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Artigo

The effect of using different energy sources and levels on carcass weight, breast muscle mass, mTORC1 and AKt gene expression in Cobb 500 broiler chicks

Efeito do uso de diferentes fontes e níveis de energia sobre o peso da carcaça, massa muscular do peito, expressão gênica mTORC1 e AKt em frangos de corte Cobb 500

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Abstract: The present study was aimed to evaluate the effect of different energy sources and levels on carcass weight, breast muscle mass mTORC1 and AKt gene expression in Cobb 500 broiler chicks. A total of 600 1-day-old Cobb 500 broiler chicks with an average weight of 39 ± 0.50 g were randomly divided into five treatments. Each treatment was further divided into four replicates. Chicks were fed by basal diet based on corn 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) for 42 days. To assess carcass characteristics two chicks of each replication were slaughtered and weighted after picking the carcasses. After DNA extraction, the UV spectrophotometric method was performed for determines the quality and quantity and Real-time PCR was performed on the corresponding cDNA synthesized from each sample. Data from this study showed that there were significant differences about carcass and breast muscle weight between treatments ($P < 0.05$). As result obtained from this study the highest mTORC1 and AKt gene expression were for T4 and T3, T2, T1 had higher gene expression compared to control respectively. In conclusion higher levels of energy are needed for Cobb 500, for both mTORC1 and AKt gene expression as well as the more carcass and breast muscle influences.

Resumo: O presente estudo teve como objetivo avaliar o efeito de diferentes fontes e níveis de energia sobre o peso da carcaça, massa muscular do peito mTORC1 e expressão gênica AKt em frangos de corte Cobb 500. Um total de 600 pintos de corte Cobb 500 com 1 dia de idade e peso

médio de 39 ± 0,50 g foram divididos aleatoriamente em cinco tratamentos. Cada tratamento foi ainda dividido em quatro repetições. Os pintinhos foram alimentados com dieta basal à base de milho e nível de energia pelo manual de instruções Cobb 500 como grupo controle, dieta basal com 3% menos energia que no controle (T1), dieta basal com 6% menos energia que no controle (T2), dieta basal à base no nível de milho e gordura de acordo com o manual de instruções Cobb 500 (T3), dieta basal à base de milho e gordura com 3% de energia superior (T4) por 42 dias. Para avaliação das características de carcaça, dois pintos de cada repetição foram abatidos e pesados após a retirada das carcaças. Após a extração do DNA, o método espectrofotométrico UV foi realizado para determinar a qualidade e quantidade e PCR em tempo real foi realizado no cDNA correspondente sintetizado de cada amostra. Os dados deste estudo mostraram que houve diferenças significativas sobre o peso da carcaça e do músculo do peito entre os tratamentos (P <0,05). Como resultado obtido neste estudo, a maior expressão gênica de mTORC1 e AKt foram para T4 e T3, T2, T1 tiveram maior expressão gênica em relação ao controle, respectivamente. Em conclusão, níveis mais altos de energia são necessários para o Cobb 500, tanto para a expressão dos genes mTORC1 quanto para a AKt, bem como mais influências na carcaça e no músculo do peito.

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Introduction

Poultry meat and poultry meat products are important components in 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 et al., 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).

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 as energy rich feeds. Fats also provide varying quantities of the essential nutrient linoleic acid (LEESON, 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.

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. Nobre et al. (1994) evaluated four energy levels and reported no significant differences in carcass yield between treatments. The mechanistic target of rapamycin (mTOR) is a kinase that regulates key cellular functions linked to the promotion of cell growth and metabolism, which is part of two protein complexes termed mTOR complex (mTORC1) and (mTORC2) and has a fundamental role in coordinating anabolic and catabolic processes in response to growth factors and nutrients.

The mammalian target of rapamycin (mTOR) in conjunction with AKt (also known asprotein kinase B) are required via the phosphatidyl inositol 3- kinase pathway for skeletal muscle cell development (ADEGOKE et al., 2012). The findings of Bottje et al. (2014) support a hypothesis that differential expression of genes associated with the AKt/ mTOR, protein ubiquitination, and proteasome pathways through modulation of transcription and protein turnover could play an important role in the phenotypic expression of feed efficiency. Over the last years, several groups observed that mTORC1 inhibition, in addition to reducing protein synthesis, deeply affects gene transcription. Many studies showed that the activation of the AKt/ mTOR signaling pathway is critical for regulating skeletal muscle development. Also, it has been reported that the mTOR pathway genes were up regulated in duodenum and liver in broilers with low residual feed intake compared to broilers with high residual feed intake (SENGUPTA et al., 2010; LEE, 2012; BOTTJE et al., 2014).

