Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Scholars Research Library

Annals of Biological Research, 2011, 2 (3): 113-120
(http://scholarsresearchlibrary.com/archive.html)

Annals of Biological Research
Scholars Research Library

ISSN 0976-1233
CODEN (USA): ABRNBW

Effect of fish oil Fed a Low-protein Diet on performance, carcass characterizes and blood indices in Broiler chicks

Navid Hosseini Mansoub

Islamic Azad University, Maragheh branch, Maragheh, Iran

ABSTRACT

The objective of this study was to assess the influence of fish oil (FO) in two different levels of protein on growing broilers and some blood parameters.The birds were fed a common control diets, in the pre-starter phase (1 to 7 d), and from d 8 on, 480 birds were assigned to 8 dietary groups and received the diets supplemented with 0, 1.5, 3, or 6 % FO with two dietary crude protein (CP) level (21.5 and 19.5% for grower & finisher phases, respectively) and 10% diluted CP (19.35 and 17.55 for grower & finisher phases, respectively), throughout a 35-d growth period.. Body weight gain (BWG), daily BWG, feed conversion ratio (FCR) and feed intake were measured. The birds slaughtered after blood sampling. The FCR were improved in the groups treated with fish oil. Highest final BW, highest daily BW gain, and best FCR were recorded for the 1.5% FO dietary group. A 10% reduction in dietary CP level decreased the weight gain and feed intake of chickens. Feed conversion ratio of chicks fed the crude protein diluted diets was higher ($P<0.05$) than chicks fed recommended CP level. In addition, with increasing levels of FO in the broiler diets, the blood glucose (G) level increased, total protein (TP), albumin (A) and globulin (GL) concentrations decreased.

Keywords*:* broiler, fish oil, growth performance, Carcass trait, blood.

INTRODUCTION

Fish oil is one of the available sources used in the diet to provide energy for fowls while it's supplementation has been shown to improve body weight gain, feed conversation ratio and safety in the poultry [1-4] The positive effect of adding fish oil to broiler diets on bird performance has been already demonstrated. Fish oil has been administered to poultry diet to enhance production performance and immune responses. Fish oil is one of the available sources used in the diet to provide energy for fowls while it's supplementation has been shown to improve body weight gain, feed conversation ratio and safety in the poultry [5-7].Fish oil contains unsaturated fatty acids with long omega-3 chains (LC-n-3 PUFA), ecosapentanoic acid (EPA20:5n-3) and

decosahexanoic acid (DHA22:6n-3) that improve health-related factors in humans and animals [8]. Numerous studies indicate that dietary fish oil declines the level of total cholesterol, low density lipoprotein (the major carrier of cholesterol in plasma) and triglycerides [9]. Also, it has been shown that oils rich in polyunsaturated fatty acids can modifies abdominal fat deposition in broiler chickens.. It is well known that low-protein diets promote higher rates of de novo hepatic lipid synthesis in chickens than high protein diets [10]. The purpose of this experiment was to study the effect of different dietary levels of fish oil (ω-3 rich) on performance parameters, carcass characteristics and blood indices in different protein levels in broiler chickens

MATERIALS AND METHODS

A number of 480 one-day-old Cobb500 broiler chickens were obtained from a commercial hatchery and fed a common feed starter (control diet, T_0) from 1 to 7 day-old. In age of one week, randomly allocated to 24 floor pens with 20 birds per pens.. Chicks were fed the experimental diets from 8 to 28 d (grower phase) and 29-42 d (finisher phase). The study was conducted as a completely randomized design, with a factorial arrangement of fixed treatment effects consisting of two dietary crude protein (CP) level, first 21.5% for grower and 19.5% for finisher [standard protein(SP)],second 10% diluted CP, 19,35% for grower and 17.55% for finisher [low protein(LP)]. Treatments were following; T_0 =0.0% fish oil (FO)+SP control; T_1 =1.5% FO+SP; T_2 =3% FO+SP; T_3 =6% FO+SP; T4=0.0 %FO+LP;T5 =1.5% FO+ LP; T6 =3% FO+ LP; T7 =6% FO+LP. The Ingredients and nutrient analysis for experimental diets is shown in Table1.

