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Effects of DL Methionine supplementation to broiler and native chicken feed on the expression of breast muscle and liver genes

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This study aimed to evaluate insulin-like growth factor I (IGF-I), growth hormone receptor (GHR) gene, cathepsin L2 (CTSL2), forkhead box O (FOXO), muscle ring finger-1 (MuRF1), muscle atrophy-induced ubiquitin ligases (atrogin-1), however, phosphatidylinositol 3-kinase in the breast muscle, Regulatory 1 (PI3KR1) was elevated in liver. The differences in expression between a broiler strains (Cobb 500) and a native Egyptian breed (Fayoumi) fed different methionine levels were studied. Three weeks old chicks were distributed in 4 treatments for each strain (control diet, T1 - 0.09% added methionine, T2 - 0.20% added methionine, T3 - 0.30% added methionine).The increase of dietary methionine levels had significant beneficial effects on most traits studied. The highest level of methionine in the diet of Cobb and Fayoumi chicks, till 6 weeks of age, caused important alteration in the mRNA expression of GHR and IGF-1 in their breast muscle. However, it was noticeable that birds fed the lowest level of methionine trended to show a higher expression of atrogin-1 in Cobb 500 strain while the higher expression of the same gene atrogin-1 was found in the intermediate level of methionine in Fayoumi strain. As for the CTSL2 & FOXO genes, they show the highest gene expression levels in the highest level of DL-methionine in both strains. The lower level of methionine resulted in the highest expression of MURF1 in Cobb 500 strains, while the highest expression of the same gene was observed in the lowest methionine level (control) of the Fayoumi strain. As for PI3KR1gene expression, the lowest level of methionine in Cobb strain shows the highest expression in liver, while the highest level of methionine shows the highest expression of the same gene in Fayoumi strain. Our results indicated that methionine addition to broilers feed improved birds performance and breast muscle growth.

Keywords: Cobb; Fayoumi; Gene expression; Methionine; Breast muscle, Liver.

INTRODUCTION

The composition of diet interferes with gene physiology and expression, causing feed conversion ratio alterations. Methionine is considered to be the first limiting amino acid for birds, mostly due to amino acid demand for muscle and feather metabolism and due to the composition of feed used. Supplementation of methionine is important to maximize bird's performance.

In livestock production systems, the important challenge for the next century is to satisfy the predicted high demand of meat because of the human population increasing. To carry out this goal, producers will have to control animal performance more precisely by improving quantification of animal requirements and

evaluating animal responses to different nutritional inputs according to their genetic potential. Meanwhile, the farming and agri-food sectors in developed countries are faced with an increasing demand by consumers for safe high-quality meat and dairy products while respecting animal health and welfare and protecting the environment. The mixing of these objectives has led to the concept of sustainable animal husbandry.

Yaqoob and Ali (2018) stated that the suitable concentration and proportion of different amino acids should be present in the diet according to broilers requirements. Under feeding of amino acids decrease the production performance and over feeding will lead to increase the cost of production and nitrogen excretion.

Lassiter et al., (2006) reported that feed cost accounts for an important proportion of animal production costs, making feed efficiency increasingly important and object of many recent studies.

Several studies have shown that broilers that produce less ATP, because of the lower efficiency of their mitochondria in producing ATP from substrates, presented worse feed efficiency (Bottje and Carstens, 2009).

The source of methionine that will be supplemented in the diet must also be taken into consideration, because various sources present different availability and bioefficacy, resulting in different bird performance (Lemme, 2002 and Payne, 2006).

Commercial methionine sources usually are DL-methionine (DL- 2-amino-4-(methylthio)-butanoic acid (99%)) and methionine hydroxy analog-free acid, MHA-FA (DL-2 -hydroxy-4-(methyl)-butanoic acid (88%). Due to chemical and physical differences between methionine sources, the absorption occurs by distinct mechanisms (Dibner, 2003), thereby expressing different biologic efficacy (Jansman et al., 2003).

Corzo et al., (2006) stated that the deficiency of methionine has been showed to impair chicken growth; thus, it is important to have accurate information on methionine requirement of chicks for formulating diets to improve their growth and production. The requirement of methionine for growth and maintenance would be predicted to vary with factors that effect maximum growth and feed intake (Chamruspollert et al., 2002). A lot of work has been done to estimate the methionine requirement of birds under different conditions such as sex, dietary nutrients and rearing environment intake (Chamruspollert et al., 2002).

The objectives of the present study were to determine the effects of different methionine levels on mRNA GHR, mRNA CTSL2, mRNA FOXO, mRNA MuRF1, mRNA atrogin-1 expressions in breast muscle and mRNA PI3KR1 expression in liver for increased meat production associated with live body weight, breast, carcass parts and internal organs of growing chicken.

These methods will help us also to increase chicken meat production on a commercial scale.

MATERIALS AND METHODS

The present study was carried out at the Poultry Experiment Station, Department of Animal Production, and the CURP Biotechnology Center, Faculty of Agriculture, Cairo University, Giza, Egypt

Experimental design, diets and Animals

Feeding Program 21-42 days old

A total of 288 birds, 144 Cobb broilers and 144 Fayoumies (Native chickens) were used for this experiment.

Twenty four cages, twelve cages for Cobb and twelve cages for Fayoumi were used. The birds were raised in battery cages, twelve birds per cage.

The birds were divided into four treatments related to methionine supplementation as follows: Control group without supplementation of methionine, T1 - 0.09% added methionine, T2 - 0.20% added methionine, T3- 0.30% added methionine). (Table 1).

Each of the two strains were divided into four treatments (control, T1, T2 and T3)

For each treatment groups, there were 3 replicates of 12 birds per cage

Slaughter performance.

In this study, live body weights (LBW), for all birds, at 42 days of age were obtained individually by using a digital scale. Slaughter traits were obtained at 6 weeks of age. Fifteen birds, from each of the Cobb 500 and the Fayoumi lines, were chosen at random 12 birds from each treatment group. Birds were weighted (LBW) and slaughtered after 8 hours of fasting (Papa, 1991). Birds were slaughtered by slitting the throat, cutting the carotid arteries, jugular veins, esophagus and trachea without severing the head (Sams, 2001). After slaughtering each bird was hanged in a bleeding funnel for 3 minutes and weighted again to obtain the blood weight. Birds were then scalded in a 68^{o}C water bath for 30

seconds, and then the feathers were removed by an automatic circular feather plucker.

The birds were then weighted again to get the feathers weight. The shanks and head, without neck, were then removed and the birds where eviscerated and chilled. Each empty chilled carcass was weighted to obtain the dressed weight. Dressing percentages were expressed as the percentage of dressed weight to LBW. The wings, with bones until the end of the humeres, were then removed from the front parts and weighted. Also, the skinless pictoralis major and minor muscles were removed to obtain breast muscles weight. The bones from the thighs and drumsticks were removed then the skinless leg muscles were weighted as leg meat. The liver, heart, gizzard (empty) and abdominal fat were weight.

