GMR Genetics and Molecular Research
Online Journal
ISSN:1676-5680

Dietary methionine effects on IGF-I and GHR mRNA expression in broilers

A.P. Del Vesco[1], E. Gasparino[1], A.R. Oliveira Neto[2], S.E.F. Guimarães[3], S.M.M. Marcato[1] and D.M. Voltolini[1]

[1]Departamento de Zootecnia, Universidade Estadual de Maringá, Maringá, PR, Brasil
[2]Evonik Degussa do Brasil, São Paulo, SP, Brasil
[3]Departamento de Zootecnia, Universidade Federal de Viçosa, Viçosa, MG, Brasil

Corresponding author: E. Gasparino
E-mail: egasparino@uem.br

Genet. Mol. Res. 12 (4): 6414-6423 (2013)
Received March 20, 2013
Accepted October 23, 2013
Published December 10, 2013
DOI http://dx.doi.org/10.4238/2013.December.10.2

ABSTRACT. This study aimed to evaluate liver and breast muscle insulin-like growth factor I (IGF-I) and growth hormone receptor (GHR) gene expression between broilers fed different methionine levels and sources. Broiler chicks were 22 to 42 days old, distributed in 5 treatments (control diet, DL1 - 0.08% DL-methionine, DL2 - 0.24% DL-methionine, MHA-FA1 - 0.11% methionine hydroxy analogue-free acid, and MHA-FA2 - 0.33% methionine hydroxy analogue-free acid). The broilers were euthanized by cervical dislocation. RNA was extracted from liver and breast muscle, followed by cDNA synthesis and amplification using qRT-PCR. DL2 methionine supplementation provided best animal performance results. GHR and IGF-I gene expression in the muscle tissue was not affected by methionine supplementation. IGF-I gene expression in the liver was higher in animals fed methionine supplementation than in animals fed control diet. IGF-I mRNA levels in broilers fed DL2 were greater than DL1 (1.56 *vs* 0.97 AU) and greater than MAH-FA1 and MAH-FA2. Broilers

fed DL2 increased significantly GHR gene expression in the liver than animals fed the control diet. Addition of methionine improved animal performance by stimulating synthesis and release of growth factor.

Key words: Broiler; Growth hormone receptor; Methionine; Insulin-like growth factor I

INTRODUCTION

Poultry productivity important characteristics such as, rate of daily weight gain and weight gain composition (protein and fat), are influenced by several biological mechanisms such as, growth hormone (GH) and the diet made available to the animal. GH is an important regulator of growth and body composition. Through GH receptor (GHR) pathway (Kita et al., 2005) GH promotes synthesis and release of IGF-I (insulin-like growth factor 1) (van Vught et al., 2008).

Diet composition interferes on gene physiology and expression, causing feed conversion ratio alterations. Methionine is considered to be the first limiting amino acid for broilers, mostly due to amino acid demand for muscle and feather metabolism and due to the composition of foods used in their diets. Supplementation of methionine is important to maximize performance of broilers. Commercial methionine sources usually are DL-methionine (DL-2-amino-4-(methylthio)-butanoic acid) and methionine hydroxy analog-free acid, MHA-FA (DL-2 -hydroxy-4-(methyl)-butanoic acid). 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).

Despite reports in literature regarding the influence of methionine supplementation on performance of broiler chickens, there are few studies showing this influence or about the influence of other nutrients on gene expression. Research on methionine interference on genes that are involved in animal growth contributes to explain different poultry performance due to physiological changes at the cellular and molecular level. This study aimed to evaluate weight gain, feed conversion, liver and breast muscle IGF-I, and GHR gene expression in broilers fed diets containing two different sources and two levels of methionine supplementation.

MATERIAL AND METHODS

One-day-old male broilers (Cobb 500) were obtained from commercial hatchery. The birds were housed at broiler barn during an initial period of 1 day to 21 days old and husbandry under the same experimental conditions.

