Dietary methionine level alters growth, digestibility, and gene expression of amino acid transporters in meat-type chickens

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ABSTRACT  Imbalance in nutrients can affect digestibility of amino acids by altering gene expression of amino acid transporters. We investigated digestibility and molecular transporters of essential amino acids in chickens fed a methionine-deficient diet. A total of 40 chicks (23 D old) were randomly assigned to either a control (0.49% methionine) or a deficient (0.28%) diet until 41 D when they were sampled for Pectoralis (P.) major, kidney, ileum, and hypothalamus for mRNA expression analysis. The ileal content was collected for apparent ileal digestibility (AID) analysis. Birds fed the deficient diet had reduced growth and worse feed efficiency compared to control. The AID of methionine was similar between both groups. The AID of other essential amino acids was higher in the deficient group than control. The change in digestibility is reflected in the mRNA expression of amino acid transporters across different tissues.

Key words: methionine, digestibility, amino acid transporters, gene expression

INTRODUCTION

Methionine is an essential amino acid required for protein synthesis and is also the precursor of S-adenosylmethionine (SAM). Methionine is involved in 5 metabolic pathways: transmethylation, transsulfuration, re-methylation, aminopropylation, and salvage. In transmethylation, methionine acts as the sole methyl donor to a variety of acceptors including nucleic acids, proteins, CpG islands in DNA and biological amines (Mato et al., 1997). Methionine is converted to homocysteine, which can be irreversibly converted to cysteine via the transsulfuration pathway where it condenses with serine to form cystathionine, a reaction catalyzed by cystathionine β-synthase followed by a conversion to cysteine by cystathionine γ-lyase (Lu, 1998). In re-methylation, homocysteine is re-methylated by 5methyltetrafolate and/or trimethylglycine (betaine) to form methionine (Finkelstein et al., 1988). In aminopropylation, decarboxylated SAM initiates the synthesis of polyamines, which are essential for cellular growth (Jänne et al., 1978). In the last pathway, salvage, methylthiodenine from the aminopropylation pathway is converted to methionine (Bottiglieri, 2002). The complexity of the methionine pathway and its interconnection with the folate, choline and arginine pathways makes it essential, especially in avian nutrition.

In poultry, most feeds are manufactured from corn and soybeans and the first limiting amino acid in a typical corn–soy diet is methionine, and as a result, diets have to be supplemented with methionine. Poultry diets deficient in methionine impair growth (Sekiz et al., 1975), increase abdominal fat (Moran, 1994), and cause alterations in immune organs (Wu et al., 2013). Dietary amino acid imbalances can alter feed intake and affect uptake of essential nutrients. It is plausible that dietary deficiency in an essential amino acid could affect digestibility, transport, and absorption of nutrients. A variety of molecular transporters expressed on cellular membranes is responsible for the cellular uptake of amino acids (Zhang et al., 2017). These transporters are also present in enterocytes and kidney cells contributing to absorption and reabsorption of amino acids, respectively. Transport of amino acids depend on the charge on the amino acid, the tissue, and the...
direction of transport (from the lumen into the cell or through the baso-lateral membrane into the blood) (Stevens, 2010).

Amino acids also act as modulators of signaling pathways that control metabolism and cell functions (Tesseraud et al., 2011), therefore, an understanding of the molecular regulation of amino acid transporters could be essential in optimizing dietary requirements. Transporters responsible for enterocyte amino acid transport include B0, B0AT, and LAT4. B0, B0AT and LAT4 are present in the apical membrane and transport cationic and neutral amino acids, respectively (Fernandez et al., 2002; Kowalczuk et al., 2008). LAT4 is present in the basolateral membrane and transports large neutral amino acids (Bodo et al., 2005; 2013). In addition to these 3, LAT1, and TAT1 are also present in the kidney where they transport large neutral and aromatic amino acids, respectively (Prasad et al., 1999; Kim et al., 2002). LAT1, TAT1, and SNAT1 are also found in skeletal muscle where SNAT1 transports L-glutamine (Varoqui and Erickson, 2002; Schiöth et al., 2013). LAT1 and SNAT1 are also expressed in the brain. CAT1 is ubiquitously expressed and transports cationic amino acids (Verrey et al., 2004; Schiöth et al., 2013). SNAT2 and SNAT7 are also ubiquitously expressed and transport L-glutamine (Melnik et al., 2006; Schiöth et al., 2013).