Liver and breast muscle tissues were collected and stored in RNA Holder® (Biotechnology Central Lab, Faculty of Agriculture, Cairo University) at -80°C for RNA extraction.

Blood sample and DNA isolation.

Individual genomic DNA was isolated from venous blood collected in anti-coagulate buffer from 15 birds from each strain Control diet 3 samples, T1 (0.09 % added DL-ME) 3 samples, T2 (0.2% added DL ME) 3 samples, T3 (0.3% added DL ME) 6 samples at 6 weeks of age. The blood was collected from the jugular veins into heparinized tubes and was stored on ice. The extraction was carried out according to the method described by Bailes et al., (2007) as follows:

Table 1: Feed Formulation (content per 100 kilogram) and Calculated analysis

Feedstuffs	Control	0.09% MET	0.20%MET	0.30% MET
Corn yellow kg	66.08	65.88	65.67	65.46
Soybean meal 44 kg	19.05	19.09	19.13	19.17
Corn gluten 60% kg	10.00	10.00	10.00	10
soybean oil kg	0.86	0.92	0.99	1.06
Lime stone kg	1.15	1.15	1.15	1.15
DiCal Ph kg	1.52	1.52	1.52	1.52
Salt kg	0.42	0.43	0.43	0.43
Premix** kg	0.30	0.30	0.30	0.30
Dl- methionine (added) gm	0.00*	0.09	0.20	0.3
L Lysine (added) kg	0.52	0.52	0.52	0.52
Anti toxins	0.10	0.10	0.10	0.10
Total	100.00	100.00	100.01	100.01
Calculated Analysis Nutrients				
Dry Matter %	89.552	89.535	89.571	89.590
Metab. Energy kcal/kg	3.086	3.086	3.086	3.086
Protein %	20.000	20.000	20.000	20.000
Calcium %	0.840	0.840	0.840	0.840
Avail. Phos. %	0.420	0.420	0.420	0.420
Sodium %	0.190	0.190	0.190	0.190
Lysine %	1.190	1.190	1.190	1.190
Methionine %	0.386	0.480	0.580	0.680
Met + Cys %	0.740	0.834	0.934	1.034

*Basal diet without Dl-Methionine supplementation

**Supplied by kilogram of diet: retinyl-acetate, 3.44mg; cholecalciferol, 50 mg; DL-a-tocopherol, 15mg;thiamine, 1.63mg; riboflavin, 4.9mg; pyridoxine, 3.26mg; cyanocobalamin, 12 mg; D-pantothenic acid,9.8mg; D-biotin, 0.1mg; menadione, 2.4mg; folicacid, 0.82mg; niacinamide, 35mg;selenium, 0.2mg; iron, 35mg; copper, 8mg; manganese, 60mg; Zn, 50mg; I, 1mg; choline: 650mg; salinomycin:60mg; avilamycin:5mg; butyl hydroxy toluene, 80mg.

Gene expression

For gene expression analysis, samples of liver and breast muscles were collected from each treatment for the Cobb 500 and Fayoumi strains at 42 days of age, and stored in RNA Holders (CURP Biotechnology, Cairo, Egypt) at -20°C until total RNA extraction.

Liquid nitrogen reagent was used for RNA extraction according to manufacturer recommendations, 1 mL/100 mg tissue. The

RNase inhibitors, Thermo RNase genejet RNA purification Kit, were used to prepare laboratory materials. Tissues were triturated using electric Polytron homogenizer (tissue + liquid nitrogen) to complete dissociation, 200 µL chloroform was added and homogenized by hand for 1 min. The samples were centrifuged at 12,000 *g* at 4°C for 15 min, liquid phase was collected and transferred to clean tube with 500 µL isopropanol in each tube. Supernatant was discarded and precipitate was washed with 1 mL 75% ethanol. Then, it was centrifuged at 12,000 *g* for 5 min and the supernatant was discarded. Pellets were dried for 15 min at room temperature and re-suspended in RNase-free ultrapure water.

Its purity and concentration were measured using a NanoDrop RNA integrity and was evaluated on 1% agarose gel stained with 10% ethidium bromide and observed using ultraviolet light. RNA samples were treated with DNase I (Invitrogen) to remove DNA residues, according to manufacturer recommendations.

Thermo RNase genejet RNA purification Kit (www.thermoscientific.com/onebio) was used to synthesize cDNA according to manufacturer recommendations. In a sterile and RNA-free tube 6 µL RNA, 1 µL oligo (dT) (50 µM oligo[dT]20) and 1 µL annealing buffer were added. Reaction was incubated for 5 min at 65°C and placed on ice for 1 min. Followed by addition of 10 µL of 2X First-Strand Reaction Mix solution and 2 µL enzyme solution, SuperScript III reverse transcriptase and RNAse inhibitor. Mixed solution was incubated for 50 min at 50°C to allow for synthesis of cDNA to occur. Solution contained cDNA was incubated for 5 min at 85°C and immediately placed on ice. Samples were stored at -20°C for later analysis.

Real-time polymerase chain reaction (RT-PCR) was performed using Fluorescent dye SYBR Green (SYBR® Green PCR Master Mix (Applied Biosystems,).

RT-PCR products were analyzed on Strategen (Applied Biosystems), Department of Genetics, Faculty of Agriculture, Ain Shams University.

Nine qRT-PCR primers were chosen that are associated with chicken live body weight and carcass parts and internal organs according to the public chicken genome database (http://www. ncbi.nlm.nih.gov/genebank/). The nucleotide sequences of the primers used in this study are presented in Table (2).

Table 2 ; The qRT-PCR primers used in this study.

Gene	Temperature (ºC)	Primer sequence (5'-3')	Product size bp
IGF-I	60°C	CATTTCTTCTACCTTGGC TCATCCACTATTCCCTTG	140
GHR	60°C	AACACAGATACCCAACAGCC AGAAGTCAGTGTTTGTCAGGG	145
Atrogin-1	60°C	ACTTTGGTTCAACGGGTCG CGGTCTTCGCTGAGCACTT	174
MuRF1	60°C	GGATGCCTTCACAGTCAGTC TGCGGAATAGTCCTCTTGG	254
CTSL2	60°C	GAAGTCAGAAAGGAAGTACAGAGG CTCTCCAGTCAACAGATCGTG	80
FOXO	60°C	ATGCGACCTCTGGTAATA AAGTGTAGGCAAATCGTC	122
PI3KR1	60°C	GCCCTCTCCTTTTCAAAT ACAGTATTAGGTTTCGGTGGC	145
β-actin	60°C	TGCTGTGTTCCCATCTATCG TTGGTGACAATACCGTGTTCA	136
GAPDH	60°C	AGAACATCATCCCAGCGTCC CGGCAGGTCAGGTCAACAAC	182

IGF-I, insulin-like growth factor-I; GHR;growth hormone receptor gene; atrogin-1; muscle atrophy-induced ubiquitin ligases ; MuRF1, muscle ring finger-1; CTSL2,cathepsin L2; FOXO, forkhead box O; PI3KR1; phosphatidylinositol 3-kinase in the breast muscle, Regulatory 1 ; GAPDH, glyceraldehyde-3- phosphate dehydrogenase.
(http://www. ncbi.nlm.nih.gov/genbank/).

