



### ABSTRACT

The present study conducted to investigate the effect of energy sources and levels on performance and breast meat amino acids profile in Cobb 500 broiler chicks. A total of 600 1-day-old Cobb 500 broiler chicks with an average weight of  $39 \pm 0.50$  g were randomly divided into five treatments. Each treatment was further divided into four replicates. Chicks were fed a basal diet based on corn and energy level was same as Cobb 500 manual as control group, the basal diet with 3% lesser energy than control (T1), the basal diet with 6% lesser energy than control (T2), the basal diet based on corn and fat level according to Cobb 500 instruction manual (T3), the basal diet based on corn and fat with 3% upper energy (T4) for 42 days. Results showed that the best and the worst performance were for T4 and T2, respectively. Also, feed intake of chicks increased significantly in T4. Body weight gain was also significantly higher in the treated group with the basal diet based on corn and fat with 3% upper energy (T4). As result relevant although the lowest feed conversion ratio was for control and T3 on 14 and 28 days but also it was at the lowest on T3 on 42 days. The breast muscle function and amino acid profiles showed that there were significant effects between arginine (Arg), glutamic acid (Glu), proline (Pro), alanine (Ala), aspartic acid (Asp), serine (Ser), glycine (Gly), isoleucine (Ilo), lysine (Lys), valine (Val) and phenylalanine (Phy) amino acids ( $P \le 0.01$ ). The highest level of amino acids (g/g DM basis) was for Glu, Asp, Arg, Lys, Ser, Phe and Pro. In conclusion, it seems that inclusion of higher energy level than broiler nutritional requirements recommendation for Cobb 500 chicks could give better performance and affect the quantity and quality of their breast meat and its amino acids profile. In order to achieve higher weights, more energy is needed than the recommendation of Cobb 500, but to have better feed conversion ratio the recommended energy level is the best.

KEY WORDS amino acids, breast meat, broiler, energy sources, performance.

# INTRODUCTION

Poultry meat is a good source of protein having high biological value, high digestibility and relatively little fat content and it is a good source of iron and some of the Bcomplex vitamins and fat-soluble organic compounds (Marcu *et al.* 2013). It is well known that this huge demand for poultry meat has put pressure on breeders, nutritionists, and farmers to improve the growth rate of birds, feed efficiency and breast meat yield. One of the major challenges to researchers is the provision of alternative feeds for monogastric animals. Corn has remained the major energy source in compounded diets for poultry. The various uses to which corn is being committed, such staple food for man, brewing and confectionary, has placed additional cost constraints on its continued use in poultry diets. The solution is to explore the use of alternative feed ingredients, hitherto under exploited by poultry farmers (Durunne *et al.* 2015). Among the alternative feedstuff which could be used as energy sources for poultry diet even though it is lower in protein and other essential nutrients (Odukwe, 1994). Poultry meat and its products are important components of the diet of developed countries and their consumption is affected by various sensory properties such as color, tenderness and flavor (Resurreccion, 2002).

These changes have driven the poultry industry to put an emphasis on the improvement of breast meat yield and muscle mass development (Abdullah and Matarneh, 2010). Energy and protein are very important nutrients for broilers like other living creatures. Energy is required for body functioning and protein is an essential constituent of all tissues of animal body. Protein having major effect on growth performance of the bird is the most expensive nutrient in broiler diets (Kamran et al. 2004). The usefulness of a protein feedstuff for poultry depends upon its ability to supply a sufficient amount of the essential amino acids that the bird requires, as well as the protein digestibility and the level of toxic substances associated with it (Scanes et al. 2004). In general, vegetable protein sources are nutritionally unbalanced and poor in certain essential amino acids and this decreases their biological value as they may not furnish the required limiting amino acids needed by birds for egg and meat production. Poultry nutritionists have paid more attention to the use of animal protein sources to create a balanced diet (Akhter et al. 2008).

Fats or oils are energy rich feeds. Fats also provide varying quantities of the essential nutrient linoleic acid (Leeson and Summers, 2001). Another important role of fats in diet is its inhibition from de novo lipogenesis in broiler chickens (Wongsuthavas et al. 2011) that could increase energy efficiency in diets. Researchers (Haunshi et al. 2012) concluded that provision of 2600 kcal/kg ME and 16% crude protein would be ideal for optimum growth of Aseel birds during juvenile phase. However, to obtain better feed conversion ratio (FCR), feeding Aseel birds with diet having 2800 kcal/kg ME and 16% CP would be ideal. It was shown that feeding different levels of soybean oil in chicks fed with a NRC recommended protein level had no significant effect on weight gain in 7-21 days (Tabeidian et al. 2005). Another researcher (Maiorka et al. 2004) showed that feed intake was not significantly influenced by the way amino acid requirements were expressed. They also noted that feed intake was not influenced by dietary energy level. However, metabolizable energy intake was higher in birds fed diets containing 3200 kcal ME/kg. Other researcher (Nobre et al. 1994) evaluated four energy levels and reported no significant differences in carcass yield between treatments.

Batal and Parsons (2004) indicated that metabolizable energy corrected for nitrogen (Men), varies among carbohydrate sources and increases with age for most carbohydrate soybean meal diets. Murugesan et al. (2013) suggested that energy is used following the pattern of production and maintenance before storage requirements and that fat pad (energy storage) may be the most sensitive indicator of dietary energy status over short term in Hybrid Line W36 laying hens. El-Yamany et al. (2008) noted that replacement of different levels of linseed oil, sun flower oil, or olive oil from poultry fat in control diet of Japanese quail improved the digestibility of all nutrients crude protein and ethyl extract while crude fiber digestibility remained affected. Additionally, no significant effect was recorded on both edible giblets (gizzard, liver and heart) and offal (blood, feather, legs, head and viscera) percentage. Brake (1990) reported that 5% addition of fat to broiler breeder diets resulted in improved egg production and reduced feed intake. Due to the effects of different sources of fat and protein levels on performance and amino acid profile on poultry meat this study was performed to investigate the effect of energy sources and levels on performance and breast meat amino acids profile in Cobb 500 broiler chicks.