Broilers (22 days old) were distributed in a randomized complete design in a 2 x 2-factorial arrangement with a control diet. A control diet and two sources and two levels of methionine composed five treatments: control diet (no methionine supplementation); DL1: 0.08% DL-methionine supplementation; DL2: 0.24% DL-methionine; MHA-FA1: 0.11% MHA-FA; MHA-FA2: 0.33% MHA-FA, five replicates with 30 birds per box, in a total of 750 birds. The experimental diets were formulated according NRC (1994) recommendation (Table 1). Correlation between amino acid concentration and lysine intake was increased by 5% to avoid essential amino acid limitation. Broilers were given *ad libitum* access to food and water.

Table 1. Experimental diets centesimal composition (expressed as fed basis).

Ingredients	Experimental diets				
	Control	DL1	DL2	MHA-FA1	MHA-FA2
Corn 7.8% crude protein	45.51	45.51	45.51	45.51	45.51
Sorghum 8.0% crude protein	20.00	20.00	20.00	20.00	20.00
Soybean meal 46.7% crude protein	24.00	24.00	24.00	24.00	24.00
Meat meal 46% crude protein	4.50	4.50	4.50	4.50	4.50
Viscera flour 59% crude protein	2.00	2.00	2.00	2.00	2.00
Soy oil	2.80	2.80	2.80	2.80	2.80
DL 99%	0.00	0.08	0.24	0.00	0.00
MHA-FA 88%	0.00	0.00	0.00	0.11	0.33
L-lysine HCl 78%	0.17	0.17	0.17	0.17	0.17
L-threonine 78%	0.08	0.08	0.08	0.08	0.08
Salt	0.34	0.34	0.34	0.34	0.34
Calcareous 38%	0.36	0.36	0.36	0.36	0.36
Inert (kaolin)	0.45	0.45	0.45	0.45	0.45
Premix[1]	0.40	0.40	0.40	0.40	0.40
Total	100.60	100.70	100.90	100.70	100.90
	Composition analysis[2] (%)				
Crude protein	19.60	19.60	19.60	19.60	19.60
Lysine digestible	1.00	1.00	1.00	1.00	1.00
Met+Cis digestible	0.52	0.59	0.75	0.59	0.75
Threonine digestible	0.68	0.68	0.68	0.68	0.68
Tryptophan digestible	0.19	0.19	0.19	0.19	0.19
Valine digestible	0.79	0.79	0.79	0.79	0.79
Isoleucine digestible	0.69	0.69	0.69	0.69	0.69
Arginine digestible	1.13	1.13	1.13	1.13	1.13
	Composition calculated (%)				
Ca	0.74	0.74	0.74	0.74	0.74
P	0.38	0.38	0.38	0.38	0.38
Na	0.19	0.19	0.19	0.19	0.19
AME (kcal/kg)	3150	3150	3150	3150	3150

Control diet (no methionine supplementation); DL1: 0.08% DL-methionine; DL2: 0.24% DL-methionine; MHA-FA1: 0.11% MHA-FA; MHA-FA2: 0.33% MHA-FA. [1]Supplied by kilogram of diet: retinyl-acetate, 3.44 mg; cholecalciferol, 50 µg; DL-α-tocopherol, 15 mg; thiamine, 1.63 mg; riboflavin, 4.9 mg; pyridoxine, 3.26 mg; cyanocobalamin, 12 µg; D-pantothenic acid, 9.8 mg; D-biotin, 0.1 mg; menadione, 2.4 mg; folic acid, 0.82 mg; niacinamide, 35 mg; selenium, 0.2 mg; iron, 35 mg; copper, 8 mg; manganese, 60 mg; Zn, 50 mg; I, 1 mg; choline, 650 mg; salinomycin, 60 mg; avilamycin, 5 mg; butyl hydroxy toluene, 80 mg. [2]Feed formulations were made based on the total amino acids of corn and soybean meal as analyzed by Evonik Degussa (Hanau, Germany). The digestibility coefficient from NRC (1994) was used to obtain digestible amino acids. AME: apparent metabolizable energy. Amino acids, crude protein and dry matter as analyzed by Evonik Degussa (Hanau, Germany).