The primers used in the IGF-I, GHR, PIK3R1, atrogin-1, and CTSL2 amplification reactions were designed based on the gene sequences deposited at www.ncbi.nlm.nih.gov according to sequence in Genebank (accession numbers: FJ977570.1, NM001001293.1, XM_424759.3, NM_001030956, NM_001168009.1, respectively) using the site www.idtdna.com (Table 2).

Primers were designed at http://www.delta.com/Home/Home.aspx (accessed March 14, 2010), using GHR, IGF-I, (FOXO), (MuRF1), (atrogin-1), (CTSL2), and (PI3KR1), gene sequences from www.ncbi.nlm.nih.gov (Table 2). The two endogenous controls, ß-actin (accession number L08165) and GAPDH (accession number NM_204305), were used, for breast muscle and liver respectively. They were selected because there amplification were more efficient and had no observed variation across treatments.

Statistical analysis

Data were analyzed as a two-way analysis of variance using the SAS software, general linear model (SAS Institute, 2008). The main effects were strain wt and methionine level. Traits analyzed were: LBW at 42 days, carcass, breast meat, leg meat wt, liver wt, gizzard wt, heart wt, shank length. The following model was used:

$Y_{ijk} = \mu + S_i + T_j + ST_{ij} + e_{ijk}$

Where:

Y_{ijk}: The k^{th} observation of the jth diet within the ith strain.

μ: The overall mean.

S_i: The effect of the i^{th} strain.

T_j: The effect of the j^{th} diet

ST_{ij}: The interaction between the i^{th} strain and the j^{th} diet

E_{ijk}: Random error associated with the individual.

All data are reported as least square means (LSM)±standard errors (SE). Mean values were separated, when significance existed, using Duncan's multiple range test (Duncan, 1955). Significance level was set at 5%.

RESULTS

The present study confirmed that methionine supplementation significantly improved bird's weight. The best results were obtained by the birds fed the highest methionine level (0.68% added ME) for most genes in both Cobb and Fayoumi strains.

In addition, the body weight of birds at different ages were obtained. Table (3)

Table (4) indicates that, as expected, there were statistically significant ($P<0.05$) variations between live body weight, carcass wt, breast meat wt and liver wt between Cobb and Fayoumi strains.

Quantitative RT-PCR was used to evaluate gene expression patterns in liver and breast muscle in response to the different diets. The data was normalized using the b-actin gene in case of breast muscles and GAPDH in case of liver, the expression of which did not change among the treatment groups.

Melting curves obtained for the genes showed no presence of nonspecific product (more than one peak), nor primer dimers (nonspecific Tm peak), thus, indicating the reliability of mRNA transcript identification revealing that IGF-I- , GHR, FOX, CTSL2, MuRF1, atrogin-1, and PI3KR1 specific primers are adequate to RT-PCR.

Relative quantification analysis was determined by the $2^{-\Delta ct}$ method (Table 5).

Broilers fed methionine supplementation showed changes in muscle IGF-I mRNA,

GHR mRNA, CTSL2 mRNA, MuRF1 mRNA, FOXO mRNA, Atrogin-1 and on liver PI3KR1 mRNA transcription.

It was observed that broilers fed the first level T1 (0.48 % total methionine) tended to show a higher expression of atrogin-1 in Cobb strain while the higher expression of the same gene atrogin-1 was found in T2 (0.58% total methionine) in Fayoumi strain. (Figure 1).

CTSL2 & FOXO genes, show the highest expression level in T3 (0.68% total methionine) in both strains Cobb and Fayoumi but CTSL2 & FOXO genes have higher expression value in Cobb than Fayoumi strain, (Figures 2 and 3).

When comparing the GHR mRNA expression in breast muscle, it was noted that the higher expression in the T3 0.68% methionine groups of both strains. However, the Cobb strain had higher expression value than the Fayoumi strain. (Figure 4).

Broilers fed the highest methionine level (0.68% methionine) showed higher level expression in Cobb strain than Fayoumi fed the same level of methionine (0.68%) of breast muscle IGF-I mRNA transcription. (Figure 5).

However, there were significant differences on the broilers fed 0.48% methionine showed a higher expression of MURF1 in Cobb strains.

However, the highest expression of the same gene (MURF1) was that of the control treatment of Fayoumi strain (0.38% methionine). (Figure 6).

Birds fed 0.68% methionine, had significant lower P1KR3 gene expression in the liver of

Fayoumi strain than those fed 0.48% in liver of Cobb strain. (Figure 7)

Table 3: Weekly Body Weight of Cobb and Fayoumi strains fed different levels of methionine from 21 days to 42 days of age.

Trait / S.O.V	N	21 days (gm)	28 days (gm)	35 days (gm)	42 days (gm)
Strain					
Cobb	140	485	788a	1111a	1547[a]
Fayoumi	140	148	198b	254b	312[b]
SE		4.27	12.19	14	23
Probability			<.0001	<.0001	<.0001
Treatment					
0.386% methionine	70	319	504a	664a	880.59a
0.486% methionine	70	312	475a	676a	924.90a
0.586% methionine	70	305	479a	684a	949a
0.686% methionine	70	328	514a	706a	963a
SE		7.01	16.24	23.21	33.87
Probability			0.4849	0.2450	0.155
Strain * Treatment					
Cobb / 0.386% M	35	486	797b	1072a	14443b
Cobb / 0.486% M	35	474	755b	1090a	1527b
Cobb / 0.586% M	35	472	774b	1136a	1609a
Cobb / 0.686% M	35	506a	825a	1146a	1608a
Fayoumi / 0.386% M	35	151c	210c	256b	317c
Fayoumi / 0.486% M	35	151c	194c	261b	322c
Fayoumi / 0.586% M	35	139d	183d	231b	288d
Fayoumi / 0.686% M	35	151c	203c	265b	318c
SE		8.55	24.3	28	46.09
Probability		<0.0001	<0.0001	<0.0001	<0.0001

SE = Standard error of means

Means within a column followed by unlike letters are significantly different (P<0·05). N= 280

Table 4: Slaughter performance at 42 days of age as influenced by strain and methionine level.