# MATERIALS AND METHODS

#### **Birds and diets**

All procedures used in this experiment were approved by the Department Science and Research Branch, Islamic Azad University, Tehran, Iran. A total of 600 1-day-old Cobb 500 commercial broiler chickens with an average weight of  $39 \pm 0.50$  g were randomly distributed into five dietary treatments with four replicates each. The birds were housed in groups of 5 in 20 pens under standard conditions of temperature, humidity and ventilation. The study was carried out at the poultry farm of Atomic Energy Organization, Karaj, Iran, for a period of 6 weeks. Chicks were fed the basal diet based on corn-soybean meal and energy level by Cobb 500 instruction manual as control group, basal diet with 3% lesser energy than control (T1), basal diet with 6% lesser energy than control (T2), basal diet based on corn and fat level according to Cobb 500 instruction manual (T3), basal diet based on corn and fat with 3% upper energy (T4). The experimental diets formulated using broiler performance and nutrition supplement Cobb 500 (2013). Diets and fresh water were provided ad libitum during the experiment (Tables 1, 2 and 3).

### **Data collection**

The live body weight gains and feed consumption of chick were measured individually, while feed conversion efficiency was performed weekly.

 Table 1
 Composition (measured in %) of the experimental diets for broiler chicks (0-14 days old)

Ingredients	Control	T1	T2	T3	T4
Corn	63.38	63.33	62.13	58.27	54.30
Soybean meal	22.57	28.86	31.48	31.90	32.21
Corn gluten meal	9.17	3	0	2.7	4.7
Soybean oil	0	0	0	2.5	4.14
Di-calcium phosphate <sup>1</sup>	2.07	2.06	2.06	2.05	2.05
Oyster shell	1.06	1.03	1.01	1.01	1.01
NaCl	0.38	0.38	0.38	0.38	0.38
DL-methionine	0.24	0.33	0.35	0.30	0.26
L-lysine	0.53	0.40	0.36	0.31	0.36
L-threonine	0.10	0.11	0.13	0.08	0.09
Mineral and vitamin premix <sup>2</sup>	0.5	0.5	0.5	0.5	0.5
Filler <sup>3</sup>	0	0	1.60	0	0
Total	100	100	100	100	100
Calculated nutrient content					
Metabolizable energy (kcal/kg)	3035	2943	2853	3035	3120
Crude protein (CP) (%)	22	22	22	22	22
Ca (%)	0.90	0.90	0.90	0.90	0.90
P (%)	0.45	0.45	0.45	0.45	0.45
Met (%)	0.65	0.67	0.67	0.67	0.67
Lys (%)	1.18	1.18	1.18	1.18	1.18
Met + Ces (%)	0.98	0.98	0.98	0.98	0.98
Thr (%)	0.86	0.86	0.84	0.86	0.86
Trp (%)	0.18	0.20	0.20	0.21	0.21
Arg (%)	1.24	1.24	1.24	1.24	1.24
Val (%)	0.91	0.91	0.91	0.91	0.91
Na (%)	0.17	0.17	0.17	0.17	0.17
K (%)	0.70	0.80	0.82	0.81	0.81
Cl (%)	0.23	0.23	0.23	0.23	0.23

<sup>1</sup> Per kg contains: Ca: 23% and P: 18.5%.

<sup>2</sup> Provided per kilogram: vitamin A: 360000 IU; vitamin D<sub>3</sub>: 800000 IU; vitamin E: 7200 IU; vitamin K<sub>3</sub>: 800 mg; vitamin B<sub>1</sub>: 720 mg; vitamin B<sub>9</sub>: 400 mg; vitamin H<sub>2</sub>: 40 mg; vitamin B<sub>2</sub>: 2640 mg; vitamin B<sub>3</sub>: 4000 mg; vitamin B<sub>5</sub>: 12000 mg; vitamin B<sub>6</sub>: 1200 mg; vitamin B<sub>12</sub>: 6 mg; Choline choloride: 200000 mg; Manganese: 40000 mg; Iron: 20000 mg; Zinc: 40000 mg; Copper: 4000 mg.

<sup>3</sup> Inert filler used to complete diet formulations to 100%.

To evaluate amino acid compositions of breast tissue on day 28, all birds were processed after a 4 h feed withdrawal period; two birds per treatment were randomly selected and killed in a commercial slaughter house according to the international animal rights.

### Evaluation amino acid composition of breast tissue

The amino acid profile of the muscle samples was determined by the method of Antoine *et al.* (2001) cited by Okarini *et al.* (2013) and Okruszek *et al.* (2013) with slight modification. The sample obtained were 3 mg protein or 5 g chicken breast meat dry weight and then 1 mL 6 N HCL was added.

The mixture was ponged with  $N_2$  and then heated in the oven at 110 °C for 24 hours. A sample was prepared by 6 N HCl hydrolysis then dissolved in 5 mL 0.01 N HCl and filtered using Millipore 0.45 µm filter. Mobile phase A was made up of 0.025 M sodium acetate buffer (pH 6, 5) 0, 5 g Na-EDTA, 90 mL methanol and 10 mL tetra hydro folate (THF) (80:10:9:1) prepared from analytical grade dissolving to 1 L water Hi Pure. The pH of the acetate buffer (A buffer) was adjusted to 6.5 using NaOH solution, and mo-

bile phase B buffer contain 95% methanol on hi pure water. The mobile phases were ultra filtered through Millipore filter having a pore diameter of 0.45 µm (WHATMAN® diameter 25 mm) and degassed by spraying for 5 minutes with pure nitrogen. Gradient elution was generated using solvent delivery module Varian Pro Star Model Number 240 (Chromatography Systems, Walnut Creek, CA 94598 USA Made in USA), was used for controlling the gradient and flow rate (1.0 mL/min) of the mobile phases. OPA-thiol reagent was made up at least 24 hours before used by dissolving 50 mg o-phthaldialdehyde in 4 mL methanol and 0.025 mL mercaptoethanol was added. The mixture was thoroughly mixed, then 0.050 mL Brij-30 and 1 mL borax buffer solution were added. The OPA-thiol reagent stored in the dark bottle at temperature 4 °C in a tightly closed container.