Due to the effects of different sources of fat and protein levels on performance, growth and breast meat mass on poultries the current study was conducted to evaluate the effect of different energy sources and levels on carcass weight, breast muscle mass mTORC1 and AKt gene expression in Cobb

500 broiler chicks.

2 Material and methods

2.1 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 ± 0.50 g were randomly distributed into five dietary treatments with four replicates each. The birds were housed in groups of 5 in 20 stand trial under standard conditions of temperature, humidity, and ventilation.

The study was carried out in the poultry farm of Atomic Energy Organization, Karaj, Iran, for a period of 42 days. Chicks were fed by basal diet based on corn 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 by using (Cobb 500 performance and nutrition supplement instruction manual, 2013) and animal and poultry feed formulation (UFFDA) software (Table 1,2 and 3).

Table 1. The composition of the experimentais diets for broiler chicks (0-14 days old).

Ingredients (%)	Control	T1	T2	T3	T4
Corn	61.25	60	58.62	56.10	54.17
Soybean meal	23.57	31.22	32.21	32.10	32.21
Corn gluten	10.52	4	4.38	4.56	4.74
Soybean oil	0	0	0	2.46	4.14
DCP	2.03	2.03	2.05	2.04	2.05
Oyster shell	1.07	1.03	1.02	1.02	1.01
Nacl	0.16	0.20	0.23	0.23	0.20
L-Methionine	0.19	0.21	0.19	0.19	0.19
L- Lysine	0.30	0.34	0.36	0.36	0.36
L-Threonine	0.47	0.33	0.30	0.30	0.29
Mineral and Vitamin premix	0.07	0.14	0.14	0.14	0.14
Total	100	100	100	100	100
Calculated nutrient content					
ME (kcal.kg)	3035	2943	2853	3035	3120
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 TFD (%)	0.65	0.67	0.67	0.67	0.67
Lys TFD (%)	1.18	1.18	1.18	1.18	1.18
Met + Ces TFD (%)	0.98	0.98	0.98	0.98	0.98
Thr TFD (%)	0.86	0.86	0.84	0.86	0.86
Trp TFD (%)	0.18	0.20	0.20	0.21	0.21
Arg TFD (%)	1.24	1.24	1.24	1.24	1.24
Val TFD (%)	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

Table 2. The composition of the experimentais diets for broiler chicks (14-28 days old).

Ingredients (%)	Control	T1	T2	T3	T4
Corn	69.22	67.7	68.8	65.31	59.31
Soybean meal	18	26.2	23.43	24	28.45
Corn gluten	8.2	0	3.24	4.2	2.9
Soybean oil	0	0	0	2	5
DCP	1.9	1.9	1.9	1.9	1.9
Oyster shell	1.05	1.05	1.05	1.05	1.05
Nacl	0.37	0.37	0.37	0.37	0.37
L-Methionine	0.22	0.30	0.27	0.25	0.25
L- Lysine	0.44	0.28	0.34	0.32	0.22
L-Threonine	0.10	0.12	0.10	0.10	0.05
Mineral and Vitamin premix	0.5	0.5	0.5	0.5	0.5
Filler		1.58	0	0	0
Total	100	100	100	100	100
Calculated nutrient content					
ME (kcal.kg)	3108	3014	2921	3108	3201
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 TFD (%)	0.53	0.53	0.53	0.53	0.53
Lys TFD (%)	1.05	1.05	1.05	1.05	1.05
Met + Ces TFD (%)	0.89	0.89	0.89	0.89	0.89
Thr TFD (%)	0.78	0.78	0.78	0.78	0.78
Trp TFD (%)	0.21	0.22	0.22	0.22	0.22
Arg TFD (%)	1.25	1.25	1.25	1.25	1.25
Val TFD (%)	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

Table 3. The composition of the experimentais diets for broiler chicks (29-42 days old).

Ingredients (%)	Control	T1	T2	T3	T4
Corn	70.18	71.47	71.5	65.08	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
DCP	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	0.5	0.5	0.5	0.5	0.5
Choline Chloride	0.16	0.16	0.16	0.16	0.16
Filler	0.06	0.06	0.06	0.05	0.06
Total	100	100	100	100	100
Calculated nutrient content					
ME (kcal.kg)	3185	3085	2990	3185	3275
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 TFD (%)	0.48	0.48	0.48	0.48	0.48
Lys TFD (%)	0.95	0.95	0.95	0.95	0.95
Met + Ces TFD (%)	0.74	0.74	0.74	0.74	0.74
Thr TFD (%)	0.65	0.65	0.65	0.65	0.65
Trp TFD (%)	0.16	0.16	0.17	0.17	0.17
Arg TFD (%)	1.02	1.02	1.03	1.03	1.03
Val TFD (%)	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

2.2 Sampling of data

To investigate the effect of using different energy sources and levels on carcass weight, breast muscle mass mTORC1 and AKT gene expression in Cobb 500 broiler chicks, and to assess carcass characteristics two chicks of each replication were selected and slaughtered and after picking the carcasses cleaned and weighed by precise digital scale. Then breasts removed and weighed and evaluated chest muscle at 14, 28 and 42 days old and breast muscle tissue obtained and flash frozen in liquid nitrogen.