Trait / S.O.V	LBW gm	Carcass wt. gm	Breast meat wt. gm	Leg meat wt. gm	Liver wt. gm
Strain					
Cobb	1677a	1104a	236a	159a	17a
Fayoumi	279b	235b	45b	31b	9b
Probability	<.0001	<.0001	<.0001	<.0001	<.0001
Treatment					
0.386% methionine	1018a	654a	138a	96a	20a
0.486% methionine	1070a	690a	145a	101a	11b
0.586% methionine	972a	645a	132a	93a	12b
0.686% methionine	1035a	678a	143a	93a	10b
Probability	0.8349	0.8362	0.7196	0.9360	0.0147
Strain * Methionine					
Cobb / 0.386% M	1616a	1056a	226a	157a	19a
Cobb / 0.486% M	1690a	1104a	233a	163a	14b
Cobb / 0.586% M	1637a	1093a	229a	160a	12b
Cobb / 0.686% M	1723a	1134a	246a	157a	15b
Fayoumi / 0.386% M	421b	252b	50b	35b	11b
Fayoumi / 0.486% M	469b	281b	56b	39b	10b
Fayoumi / 0.586% M	309b	197b	36b	26b	8b
Fayoumi / 0.686% M	348b	217b	41b	29b	9b
SE (overall)	130	84	17.5	9.9	2.1
Probability	0.0039	0.0067	0.0040	0.0020	<.000

SE = Standard error of means

Means within a column followed by unlike letters are significantly different (P<0·05).

Table 5: Differences in delta Cycle Threshold (∆CT) of genes studied as affected by strain and treatment measured at 6 weeks of age.

Trait /S.O.V	∆CT Atrogin	∆CT CTS	∆CT FOXO	∆CT GHR	∆CT IGF	∆CT MURF	∆CT PI3KR1
Strain							
Cobb	3.35a	2.28a	4.0a	2.34a	5.91a	0.95a	6.7b
Fayoumi	1.95b	2.44a	4.3a	2.53a	5.5a	1.37a	7.3a
SE	1.62	1.78	1.87	1.93	2.45	1.46	1.15
Probability	0.040	0.96	0.39	0.65	0.82	0.48	0.05
Treatment							
0.386% M	3.73a	3.96 a	6.71a	4.41a	6.51	1.16	7.18ab
0.486% M	1.53b	2.7ab	5.1a	2.9a	6.91	0.26	6.02b
0.586% M	2.50ab	3.08ab	5.8a	4.01a	6.7	1.11	7.86a
0.686% M	2.74ab	1.04b	1.54b	0.39b	4.22	1.63	7.08ab
SE	4.91	13.39	47.9	30.12	14.92	2.49	3.47
Probability	0.01619	0.017	<0.0001	0.0008	0.08	0.345	0.007
Strain* Treat							
Cobb / 0.386% M	5.40a	4.5	7.4ab	5.46	6.6	0.1154	6.69ab
Cobb /0.486% M	0.26bc	1.63	4.1ab	2.63	6.8	1.25	4.58ab
Cobb / 0.586% M	3.96ac	3.93	7.8a	5.13	6.4	0.68	7.556a
Cobb /0.686% M	3.56ac	0.7	1.0b	-0.76	4.8	3.63	7.558a
Fayoumi / 0.386%	2.1ac	3.4	6.03ab	3.36	6.4	1.479	7.67a
Fayoumi / 0.486%	2.8ac	3.7	6.0ab	3.3	7.0	0.608	7.47a
Fayoumi / 0.586% M	1.03b	2.2	3.86ab	2.9	6.9	1.38	8.1a
Fayoumi /0.686% M	1.9ac	1.3	2.05ab	1.5	3.6	3.27	6.61ab
SE	10.84	4.62	11.5	10.19	1.13	3.34	5.06
Probability	0.0179	0.256	0.0403	0.0686	0.9034	0.226	0.025

SE = Standard error of means

Means within a column followed by unlike letters are significantly different (P<0·05).

Table (6): Effect of methionine (Met) levels on the relative mRNA levels in breast muscle and liver of Cobb and Fayoumi strains at 42 days of age.

Gene	Strain	Met. Level %	Average	Fold Change
Atrogin -1	Cobb	0.38% M	5.4	1.00
		0.48%	0.26	35.1
		0.58%	3.96	2.70
		0.68%	3.56	3.56
	Fayoumi	0.38% M	2.06	1.00
		0.48%	2.8	0.6
		0.58%	1.03	2.05
		0.68%	1.91	1.11
CTSL2	Cobb	0.38% M	4.46	1
		0.48%	1.63	7.1
		0.58%	3.93	1.44
		0.68%	0.7	13.6
	Fayoumi	0.38% M	3.46	1.00
		0.48%	3.76	0.81
		0.58%	2.23	2.35
		0.68%	1.38	4.24
FOXO	Cobb	0.38% M	7.4	1.00
		0.48%	4.13	9.62
		0.58%	7.86	0.72
		0.68%	1.57	56.69
	Fayoumi	0.38% M	6.03	1.00
		0.48%	6	1.02
		0.58%	3.86	4.49
		0.68%	2.05	15.82
GHR	Cobb	0.38% M	5.46	1.00
		0.48%	2.63	7.13
		0.58%	5.13	1.26
		0.68%	-0.76	75.24
	Fayoumi	0.38% M	3.36	1.00
		0.48%	3.3	1.05
		0.58%	2.9	1.38
		0.68%	1.55	3.52
IGF-1	Cobb	0.38% M	6.63	1.00
		0.48%	6.83	0.87
		0.58%	6.46	1.12
		0.68%	4.81	3.52
	Fayoumi	0.38% M	6.4	1.00
		0.48%	7	0.66
		0.58%	6.9	0.71
		0.68%	3.63	6.81
MURF1	Cobb	0.38% M	2	1.00
		0.48%	-0.66	6.35
		0.58%	0.66	2.52
		0.68%	1.38	1.53
	Fayoumi	0.38% M	0.33	1.00
		0.48%	1.2	0.55
		0.58%	1.56	0.43
		0.68%	1.88	0.34
PI3KR1	Cobb	0.38% M	6.69	1.00
		0.48%	4.58	4.33
		0.58%	7.55	0.55
		0.68%	7.55	0.55
	Fayoumi	0.386% M	7.67	1.00
		0.486%	7.47	1.15
		0.586%	8.17	0.71
		0.686%	6.61	2.08