Methionine-deficient diets produce an imbalance of nutrients, which can affect the digestibility of essential amino acids by the alteration on gene expression of amino acid transporters. The objective of this study was to investigate intestinal growth, ileal digestibility, and the dynamics of amino acid transporters in meat-type birds fed a methionine deficient diet.

**MATERIALS AND METHODS**

**Animals and Diets**

Experimental protocols complied with the regulations and guidelines of the Animal Care and Use committee of the University of Georgia, Athens, GA. A total of 102 day-old Cobb500 broiler male parent were raised on the floor until 22 D of age and they were fed with recommended starter (1 to 10 D) and grower (11 to 22 D) diets. Hereafter, 40 chickens weighing approximately 1 kg were selected at random, transferred to individual cages (L = 30.48 cm × B = 60.96 cm × H = 45.72 cm) equipped with feeder and nipple drinker and fasted overnight. The birds were fed on a diet of mainly corn and soybean meal containing 18% crude protein and 3,180 Kcal ME/kg. Each cage was randomly assigned to 1 of 2 treatments, control (0.49%) and basal (deficient) (0.28%) methionine diet. The diet was formulated based on Cobb500 recommendations (www.cobb-vantress.com). There were 20 birds per treatment. Feed and water were provided ad libitum. The analyzed amino acid composition of the basal diet is presented in Table 1. The diets contained 0.3% titanium (Ti) oxide. Individual BW and feed intake (FI) were measured every 3 D until day 41 when all birds were euthanized by cervical dislocation. Feed conversion ratio (FCR) was calculated as FI per BW gain. The amino acid consumption was calculated from the amount of amino acid in the diet and the amount of feed consumed. Ileum, kidney, hypothalamus, and Pectoralis (P) major samples from 5 birds in each group were taken at random and snap frozen in liquid nitrogen and stored at –86°C for molecular analysis. The ileum contents were removed and dried in a forced air oven at 75°C for amino acid analysis. Samples of the duodenum, were excised and stored in formalin for histopathology analysis.

**Apparent Digestibility**

The essential amino acid profile of the diets and ileal content of 10 randomly sampled birds from each

| Table 1. Ingestion composition and calculated and analyzed nutrients of basal diet. |
|---------------------------------|-----------------|
| Ingredient                      | (%)             |
| Corn, 815%                      | 66.557          |
| Soybean meal, 47.47%            | 25.245          |
| Soybean fat                     | 3.967           |
| Dicalcium phosphate             | 1.273           |
| Limestone (CaCO3)               | 1.104           |
| Vitamin premix                  | 0.249           |
| Mineral premix                  | 0.075           |
| Salt (NaCl)                     | 0.348           |
| L-Lysine HCl                    | 0.220           |
| Threonine                       | 0.089           |
| L-Valine                        | 0.070           |
| Isoleucine                      | 0.050           |
| Coccidiostat                    | 0.050           |
| Titanium oxide                  | 0.298           |
| Solka-flock                     | 0.398           |

**1**Vitamin mix provided the following (per kg of diet): thiamin-mononitrate, 2.4 mg; niacin acid, 44 mg; riboflavin, 4.4 mg; D-Ca pantothenate, 12 mg; vitamin B12 (cobalamin), 12.0 μg; pyridoxine-HCl, 2.7 mg; D-biotin, 0.11 mg; folic acid, 0.55 mg; menadione Na bisulfate complex, 3.34 mg; choline chloride, 220 mg; cholecalciferol, 1,100 IU; transretinyl acetate, 5,500 IU; all-rac-tocopherol acetate, 11 IU; and ethoxyquin, 150 mg.

**2**Trace mineral mix provides the following (per kg of diet): Mn (MnSO4 *H2O*), 60 mg; Fe (FeSO4 *7H2O*), 30 mg; Zn (ZnO), 50 mg; Cu (CuSO4 *5H2O*), 5 mg; and I (ethylene diamine dihydroiodide), 1.5 mg.