The broilers were weighed on the beginning of trial (22 days old) and by the end of trial (42 days old). Feed intake was calculated from amount of feed offered and subtracted from orts by the end of experiment. Feed conversion was obtained by dividing feed intake by weight gain. Mortality was included to calculate feed conversion.

Six animals from each treatment (N = 6) were euthanized by cervical dislocation by the end of trial. Liver and muscle breast (pectoralis superficialis) tissues were collected and stored in RNA Holder® (BioAgency Biotechnology, Brazil) at -20°C for posterior RNA extraction.

Trizol® reagent (Invitrogen, Carlsbad, CA, USA) was used for RNA extraction according to manufacturer recommendations, 1 mL/100 mg tissue. The RNase inhibitors, RNase AWAY® (Invitrogen), were used to prepare laboratory material. Tissue was triturated using electric Polytron homogenizer (tissue + Trizol) 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. Solution was centrifuged at 12,000 *g* for 5 min and the supernatant was discarded. Pellet was dried for 15 min and material resuspended in RNase-free ultrapure water. Spectrophotometer wavelength 260 nm measured total RNA concentration. RNA integrity 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.

SuperScrippt™ III First-Strand Synthesis Super Mix kit (Invitrogen Corporation, Brazil) 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 occur synthesis of cDNA. Solution contained cDNA was incubated for 5 min at 85°C and immediately placed on ice. Samples were stored at -20°C for posterior analysis.

Real-time polymerase chain reaction (RT-PCR) was performed using Fluorescent dye SYBR Green (SYBR® Green PCR Master Mix (Applied Biosystems, Carlsbad, CA, USA). RT-PCR products were analyzed on StepOnePlus v.2.2 (Applied Biosystems).

Primers were designed at http://www.idtdna.com/Home/Home.aspx (accessed March 14, 2010), using GHR and IGF-I gene sequences from www.ncbi.nlm.nih.gov (accession Nos. NM001001293.1 and FJ977570.1, respectively) (Table 2). We tested two housekeeping genes, the β-actin and GAPDH genes, and used the gene for β-actin (accession No. L08165), because it presented better efficiency in the reaction. All analyzes were performed in a volume of 25 µL and in duplicates.

Table 2. qRT-PCR primers.

Gene	Amplicom (bp)	Annealing temperature (°C)	Primer sequence (5'-3')
GHR	145	60	AACACAGATACCCAACAGCC AGAAGTCAGTGTTTGTCAGGG
IGF-I	140	60	CACCTAAATCTGCACGCT CTTGTGGATGGCATGATCT
β-actin	136	60	ACCCCAAAGCCAACAGA CCAGAGTCCATCACAATACC

Relative quantification analysis was determined by the $2^{-\Delta CT}$ method. Statistical analyses were determined by the Bayesian inference. The resistance (Yi) normal distribution Yi - normal (μ_i, σ_i^2), i = 1, 2,..., n_j was considered, where j = treatments; μ_i and σ_i^2 were considered non-informative *a prior* probability distributions: μ_i - normal (0.10^6) and σ_i^2 - gamma $(10^3, 10^3)$, respectively. Multiple comparisons were performed between the *posteriori* probability distributions. Treatments whose credibility intervals for the mean differences do not include the value zero were considered to be significant at the 5% level. The marginal *posteriori* distributions for all parameters were obtained by the Brugs program (R Development Core Team, 2011) package. MCMC (Markov Chain Monte Carlo) process 110,000 values, assuming 10,000 initial values of sampling disposal period, final sample contained 100,000 values. Chains convergence was checked by package CODA R by the Heidelberger and Welch (1983) criterion.

RESULTS

Melting curves 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 IGF-I- and GH-specific primers adequate to RT-PCR. Housekeeping β-actin showed great efficiency as endogenous control showing no statistical difference among treatments.

Credibility interval 95% reveals that gene expression profile of liver showed differences between treatments (Table 3). However, IGF-I and GHR genes expression in the muscle tissue showed no differences among treatments.

Table 3. *Posteriori* distributions estimative of contrasts among treatments in the liver IGF-I and GHR genes expression (AU).