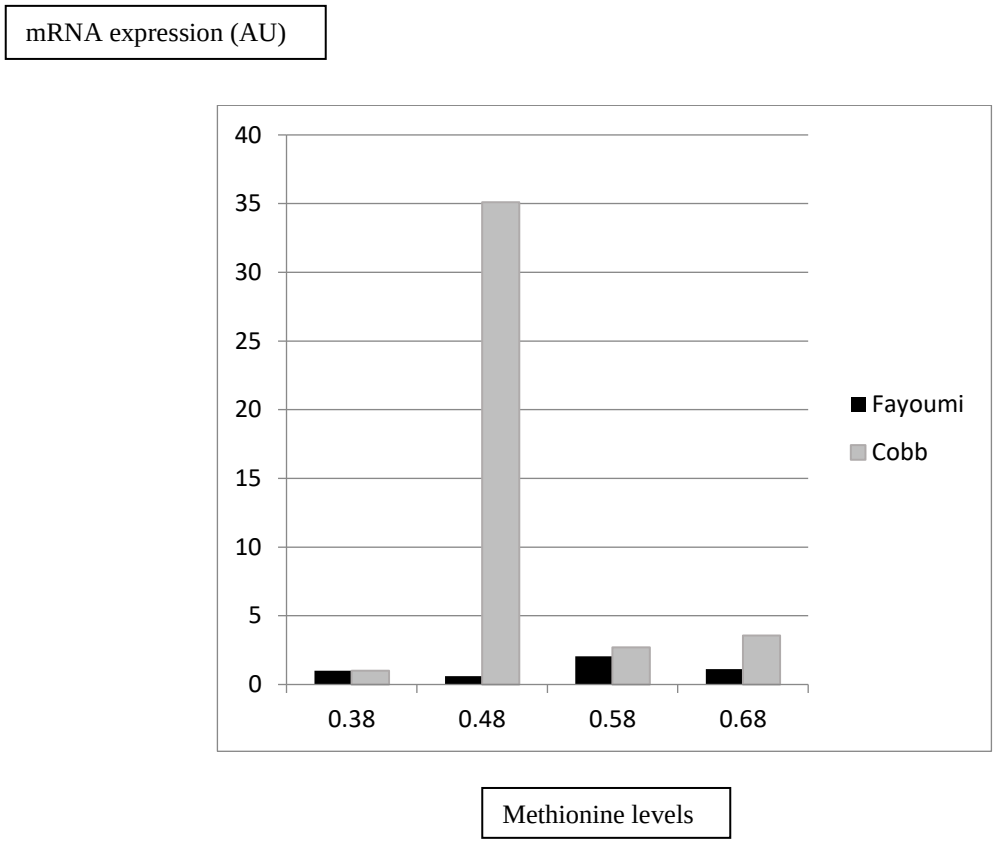

Figure 1: Expression of Atrogin -1 gene in breast muscle of Cobb and Fayoumi strains fed control and three levels of methionine diets.

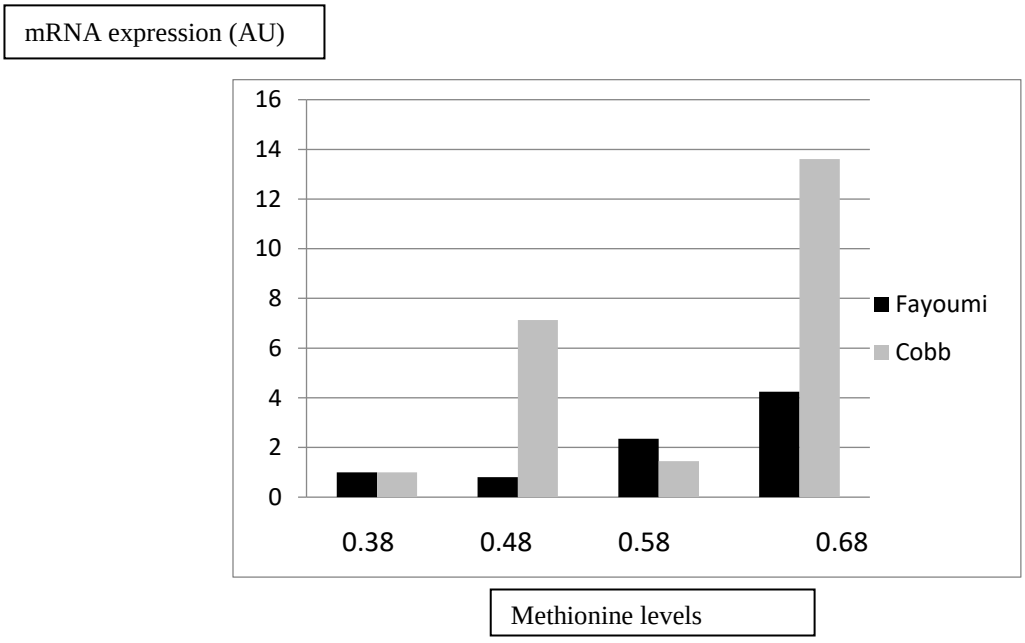

Figure 2: Expression of CTSL2 gene in breast muscle of Cobb and Fayoumi strains fed control and three levels of methionine diets

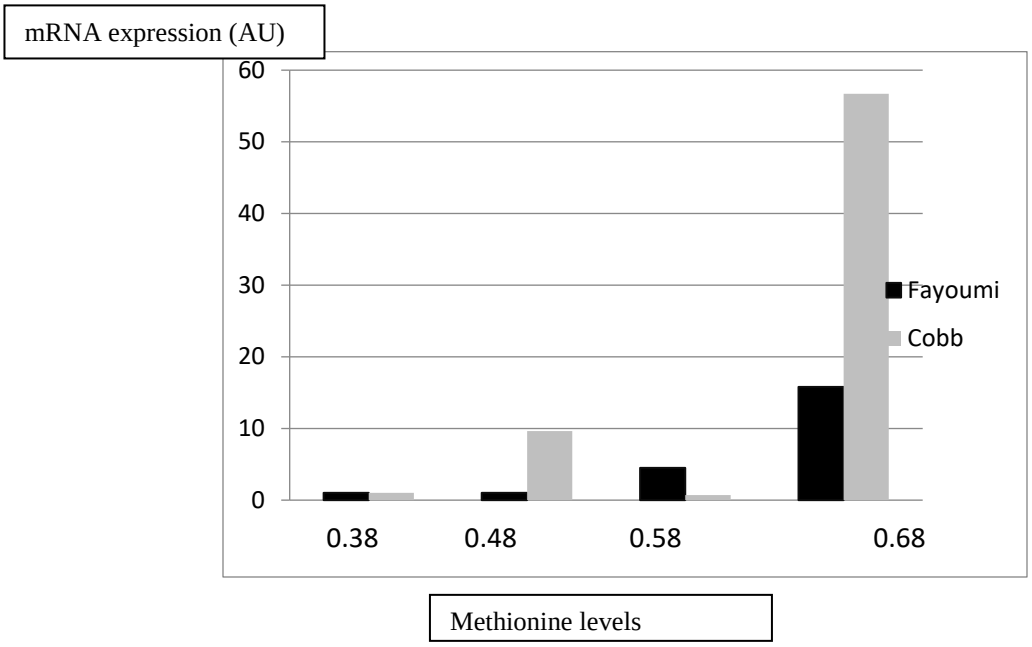

Figure 3: Expression of FOXO gene in breast muscle of Cobb and Fayoumi strains fed control and three levels of methionine diets