**3**Analyzed dietary methionine for the control diet was 0.49%.
treatment group were analyzed using standardized method 982.30 E(a, b, and c) (AOAC, 2006). The moisture content of the feed was 10% and that of the ileum content ranged from 1 to 3% with an average of 1.9%. Dietary and ileum content Ti were determined by a method described by Meyers et al. (2004). The apparent ileal digestibility (AID) of amino acid was calculated as (Edwards and Gillis, 1959):

**Apparent Digestibility**

$$\text{Apparent Digestibility} = 100 - \left[ 100 \left( \frac{\% \text{ Ti in diet}}{\% \text{ Ti in ileum}} \right) \times \left( \frac{\% \text{ aa in ileum}}{\% \text{ aa in diet}} \right) \right]$$

The amino acids analyzed were methionine, lysine, threonine, valine, tryptophan, leucine, isoleucine, phenylalanine, arginine, histidine, glycine, and cysteine and were expressed on an “as in” basis.

**Duodenum Morphology**

The duodenum, skeletal muscle from the superficial pectoral muscle, spleen, liver, and bursa of each bird (N = 10 per group) were collected and fixed in 10% buffered formalin. After fixation, samples were trimmed, routinely processed (Tissue-Tek VIP Sakura, Torrance, CA), embedded in paraffin (Leica EG1150), sectioned at 4 microns (Leica RM2255), and stained with hematoxylin and eosin (Leica Autostainer XL). Slides were examined by light microscopy (Leica DMR). Duodenum samples were used for villus height and crypt depth measurements using photomicrographs (Leica DC 500 camera) and Image J (NIH download) program for measurements. A total of 3 intact villi and the 3 corresponding crypts were randomly sampled thrice and measured in microns and averaged for each duodenum. Villus height/crypt depth ratios were calculated. All tissues were examined for microscopic changes and scores were assigned to major alterations using the scoring method of Henry et al. (1980).

**mRNA Isolation, cDNA Preparation and Real Time PCR (RT-PCR) Analysis of Amino Acid Transporters**

Ileum, kidney, P. major, and hypothalamus samples (5 per treatment group) were ground in liquid nitrogen and total RNA was extracted using Trizol reagents (Invitrogen, Carlsbad, CA), purified with RNeasy Mini Kit (Qiagen, Valencia, CA), and treated with RNase-Free DNASE Set (Qiagen, Valencia, CA) according to the manufacturer’s instructions. The RNA was suspended in diethyl pyrocarbonated (DEPC) treated water and sample purity and concentration were measured on a NanoDrop 2000 Spectrophotometer (Thermo Scientific, Wilmington, DE) and stored at −86°C. The purity of the extracted RNA was assessed by UV absorbance and the OD_{260/280} ratios for all samples were >1.9. The RNA concentrations ranged from 1 to 2 μg/μL. A total of 2 μg of total RNA was reverse transcribed with high capacity cDNA reverse transcription kit according to manufacturer’s protocol (Applied Biosystems, Foster City, CA) using a Gradient Mastercycler (Eppendorf, Hauppauge, NY) for 10 min at 25°C, 120 min at 37°C, 5 min at 85°C, and overnight at 4°C. cDNA samples were stored at −25°C. Complementary DNA (cDNA) samples were diluted 1:1 prior to RT-PCR analysis. Each reaction consisted of 1 μL diluted cDNA, 0.3 μL forward primer (10 μM), 0.3 μL reverse primer (10 μM), 8.4 μL DEPC water, and 10 μL Fast SYBR Green Master Mix (Applied Biosystems, Carlsbad, CA). Primer sequences used for the RT-PCR assays are listed in Table 2. The qPCR conditions were 95°C for 20 s, followed by 40 cycles of 95°C for 3 s and 60°C for 30 s. In addition, at the end of each reaction, a melting temperature curve of each qPCR reaction was determined. The qPCR was run in triplicates using StepOne Plus (Applied Biosystems, Carlsbad, CA). The gene expression differences between the birds on dietary methionine deficient and controls for Solute Carrier Family 7 (Amino Acid Transporter Light Chain, B<sup>0</sup> + System, b<sup>0</sup>+AT) Member 9 (SLC7A9), SLC Family 6 (Neutral Amino Acid transporter B(0) System, B<sup>0</sup>AT) Member 9 (SLC6A19), SLC Family 7 (Cationic Amino Acid Transporter, Y+ System, CAT1) Member 1 (SLC7A1), SLC Family 7 (Amino Acid Transporter Light Chain, L System, LAT1) Member 5 (SLC7A5), LAT 4 (SLC43A2), SLC Family 38 (Sodium-coupled Neutral Amino Acid Transporter, SNAT1) member 1 (SLC38A1), SNAT2 (SLC38A2), SNAT7 (SLC38A7), and SLC Family 16 (Aromatic Amino Acid Transporter, TAT1) Member 10 (SLC16A10) were analyzed according to the 2<sup>−ΔΔCT</sup> method (Livak and Schmittgen, 2001). Beta-actin was used as an internal standard. Differential mRNA expression was expressed as deficient/normal methionine level diets. LAT4 and B<sup>0</sup>AT1 were not expressed in the P. major, and B<sup>0</sup>AT1 was not expressed in hypothalamus.

