local hatchery and randomly assigned to 2 experimental treatments. There were 2 diets; each was fed to 8 replicate pens of 26 chicks each. Feed and water were supplied *ad libitum*. A light/dark cycle of 23:1 h and a room temperature of 25 °C was maintained throughout the 35 day experimental period.

Birds were fed with isocaloric and isonitrogenous finisher diets (19 % crude protein, 3.200 kcal/kg ME). The oil sources were olive oil sediments (OOS) and soybean oil soap stock (SOY). They were added to the finisher diet (day 22) at 30 g/kg diet. The experimental diets (**Table 1**) were formulated to meet NRC [12] broilers nutrient requirements. Chicks were weighed on a weekly basis and feed consumption in each pen was recorded on the same days. FCR was calculated for each period (g feed/g gain). Birds' mortalities were recorded.

Blood collection and analysis

Initial (i.e. baseline) blood samples were taken after all birds had been fed the control diet for 21 day. Blood samples were then taken after 7 and 14 day of consumption of oil supplemented diets (i.e. at 28, 35 day of age). Prior to blood sampling, feed was withdrawn for 10 h to decrease the effects of feeding on blood parameters. At the end of the trial 8 birds from each replicate, with body weight similar to the mean replicate body weight, were selected for blood sampling for measurement of serum TG, COL, HDL, LDL and VLDL. Blood from the wing vein was collected in a test tube to obtain serum. The collected blood samples were centrifuged at 3000 g for 10 min and the serum was decanted into aseptically treated vials and stored at -20 °C for later analysis. Serum TG, COL and HDL were measured spectrophotometrically by using commercial kits and an enzymatic method. LDL and VLDL levels were estimated using the Friedewald equation [13].

Carcass measurements

At the end of the experiment (40 day), 5 chicks from each replicate were slaughtered, and after bleeding, fat pad, breast, thigh, liver and heart were weighed and presented as a percentage of hot carcass weight but with carcass being presented as a percentage of live weight.

Water holding capacity

The frozen broiler carcasses were thawed in a refrigerator (4 °C) for 48 h. One gram of the minced breast meat of broiler was placed on a round filter paper. The filter paper with meat was placed into a centrifuge tube and centrifuged for 10 min. The released water absorbed into the filter paper was weighed and calculated as a percentage of the initial moisture of meat.

Microbiological analysis

The breast meat (10 g) with 100 ml saline solution (0.85 %, NaCl) was homogenized for 2 min using a stomacher homogenizer and the homogenate was serially diluted 10-fold with a saline solution. Each diluent (100 µl) was spread in triplicate on each agar plate and the plates were incubated at 37 °C for 24 h. Colony forming units (CFU) per gram were counted, at a dilution giving 30 - 300 CFU per plate.

Statistical analysis

Analysis of variance was performed using the raw data, and the mean values and standard errors of the means (SEM) were obtained using the Statistical Analysis System (SAS) [14]. Differences among the means (of the replicates) were determined by the Duncan's multiple range test with a significance defined at $p < 0.05$.

Table 1 The experimental finisher diets fed to broilers and their chemical composition.

	Treatments	
	OOS	SOY
Ingredient composition, g/kg (air dry basis)		
Corn	633	633
Soybean meal	290	290
Oil source	30	30
Limestone	80	80
Salt	30	30
Methionine	15	15
lysine	5	5
DCP	16.0	16
Premix*	5.9	5.9
Nutrients composition, g/kg (air dry basis)		
Dry matter	89.01	89.32
Crude protein	18.26	18.40
Crude fiber	3.08	3.08
Crude fat	5.83	6.32
Ash	5.97	5.72
Calcium	1.14	1.10
Phosphorous (available)	0.64	0.65
ME, kcal/kg	3195	3195
Mn	236	235
Fatty acids, g/kg DM		
18:0	3.4	2.6
18:1	28.3	62.0
18:2	48.0	18.0
18:3	1.3	2.8

*Premix contents per 1 kg diet: Vitamin (A) 12000 IU, vitamin (D3) 1500 IU, vitamin (E) 50 mg, vitamin (K3) 5.0 mg, vitamin (B1) 3 mg, vitamin (B2) 6 mg, pantothenic 11.20 g, niacin 25 mg, vitamin (B6) 5 mg, vitamin (B12) 0.03 mg, folic acid 1 mg, biotin 0.05 mg, choline 400 mg, anti-oxidant 125.00 g, manganese 80 mg, zinc 50 mg, iron 20 mg, copper 15 mg, iodine 1.2 mg, cobalt 0.2 mg, Selenium 0.2 mg, wheat enzyme 90 mg, phytase 750 PU, salinomycin 60 mg.