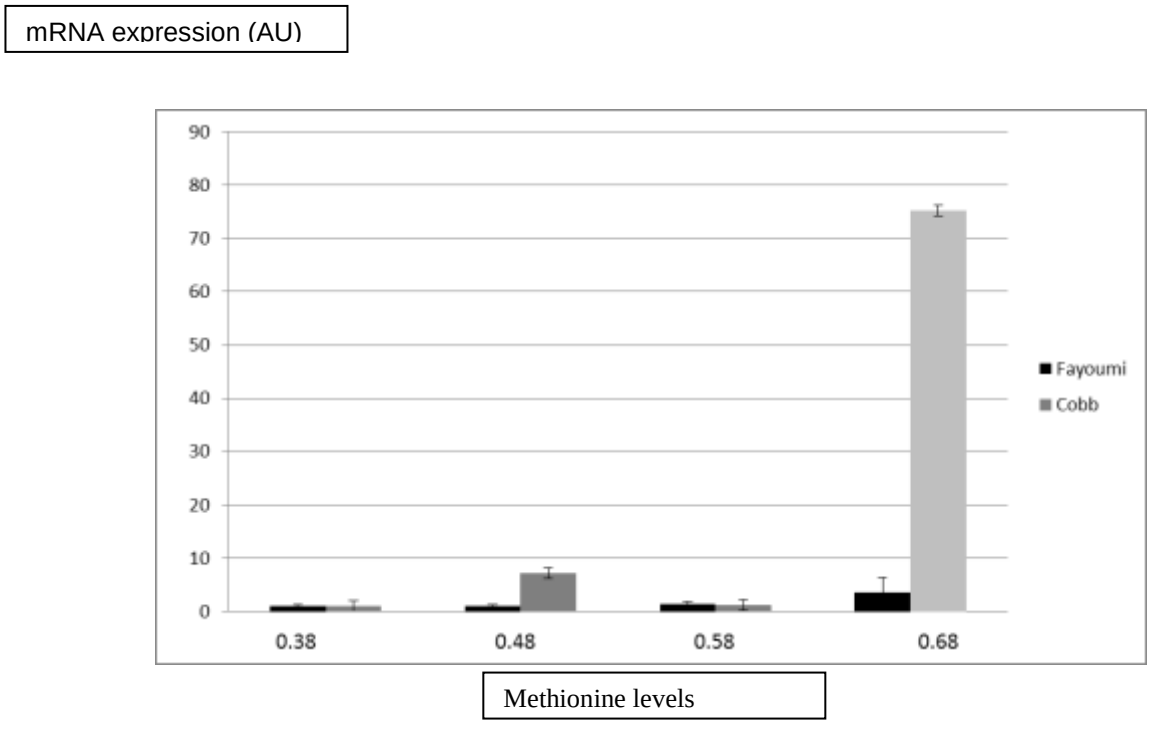

Figure 4: Expression of GHR gene in breast muscle of Cobb and Fayoumi strains fed control and three levels of methionine diets.

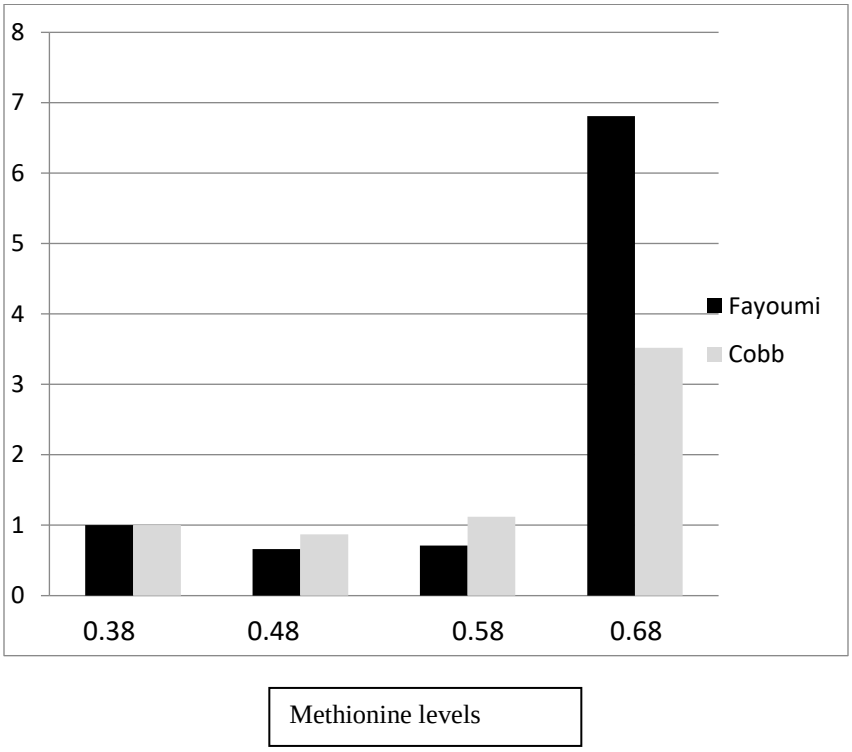

Figure 5: Expression of IGF-1 gene in breast muscle of Cobb and Fayoumi strains fed control and three levels of methionine diets.