**Statistical Analysis**

Statistical analysis between treatments for BW, FI, FCR, amino acids consumed, apparent digestibility, duodenal anatomy, and gene expression between the dietary methionine deficient and control groups were analyzed with generalized linear model procedure in SAS 9.4 software (SAS, 2013) using the following model:

$$y_{ij} = \mu + t_j + e_{ij},$$

where $y_{ij}$ is the mRNA abundance of an amino acid transporter for bird $i$ under treatment $j$ ($j = 1$ (control), 2 (deficient)), $\mu$ is an overall mean, $t_j$ is the effect of treatment $j$, and $e_{ij}$ is the random error term. Statistical significance between treatment levels was assessed using $t$ test in SAS 9.4 software (SAS, 2013) at a probability value of <0.05.
**RESULTS**

The feed intake, body weight gain (BWG), and FCR for the control and birds fed methionine deficient diet are presented in Table 3. The differences in BWG and FCR for the 2 groups were significant ($P < 0.05$). The birds on the deficient diet consumed about 2% more feed and had about 13% decreases in BWG compared to the birds on the control diet resulting in a better FCR compared to the control group. The duodenal villi height, crypt depth, and villi: crypt ratio was similar between the methionine deficient and control birds with less than 3% compared to the control group. The duodenal villi height, crypt depth, and villi height:crypt depth ratio was similar between the methionine deficient group and the control birds (Table 2). The mRNA expression of some amino acid transporters was influenced by dietary methionine levels and the tissue. The amino acids transporter expression between the methionine deficient and control groups in the ileum, kidney, and hypothalamus are presented in Figure 2. The LAT4 transporter was upregulated in the ileum, kidney, and hypothalamus of the methionine deficient birds compared to the control birds. The TAT1 transporter was downregulated in the kidney in the methionine deficient group compared to the controls. The TAT1 transporter was upregulated in the ileum, kidney, and hypothalamus of the methionine deficient birds compared to the control group. In general, the differences in BWG and FCR for the control and birds fed methionine deficient diet and control is presented in Figure 1. Even though the AID of methionine in the deficient birds was lower than the control birds the difference was not statistically significant. The digestibility of lysine, threonine, valine, tryptophan, isoleucine, arginine, histidine, glycine, and cysteine were significantly higher ($P < 0.05$) in the methionine deficient birds compared to the control birds. The AID of the aforementioned amino acids increased by 3 to 7%. Even though the digestibility of leucine ($P = 0.07$) and phenylalanine ($P = 0.07$) were numerically higher in the deficient group, they were not statistically significant from the control group. The increase in AID of leucine and phenylalanine in the deficient group were less than 3% compared to the control group. The duodenal villi height, crypt depth, and villi height:crypt depth ratio was similar between the methionine deficient group and the control birds (Table 2). The mRNA expression of some amino acid transporters was influenced by dietary methionine levels and the tissue. The amino acids transporter expression between the methionine deficient and control groups in the ileum, kidney, and hypothalamus are presented in Figure 2. The LAT4 transporter was upregulated in the ileum, kidney, and hypothalamus of the methionine deficient birds compared to the control birds. The TAT1 transporter was downregulated in the kidney in the methionine deficient group compared to the controls. The b$_0$,+AT transporter was upregulated in the ileum and kidney of the methionine deficient group compared to the control group. In the P. major, SNAT1, SNAT2, SNAT7, and CAT1 transporters were upregulated in the methionine deficient group compared to the control group in the hypothalamus, the SNAT2, SNAT7, CAT1, and LAT4 were upregulated in the methionine deficient group compared to the control group. In general, the significant differentially expressed transporters were all upregulated in the methionine deficient group when compared to the control group, except for TAT1 and