Results

Broilers' performance

Type of oil had a significant effect on feed intake, final body weight (FBW) and FCR. Chicks fed with the OOS consumed more ($p < 0.05$) feed during the finishing period (i.e. 22 to 40 day), had heavier FBW ($p < 0.05$) compared to birds fed SOY and better FCR ($p < 0.05$) was observed in birds fed the OOS (**Table 2**).

Carcass and visceral organs

Heavier ($p < 0.05$) carcass weights were observed in chicks fed OOS compared to those fed SOY. Similar results on the dressing proportions was observed in OOS. The gizzard, liver, heart and giblets values were higher ($p < 0.05$) in birds fed the OOS compared to these tissues in SOY fed birds (**Table 3**). However, fat pad percentage in the birds fed the conventional oil (i.e. SOY) was higher than OOS.

Water holding capacity

Carcasses of broilers fed the OOS had higher ($p < 0.05$) WHC compared to those in SOY fed broilers. The OOS resulted in more than 100 % improvement in WHC compared to the traditionally used SOY (**Table 3**).

Colony forming units

The type of oil had a significant effect on the total CFU in broiler carcasses (**Table 3**). The carcasses of SOY fed broilers had more ($p < 0.05$) CFU count compared to that of birds fed the OOS diet.

The blood lipids profile

The effect of oil supplements on blood metabolites is shown in **Table 4**. Compared to these levels at day 21, prior to oil supplementation, only the OOS had significant effects on all tested metabolites. At day 28 and 35 the OOS increased ($p < 0.05$) levels of HDL and decreased ($p < 0.05$) levels of LDL, TG, COL and VLDL compared to SOY.

Table 2 Effect of supplemental oils on feed intake (FI) and feed conversion ratio (FCR) of broilers (\pm SD).

Variable	Treatments		P value
	OOS	SOY	
Feed intake (0 - 28 day)	1880 \pm 15.8	1821 \pm 17.2	0.05
Body weight at 28 day	1245 ^a \pm 13.5	1167 ^b \pm 14.9	0.05
FCR ¹	1.51 ^b \pm 0.01	1.56 ^a \pm 0.03	0.05
Feed intake (0 - 35 day)	2771 ^a \pm 19.0	2666 ^b \pm 20.7	0.05
Body weight at 35 day	1765 ^a \pm 15.2	1643 ^b \pm 15.0	0.05
FCR	1.57 ^b \pm 0.2	1.62 ^a \pm 0.02	0.05
Feed intake (0 - 40 day)	3483 ^a \pm 22.9	3362 ^b \pm 23.9	0.05
Body weight at 40 day	2261 ^a \pm 17.8	2154 ^b \pm 18.0	0.05
FCR	1.54 ^b \pm 0.02	1.56 ^a \pm 0.01	0.05

Rows of different letters are significantly different ($p < 0.05$).

¹feed conversion ratio (g feed: g/gain).

Table 3 Effect of supplemental oils on dressing percentages, visceral organs, broiler water holding capacity and colony forming units (\pm SD).

Parameter	OOS	SOY	P value
Live weight	2261 ^a \pm 23.9	2154 ^b \pm 21.6	0.05
Hot carcass weight	1739 ^a \pm 21.9	1549 ^a \pm 17.8	0.05
Dressing %	76.9 ^a \pm 3.2	71.9 ^b \pm 0.3	0.05
Fat pad %	1.2 ^c \pm 0.01	1.8 ^b \pm 0.01	0.05
Gizzard	47.3 ^a \pm 1.9	35.0 ^b \pm 1.6	0.05
Liver + Heart	64.8 ^a \pm 2.9	50.3 ^b \pm 2.0	0.05
Giblets %	5.2 ^a \pm 0.7	3.8 ^b \pm 0.2	0.05
W.H.C ¹ %	74.4 ^a \pm 3.6	33.2 ^b \pm 1.8	0.05
CFU ² /1g	9*10 ^{4b}	191*10 ^{4a}	0.05

Rows of different letters are significantly different ($p < 0.05$).

Table 4 Effect of supplemental oils on cholesterol (COL), triglycerides (TG) and high density lipoprotein (HDL) and very low density lipoprotein (VLDL) of broilers (\pm SD).