Birds were weighed every week and data on weekly food intake, food conversion ratio (FCR) (food intake/ weight gain) were recorded in each replicate group upto 42 days. Broilers slaughtered (two male and two female) and carcass yield, abdominal fat, breast, thighs, liver and spleen were characterized as a percentage of live weight.

For evaluate some blood parameters such as triglycerides, cholesterol, glucose (G), total protein (TP), albumin (A) and globulin (GL) concentrations, blood sampling were performed in ages of 21 and 41 days. Blood samples were taken from the wing vein by injection into the vacuum tubes and were collected in non-heparinized tubes by puncturing the brachial vein.

All data (pen means) were analyzed using a completely randomized design and were subjected to ANOVA using the GLM procedures of SAS software [11]. Means were compared by the least significant difference (LSD) procedure of the same statistical package and then Statistical significance was considered at a *P* of -0.05.

RESULTS

Growth performance

The effects of diet supplementing with FO on the performance are shown in Table 2 and Table 3. Inclusion of fish oil to diet significantly affected body weight in the 5^{th} and 6^{th} week ($p<0.05$). Body weights increased upto 3% fish oil supplement but the final weight was significantly decreased in group T_3 (broilers fed from 6% fish oil). Also, feed intake decreased by diet supplementing with 6% FO. A 10% reduction in dietary crude protein level decreased the final body weight and daily weight gain ($P<0.05$) of chickens also affected the daily feed intake and decreased it. Daily weight gain and FCR were improved in FO treatments in compared to control and the best results were observed in groups T_1 and T_2 (1.5 and 3% FO, respectively). In the total period, the highest feed consumption rate was observed in group T_7 and was lowest in group T_1.

Table 1: diet components for different study groups and chemical composite analysis

	Growth diet(SP)				Final diet(SP)				Growth diet(LP)				Final diet (LP)			
	T0	T1	T2	T3	T0	T1	T2	T3	T4	T5	T6	T7	T4	T5	T6	T7
Yellow corn	56.9	56.5	56	50.2	63	62.5	62.5	56.47	63.2	62.82	62.75	56.91	66	66.5	66.8	61
Soybean meal	33.5	30.3	33.5	36.20	28.17	27.9	27.2	29.20	27.12	26.8	26.3	28.62	22.6	22.6	22.45	24.5
Corn gluten	2.9	3	3	1.94	1.77	2.07	2.54	2	1.87	2.21	2.63	2.1	2.7	2.7	2.7	2.1
Inert	0	0	0	1.64	0.4	0.37	0.6	2.65	0.4	0.37	0.6	2.65	1.67	1.17	1.02	2.85
Fish oil	0	1.5	3	6	0	1.5	3	6	0	1.5	3	6	0	1.5	3	6
Oyster shell	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1
Dicalcium phosphate	2	2	2	2	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.5	1.5	1.5	1.5
Salt	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Vitamin/mineral premix[3]	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
DL-Methionine	0.1	0.1	0.1	0.1	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.09	0.09	0.09	0.09
L-Lysine	0.0	0.0	0.0	0.0	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.04	0.04	0.04	0.04
Animal fat	2.75	1.7	0.25	0.02	3	0.5	0.5	0.02	3	0.5	0.5	0.02	3.5	0.5	0.5	0.02
Vitamin E	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.01	0.10	0.10	0.01	0.01	0.10	0.10	0.01	0.01
Total	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Calculated nutrient content																
Crude fat	0.06	0.07	0.07	0.07	0.06	0.07	0.07	0.08	0.06	0.07	0.07	0.08	0.07	0.08	0.08	0.08
Dry matter	89.03	89.97	88.80	89.00	89.00	89.07	89.57	88.57	89.00	89.07	89.57	88.57	0.10	0.10	0.01	0.01
Moisture	10.97	11.03	11.20	11.00	11.00	10.93	10.43	11.43	11.00	10.93	10.43	11.43	100	100	100	100
ME (Kcal/Kg)	3000				3050				3050				3100			
Protein (%)	21.5				19.5				19.35				17.55			
Calcium	0.81				0.83				0.83				0.84			
Available P	0.40				0.41				0.41				0.32			
Lysine	1.19				1.18				1.18				1.04			
Methionine	0.48				0.49				0.49				0.33			
Methionine+ cystine	0.81				0.73				0.73				0.65			