The amino acid chicken breast meat were analyzed by cation exchange ICI Instrument high performance liquid chromatography (HPLC) with column Ultra Techsphere ODS 3  $\mu$  particle size, 4.6 mm  $\times$  7.5 cm PARKER 316 (SGE PTY. LTD Victoria Australia) and Ophthaldialdehyde (OPA) precolumn derivatisation.

Table 2 Composition (measured in %) of the experimental diets for broiler chicks (14-28 days old)

Ingredients	Control	T1	T2	Т3	T4
Corn	69.22	68.8	67.7	65.31	59.31
Soybean meal	18	23.43	26.2	24	28.45
Corn gluten meal	8.2	3.24	0	4.2	2.9
Soybean oil	0	0	0	2	5
Di-calcium phosphate <sup>1</sup>	1.9	1.9	1.9	1.9	1.9 1.05
Oyster shell	1.05	1.05	1.05	1.05	
NaCl	0.37	0.37	0.37	0.37	0.37
L-methionine	0.22	0.27	0.3	0.25	0.25
L-lysine	0.44	0.34	0.28	0.32	0.22
L-threonine	0.10	0.10	0.12	0.10	0.05
Mineral and vitamin premix <sup>2</sup>	0.5	0.5	0.5	0.5	0.5
Filler <sup>3</sup>	0	0	1.58	0	0
Total	100	100	100	100	100
Calculated nutrient content					
Metabolizable energy (kcal/kg)	3108	3014	2921	3108	3201
Crude protein (CP) (%)	19	18.4	17.8	19	19.5
Ca (%)	0.84	0.84	0.84	0.84	0.84
P (%)	0.42	0.42	0.42	0.42	0.42
Met (%)	0.53	0.53	0.53	0.53	0.53
Lys (%)	1.05	1.05	1.05	1.05	1.05
Met + Ces (%)	0.89	0.89	0.89	0.89	0.89
Thr (%)	0.78	0.78	0.78	0.78	0.78
Trp (%)	0.21	0.22	0.22	0.22	0.22
Arg (%)	1.25	1.25	1.25	1.25	1.25
Val (%)	0.91	0.91	0.91	0.91	0.91
Na (%)	0.19	0.19	0.19	0.19	0.19
K (%)	0.72	0.81	0.85	0.81	0.86
Cl (%)	0.35	0.33	0.32	0.32	0.31

<sup>1</sup> Per kg contains: Ca: 23% and P: 18.5%.

<sup>2</sup> Provided per kilogram: vitamin A: 360000 IU; vitamin D<sub>3</sub>: 800000 IU; vitamin E: 7200 IU; vitamin K<sub>3</sub>: 800 mg; vitamin B<sub>1</sub>: 720 mg; vitamin B<sub>9</sub>: 400 mg; vitamin B<sub>2</sub>: 2640 mg; vitamin B<sub>3</sub>: 4000 mg; vitamin B<sub>5</sub>:12000 mg; vitamin B<sub>6</sub>: 1200 mg; vitamin B<sub>12</sub>: 6 mg; Choline choloride: 200000 mg; Manganese: 40000 mg; Iron: 20000 mg; Zinc: 40000 mg; Copper: 4000 mg.

<sup>3</sup> Inert filler used to complete diet formulations to 100%.

The amino acid standard (L-alanine, L-arginine, Laspartic acid, L-glutamic acid, glycine, L-histidine, Lisoleucine, L-leucine, L-lysine, L-methionine, Lphenylalanine, L-serine, L-threonine, L-lysine, L-valine, cysteine and phenylalanine (PIERCE, Rockford Illinois 61105, USA) were used. Amino acids were analyzed in three replicates and each replicate of sample was obtained from 3 different whole chicken breast meats (Musculus Pectoralis Superficial is, left and right breast muscles of each bird).

The amino acid composition was expressed as g of amino acid per 100 g of raw breast meat. Membrane (WHATMAN® diameter 25 mm) followed by adding potassium-borate buffer (pH 10.4). A 5  $\mu$ L quantity of hydrolyzes protein sample was added to 25  $\mu$ L of OPA reagent and then injected after 1 minute of derivatization.

#### Statistical analysis

Data analysis was performed by using the general linear model procedure and the comparison of means was made through Duncan's, (1995) multiple range tests by using SAS 9.1 software (SAS, 2004).

# **RESULTS AND DISCUSSION**

The effect of the energy source on performance of broiler chicks is shown in Table 4. Data from this study showed that there were significant differences between treatment about feed intake (FI), body weight (BW) and FCR at 14.28 and 42 days old (P $\leq$ 0.01). Using of T1, T2 and T3 at 14 and 28 days old had decreased BW and FI compared to the control, but at 42 days old T1, T2, T3 and T4 increased FI and BW. According to the Table 4 data the feed conversion ratio of broiler chicks significantly (P<0.01) increased by feeding treatments. It was observed that the performance parameter showed significant (P $\leq$ 0.01) differences throughout the experimental period.

As result revealed from Table 5 amino acid compositions of breast muscles were varied by using different levels of energy and protein (P $\leq$ 0.01). Result showed that most of Arg obtained in the T4, T3 and T2, respectively. The results showed that the amount of energy and its kind could affect Arg content. Glu numerically at highest in group T4 with the highest energy levels and was based on corn and soybean oil.