	HDL (mg/dl)	LDL (mg/dl)	TG (mg/dl)	COL (mg/dl)	VLDL (mg/dl)
Pre treatment	69.3 ^b \pm 3.0	38.6 ^a \pm 2.8	49.4 ^a \pm 3.0	139.3 ^a \pm 7.1	9.9 ^a \pm 0.9
28 day					
OOS	75.0 ^a \pm 3.1	31.0 ^b \pm 1.5	39.1 ^b \pm 2.1	132.4 ^b \pm 5.8	8.1 ^b \pm 0.6
SOY	70.5 ^b \pm 2.9	39.5 ^a \pm 2.0	47.8 ^a \pm 2.8	140.5 ^a \pm 6.9	9.6 ^a \pm 0.5
35 day					
OOS	79.5 ^a \pm 3.5	29.0 ^b \pm 1.9	37.5 ^b \pm 2.0	130.5 ^b \pm 5.4	8.5 ^b \pm 0.4
SOY	68.3 ^b \pm 2.7	35.5 ^a \pm 1.9	49.5 ^a \pm 2.3	141.7 ^a \pm 7.0	9.9 ^a \pm 0.4
P value	0.05	0.05	0.05	0.05	0.05

Columns of different letters are significantly different ($p < 0.05$)

Discussion

The type of oil had a significant effect on FI, FBW and FCR. Chicks fed the OOS consumed more feed, had heavier final weights and better FCR compared to birds fed SOY. Crespo and Esteve-Garcia [15] and Viveros *et al.* [16] reported different results where supplemental fats had no effects on these parameters. However, Atteh and Leeson [17] reported that the saturation degree of a dietary fat can influence FI, weight gain and FCR which results from better availability of energy from unsaturated fatty acids. Vegetable oil resulted in higher metabolizable energy through decreasing fecal energy [8]. Moreover, digestion and absorption of dietary fat depends on the age of birds.

Fats are often used in broiler diets to improve the energy density. Feed conversion ratio can be improved by reducing feed intake without affecting daily gain as a result of the increased energy concentration [1,2].

Heavier carcass weights were observed in chicks fed the OOS compared to carcasses of birds fed the SOY. The dressing proportions were higher in birds fed the OOS compared to that of SOY fed birds. The organs (i.e. liver, heart, gizzard) average weights were higher in birds fed SOY. Opposite results were reported for broilers when fed diets rich in polyunsaturated fatty acids (PUFA), as had lower abdominal fat deposition and visceral tissues compared with feeding diets with predominantly saturated or monounsaturated fats [15,18].

Compared to the baseline levels of blood fat parameters prior to oil supplementation, LDL, CHOL, TG and VLDL levels at 28 and 35 day were reduced by feeding OOS. However, SOY had no effect on these parameters. Levels of HDL for the same periods were reduced by OOS. In contrast, previous research showed that different types of oil has similar effects on the content of triglycerides and total cholesterol in the blood serum of the birds of all the experimental groups [19,20]. HDL, beneficial for the body and conditioning the transport of cholesterol from the peripheral tissues to the liver, is the main fraction of lipoproteins in the blood of the birds [21].

Level and type of fat in the diet influence the biochemical parameters of the blood, which are sensitive indicators of the state of health of the animals and reflects the intensity of metabolic processes taking place [19,20,22].

WHC of muscles is very important as a tool that affects meat quality. Lipid oxidation has been known to cause quality problems by forming off-odor and off-flavor compounds and decreasing nutritive values in meat. Dietary addition of unsaturated fatty acids may be related to an increased level of lipid oxidation. In this study, the WHC was affected by the type of oil. Meat of broilers fed the OOS had higher WHC. The OOS caused more than 100 % improvement in WHC compared to the traditionally used SOY. WHC, or the ability of meat to retain water, is influenced by several factors. Those factors include production of lactic acid and loss of adenosine triphosphate, which influences rate and extent of pH decline, protein oxidation, and changes in cell structure associated with proteolytic enzyme activity [23].

Previous research has identified the occurrence of pale, soft, and exudative meat as a leading cause of reduced WHC in the poultry industry. WHC values in this study ranged from 34 to 77 % which means low denaturation levels of carcass protein. Occurrences ranging from 5 to 50 % have been reported in commercial processing plants [24-28].