For each kg of the diets; vitamin A, 9,000,000 IU; vitamin D3, 2,000,000 IU; vitamin B1, 1,800 mg; vitamin B2, 6,600 mg; vitamin B3, 10,000 mg; vitamin B6, 3,000 mg; vitamin B12,15 mg; vitamin E, 18,000 mg; vitamin K3, 2,000 mg; vitamin B9, 1,000 mg; vitamin B5, 30,000 mg; folic acid, 21 mg; nicotinic acid, 65 mg; biotin, 14 mg; choline chloride, 500,000 mg; Mn, 100,000 mg; Zn, 85,000 mg; Fe, 50,000 mg; Cu, 10,000 mg; I, 1,000 mg; Se, 200 mg.

Table 2: Effects of fish oil and crude protein concentration on mean body weight in different weeks (g)

Treatment	Week 2	Week 3	Week 4	Week 5	Week 6
T_0	359.54^{a}	753.25^{a}	1248.78^{a}	1845.16 ^{a}	2321.08 ^{ab}
T_1	363.93^{a}	763.72^{a}	1255.96^{a}	1828.75 ^{ab}	2432.89 ^{a}
T_2	360.23^{a}	758.24^{a}	1257.31^{a}	1805.84 ^{cb}	2393.01 ^{ab}
T_3	359.55^{a}	746.68^{a}	1242.25^{a}	1800.90 ^{c}	2290.38 ^{b}
T4	291.65^{b}	685.87^{b}	1123.71^{b}	1681.32^{b}	2012.12^{c}
T5	292.52^{b}	684.65^{b}	1128.76^{b}	1682.56^{b}	2014.31^{c}
T6	270.31^{ab}	678.75^{ab}	1119.91^{ab}	1661.12^{ac}	1991.51^{c}
T7	268.91^{ab}	669.23^{ab}	1114.11^{ab}	1657.76^{ac}	1982.92^{ac}
SE	4.37	9.12	13.17	20.49	17.15

^{ac}: Values in the same row and variable with no common superscript differ significantly($P<0.05$)

Table 3: . Effects of fish oil and crude protein concentration on daily weight gain, feed intake and FCR in different groups

Experimental diets	Body weight gain (g/bird/day)	Daily feed intake (g/bird/day)	FCR (g:g)
T0	55.27 ^{b}	98.73 ^{ab}	1.79^{a}
T1	57.98^{a}	99.94^{ab}	1.71 ^{b}
T2	55.96 ^{b}	98.91 ^{ab}	1.74 ^{b}
T3	54.54 ^{b}	95.66 ^{b}	1.75 ^{b}
T4	51.18^{c}	94.87^{b}	1.88^{c}
T5	52.34^{ab}	95.67^{b}	1.94^{ab}
T6	49.12^{c}	93.05^{ab}	1.95^{ab}
T7	48,97^{c}	92.85^{ab}	2.09^{ac}
SE	0.39	0.77	0.01

^{ac}: Values in the same row and variable with no common superscript differ significantly($P<0.05$)

Table 4. Effects of fish oil and crude protein concentration on some blood parameters (mg dL^{-1}) .

Variable (mg/dl)	Tr	Chol	G	TP	A	GL
In 21 d						
T_0	112.31	114.74	88.02	3.79	2.02	1.78
T_1	118.11	114.98	98.88	3.78	2.06	1.73
T_2	104.52	113.58	120.05	3.70	2.06	1.69
T_3	101.23	111.96	121.12	3.54	1.94	1.59
T_4	95.88	104.70	73.37	3.01	1.91	1.51
T_5	96.24	103.57	76.45	2.58	1.90	1.50
T_6	90.11	100.23	86.34	2.50	1.92	1.45
T_7	88.78	99.81	90.51	2.48	1.85	1.41
SE	1.21	0.55	2.51	0.114	0.064	0.061
Significance	**	*	*	**	NS	*
In 42 d						
T_0	111.53	114.60	110.75	3.85	2.10	1.72
T_1	106.62	105.56	111.60	3.74	2.08	1.65
T_2	113.62	104.52	123.10	3.65	1.90	1.66
T_3	99.85	98.11	131.20	3.43	1.82	1.65
T_4	92.75	91.41	106.91	3.12	1.79	1.51
T_5	91.19	90.85	109.94	3.19	1.69	1.49
T_6	93.77	89.09	110.59	3.23	1.61	1.41
T_8	90.22	88.13	111.32	3.20	1.60	1.40
SE	2.28	2.53	3.38	0.058	0.033	0.035
Significance	**	*	*	*	**	NS