2.3 Polymerase chain reaction (PCR)

Detailed procedures for isolation of RNA, and microarray hybridization, data collection and analysis have been previously reported by (KONG et al., 2011). RNA was extracted from breast muscle from broilers individually that had been flash frozen in liquid nitrogen and stored at -80°C previously (BOTTJE et al., 2009). After extracting DNA, the UV spectrophotometric method was performed for determines the quality and quantity of that. (NANODROP, 2000; Thermo scientific. USA).

In order to assess the quality of DNA, 1 micro gram of sample were used by horizontal electrophoresis on Agarose gel (1%). A transcript of the target gene sequence, direct and reverse Oligo nucleotide primers were designed by Oligo Primer Analysis Software v. 7 (Table 4) then sequencing primers designed using the BLAST software in search of the human genome was sequenced and Real-time PCR was performed on the corresponding cDNA synthesized from each sample.

Table 4. The sequence of primers

Primers	**Sequence**
mTORC1 F	5-GGTGATGACCTTGCCAAACT -3
mTORC1 R	5-CTCTTGTCATCGCAACCTCA -3
Akt 1 F	5-CAATTTGCCTCCCTACCTCA -3
Akt 1 R	5-ATGGATGGGAAGGAGCTACAA -3
18S rRNA F	5-ATTCCGATAACGAACGAGACT-3
18S rRNA R	5-GGACATCTAAGGGCATCACA-3

2.4 Data analysis

Data analysis was performed by using comparative evaluation. Sample spreadsheet of data analysis using the $^{2\text{-}\Delta\Delta}$CT method. The fold change in expression of the target gene (fos–glo–myc) relative to the internal control gene (b-actin) at various time points was studied. The samples were analyzed using real-time quantitative PCR and the CT data were imported into Microsoft (excel, 2010).

The mean fold change in expression of the target gene at each time point was calculated using equation by (Lyer, 1999), where $^{2\text{-}\Delta\Delta}$ $CT = (CT,_{Target} - CT,_{Actin})_{Timex} - (CT,_{Targe} - CT,_{Actin})_{Time0}$. The mean CT at time zero are shown (colored boxes) as is a sample calculation for the fold change using $^{2\text{-}\Delta\Delta}$ CT (LIVAK AND SCHMITTENGENT, 2001).

2.5 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 ,2001).

3 Results

3.1 Carcass weight and breast muscle mass

As result relived from table 5 the source of energy and its levels had significant effects on Carcass and breast muscle and there were significant differences between treatment compared to the control (P <0.05). At 14, 28 and 42 days old T4 (basal diet based on corn and fat with 3% upper energy) had a better carcass and breast muscle weight.

Table 5. The effect of the energy source on carcass and breast muscle of broiler chicks (gr)

Treatments	14 days old		28 days old		42 days old	
-------	Carcass	Breast muscle	Carcass	Breast muscle	Carcass	Breast muscle
Control	240.6 ±3.46^{a*}	57.68±0.83^{a}	910 ±10.55^{b}	218.375 ±2.5^{b}	1558.46^{2} ±8^{b}	374.05 ±1.9^{b}
T1	225.9 ±0.45^{b}	54.17±0.11^{b}	840.143±12.19^{c}	201.538±2.94^{c}	1507.098±3.17^{c}	361.462 ±0.74^{c}
T2	211.75±0.8^{c}	50.750±0.21^{c}	768.425 ±0.92^{d}	184.338±0.23^{d}	1462.082±3.18^{d}	350.7 ±0.74^{d}
T3	234.25±3.3^{ab}	56.338±0.83^{ab}	882.375 ±4.48^{b}	212.95 ±0.97^{b}	1560.162±17.29^{b}	374.52 ±4.14^{b}
T4	240±1.47^{a}	57.65±0.38^{a}	953.250 ±6.33^{a}	228.838±1.52^{a}	1645.780 ±9.52^{a}	395.2±2.25^{a}

*Means within row with no common on letter are significantly different (p<0.01)

3.2 The gene expression of mTORC1 and Akt

The effect of the different energy source and levels on mTORC1 and AKt gene expression is shown in Table 6. Data showed that there were significant differences about mTORC1 and AKt gene expression between treatments. The highest mTORC1 and AKt gene expression was for T4 and T3, T2, T1 had higher gene expression compared to control respectively.