Higher carcass contamination was detected in carcasses of birds fed the SOY. It is known that microbes that contaminate broiler carcass can survive in the environment, especially in untreated water, but it is primarily transferred onto poultry carcasses via fluid and feces from the gastrointestinal tract of the birds, due to the high numbers of the organism found in these fluids. The organism then attaches to the skin of the broiler and perseveres to final products [29]. The majority of viable microbial cells found on poultry skin have been trapped in either the surface water layer or entrapped with water in skin crevices or feather follicles [30]. Results of this study showed that OOS had a strong effect on microbial population of broiler carcasses compared to SOY. These results are in agreement with those by Saxena and Vyas [31] who reported that essential oils inhibited the growth of pathogenic bacteria when fed at high levels. On the contrary, Ismail [32] showed that low levels of essential oils decreased the coliform bacteria counts in the cecal intestine of broilers. In comparison with other parts of the gastrointestinal tract, the caecum provides a stable environment for microorganisms, thus resulting in a large microbial population due to the slower transit time. Intestinal microflora plays an important role in the health status of host animals. In general, intestinal bacteria may be divided into species that exert either harmful (pathogenic) or beneficial effects on host health. Therefore, a common approach to maintain host health is to increase the number of desirable bacteria in order to inhibit the colonization of invading pathogens [33]. Decreased CFU in broilers given dietary fats, especially the OOS, may be explained by the fact that OOS has an antibacterial activity against different bacteria [34].

Conclusions

It can be concluded that feeding OOS results in several advantages. Most of the tested parameters (FCR, WHC, CFU) were positively affected by OOS supplementation. However, more research is needed to investigate the effects of the feeding level and duration of feeding of these sources.

Acknowledgements

The authors thank the deanship of graduate studies of An Najah National University for support.

References

- [1] Y Pinchasov and I Nir. Effect of dietary polyunsaturated fatty acid concentration on performance, fat deposition and carcass fatty acid composition in broiler chickens. *Poultry Sci.* 1992; **71**, 1504-12.
- [2] JR Scaife, J Moyo, H Galbraith, W Michie and V Campbell. Effect of different dietary supplemental fats and oils on the tissue fatty acid composition and growth of female broilers. *Brit. Poult. Sci.* 1994; **35**, 107-18.
- [3] RH Harms, GB Russell and DR Sloan. Performance of four strains of commercial layers with major changes in dietary energy. *J. Appl. Poult. Res.* 2000; **9**, 535-41.
- [4] M Ozdoga and M Aksit. Effects of feeds containing different fats on carcass and blood parameters of broilers. *J. Appl. Poult. Res.* 2003; **12**, 251-6.
- [5] HJ Witt and A Hugo. Effect of dietary lipid sources on production performance of broilers. *S. Afr. J. Anim. Sci.* 2009; **39**, 45-8.
- [6] M Ozdogan, A Alcicek and M Aksit. Effect of dietary fat in summer season on performance and cost efficiency in broilers. *Archiv fur Geflugelkunde* 2004; **68**, 115-9.
- [7] H Takeuchi, T Matsuo, K Tokuyama, Y Shimomura and M Suzuki. Diet-induced thermogenesis is lower in rats fed a lard diet than in those fed a high oleic acid safflower oil diet, a safflower oil diet of a linseed oil diet. *J. Nutr.* 1995; **125**, 920-5.
- [8] W Zollitsch, W Knaus, F Aichinger and F Lettner. Effects of different dietary fat sources on performance and carcass characteristics of broiler. *Anim. Feed Sci. Tech.* 1996; **66**, 63-73.
- [9] A Mossab, JM Hallouis and M Lessire. Utilization of soybean oil and tallow in young turkeys compared with young chickens. *Poultry Sci.* 2000; **79**, 1326-31.
- [10] LB Crew, RH Machemer, RW Sharp and DC Foss. Fat absorption by very young chick. *Poultry Sci.* 1972; **51**: 738-42.
- [11] M Ozdogan and M Sari. The effects of mixed fat in broiler rations on the performance in summer season. *J. Adnan Menders Univ. Agric. Facul.* 2006; **3**, 5-50.
- [12] National Research Council. *Nutrient Requirements of Poultry*. 9th ed. National Academy Press, Washington, 1994.
- [13] WT Friedewald, RI Levy and DS Fredrickson. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin. Chem.* 1972; **18**, 499-502
- [14] Institute Inc. *SAS User's Guide*. Cary, SAS institute Inc, NC, 2000.
- [15] N Crespo and E Esteve-Garcia. Nutrient and fatty acid deposition in broilers fed different fatty acid profiles. *Poultry Sci.* 2002; **81**, 1533-42.
- [16] A Viveros, LT Ortiz, ML Rodriguez, A Rebole, C Alzueta, I Arija, C Centeno and A Brenes. Interaction of dietary high-oleic-acid sunflower hulls and different fat sources in broiler chickens. *Poultry Sci.* 2009; **88**, 141-51.
- [17] JO Atteh and S Leeson. Effects of dietary fatty acids and calcium levels on performance and mineral metabolism of broiler chickens. *Poultry Sci.* 1983; **62**, 2412-9.
- [18] M Sanz, A Flores, P Perez De Ayala and CJ Lopez-Bote. Higher lipid accumulation in broilers fed saturated fats than in those fed unsaturated fats. *Brit. Poult. Sci.* 1999; **40**, 95-101.
- [19] VA Bowes, RJ Julian and T Stirtzinger. Comparison of serum biochemical profiles of male broilers with female broilers and white leghorn chickens. *Can. J. Vet. Res.* 1989; **53**, 7-11.
- [20] A Meluzzi, G Primiceri, R Giordani and G Fabris. Determination of blood constituents reference values in broilers. *Poultry Sci.* 2009; **71**, 337-45.
- [21] ED Peebles, JD Cheaney, JD Brake, CR Boyle, MA Latour and CD McDaniel. Effects of added lard fed to broiler chickens during the starter phase. 2. Serum lipids. *Poultry Sci.* 1997; **76**, 1648-54.
- [22] JG Ross, G Christie and WG Halliday. Haematological and blood chemistry "comparison values" for clinical pathology in poultry. *Vet. Rec.* 1978; **102**, 29-31.
- [23] E Huff-Lonergan and SM Lonergan. Mechanisms of water holding capacity of meat: The role of postmortem biochemical and structural changes. *Meat Sci.* 2005; **71**, 194-204.