Ingredients	Control	<b>T1</b>	T2	Т3	Τ4
Corn	70.18	71.47	71.5	65.07	62.4
Soybean meal	19.3	20	24.2	25.8	25
Corn gluten	6.23	4.2	0	1.5	3.5
Soybean oil	0	0	0	3.5	5
Di-calcium phosphate <sup>1</sup>	1.7	1.7	1.7	1.7	1.7
Oyster shell	0.92	0.92	0.92	0.90	0.90
NaCl	0.32	0.32	0.32	0.32	0.32
L-methionine	0.18	0.22	0.27	0.22	0.20
L-lysine	0.36	0.36	0.27	0.21	0.22
L-threonine	0.09	0.09	0.10	0.06	0.04
Mineral and vitamin premix <sup>2</sup>	0.5	0.5	0.5	0.5	0.5
Choline chloride	0.22	0.22	0.22	0.22	0.22
Filler <sup>3</sup>	0	0	0	0	0
Total	100	100	100	100	100
Calculated nutrient content					
Metabolizable energy (kcal/kg)	3185	3085	2990	3185	3275
Crude protein (CP) (%)	18	17.4	16.8	18	18.5
Ca (%)	0.76	0.76	0.76	0.76	0.76
P (%)	0.38	0.38	0.38	0.38	0.38
Met (%)	0.48	0.48	0.48	0.48	0.48
Lys (%)	0.95	0.95	0.95	0.95	0.95
Met + Ces (%)	0.74	0.74	0.74	0.74	0.74
Thr (%)	0.65	0.65	0.65	0.65	0.65
Trp (%)	0.16	0.16	0.17	0.17	0.17
Arg (%)	1.02	1.02	1.03	1.03	1.03
Val (%)	0.73	0.73	0.74	0.74	0.74
Na (%)	0.16	0.16	0.16	0.16	0.16
K (%)	0.6	0.6	0.7	0.71	0.7
Cl (%)	0.29	0.29	0.28	0.28	0.28

Per kg contains: Ca: 23% and P: 18.5%

<sup>2</sup> Provided per kilogram: vitamin A: 360000 IU; vitamin D<sub>3</sub>: 800000 IU; vitamin E: 7200 IU; vitamin K<sub>3</sub>: 800 mg; vitamin B<sub>1</sub>: 720 mg; vitamin B<sub>9</sub>: 400 mg; vitamin H<sub>2</sub>: 40 mg; vitamin B<sub>2</sub>: 2640 mg; vitamin B<sub>3</sub>: 4000 mg; vitamin B<sub>5</sub>:12000 mg; vitamin B<sub>6</sub>: 1200 mg; vitamin B<sub>12</sub>: 6 mg; Choline choloride: 200000 mg; Manganese: 40000 mg; Iron: 20000 mg; Zinc: 40000 mg; Copper: 4000 mg and Iodine: 400 mg.

<sup>3</sup> Inert filler used to complete diet formulations to 100%

All the treatments were significantly different in terms of Pro amino acid. Content of Ala and Ser had decreased significantly by using T1, T2, T3 and T4, respectively. Data showed that Asp increased significantly by treated groups. The differences between chicken groups studied were increased significantly for Gly (P≤0.01). The data obtained in this study showed that Leu remained unaffected and Ilu increased for the treatments T3 and T4, while, Lys and Val decreased compared to the control. The Phe content was increased by treatment except for T3 and T4. There were no significant differences for Tyr, Trp, His, Met, Cys, Thr, Gln, Asn amino acids profile between treatments.

Recent research showed that energy and protein manipulation in broiler's diet could have been able to change performance and carcass characteristics of broilers. In current study, using different levels of energy and protein could affect performance and amino acid profile in experimental Cobb 500 broilers. In our study, varying levels of energy and protein affected performance.

We observed significant depression in BW (for the first 28 days) for T1 and T2 while, FI presented the same results only for the last period (29-42 days) and was the highest for T4. Additionally, FCR was the highest for T2 in all ages. These results indicate that as dietary energy level increases BW and FI also increase, but FCR requires.

Recent studies by Eits et al. (2003) showed that increasing levels of a well balanced protein up to 27.0% considerably improved the performance of birds. Additionally, data provided by Temim et al. (2000) showed improved performance when raising the amino acid profile. While Ajuyah et al. (1993) noted that an increase in the saturation of the diet decreased the weight gain and final weights, although controversial results have been reported elsewhere. Furthermore, Palmer (1993) showed that the improvement of performance and growth in poultry may be due to the inclusion of some fatty acids which may affect muscle protein synthesis and protein deposition through a prostaglandin-depend mechanism.

Treatments	14 days old			28 days old			42 days old		
	BW (g)	FI (g)	FCR	BW (g)	FI (g)	FCR	BW (g)	FI (g)	FCR
Control	341.80 <sup>a</sup>	401.0 <sup>b</sup>	1.17 <sup>e</sup>	1279.6 <sup>b</sup>	1757.5 <sup>bc</sup>	1.36 <sup>e</sup>	3617.5 <sup>b</sup>	2160.3 <sup>b</sup>	1.67 <sup>d</sup>
T1	321.87 <sup>b</sup>	407.5 <sup>b</sup>	1.26 <sup>b</sup>	1199.2°	1872.5 <sup>ab</sup>	1.55 <sup>b</sup>	3649 <sup>b</sup>	2093.2°	1.74 <sup>b</sup>
T2	303.85°	405.5 <sup>b</sup>	1.32 <sup>a</sup>	1086.1 <sup>d</sup>	1857 <sup>ab</sup>	1.62 <sup>a</sup>	3722.5 <sup>ab</sup>	2030.7 <sup>d</sup>	1.82 <sup>a</sup>
Т3	329.97 <sup>ab</sup>	404.2 <sup>b</sup>	1.22 <sup>d</sup>	1243.7 <sup>b</sup>	1717.7 <sup>°</sup>	1.37 <sup>d</sup>	3670 <sup>b</sup>	2163.1 <sup>b</sup>	1.65 <sup>e</sup>
T4	342.70 <sup>a</sup>	428.5 <sup>a</sup>	1.24 <sup>c</sup>	1337.8ª	1906.2ª	1.42 <sup>c</sup>	3826.5ª	2260.5ª	1.69 <sup>c</sup>
SEM	7.182	4.902	0.025	42.388	35.99	0.054	38.46	36.58	0.031

 Table 4
 The effect of the energy source on performance of broiler chicks

BW: body weight; FI: feed intake and FCR: feed conversion ratio.