Table 6. The effect of the energy source on mTORC1 and AKt gene expression

Treatments	AKt	mTORC1
Control	1±0.11[b]	1±0.86[b]
T1	0.5158±0.0057[c]	0.4580±0.0057[c]
T2	0.4052±0.0057[c]	0.2615[c]
T3	1.042±0.057[b]	1.058±0.023[b]
T4	2.230±0.11[a]	2.727±0.13[a]

*Means within row with no common on letter are significantly different (p<0.01)

4 Discussion

In this study we showed that carcass and breast muscle weight influenced by treatments**.**

The protein and lipid quantity of breast muscles is influenced by genetic and non-genetic factors (BOGOSAVLJEVI et al., 2010). Nutrition is an external factor with major influence on the chemical composition of broiler meat. Thus, diets with low protein and energy had determined reduced meat protein content, while the lipids content of the muscles had increased (MARCU et al., 2009).

Marcu et al. (2014) showed that at the trial group, high levels of dietary proteins and energy has significantly influenced pH value and the thickness of myocytes in the muscle, as compared with control group. Abdullah et al. (2010) demonstrated that lighter carcasses had a higher ($P < 0.001$) thawing loss percentage, with breast muscle from males having a higher ($P < 0.01$) thawing loss percentage than that from females.

Scheurmann et al. (2004) showed that broilers have higher myofiber number in the breast muscles than Leghorn type chickens, and that high breast yield of broiler strains may be due to increased myofiber number. Higher muscularity of broilers, as compared with Leghorns, was not attributed to lower expression of myostatin during embryonic development.

Results from the Poorghasemi et al. (2013) study suggested that the supplementation with a combination of vegetable and animal fat sources in broiler diet supported

positively growth performance and carcass p arameters.

Tumova et al. (2009) reported that chicks fast growing had greater number of giant fibers in pectoral muscle, fibers which have a cross section area three to five times larger than normal. The giant fibers from the breast muscle in broiler chickens are a side effect at genetic selection for increased the muscles mass in superficial pectorals (MIRAGLIA et al., 2006).

Jafarnejad et al. (2011) demonstrated that there was no significant difference between using unlimited fat diet and limited one on both body weight and feed conversion ration. In 0-7 day old, feeding diets didn't show any significant difference in feeding conversion but in 7–21-day old chicks, feeding diets with higher energy level significantly decreased feed conversion ration especially at the 2nd and 3rd week ($p<0.001$) but there was no significant difference in feed conversion ration with higher and lower levels of protein.

Rosebrough et al. (1999) showed that dietary fat addition to diets containing low crude protein levels did not decrease lipogenesis to the degree noted when added to a diet containing a higher level of crude protein. As result relevant form this study mTORC1 and AKt gene expression were differ by using experimentais diets and by using T4 (basal diet based on corn and fat with 3% upper energy) mTORC1 and AKt gene expression were increased significantly.

The findings of Bottje et al. (2014) supported a hypothesis that differential expression of genes associated with the Akt/mTOR, protein ubiquitination, and proteasome pathways through modulation of transcription and protein turnover could play an important role in the phenotypic expression of feed efficiency. Confirmation of this hypothesis will require a thorough assessment of protein expression as well as protein and

enzyme activity measurements associated with these pathways in the low and high feed efficiency broiler phenotypes.

Zhou et al. (2015) study revealed the overall differences of gene expression in the abdominal fat from high feed intake and low feed intake chicks, and the results suggest that the divergent expression of lipid metabolism genes represents the major differences. RNA sequence analysis in Zhou et al. (2015) study revealed that genes involved in de novo triglyceride synthesis, cholesterol synthesis, lipid transport, and lipid stabilization were up-regulated, whereas

genes involved in lipid hydrolysis and lipid reverse efflux were down-regulated in the abdominal.

5 Conclusion

In conclusion in current study higher levels of energy are needed for Cobb 500, for both mTORC1 and AKt gene expression as well as the more carcass and breast muscle influences. Confirmation of increased activity of the Akt/mTOR pathway and the role of the protein ubiquitination pathway in broilers will require additional investigation such as protein expression and protein activity measurements. Mechanistic studies could also be accomplished with gene knockdown models, but these are currently not available in poultry. Future studies are needed for more explanation.

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