	Contrast	Average	Standard deviation	Median	$p_{2.5\%}$	$p_{97.5\%}$
IGF-I	Δ1	-0.71*	0.07	-0.71	-0.84	-0.57
	Δ2	-1.30*	0.09	-1.30	-1.47	-1.12
	Δ3	-0.51*	0.07	-0.51	-0.65	-0.37
	Δ4	-0.62*	0.13	-0.62	-0.88	-0.36
	Δ5	-0.59*	0.07	-0.59	-0.73	-0.44
	Δ6	0.19*	0.05	0.19	0.08	0.30
	Δ7	0.09	0.12	0.09	-0.15	0.33
	Δ8	0.78*	0.08	0.78	0.63	0.94
	Δ9	0.68*	0.13	0.68	0.41	0.94
	Δ10	-0.10	0.12	-0.10	-0.35	0.14
GHR	Δ1	-1.61	1.16	-1.61	-3.77	0.50
	Δ2	-1.42*	0.51	-1.42	-2.35	-0.49
	Δ3	-0.63	0.19	-0.63	-1.99	0.73
	Δ4	-0.57	0.28	-0.57	-1.48	0.33
	Δ5	0.19	0.02	0.19	-2.10	2.49
	Δ6	0.98	1.32	0.98	-1.50	3.47
	Δ7	1.04	1.23	1.03	-1.22	3.33
	Δ8	0.79	0.81	0.79	-0.77	2.36
	Δ9	0.84	0.65	0.84	-0.35	2.04
	Δ10	0.05	0.79	0.05	-1.51	1.61

Δ1 = contrast between control diet and DL1; Δ2 = contrast between control diet and DL2; Δ3 = contrast between control diet and MHA-FA1; Δ4 = contrast between control diet and MHA-FA2; Δ5 = contrast between DL1 and DL2; Δ6 = contrast between DL1 and MHA-FA1; Δ7 = contrast between DL1 and MHA-FA2; Δ8 = contrast between DL2 and MHA-FA1; Δ9 = contrast between DL2 and MHA-FA2; Δ10 = contrast between MHA and MHA-FA1-FA2. *Statistical significant difference 5%.

Methionine supplementation of any source and concentration showed better assets for weight gain, DL-2 provided the highest results. No weight gain differences were found between animals fed MHA-FA1 and MHA-FA2 supplementation. Feed conversion was improved with methionine supplementation; however, there were no, within each level, differences between sources supplementation (Table 4).

Broilers fed methionine supplementation showed no changes on muscle IGF-I mRNA transcription. However, there were significant differences on gene expression profile in the liver in animals fed with the control diet compared to those receiving some source of methionine supplementation. DL2 caused greater mRNA IGF-I expression than DL1 (1.56 *vs* 0.97 AU), and greater than MHA-FA1 and MHA-FA2 supplement diets (Figure 1A).

When comparing the GHR mRNA expression in liver and muscle, we noted that higher expression in the liver occurs independent of the diet given. Broilers fed DL2 showed significant higher gene expression in the liver than animals fed control diet (Figure 1B).

Table 4. Performance of broilers in the period of 22-42 days of age.

	Body weight 22 days (g)	Body weight 42 days (g)	Weight gain* (g)	Feed consumption (g)	Feed conversion* (g/g)	Mortality (%)
Control	987 ± 14.28	2597 ± 34.70	1610 ± 29^{c}	3031 ± 41.23	1.88 ± 0.02^{a}	1.30 ± 0.04
DL1	976 ± 14.07	2769 ± 32.94	1793 ± 23^{b}	3026 ± 33.64	1.68 ± 0.01^{b}	1.28 ± 0.02
DL2	975 ± 13.21	2802 ± 27.83	1827 ± 45^{a}	2893 ± 31.28	1.58 ± 0.02^{c}	1.03 ± 0.03
MHA-FA1	969 ± 14.77	2692 ± 30.12	1723 ± 46^{b}	2983 ± 40.12	1.73 ± 0.03^{b}	1.15 ± 0.03
MHA-FA2	969 ± 13.86	2747 ± 29.96	1778 ± 40^{b}	2870 ± 31.71	1.61 ± 0.01^{c}	1.19 ± 0.01

*Weight gain and feed conversion in broilers fed control diet or two sources and two concentrations of methionine diets. Results are averages *a posteriori* and standard deviation. Different letters represent statistical differences with 95% credibility interval.