The means within the same row with at least one common letter, do not have significant difference (P>0.01).

Table 5 The effect of the energy source on breast muscles amino acid composition of broiler chicks at 28 days old (g/100 g dry weight)

Treatments	Control	T1	T2	Т3	<b>T4</b>	SEM
Amino acids			14	15	17	GEWI
Arg	3.818 <sup>c*</sup>	4.645 <sup>bc</sup>	5.807 <sup>a</sup>	5.108 <sup>ab</sup>	5.960 <sup>a</sup>	0.392
Glu	7.237 <sup>b</sup>	6.150 <sup>c</sup>	5.310 <sup>c</sup>	7.930 <sup>ab</sup>	8.820 <sup>a</sup>	0.623
Pro	3.110°	2.645 <sup>d</sup>	2.000 <sup>e</sup>	3.860 <sup>b</sup>	4.337 <sup>a</sup>	0.417
Ala	4.520 <sup>a</sup>	3.833 <sup>b</sup>	3.310 <sup>c</sup>	2.602 <sup>d</sup>	2.140 <sup>e</sup>	0.424
Asp	4.210 <sup>d</sup>	4.878 <sup>c</sup>	6.480 <sup>a</sup>	5.763 <sup>b</sup>	6.250 <sup>ab</sup>	0.426
Ser	4.720 <sup>a</sup>	4.128 <sup>b</sup>	3.528°	2.812 <sup>d</sup>	2.320 <sup>e</sup>	0.432
Gly	2.140 <sup>c</sup>	2.282 <sup>c</sup>	3.752 <sup>a</sup>	3.208 <sup>b</sup>	3.600 <sup>a</sup>	0.333
Ilu	2.523 <sup>b</sup>	1.962 <sup>c</sup>	1.620 <sup>c</sup>	2.873ª	3.145 <sup>a</sup>	0.282
Leu	1.425 <sup>a</sup>	1.407 <sup>a</sup>	1.380 <sup>a</sup>	1.563ª	1.257 <sup>a</sup>	0.049
Lys	4.940 <sup>a</sup>	4.450 <sup>b</sup>	3.720 <sup>c</sup>	3.120 <sup>d</sup>	2.642 <sup>e</sup>	0.419
Val	2.580 <sup>a</sup>	2.120 <sup>b</sup>	1.153 <sup>d</sup>	1.688 <sup>c</sup>	1.263 <sup>d</sup>	0.266
Phe	3.230 <sup>b</sup>	3.923 <sup>a</sup>	4.210 <sup>a</sup>	2.608 <sup>c</sup>	2.247 <sup>c</sup>	0.373
Tyr	1.083	1.103	1.045	1.070	1.082	0.01
Trp	0.418	0.395	0.365	0.443	0.407	0.012
His	0.565	0.585	0.520	0.458	0.492	0.023
Met	0.860	1.240	2.120	1.610	1.750	0.216
Cys	0.560	0.620	0.710	0.500	0.410	0.012
Thr	0.135	0.185	0.213	0.160	0.172	0.012
Gln	0.115	0.130	0.137	0.165	0.180	0.012
Asn	0.175	0.262	0.335	0.332	0.280	0.03

The means within the same row with at least one common letter, do not have significant difference (P>0.01).

While Marcu *et al.* (2013) demonstrated that the economic efficiency of Cobb 500 broiler chickens growth was positively influenced by the growth performance and the recorded viability. Furthermore, Danicke *et al.* (1997) reported that the performance was higher for broilers that received a soybean oil in diet that was supplemented with an enzyme compared with unsupplemented tallow based diet, while significant interactions were reported between dietary fat type and carbohydrate addition (Danicke *et al.* 2000).

The results of present study revealed that FI was affected by energy level in all three periods. These results are not completely in accordance with the results obtained by Abudabos (2014) that showed during the starter period, FI was not affected by fat source, energy level or enzyme supplementation or their interactions (P>0.05) but FI was influenced by the energy level of the diet at finisher period (P<0.05).

These differences might be the result of differences in energy level and fat content of the diets, because Pesti (1982) mentioned that chicks fed diets with soybean or corn oil consumed more ME than chicks fed comparable diets that had a low fat content. Also, Murugesan et al. (2013) showed that there were no significant interactions in BW, FI and FE among dietary and challenge treatments. They showed that direct comparison of the excess energy contributed by the 3% diets provided an average of 69% increase over the energy value derived from the equations. They mentioned that the vegetable oils were fairly consistent, although the pure soybean oil resulted in slightly higher apparent metabolizable energy corrected for nitrogen (AMEn) than the crude corn oil. The poultry fat resulted in an AMEn value lower than the vegetable oils, in line with previous reports for fat of this nature. The soy methyl esters resulted in significantly higher dietary energy, resulting in an AMEn value slightly higher than the corn oil.

According to our results and the differences with the previously mentioned experiments, we can conclude that different energy sources and levels have the potential to affect the performance of broilers. Furthermore, our results showed that amino acid compositions of breast muscles were varied by using different levels of energy and protein (P $\leq$ 0.01).