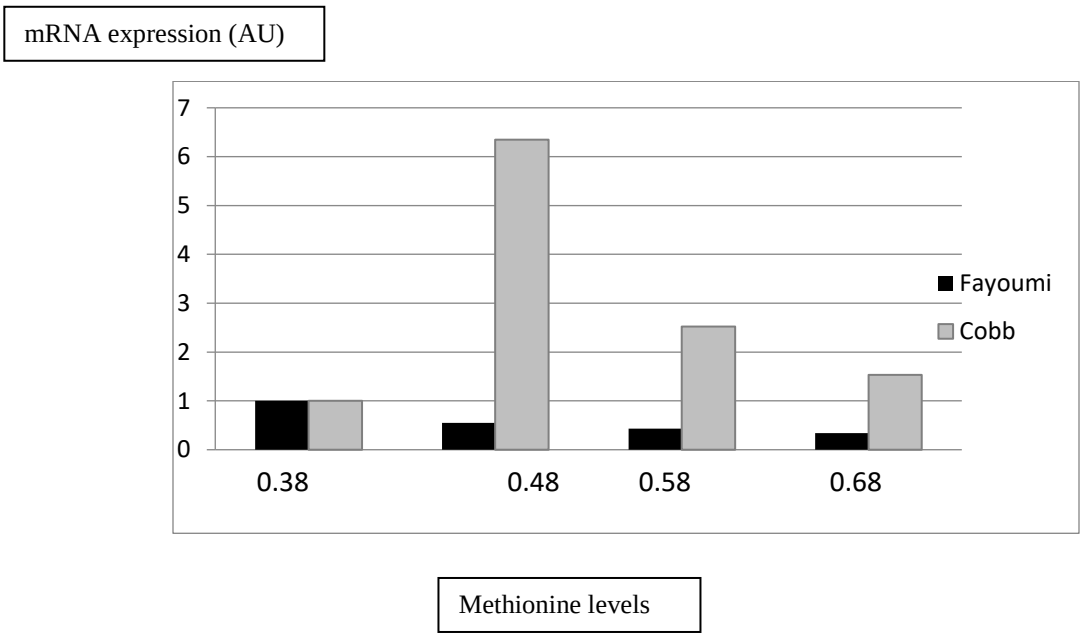

Figure 6: Expression of MURF1 gene in breast muscle of Cobb and Fayoumi strains fed control and three levels of methionine diets.

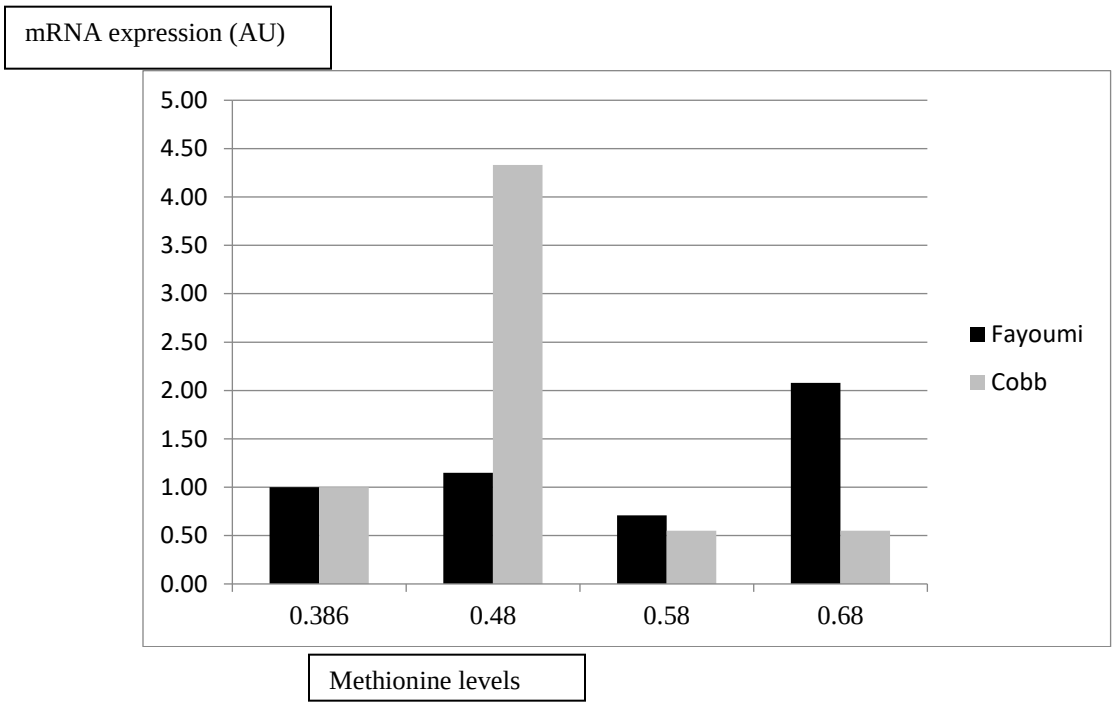

Figure 7: Expression of PI3KR1 gene in liver of Cobb and Fayoumi strains fed control and three levels of methionine diets

DISCUSSION

Energy production by the body is controlled by several mechanisms, involves genetic and nutritional factors. Relative to the feeds macro-ingredients, protein presents the highest oxidation rate, followed by carbohydrates and fat. Thus, the protein composition of feeds may have an important influence on broiler energy metabolism and body composition (Collin et al., 2003).

Amino acids act not only in translation initiation and on elongation factors, but amino acids also act on signaling pathways for the synthesis of proteins that were previously thought to be influenced only by hormones (Kimball and Jefferson, 2006). In addition, expression of genes involved in protein degradation can be reduced not only by growth factors, but also by effectiveness of dietary methionine (Tesserau et al., 2007).

Methionine supplementation is a nutritional factor that enhances animal performance parameters, such as weight gain, feed efficiency, noble cuts yield, and egg production and quality (Daskiran et al., 2009; Kauomar et al., 2011; Waldroup et al., 2006). Studies have indicated better performance on animals fed methionine supplementation (Waldroup et al., 2006; Kauomar et al., 2011).

Animal growth is mostly a result of protein deposition, which is regulated from the balance between protein synthesis and degradation. It is suggested that these two distinct pathways are products of the same biological route, and the hormonal concentration and diet are factors that can determine which of these pathways will prevail (Sacheck et al., 2004).

The results of the present study confirmed that heavy breeds had better performance than light breeds when both were fed a control diet, as reported previously (Mendes et al., 2011, Sklan et al., 2003)

It was observed that DL-methionine supplementation, significantly improved the performance of broiler, which is partially due to the fact that methionine induce growth by means of growth factors (Stubbs et al., 2002) and due to its effect on protein synthesis (Kimball and Jefferson, 2006) and breakdown (Tesseraud et al., 2007).

High-Met diets improved the performance of light strain, similar to that of heavy strain,

indicating that Met level used in the control diets in the present study was adequate for the heavy and light chicks. Similar results were obtained by Leandro et al., (2006)

In this study, we assessed the expression of PI3KR1 genes in the liver because a study performed by a research group showed that the liver is the primary site of IGFI production (Del Vesco et al., 2013). The expressions of the CTSL2 and atrogin-1 genes was performed in the muscle because results from the literature indicate the importance of the ubiquitin-proteasome pathway (Tesseraud et al., 2007, Gomes-Marcondes et al.,2002) and the role of lysosomal degradation in muscular atrophy (Lee et al., 2012)

Hormonal growth regulation includes a complex series of interactions between different hormones, with the somatotropic axis (GH, GHR, and IGF-I) considered to be the most significant.