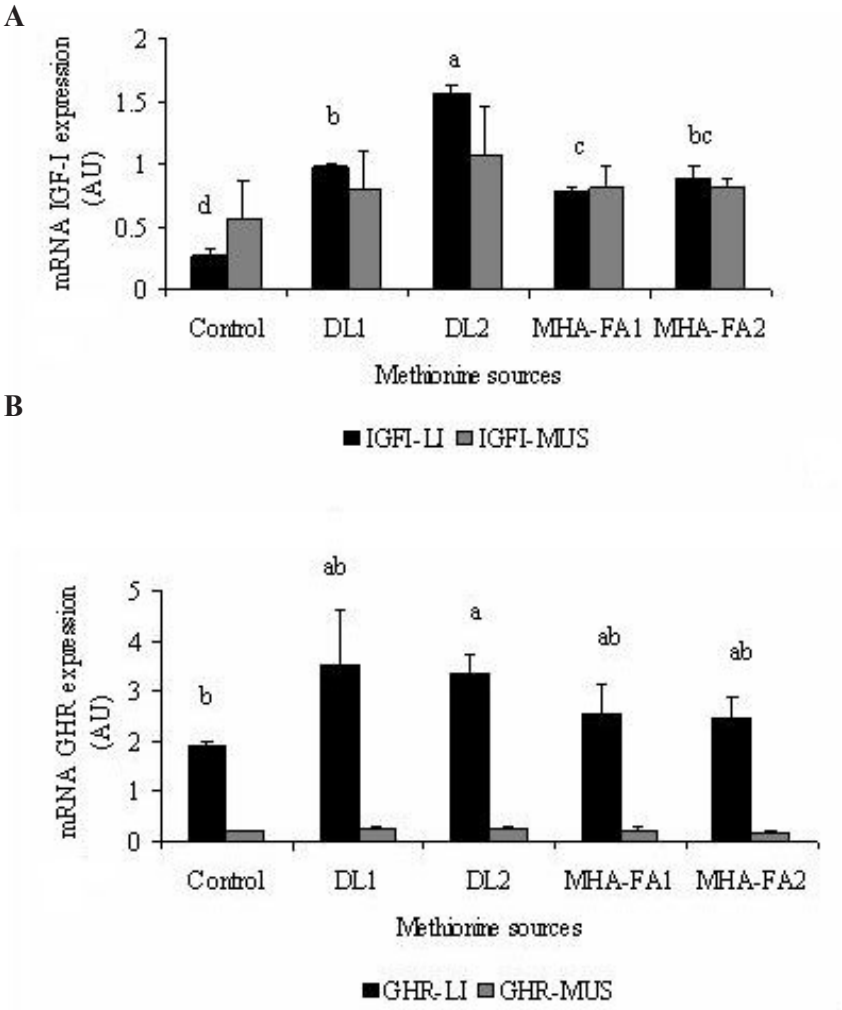

Figure 1. IGF-I (**A**) and GHR mRNA (**B**) expression in the liver (LI) and muscle (MUS) of broilers fed control diet or two sources and two concentrations of methionine diets. Results are averages *a posteriori* and standard deviation represented by vertical bars. Different letters represent statistical differences with 95% credibility interval.

DISCUSSION

Studies have described better performance on animals fed methionine supplementation (Waldroup et al., 2006; Kauomar et al., 2011). Many of these studies compared the effectiveness of two major commercial sources of supplementation available on the market, DL-methionine and methionine hydroxy analog, on characteristics such as weight gain and feed efficiency (Lemme et al., 2002; Bunchasak et al., 2006; Payne et al., 2006). Differences between MHA-FA and DL-methionine are due to chemical composition of the two sources: MHA-FA formulation is composed not only by monomers of methionine, but 12% water and impurities, while DL-methionine is composed of a 99% amino acid monomer powdery substance (Lemme et al., 2007).