^{ab}: Values in the same row and variable with no common superscript differ significantlyTr = triglyceride, Chol= cholesterol,G= glucose, TP= total protein, A= albomin, GL= glubolin. * : $P<0.05$, **: $P<0.01$, NS: Not Significant.

Feed conversion ratio of chicks fed the crude protein diluted diets was higher (P<0.05) than chicks fed recommended 21% CP level.

3.1. Blood parameters

Data presented in Table 4 show the effects of diet supplementing with fish oil and protein on serum biochemical values of broilers at 21 and 42 days of age. Dietary fish oils altered selected blood parameters in broiler Chicks. Blood amounts of cholesterol and triglyceride were decreased after a 35-d trial period. In the ages of 21 and 42 days, significant decrease was observed in triglyceride content (P<0.01). In samples of mentioned days also, glucose (G) content was increased (P<0.05) in both groups with different levels of protein. But the amounts of total protein (TP), albumin (A) and globulin (GL) concentrations did showed decline.

DISCUSSION

Growth performance

Growth performance of broilers fed from FO were improved probably because of the dietary fat composition with type of long-chain n-3 FA that makes it possible to increase diet digestibility and to stimulate growth and feed efficiency (Table 2 and Table 3). Dobrzansiki et al. [12] reported an increased daily weight gain compared to the control group by adding 3% fish oil to the base diet. Alparslan and ÖZdogan [6] did not detect significant difference in body weights by adding 2% and 4% fish oil to the base diet; however, the presence of 2% fish oil in the diet lead to higher value of body weight. Results related to performance parameters in the present experiment is in agreement with the findings of Newman et al. [13], lopez ferrer et al. [14] and Farhoomand and Chekani-azer [7] have reported that the digestibility of fat increases as the degree of unsaturation increases. Therefore, the good performance of FO-fed broiler may be related to the FA composition of the FO. It is well known that excessive dietary amino acids promote hepatic lipogenic enzymes and lipogenesis [15]. On the other hand, it seems that low-protein diets stimulate higher rates of de novo hepatic lipid synthesis in chickens than high protein diets [10].

The percentage of fish oil in the diet seems to indicate decrease or increase in food consumption. Low percentage (3-8 grams per Kg or less than 1%) of auto oxidated fish oil [16] or Ethyl ester fish oil or Glyceryl ester fish oil [5] leads to an improved food consumption rate and high percentage (2% and 4%) results in lower food consumption rate in the final period [6] Lopez-Ferrer et al [17] showed that 4% fish oil does not have negative effect on food consumption. However, other studies show that even high-percentage menhaden fish oil (8.2%) results in an increased food consumption compared to the control group. But the amount of this increase is lower in compared to effect of lower-percentage fish oil [4]. Hence, the use of saturated fatty acids in combination with unsaturated fatty acids results in their better digestion due to their synergist effect. The appropriate proportion of these fatty acids can be effective in different ages of birds with regard to appropriate digestion of fat. The feed conversion ratio of chicks fed diets containing less crude protein increased, because of the adverse effects of a protein deficiency on body weight gain. In poultry, 60 to 65% of the ingested dietary N is excreted via excreta [18-19]. This means that only 35 to 40% of dietary protein is retained by animals. Low protein diets supplemented with synthetic amino acids resolved this problem. Although acceptable productive performance can be achieved by feeding lowprotein diets, this will be associated whit an increase in whole-body fat deposition [18-19].

In our study, group T_1 showed better final weight, better daily weight gain and improved feed efficiency. It effect could be due to the appropriateness and synergist effects between the

saturated and the unsaturated fatty acids, though other factors such as free fatty acids and the diet composition in terms of fatty acids have influence on fat digestion.