Furthermore, our results showed that amino acid compositions of breast muscles were varied by using different levels of energy and protein (P≤0.01). However, it did not correlate with trypsin inhibitor activity levels, indicating that other factors also affect amino acid digestibility of soybean meal. Furthermore Foltyn et al. (2013) reported that the replacement of soybean meal by raw full-fat soybean decreased amino acids coefficients of ileal apparent digestibility when the level of extruded full fat soybean was higher than 40 g/kg in feed, while Makkink et al. (1994) also reported that trypsin activity in the jejunum is affected by protein sources. Arg and Lys are amino acids that specifically cause the release of trypsinogen in a pancreatic tissue homogenate, because they contain sites of tryptic cleavage (Niederau et al. 1986). Clarke and Wiseman (2015) demonstrated that the coefficients of ileal apparent digestibility for individual amino acids varied widely among soybean samples.

Haunshi *et al.* (2012) determined that to obtain better FCR, feeding Aseel birds with diet having 2800 kcal/kg ME and 16% CP would be ideal. These studies further strength our hypothesis that energy requirements must be re-evaluated.

According to Nobakht et al. (2011) showed that digestibility of dietary fats is affected by its fatty acid profile. As unsaturated fats contain higher metabolizable energy, Crespo and Esteve Garcia (2002) have found better utilization of unsaturated compared to saturated fats. Some evaluations by Yu and Sim (1987) and Nash et al. (1996) did not find any differences in the performance of birds fed different oil sources. Nwoche et al. (2001) evidenced that 4% dietary inclusion of palm oil as the best inclusion level that will bring about an optimum growth in broilers, our results showed that FI is not affected by protein source for the first 28 days for the low energy level diets, something which is not true for the higher energy level diets. Similarly, Dari and Penz (1996) showed that feed intake was not significantly influenced by the way amino acid requirements were expressed, which is consistent with previously published results from experiments comparing diets based on alternative ingredients with low amino acid digestibility to diets based on ingredients with high amino acid digestibility (corn and soybean meal) and to diets with low digestibility ingredients supplemented with synthetic amino acids. Additionally, Maiorka et al. (2004) suggest that formulation based on digestible amino acids is necessary if the diets contain protein sources that are not reliable in terms of amino acid digestibility. The response to formulation based on digestible amino acids was minimized when birds received the low energy level diet (2900 kcal ME/kg). These results are in line with our study and confirm our hypothesis that energy requirements must be re-evaluated.

Rohaeni (2015) suggested that feed treatment provides major effects on daily weight gain, feed intake and feed conversion and the best feed that generates the highest daily weight gain, carcass percentage and feed conversion is feed which contains 19% and 22% protein levels derived from an animal based protein source and feed intake using a plant based protein source, soybean meal which contains a 22% protein level is not recommended. This is consistent with studies reported by Kartikasari (2000) that the higher the protein level the feed contains, the more increasing the weight gain.

Saima *et al.* (2008) concluded that quality of feed ingredients impose direct effect on their available amino acids profile. Dozier *et al.* (2008) showed that feeding the high amino acid diets decreased feed consumption, improved feed conversion and increased total breast meat yield. Many researchers mentioned that dietary fat supplementation has been shown to improve feed conversion and decrease feed consumption of broiler chicks (Dale and Fuller, 1980; Saleh *et al.* 2004).

Our results showed that the amount of energy and its kind could affect the breasy muscle amino acids content for many amino acids such as Arg, Pro, Ala and Ser. Similarly, Leeson et al. (1996) showed that the increase in feed consumption associated with low energy diets can affect growth and meat yield with a concurrent increase in energy and amino acids intake and all other needed nutrients for tissue assimilation. Additionally, Vieira et al. (2004) evidenced that optimum dietary digestible Methionine + cysteine level depends on dietary protein level and should therefore be related to the protein content. Furthermore, in accordance with our results, Nahashon et al. (2005) showed that on 3100 and 3150 kcal of ME/kg of diet at 0 to 4 weeks exhibited greater (P<0.05) BW gain, greater carcass and breast weights (P<0.05) and lower (P<0.05) feed consumption and feed conversion ratios than those on a diet with 3050 kcal of ME/kg. Mean feed consumption of birds fed 25% crude protein diets was higher (P<0.05) than those on other dietary crude protein concentrations. In agreement with our results, Sturkie (1976) showed that birds on highenergy diets, often due to relatively high fat content, have on average lower feed consumption due to reduced rate of passage of digesta through the gastrointestinal tract. The higher feed intake for birds on higher crude protein (CP) found in our experiment is diets is consistent with the findings by Sengar (1987) but quite contrary to the report of Waldroup (1990) in which low CP diets significantly depressed appetite.

## CONCLUSION

The results showed that the higher energy level than nutritional needs based on Cobb 500 broiler chickens requirements as specified in the manual, was effective on the expression of some amino acids and the quantity and quality of carcass meat, particularly breast muscle are affected. These effects indicate that the current nutritional recommendations are not sufficient for realizing the full genetic potential of current broiler strains for body weight gain but in terms of feed conversion ratio the most appropriate level of energy obtained from Cobb 500 broiler chickens requirements manual.

### ACKNOWLEDGEMENT

The authors sincerely acknowledge the economical help provided by the Nuclear Science and Technology Research Institute, Atomic Energy Organization of Iran for assistance to run this test.