This study reveals that both supplementation sources improved animal performance greater than the control diet. DL2 supplementation achieved highest weight gain (1827 g) and improved feed efficiency. Better feed conversion is associated with higher concentrations of methionine (Table 4). No significant difference between the commercial sources was observed within each level of supplementation. The fact that no differences were observed between the two sources may be due to the level of protein contained in the diet. Small differences in performance between the sources of amino acids are difficult to detect when there is a high protein concentration in diet (Visentini et al., 2005).

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).

GH secretion is regulated by somatotropic action, the presence of GH in the body leads to the synthesis and release of IGF-I through GHR pathway. Specific GHR binding site is followed by conformational changes that stimulates various signalling pathways, including the route that involves Janus kinase 2 (JAK2), which leads to different cellular responses such as synthesis and release of IGF-I (Kita et al., 2005). IGF-I plays an important role in poultry's growth metabolism, thus, lowest levels of IGF-I decreases growth rates (Scanes, 2009).

Higher amounts of IGF-I mRNA in the liver were observed in animals fed methionine supplementation. Related studies revealed nutrient interferences in gene expression that are associated with growth metabolic pathways. Deficient diets of essential amino acids, even when containing appropriate protein, lead to reduction in mRNA expression of IGF-I and to lower plasma levels of this hormone (Kita et al., 2002). In sheep liver, IGF-I gene expression decreased in animals fed low-concentration methionine diet. IGF-I release mechanism is induced by GH and it is interfered by methionine concentrations. Animals fed low-content total amino acid diet showed no reduction on IGF-I release, suggesting a selective methionine-inhibitory response to GH transcription factor interference in liver IGF-I gene expression (Stubbs et al., 2002).

It is also important to evaluate the sources of methionine supplementation; this study observed higher gene expression in animals fed DL2 supplementation. It is suggested that protein quality and the availability interfere on IGF-I mRNA levels (Miura et al., 1992). These authors observed an increased response on IGF-I mRNA transcription in the liver of mice fed high-quality protein diet (casein-replaced gluten); it was also observed an increase on gene expression in the liver of mice fed methionine supplementation instead of soy protein.

In this study, it can also be observed significant differences between the sources of supplementation, DL-methionine supplementation increased IGF-I gene expression. The concentration of IGF-I is affected not only by the restriction of methionine, but is also dependent on the level of deficiency of this amino acid (Carew et al., 2003). Thus, IGF-I mRNA transcription is also influenced by the amount and available source of methionine. The regulatory mechanisms of plasma IGF-I levels are more susceptible to dietary amino acids than IGF-I mRNA transcription-regulatory mechanisms. Therefore, great restriction is needed to occur differences in gene expression (Katsumata et al., 2002).

Increased expression of IGF-I mRNA in muscle compared to liver was observed in broilers fed diet without methionine supplementation and in broilers fed lower levels of MHA-FA. Katsumata et al. (2002) observed lower levels of mRNA in the liver of animals fed low concentration of amino acids. An opposite pattern was observed in the muscle, suggesting that IGF-I concentration in nonhepatic tissue is associated with low levels in the liver. However, analysis of IGF-I mRNA transcription in longissimus dorsi muscle showed no alteration on gene expression in pigs fed different amino acid concentration. In this study, broilers fed different methionine concentrations showed no effect on IGF-I mRNA transcription in muscle tissue. Since the liver is the major site of synthesis of this hormone (Schwander et al., 1983), this may have been under greater influence of diet, possibly by transcriptional and translational signals.

In this study, we observed the effect of methionine supplementation on GHR gene expression in liver; it can be seen that the consumption of the basal diet led to significant reduction in the amount of mRNA GHR in the liver (Brameld et al., 1999; Katsumata et al., 2002). When analyzing the gene expression within each source, it was observed that the lowest level of methionine tended to a greater amount of mRNA GHR. An inverse pattern revealed greater IGF-I mRNA transcription in the liver of broilers fed high methionine concentration. The GHR expression may be reduced directly by the amount of GH in this organism, but also by the influence of GH that can occur via IGF-I (Kim et al., 2010). Therefore, diets with higher methionine concentrations increased IGF-I concentrations and decreased GHR gene expression. 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 signalling of mechanisms that regulate growth.