The expression of IGF mRNA was lower in Fayoumi strain receiving 0.48% methionine than that of Cobb strain receiving the same diet.

The effect of GH on IGF-I is caused by the GH receptor (GHR) because GH-GHR binding is needful to stimulate IGF-I synthesis and release.

The expression of cathepsin L2 gene was also greater in animals with high supplementation of methionine than in animals with lower supplementation of methionine. This was observed in both strains receiving 0.68% methionine in their diets, the mRNA expression was higher expressed in Cobb strain.

The levels of IGF-I mRNA in breast muscle were lower in the light strain (Fayoumies) than in the heavy (Cobb) when both were fed the control diets, supporting the hypothesis that IGF-I mRNA may participate in the setting of muscle growth rate during development (Guernec et al., (2003)

The increase of dietary methionine levels had significant effects on most traits studied. The highest inclusion of methionine in (0.68%) in the diet of Cobb 500 and Fayoumi chicks till 6 weeks of age caused variations in the GHR and IGF-1 mRNA expression in breast muscle. This finding is in agreement with the results of Del Vesco (2013).

The statistical analysis showed that the diet with the inclusion of methionine promoted (Table 3) significantly better growth ($p<0.05$) until broilers were 42 days of age, this is in agreement with the findings of (Chao Wen et al., 2013).

In the current study, the expression of PI3KR1 gene has been observed in the liver because a study performed revealed that the liver is the primary site of IGFI production (Del Vesco., 2013). Also the FOXO family is necessary to activate the MURF-1 and atrogin-1 enzymes that act on the degradation pathway of the ubiquitin proteasome (Gomes-Marcondes and Tisdale (2002).

The degradation route of the ubiquitin proteasome consists of enzymatic actions that caused the release of amino acids after the breakdown of proteins linked to ubiquitin. Moreover, binding between IGF-I and its receptor results in auto-phosphorylation and conformational changes that produce a signaling cascade including many proteins. Among these are the phosphatidylinositol 3-kinase (PI3KR1), phosphoinositide-dependent kinase 1 (PDK1), and PKB/Akt (Nakashima et al., 2006).

The results presented in Table 5, showed parallel changes in the expression of atrogin-1, P1KR13 and FOXO4 mRNA in Cobb and Fayoumi in response to feeding different methionine levels. These findings were reported earlier Luo et al., (2010).

Previous research has shown the regulatory effect of FOXO4 on the expression of atrogin-1. Also methionine supplementation interferes with growth factors regulating such as, GHR and IGF-I. (Moylan et al., 2008)

Lu et al., (2008) observed an inverse pattern expression of GHR mRNA and GH mRNA transcription, suggesting that these two genes may play a transmitting inverse expression pattern in the profile of signaling of mechanisms that regulate growth. The improvement of breast meat yield by high-Met diets has been reported previously. (Hickling et al., 1990, Ahmed & Abbas 2011). In our study, we noticed (Table 5) an increase of methionine levels to reach the 0.68% by supplementing diets with DL-methionine was useful and did not induce any damage to the birds. Furthermore, higher amounts of methionine were associated with greater GHR, IGF-I, atrogin-1, FOXO, PI3KR1, and CTSL gene expression (Table 6). Although, there were no variation in MURF gene expression in Fayoumi strain. However, the higher methionine supplementation level yielded decreased MURF gene expression in Fayoumi strain. Lu et al., (2008) observed an inverse pattern expression of GHR mRNA and GH mRNA transcription, suggesting that these two genes may play a transmitting inverse expression pattern in the profile of signaling of mechanisms that regulate growth.

According to results reported in the literature Del Vesco et al., 2013 GHR mRNA transcription is influenced by diet, although with different patterns in distinct tissues. Results from our study supports

the above reports. Methionine supplementation increased GHR mRNA transcription in the muscle tissue.

In addition to stimulating protein synthesis through a positive effect on IGF-I gene expression, higher level of methionine in the diets may also stimulate proteins other than PI3KR1 and upstream of atrogin-1, thereby signaling less degradation. Due to the complexity and numerous factors included in the sensitive relationship between protein synthesis and degradation, different studies have been done to evaluate environmental and dietary nutrient effects (Willemsen et al., 2011 and Morand et al., 1997) on gene expression included in this metabolism.

The expression of mRNA PI3KR1 in the liver is influenced by methionine level in the diet. This trail revealed highest amounts of IGF-I mRNA and GHR mRNA, and best broiler growth performance in animals fed the highest level of methionine (0.68%).

Moreover, in our study, we found that a methionine level of 0.386% led to increased expression of the MURF gene in the Fayoumi strain.

The reduction in the expression of atrogin-1 mRNA without any change in that of MuRF1 in the breast muscle of Fayoumi chicks fed high-Met diets marks that Met may develop the muscle growth of light strains by preventing the down-regulation of protein synthesis but not proteolysis**.** (Foletta et al., 2011)

Methionine supply has been reported to modulate the expression of atrogin-1 in quail muscle fibroblasts (Tesseraud et al., 2007**)**. In other studies, the expression of atrogin-1 mRNA has been reported to be increased in chickens fed low-lysine diets (Tesseraud et al., 2009**)** or subjected to fasting (Nakashima et al., (2006**)**, showing that the expression of atrogin-1 is affected by nutritional status.

No difference in the expression of FOXO1 mRNA due to different methionine levels suggests that the response to dietary Met is isoform specific, with FOXO4 being more sensitive. This may be explained by the differential expression level of these isoforms between different organs; for example, FOXO4 is highly expressed in muscle, whereas FOXO1 is highly expressed in adipose tissue (Burgering, 2008).

CONCLUSION

In conclusion, high level of Methionine supplementation (0.58% and 0.68%), in the present study, improved breast meat weight consequently, increasing body weight.

The expression of mRNA atrogin -1, GHR, IGF-1, CTSL2, MURF1, FOXO genes in breast muscle and expression of mRNA PI3KR1 in liver are affected by methionine levels in diet.

Furthermore, supplementation of methionine may stimulate protein deposition, by that means ensuring not only higher genes expression related to synthesis but also lower genes expression related to degradation.

Further studies should be performed to confirm our results.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

Prof.Dr.F. K. R. Stino designed and performleed the experiments and also wrote the manuscript.Prof Dr.S.El-Assal performed animal treatments, flow cytometry experiments, tissue collection, and data analysis. Prof.Dr.F. K. R. Stino and Prof Dr.S.El-Assal designed experiments and reviewed the manuscript. All authors read and approved the final version.

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