With regard to the results of current study and other similar studies in this field, factors such as the percentage and or types of fish oil, it's flavor (indicative of oxidation, aldehyde and ketone-body levels) and other components of the diet can be considered effective on food consumption rate and the performance of broilers.

Serum biochemical values

Diets containing omega-3 and omega-6 fatty acids decrease the plasma cholesterol and triglyceride levels compared to diets with saturated fatty acids [20]. This differences may be due to alteration of fluidity and composition of plasma cell walls. By adding fish oil to broiler diet, amount of the triglyceride, cholesterol, total protein, albomin, and glubolin were decreased and glucose was increased. These changes could be due to fish oil and enrichment of diet with omega-3 fatty acids [6,21].

Derease the cholesterol and triglyceride contents in serum of birds can be resulting presence polyunsaturated fatty acids from direct depot from diet (that is more appropriate) or conversion from precursors by denovo synthesis (desaturation and elongation) in liver and tissue . But, several factors influence the activities of desaturases and elongases [22]. Trans fats, saturated fatty acids, and cholesterol interfere with EFA metabolism and promote inflammation, atherosclerosis and coronary heart disease [23]. This suggests that trans fats, saturated fats, and cholesterol have pro inflammatory actions whereas EFAs and PUFAs possess anti-inflammatory properties.

Crespo and Esteve Garcia [9] were reported that omega-3 fatty acids reduce the blood VLDL levels, acting to lower the circulating free LDL concentration and also, reduce the rate of triglyceride synthesis in the liver. Ozdogan and Aksit [24] found that the marin and vegetable origins rich in LC -3 PUFA improve animal growth and product quality which more important reason can be have lesser blood HDL and LDL values and thus lesser Chol and Tr contents of body [25]. Researchers were showed that low HDL and high LDL are values associated with atheroschlorosis and coronary heart disease [9,26]

Diet supplementation with FO increased the blood glucose content (Table 4) and the highest value was observed in the group 3% FO (O_3). Researchers previously has been reported that diet rich in polyunsaturated fatty acid increase the amount of serum glucose because of decline insulin secretion [27-28]. Mori et al., [29] were reported that with feeding Dietary fish and fish oil / meal to human and animals, decreased blood pressure, G value were higher ($P<0.05$). Long chain n-3 enrichment of a high-saturated fat diet exerts a rapid effect to lower insulin secretion from the islets of langerhans and raising the plasma glucose concentration [9].

One of the key reason as to why omega 3 fish oil has such a powerful effect on fat and carbohydrate metabolism is that the insulin levels secretion can be changed to FO or the omega-3 PUFAs thereof [9] Insulin is a hormone that reduces the use of fat for fuel. While also promoting fat storage in the presence of excess calories. It inhibits the action of hormone sensitive lipase, which is responsible for beraking down stored fat and preparing in for use as energy. In addition, insulin activites an enzyme, which, along with fatty acid synthesis, is responsible for converting carbohydrate into fat [30]. Therefore, the drop in insulin levels when diet supplemented with fish oil, would have allowed more fat to be used for energy accompanying raise blood glucose.

The serum TP, A and GL concentrations of the birds fed dietary FO were significantly decreased in compared to the birds of group control. (Table 4). Touchburn et al. [31] by study on glucose/ insulin handstand and dietary protein in broiler reported that diet supplementation with fats rich in PUFA, TP, A and GL levels of serum were dropped. Because, fats for transmission in blood must were mixed with proteins in the form of complex compositions of hydrophile lipoprotein and with consider to dencity of pure lipids is less from watter. Therefore, decrease the protein dencity can be due to increase lipid/protein ratio [32].