According to results found in literature, GHR mRNA transcription is influenced by diet, although with different patterns in distinct tissues. Results from this study corroborate the above hypothesis, considering that methionine supplementation showed no GHR mRNA transcription effect in the muscle tissue but showed interference on GHR mRNA transcription in the liver. Pigs fed low-concentration lysine diets decreased GHR mRNA transcription in the liver. However, in muscle tissue an inverse pattern was observed, GHR mRNA transcription increased in pigs fed low-concentration lysine diets (Katsumata et al., 2002). The GH performs different functions in muscle and liver, thus, the expression of GHR, regulated by nutritional status, can also occur with distinct mechanisms in these tissues (Dauncey et al., 1994).

Methionine supplementation and high IGF-I levels stimulate protein synthesis and decrease protein degradation, improving protein deposition and growth. Methionine supplementation interferes in growth factor regulation such as, GHR and IGF-I, and this action of methionine may be related to the activity of mTOR (mammalian target of rapamycin). mTOR is an enzyme involved in protein synthesis regulation by mechanisms dependent or not on insulin (Stubbs et al., 2002). 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 improves weight gain and feed conversion in broilers regardless of source or concentration. The expression of mRNA IGF-I and mRNA GHR in the liver is influenced by methionine addition on the diet. This trail revealed highest amounts of IGF-I mRNA and GHR mRNA, and best broiler growth performance in animals fed DL2 supplementation.

ACKNOWLEDGMENTS

The authors are grateful to Procad-CAPES and CNPq for financial support.

REFERENCES

Brameld JM, Gilmour RS and Buttery PJ (1999). Glucose and amino acids interact with hormones to control expression of insulin-like growth factor-I and growth hormone receptor mRNA in cultured pig hepatocytes. *J. Nutr.* 129: 1298-1306.

Bunchasak C, Sooksridang T and Chaiyapit R (2006). Effect of adding methionine hydroxy analogue as methionine source at the commercial requirement recommendation on production performance and evidence of ascites syndrome of male broiler chicks fed corn-soybean based. *Int. J. Poult. Sci.* 5: 744-752.

Carew LB, McMurtry JP and Alster FA (2003). Effects of methionine deficiencies on plasma levels of thyroid hormones, insulin-like growth factors-I and -II, liver and body weights, and feed intake in growing chickens. *Poult. Sci.* 82: 1932-1938.

Dauncey MJ, Burton KA, White P, Harrison AP, et al. (1994). Nutritional regulation of growth hormone receptor gene expression. *FASEB J.* 8: 81-88.

Dibner JJ and Knight CD (1984). Convertion of metabolism of 2-hydroxy-4-(methylthio) butanoic acid to L- methionine in the chick: a stereospecific pathway. *J. Nutr.* 114: 1716-1723.

Heidelberger P and Welch P (1983). Simulation run length control in the presence of an initial transient. *Oper. Res.* 31: 1109-1144.

Jansman AJM, Kan CA and Wiebenga J (2003). Comparison of the Biological Efficacy of DL-Methionine and Hydroxy-4-Methylthiobutanoic Acid (HMB) in Pigs and Poultry. (CVB, Central Bureau for Livestock Feeding), Documentation Report No. 29: 55. Centraal Veevoederbureau, Lelystad.

Katsumata M, Kawakami S, Kaji Y, Takada R, et al. (2002). Differential regulation of porcine hepatic IGF-I mRNA expression and plasma IGF-I concentration by a low lysine diet. *J. Nutr.* 132: 688-692.

Kauomar N, Farhoomand P and Ebrahzadehim SK (2011). Effects of chromium methionine supplements on the performance and serum metabolites of broiler chickens. *J. Food Agr. Environ.* 9: 292-294.