**Table 2. Gene, NCBI Reference and real-time polymerase chain reaction primer sequences.**

| Gene name | Solute carrier | Description/function | NCBI reference | Forward/reverse primer |
|-----------|---------------|----------------------|----------------|------------------------|
| b$_0$+AT  | SLC7A9        | Apical exchange of extracellular cationic amino acids and cystine for neutral | NM_001199133.1 | GATCCCTGGAGGGCTGAAATCACCTCTGGTCTT |
| B0AT      | SLC6A19       | Apical resorption of neutral amino acids | XM_419056.4 | CTGCTGGGTTTTGCTCATCTAT |
| CAT1      | SLC7A1        | Transport of cationic amino acids | EU360441.1 | GGCACACTGATGCTGATAT |
| LAT1      | SLC7A5        | Uptake of large neutral amino acids | NM_001030579.2 | CTCATGCGCCCTTCATCAATC |
| LAT4      | SLC43A2       | Basolateral transport of phenylalanine, leucine, isoleucine, and methionine | NM_415803.4 | GACTCGAGACATCCUCATAAT |
| SNAT1     | SLC38A1       | Transport of L-glutamine | NM_001199603.1 | GACCGAGAAGACAGGAAGAG |
| SNAT2     | SLC38A2       | Transport of L-glutamine | NM_001305439.1 | TGAAGACAGACATCCAAAAAG |
| SNAT7     | SLC38A7       | Transport of L-glutamine | XM_414044.4 | CAGACCTCCCTTTGCTTTC |
| TAT1      | SLC16A10      | Basolateral transport of aromatic amino acids | XM_419783.4 | GACCACTGCAAACCTCTGTAT |
| $\beta$-actin |             |                       | NM_205518.1 | CAGGACTCCATAACCAAGAG |

**Table 3. Performance variables** (feed intake (FI), body weight gain (BWG), and feed conversion ratio (FCR)) and duodenal measurements ($\pm$ SD) in broilers fed with different levels of dietary methionine.

| Variable | Control | Methionine deficient | $P$ value |
|----------|---------|----------------------|-----------|
| FI (kg)  | 2.86 ± 0.18 | 2.93 ± 0.23 | 0.3828 |
| BWG (kg) | 1.77 ± 0.13$^a$ | 1.54 ± 0.16$^b$ | 0.0008 |
| FCR (kg/kg) | 1.62 ± 0.04$^a$ | 1.91 ± 0.18$^b$ | <0.0001 |
| Duodenal morphology | | | |
| Villi height ($\mu$) | 2.744 ± 193 | 2.675 ± 348 | 0.4270 |
| Crypt depth ($\mu$) | 224 ± 32 | 224 ± 45 | 0.9987 |
| Villi: crypt ratio | 12 ± 2 | 12 ± 3 | 0.7664 |

$^a$Based on N = 20 per treatment.

$^b$Based on N = 10 per treatment.

$^a$Means within row with different superscript are significantly different at $P < 0.01$. 
DIETARY METHIONINE AND AMINO ACID TRANSPORTERS

Figure 1. Apparent ileal digestibility of essential amino acids and cysteine in broilers fed diets with different methionine levels. *(P < 0.05), **(P < 0.01), and +(P < 0.10).

LAT1 in the kidney, which were downregulated in the methionine deficient group compared with the controls.

DISCUSSION

From the performance data, it is apparent that feeding a diet deficient in methionine affects growth and feed efficiency. Methionine is an essential amino acid, and it is also the first limiting amino acid in a typical corn–soy diet (Sekiz et al., 1975). Even though the birds on the methionine deficient diet consumed about the same amount of feed as the control birds, they grew less and that affected their FCR. Thus, it is essential for the dietary methionine requirement of growing birds to be met to support normal tissue growth. In the body, methionine is converted to SAM, a process which provides methyl for methylation of hormones, amines, and DNA (Bottiglieri, 2002; Stipanuk, 2004). Methionine is a major supplier of methyl for methylation which makes it even more essential. Suboptimal dietary methionine has been shown to cause hypomethylation (Ehrlich, 2009). It is expected that the birds on the deficient diet will generate less methyl for global methylation and as a result may suffer from hypomethylation (Ehrlich, 2009) and low grade inflammation (Aggrey et al., 2016). DNA methylation plays a role in regulating gene activity (Fu et al., 2014). Benight et al. (2009) have linked methionine metabolites to the modulation of colonic inflammation. Thus, optimal levels of dietary methionine is not only required for normal growth, but also for proper cellular and molecular functions, and the modulation of the immune system.