# REFERENCES

- Abdullah A.Y. and Matarneh S.K. (2010). Broiler performance and the effects of carcass weight, broiler sex, and post chill carcass aging duration on breast fillet quality characteristics, J. Appl. Poult. Res. 19, 46-58.
- Abudabos A.M. (2014). Effect of fat source, energy level and enzyme supplementation and their interactions on broiler performance. *South African J. Anim. Sci.* **44**, 280287.
- Ajuyah A.O., Hardin R.T. and Sim J.S. (1993). Effect of dietary full-fat flax seed with and without antioxidant on the fatty acid composition of major lipid classes of chicken meats. *Poult. Sci.* 72, 125-130.
- Akhter S., Khan M., Anjum M., Ahmed S., Rizwan M. and Ijaz M. (2008). Investigation on the availability of amino acids from different animal protein sources in golden cockerels. J. Anim. Plant. Sci. 18, 53-54.
- Antoine F.R., Wie C.I., Littell R.C., Quinn B.P., Hogle A.D. and Marshall M.D. (2001). Free amino acid in dark- and whitemuscle fish as determined by o-phthaldialdehyde precolumn derivatization. J. Food Sci. 66, 72-77.
- Batal A.B. and Parsons C.M. (2004). Utilization of various starch sources as affected by age in the chick. *Poult. Sci.* 83, 1140-1147.
- Brake J. (1990). Effect of four levels of added fat on broiler breeder performance. *Poult. Sci.* **69**, 1659-1663.
- Clarke E. and Wiseman J. (2015). Effects of variability in trypsin inhibitor content of soya bean meals on true and apparent ileal digestibility of amino acids and pancreas size in broiler chicks. *Anim. Feed Sci. Technol.* **121**, 125-138.

- Cobb 500. (2013). Cobb Broiler Performance and Nutrient Supplement Guide. Cobb-Vantress Inc., Siloam Springs, Arkansas.
- Crespo N. and Esteve-Garcia E. (2002). Nutrient and fatty acid deposition in broilers fed different dietary fatty acid profiles. *Poult. Sci.* **81**, 1533-1542.
- Dale N.M. and Fuller H.L. (1980). Effect of diet composition on feed intake and growth of chicks under heat stress. II. Constant vs. cycling temperatures. *Poult. Sci.* 59, 1434-1441.
- Danicke S., Simon O., Jeroch H. and Bedford M. (1997). Interactions between dietary fat type and xylanase supplementation when rye-based diets are fed to broiler chickens. 1. Physicochemical chyme features. *Br. Poult. Sci.* 38, 537-545.
- Danicke H., Jeroch H., Bottcher W. and Simon O. (2000). Interations between dietary fat type and enzyme supplementation in broiler diets with high pentosan contents: effects on precaecal and total trac digestibility on fatty acids, metabolizability of gross energy, digesta viscosity and weights of small intestine. *Anim. Feed Sci. Technol.* 84, 279-294.
- Dari R.L. and Penz J.R. (1996). The use of digestible amino acid and ideal protein concept in diet formulation for broilers. *Poult. Sci.* **75**, 67-73.
- Dozier W.A., Kidd M.T. and Corzo A. (2008). Dietary amino acid responses of broiler chickens. J. Appl. Poult. Res. 17, 157-167.
- Duncan D.B. (1995). Multiple range and multiple f-test. *Biometrics*. **11**, 1-42.
- Durunne C.S., Udedibie A.B.I. and Uchegbu M.C. (2015). Effect of dietary in clusion of Aanthoata macrophyla meal on the performance of starter chicks. *Nigerian J. Anim.* 32, 268-273.
- EI Yamany A.T., Hewida M.H., Abdel-Samee D. and EL-Ghamry A.A. (2008). Evaluation of using different levels and Sources or oil in growing Japanese quail diets. *American-Eurasian J. Agric. Environ.* 3, 577-582.
- Eits R.M., Kwakkel R.P., Verstegen M.W.A. and Emmans G.C. (2003). Responses of broiler chickens to dietary protein: effects of early life protein nutrition on later responses. *Br. Poult. Sci.* **44**, 398-409.
- Foltyn M., Rada V., Lichovnikova M., Safarik I., Lohnisky A. and Hampel D. (2013). Effect of extruded full-fat soybeans on performance, amino acids digestibility, trypsin activity, and intestinal morphology in broilers. *Czech J. Anim. Sci.* 58(10), 470-478.
- Haunshi S.A., Panda K., Rajkumar U., Padhi M.K., Niranjan M. and Chatterjee R.N. (2012). Effect of feeding different levels of energy and protein on performance of aseel breed of chicken during Juvenile Phase. *Trop. Anim. Health Prod.* 44, 1653-1658.
- Kamran Z., Mirza M.N., Haq A.U. and Mahmood S. (2004). Effect of decreasing dietary protein levels with of decreasing dietary protein levels with optimum amino acids profile on the performance of broilers. *Pakistan Vet. J.* 24, 165-168.
- Kartikasari L.R. (2000). The chemical compotion and the study of fatty acids of breast meat of broiler treated with methionine supplementation in the low protein containing diets. *Bullet. Anim. Sci.* 25(1), 33-39.
- Leeson S., Caston L. and Summers J.D. (1996). Broiler response to diet energy. *Poult. Sci.* 75, 529-535.