REFERENCES

[1] H.W. Hulan, R.G. Ackman, W.M. Ratnayake, F.G. Proudfoot ; *Canadian Journal of Animal Science*.**1988**, 68,533–547.
[2] K.L. Fritsche, N.A. Cassity, S.C. Huang ; *Poultry Sci*. **1991**, 70,611-617
[3] Fritsche K. L., Cassity N. A., Huang S.-C. *J. Nutr*. **1992**; 122:1009-1018
[4] S. Lo´pez-Ferrer, M.D. Baucells, A.C. Barroeta, M.A. Grashorn ; *Poultry Science*.**1999**, 78, 356-365.
[5] M.Schreiner, H.W. Hulan,E. Razzazi-Fazeli, J. Bohm, R Moreira; *Journal of the Science of Food and Agriculture*.**2005**, 85, 216-226
[6] G. Alparslan and M. Özdogan; *International Journal of Poultry Science* **2006**, 5 (5), 415-419.
[7] P. Farhoomand, and S. Chekani-azer; *J. Appl. Poult. Res*. **2009** 18,508-513.
[8] N. Crespo, E Esteve-Garcia; *Poultry Science* **2001**, 80: 71-78.
[9] N. Crespo,E Esteve-Garcia; *Poultry Science* **2003**, 82,1134–1139.
[10] E.E. Donaldson, *Poult. Sci*.,**1985**, 64, 1199– 1204
11. SAS software, Ver. 9.1. Sas Institute, USA.
[12] Z. Dobrzański,D. Jamroz,P Bykowski, T Trziszka; *Electronic Journal of Polish Agriculture Universities*.**2001**, 1, 43-51
[13] R.E.Newman,J.A. Downing, W.L Bryden,E, Fleck, W.A. Buttemer ,L.H. Storlien; *Proceeding of Nutrition Society of Australia*,**1998**, 22: 54.
[14] S. Lo´pez-Ferrer,M.D. Baucells,A.C. Barroeta, J Galobart, M.A. *Poultry Science*,**2001**, 80: 753–761.
[15] A. Katsurada, N. Iritani, H. Fukuda, T. Noguchi , T. Tanaka; *Biochim. Biophys. Acta*,**1986**, 877, 350–358
[16] J.Koreleski,S. Świątkiewicz; *Journal of Animal and Feed Sciences* **2006**,15, 631–640.
[17] S. Lo´pez-Ferrer ,M.D. Baucells, A.C. Barroeta, J. Galobart ,M.A, *Poultry Science*,**2001**, 80: 753–761.
[18] V.A. Aletor, E. Hamid, E. Niess, E. Pfeffer; *J. Sci. Food Agric*.,**2000**, 80, 547–554
[19] V.A. Aletor, F.X. Roth, B.R. Paulicks, D.A. Roth-Maier; *Arch. Geflu¨gelkd*,**2002**, 66, 21–30
[20] R.E. Newman, J.A. Downing, W.L. Bryden, E. Fleck, W.A. Buttemer, L.H. Storlien, *Proc Nutr Soc Aust*, **1998**, 22,54.
[21] H. Manilla, A.F. Husveth, K. Nemeth, *Acta Agraria Kaposvariensis*.**1999**, 3: 47-57.
[22] H.W. Cook ; *Lipids* **1981**,16:920-6.
[23] E. Lopez-Garcia,M.B. Schultze,J.B. Meigs,J.E Manson,N. Rifai,M.J. Stampfer, W.C. Willett, F.B. Hu ; *J Nutr* **2005**,135,562,6.
[24] M. Özdogan, A. Aksit; *J. Appl. Poult. Res*,**2003**, 12, 251-256.
[25]S.M. Grundy ; *Arterioscler. Thromb*.**1991**, 11,1619–1635.
[26] R. Couderc, M. Machi ; *Ann-Biol-Clin*,***1999***, 57(2),157-67.
[27] V. Grill, and E. Qvigstad;Fatty acids and insulin secretion.Br. J. Nutr.**2000**, 83(1): 579–584.
[28] L.H. Storlien, J. A. Higgins, T.C. Thomas, M. A. Brown. H. Q. Wang. X. F. Huang, P. L. Else; *Br. J. Nutr*. **2000**,83(1): 85-90.

[29] T.A. Mori, O. Michael, J.M..Michael ; *American Journal of Clinical Nutrition* .**1999**, 70(10): 817-825.
[30] J. Mark,F. Holness, D. Smith, K Gemma, K. Greenwood, , C. Mary, C. Sugden ; *the American Diabetes Association.* **2004**, 53, 166-171.
[31] S. Touchburn, J. Simon, B. Leclercq. ; *J. Nutr*. **1981**,111:325–335.
[32] J.M. Olomu, and V. E. Baracos ; *Poultry Sci.* **1991**,70,1403–1411.