Growing birds fed a methionine deficient diet had higher AID of other essential amino acids. The increased higher AID of essential amino acids could be due to the increase in the apparent absorption coefficient of nitrogen observed in chickens fed methionine deficient diets (Pisano et al., 1959). Cysteine is not considered to be an essential amino acid, and is expected to be supplied through the transsulfuration route of the methionine pathway. The AID of cysteine was higher in the deficient group compared to the control, but the AID of methionine+cysteine was similar between the 2 groups due to the slightly lower but non-significant difference in the AID of methionine. The composition of the digestible total sulfur amino acids may be more important than its absolute value as we show that despite similar digestible methionine+cysteine between the 2 groups, the control group had better growth and FCR compared to the group fed a diet deficient in dietary methionine. It should be pointed out that the differences in AID between the dietary methionine deficient group and the controls were not due to concomitant changes in the duodenal morphology. The duodenal villi height, crypt depth, and villi height: crypt depth ratios were not statistically different between the 2 experimental groups.

Feeding a methionine deficient diet led to the upregulation of b0,+AT and LAT4 and downregulation of TAT1 expressions in the ileum compared to those fed a normal diet. Amino acid transporters can be expressed in different locations of the intestinal epithelium, which could reflect on whether they are transporting amino acids from the intestinal lumen across the villi into the epithelial cell or from the epithelial cell into circulation. The b0,+AT transporter has been shown to be co-expressed with the heavy subunit rBAT (SLC3A1) (Verrey et al., 2000). Increased expression of b0,+AT in the ileum of the methionine deficient birds could enhance the exchanges of extracellular amino acids and cystine (Fortiadis et al., 2013). Zhang et al. (2017) examined expression of amino acid transported in chickens fed different methionine isomers. They reported an increase in mRNA expression in the ileum of broiler chickens.
The expression of amino acid transporter mRNA in ileum, kidney, Pectoralis major, and hypothalamus of broilers fed with different levels of methionine. *(P < 0.05), **(P < 0.01), and + P < 0.10.

The system L amino acid transport activity induced by LAT4 is sodium-, chloride-, and pH-independent, is not trans-stimulated and shows 2 kinetic components. The low affinity component of LAT4 induced activity is sensitive to the sulfhydryl-specific reagent N-ethylmaleimide but not that with high affinity (Bodoy et al., 2005). The LAT4 transporter was upregulated in the ileum and the kidney of the methionine deficient birds compared to the control. At the basolateral membrane of enterocytes, LAT4 has been shown to mediate the transport of phenylalanine, leucine, isoleucine, and methionine, contributing to efflux of...
amino acids after their luminal uptake from the intestinal lumen (Bodoy et al., 2005; Guetg et al., 2015). The upregulation of LAT4 in the kidney may increase the efflux of neutral amino acids (Guetg et al., 2015). Guetg et al. (2015) reported that the LAT4 gene was not expressed in the liver and skeletal muscle of mouse. In the current study, the LAT4 gene was not expressed in the P. major of broiler chickens. From a mouse knock-out model, it was concluded that LAT4 is localized in the basolateral epithelia and is necessary to mediate a balance of its amino acid substrates between the extracellular space and the cytosol (Guetg et al., 2015).

In the kidney, 2 basolateral transporter genes, LAT1 and LAT1 were downregulated in the methionine deficient birds compared to their control counterparts. The LAT1 gene is co-expressed with 4F2hc (SLC3A2) and they mediate the Na\(^+\)-independent obligatory exchange (1:1 stoichiometry) between an efflux of large neutral amino acids as leucine, isoleucine, and methionine, and an influx of aromatic amino acids (Fotiadi et al., 2013). Thus, in the kidney of the dietary deficient methionine birds, expression levels of LAT1 and LAT1 could suggest an effort to maintain a balance of aromatic amino acids.