Kim JW (2010). The endocrine regulation of chicken growth. *Asian Australas. J. Anim. Sci.* 23: 1668-1676.

Kimball SR and Jefferson LS (2006). Signaling pathways and molecular mechanisms through which branched-chain amino acids mediate translational control of protein synthesis. *J. Nutr.* 136: 227S-231S.

Kita K, Nagao K and Okumura J (2005). Nutritional and tissue specificity of IGF-I and IGFBP-2 gene expression in growing chickens - A Review. *Asian Australas. J. Anim. Sci.* 18: 747-754.

Kita K, Nagao K, Taneda N, Inagaki Y, et al. (2002). Insulin-like growth factor binding protein-2 gene expression can be regulated by diet manipulation in several tissues of young chickens. *J. Nutr.* 132: 145-151.

Lemme A, Petri A and Redshaw M (2007). What's new about commercial methionine sources in poultry. Degussa AminoNews™ - Review: 1-35.

Lemme A, Hoehler D, Brennan JJ and Mannion PF (2002). Relative effectiveness of methionine hydroxy analog compared to DL-methionine in broiler chickens. *Poultry Sci.* 81: 838-845.

Lu FZ, Wang XX, Pan QX, Huang RH, et al. (2008). Expression of genes involved in the somatotropic, thyrotropic, and corticotropic axes during development of Langshan and Arbor Acres chickens. *Poultry Sci.* 87: 2087-2097.

Miura Y, Kato H and Noguchi T (1992). Effect of dietary proteins on insulin-like growth factor-1 (IGF-1) messenger

ribonucleic acid content in rat liver. *Br. J. Nutr.* 67: 257-265.
National Center for Biotechonology Information (2010). National Center for Biotechnology Information [Home Page]. Available at [http://www.ncbi.nlm.nih.gov]. Accessed March 20, 2010.
National Research Council (NRC) (1994). Nutrient Requirements of Poultry. 9th rev. ed. National Academy Press, Washington.
Payne RL, Lemme A and Seko H (2006). Bioavailability of methionine hydroxy analog-free acid relative to DL-methionine in broilers. *Anim. Sci. J.* 77: 427-439.
Sacheck JM, Ohtsuka A, McLary SC and Goldberg AL (2004). IGF-I stimulates muscle growth by suppressing protein breakdown and expression of atrophy-related ubiquitin ligases, atrogin-1 and MuRF1. *Am. J. Physiol. Endocrinol. Metab.* 287: E591-E601.
Scanes CG (2009). Perspectives on the endocrinology of poultry growth and metabolism. *Gen. Comp. Endocrinol.* 163: 24-32.
Schwander JC, Hauri C, Zapf J and Froesch ER (1983). Synthesis and secretion of insulin-like growth factor and its binding protein by the perfused rat liver: dependence on growth hormone status. *Endocrinology* 113: 297-305.
Stubbs AK, Wheelhouse NM, Lomax MA and Hazlerigg DG (2002). Nutrient-hormone interaction in the ovine liver: methionine supply selectively modulates growth hormone-induced IGF-I gene expression. *J. Endocrinol.* 174: 335-341.
Tesseraud S, Métayer-Coustard S, Boussaid S, Crochet S, et al. (2007). Insulin and amino acid availability regulate atrogin-1 in avian QT6 cells. *Biochem. Biophys. Res. Commun.* 357: 181-186.
van Vught AJ, Nieuwenhuizen AG, Brummer RJ and Westerterp-Plantenga MS (2008). Effects of oral ingestion of amino acids and proteins on the somatotropic axis. *J. Clin. Endocrinol. Metab.* 93: 584-590.
Visentini P, Lopes J, Toledo GS and Costa PT (2005). Níveis de substituição da DL-metionina pela metionina hidroxi análoga em base equimolar, em dietas para frangos de corte. *Cienc. Rural* 35: 1400-1405.
Waldroup PW, Motl MA, Yan F and Fritts CA (2006). Effects of betaine and choline on response to methionine supplementation to broiler diets formulated to industry standards. *Poultry Sci.* 15: 58-71.