- [24] S Barbut. Estimates and detection of the PSE problem in young turkey breast meat. *Can. J. Anim. Sci.* 1996; **76**, 455-7.
- [25] RD McCurdy, S Barbut and M Quinton. Seasonal effect on pale soft exudative (PSE) occurrence in young turkey breast meat. *Food Res. Int.* 1996; **29**, 363-6.
- [26] CM Owens, EM Hirschler, SR McKee, R Martinez-Dawson and AR Sams. The characterization and incidence of pale, soft, exudative turkey meat in a commercial plant. *Poultry Sci.* 2000; **79**, 553-8.
- [27] RL Woelfel, CM Owens, EM Hirschler, R Martinez-Dawson and AR Sams. The characterization and incidence of pale, soft, and exudative broiler meat in a commercial processing plant. *Poultry Sci.* 2002; **81**, 579-84.
- [28] RL Woelfel and AR Sams. Marination performance of pale broiler breast meat. *Poultry Sci.* 2001; **80**, 1519-22.
- [29] RD Benefield. 1997, Pathogen Reduction Strategies for Elimination of Food Borne Pathogens on Poultry during Processing. M.S. Thesis. Auburn University, Alabama, USA.
- [30] W Chantarapanont, M Berrang and JF Frank. Direct microscopic observation and viability determination of *Campylobacter jejuni* on chicken skin. *J. Food Prot.* 2003; **66**, 2222-30.
- [31] A.P Saxena and KM Vyas. Antimicrobial activity of seeds of some ethnomedicinal plants. *J. Econ. Taxonomic Bot.* 1986; **8**, 291-300.
- [32] ZSH Ismail. Effects of dietary black cumin growth seeds (*nigella sativa* L.) or its extract on performance and total coliform bacteria count on broiler chicks. *Egypt. Poultry Sci.* 2011; **31**, 139-48.
- [33] FC Guo, BA Williams, RP Kwakkel, HS Li, XP Li, JY Luo, WK Li and MW Verstegen. Effects of mushroom and herb polysaccharides, as alternatives for an antibiotic, on the cecal microbial ecosystem in broiler chickens. *Poultry Sci.* 2004; **83**, 175-82.
- [34] FR Durrani, N Chand, K Zaka, A Sultan, FM Khattak and Z Durrani. Effect of different levels of feed added black seed (*Nigella sativa* L.) on the performance of broiler chicks. *Pakistan J. Biol. Sci.* 2007; **10**, 4164-7.