- Leeson S. and Summers J.D. (2001). Nutrition of the Chicken. Published by University Books, Ontario, Canada.
- Maiorka A., Dahlke F., Santin E., Kessler A.M. and Penzjr A.M. (2004). Effect of energy levels of diets formulated on total or digestible amino acid basis on broiler performance. *Rev. Bras. Cienc. Avic.* 17(1), 1-5.
- Makkink C.A., Negulescu G.P., Guixin Q. and Verstegen M.W.A. (1994). Effect of dietary protein source on feed intake, growth, pancreatic enzyme activities and jejuna morphology in newly weaned piglets. *British J. Nutr.* **72**, 353-368.
- Marcu A., Vacaru-Opris Dumitrescu I.G., Marcu A., Petculescu Ciochina L., Nicula M., Dronica D. and Kelciov B. (2013).
  Effect of diets with different energy and protein levels on breast muscle characteristics of broiler chickens. *Anim. Sci. Biotechnol.* 46(1), 1-7.
- Murugesan G.R., Kerr B.J. and Persia M.E. (2013). Evaluation of energy values oil sources when fed to broiler chicks. *Anim. Indust. Rep.* 659(1), 55.
- Nahashon S.N., Adefope N., Amenyenu A. and Wright D. (2005). Effects of dietary metabolizable energy and crude protein concentrations on growth performance and carcass characteristics of french guinea broilers. *Poult. Sci.* 84, 337-344.
- Nash D.M., Hamilton R.M.G., Sanford K.A. and Hulan H.W. (1996). The effect of dietary menhaden meal and storage on the omega-3 fatty acids and sensory attributes of egg yolk in laying hens. *Canadian J. Anim. Sci.* **76**, 377-383.
- Niederau C., Grendell J.H. and Rothman S.S. (1986). Digestive end products release pancreatic enzymes from particulate cellular pools, particularly zymogen granules. *Biochim. Biophys. Acta.* **81**, 281-291.
- Nobakht A., Tabatabaei S. and Khodaei S. (2011). Effects of different sources and levels of vegetable oils on performance, carcass traits and accumulation of vitamin E in breast meat bbroilers. *Curr. Res. J. Biol. Sci.* **3(6)**, 601-605.
- Nobre R.T.R., Silva D.J., Fonseca J.B., Silva M.A. and Lana G.R.Q. (1994). Efeito do nível de energia sobre a qualidade da carcaça de diferentes grupos genéticos de frangos de corte. *Revist. Brasileira Zootec.* **24**, 603-614.
- Nwoche E.C., Ndubuisi E.C. and Iheukwumere F.C. (2001). Effect of dietary palm oil on the growth performance of broiler chicks. Pp. 23-27 in Proc. 6<sup>th</sup> Ann. Conf. Anim. Sci. Assoc. Maiduguri, Nigeria.
- Odukwe C.A. (1994). The feeding value of composite cassava root meal for broiler chicks. Ph D. Thesis. University of Nigeria, Nsukka, Nigeria.
- Okarini I.A., Purnomo H., Aulanni A.M. and Radiati L.E. (2013). Proximate total phenolic, antioxidant activity and amino acids profile of bali indigenous chicken, spent laying hen and broiler breast fillet. *Int. J. Poult. Sci.* **12(7)**, 415-420.
- Okruszek A., Woloszyn J., Haraf G., Orkusz A. and Werenska M. (2013). Chemical composition and amino acid profiles of goose muscles from native polish breeds. *Poult. Sci. Assoc.* 92, 1127-1133.
- Palmer R.J. (1993). Prostaglandins and the control of muscle protein synthesis and degradations leukotrienes essent, fatty

acids. Poult. Sci. 72, 95-99.

- Pesti G.M. (1982). Characterization of the response of male broiler chickens to diets of various proteins and energy contents. *Br. Poult. Sci.* 23, 527-537.
- Resurreccion A.V.A. (2002). Sensory aspects of consumer choices for meat and meat products. *Meat Sci.* 66, 11-20.
- Rohaeni E.S. (2015). The effects of the protein level from soybean meal and poultry meat meal on the growth of broiler chickens. *Livest. Res. Rural Dev.* Available at: <u>http://www.lrrd.org/lrrd27/5/roha27084.html</u>.
- Saima M., Akhter M., Khan Z.U., Anjum M.I., Ahmed S., Rizwan M. and Ijaz M. (2008). Investigation on the availability amino acids from different animal protein in golden cockerels. J. Anim. Plant. Sci. 18, 2-3.
- Saleh E.A., Watkins S.E., Waldroup A.L. and Waldroup P.W. (2004). Consideration for dietary nutrient density and energy feeding programs for growing large male broiler chickens for further processing. *Int. J. Poult. Sci.* **3**, 11-16.
- SAS Institute. (2001). SAS<sup>®</sup>/STAT Software, Release 9.1. SAS Institute, Inc., Cary, NC. USA.
- Scanes C.G., Brant G. and Ensminger M.E. (2004). Turkeys and turkey *meat*. Publisher: Pearson Prentice Hall, Upper Saddle River, New Jersey.
- Sengar S.S. (1987). Feed intake and growth rate pattern in White Leghorn chicks maintained on different planes of nutrition. *Poult. Advisor.* **20**, 23-27.
- Sturkie P.D. (1976). Alimentary canal: anatomy, prehension, deglutition, feeding, drinking passage of ingesta and motility. Pp. 185-195 in Avian Physiology. P.D. Sturkie, Ed. Springer Verlag, New York.
- Tabeidian A., Sadeghi G.H. and Pourreza J. (2005). Effect of dietary protein levels and soybean oil supplementation on broiler performance. *Int. J. Poult. Sci.* 4, 799-803.
- Temim S., Chagneau A.M., Guillaumin S., Michel J., Peresson R. and Tesseraud S. (2000). Does excess dietary protein improve growth performance and carcass characteristics in heatexposed chickens? *Poult. Sci.* 79, 312-317.
- Vieira S.L., Lemme A., Goldenberg D.B. and Brugalli I. (2004). Responses of growing broilers to diets with increased sulfur amino acids to lysine ratios at two dietary protein levels. *Poult. Sci.* 83, 1307-1313.
- Waldroup P.W., Tidwell N.M. and Izat A.L. (1990). The effect of energy and amino acid levels on performance and carcass quality of male and female broilers grown separately. *Poult. Sci.* 69, 1513-1521.
- Wongsuthavas S., Yuangklang C., Vasupen K., Mitchaothai J., Alhaidary A., Mohamed H. and Beynen A.C. (2011). Fatty acid metabolism in broiler chickens fed diets either rich in linoleic or alpha-linolenic acid. *Asian J. Anim. Vet. Adv.* 6, 282-289.
- Yu M.M. and Sim J.S. (1987). Biological incorporation of N-3 polyunsaturated fatty acids into chicken eggs. *Poult. Sci.* 63, 195-212.