The amino acids transporter expression in the P. major was different from that of the ileum and the kidney where the differentially expressed transporter genes were sodium-coupled neutral amino acid transporters SNAT1, SNAT2, and SNAT7 and CAT1, which were all upregulated in the dietary methionine deficient birds compared to the controls. The SNAT1, SNAT2, and SNAT7 transport L-glutamine in order to maintain homeostasis. The transport process performed by SNAT1/2 is highly energized such that glutamine, glycine, proline, and alanine reach high transmembrane gradients and constitute major components of the intracellular amino acid pool (Franchi-Gazzola et al., 2006). SNAT1/2 activity therefore influences the cell content of most amino acids, thus determining the overall size and the composition of the intracellular amino acid pool (Franchi-Gazzola et al., 2006). In L6 rat skeletal cells, total amino acids deprivation leads to upregulation of SNAT2. Herein, we show that dietary methionine deficiency alone can also lead to increased expression of SNAT2 in the P. major muscle (Kashiwagi et al., 2009). It has also been shown that SNAT7 transporter has L-glutamine as the preferred substrate but also transports other amino acids with polar side chains, as well as L-histidine and L-alanine (Hågglund et al., 2011). Imbalance in dietary amino acids, especially methionine and phenylalanine leads to increased feed intake and increased nitrogen excretion (Deshpande et al., 1958). The dietary imbalance in amino acids could lead to increased excretion of nitrogen especially in the birds fed the methionine deficient diet. In birds, glutamine is an intermediate in the detoxification of nitrogen and the production of uric acid via the purine biosynthesis and salvage pathways (Aggrey et al., 2014). Thus, the upregulation of SNAT1, SNAT2, and SNAT7 could be in response to increased transport of glutamine as a result of increased skeletal nitrogen that required excretion. CAT1 is a high-affinity, sodium-independent transporter involved in the transport of the cationic amino acids arginine, lysine, and ornithine in non-hepatic tissues (Verrey et al., 2004). The cationic amino acid transporters mediate the bidirectional transport of cationic amino acids, thus supporting important metabolic functions, such as synthesis of proteins, nitric oxide synthesis, polyamine biosynthesis, and inter-organ amino acid flow (Fernandez et al., 2003).

In the current study, we show that a diet deficient in methionine can increase the mRNA expression of CAT1 in the skeletal muscle. Increase in CAT expression has been shown to parallel arginase levels, shifting the metabolism of arginine from nitric oxide synthesis to arginase-dependent production of ornithine (Visiagalli et al., 2010). Ornithine is a precursor of the polyamine putrescine. Spermidine is synthesized from putrescine, and spermine is synthesized from spermidine. The synthesis of both spermidine and spermine require decarboxylated SAM from the aminopropyla sub-pathway of the methionine cycle. The shift of arginase metabolism from nitric oxide synthesis to ornithine alludes to the importance of polyamines and the interaction between methionine and arginine pathways in support of protein biosynthesis.

In the hypothalamus, the SNAT1, SNAT7, LAT1, LAT4, and CAT1 transporters were upregulated in the birds fed methionine deficient diet compared to the controls. SNAT1 is highly expressed on brain ependymal cells and also in neurons and it exhibits high affinity for glutamine, its principal substrate in the brain (Varoqui and Erickson, 2002). The glutamine transporters accumulate glutamine inside the neuronal cytosol using the electrochemical gradient of Na\(^+\) ions, which is essential to the glutamate–glutamine cycle in the brain (Albers et al., 2001). The upregulation of SNAT1 and SNAT7 in the hypothalamus in response to the deficiency in dietary methionine could possibly be an attempt to maintain glutamine homeostasis and transport of nitrogen. The SNAT7 transporter has been suggested to play a role in both excitatory and inhibitory neurotransmission in addition to its function in the glutamate cycle (Hågglund et al., 2011). In the hypothalamus, LAT1 and LAT4 could putatively act as amino acid exchangers and be involved in the transport of L-3,4-dihydroxyphenylalanine across the blood–brain barrier (Geier et al., 2013), and also play a role in neuronal cell proliferation in the brain (Geier et al., 2013). Also, similar to the P. major, the upregulation of CAT1 in the hypothalamus may be to shift the metabolism of arginine from nitric oxide to the synthesis of polyamines.

**CONCLUSIONS**

In all, methionine deficient diet does not only affect protein synthesis and feed efficiency, but also increases
the digestibility of other essential amino acids other than methionine itself. It also changes the dynamics of amino acid transporters to reflect their availability. The differences in digestibility of other essential amino acids other than methionine in birds fed a methionine deficient diet changed the dynamics of amino acid transport gene expressions in the ileum, kidney, P. major muscle, and the hypothalamus. Dietary deficiency in methionine particularly affects arginine metabolism, which is evidenced by increased expression in the arginine transporter which putatively shifts arginine metabolism from nitric oxide to polyamine synthesis. Neutral amino acid transporters are differentially expressed in response to the changes in available amino acids to maintain influx and efflux homeostasis. Amino acids digestibility and their influence on nutrient transport can affect nutrient availability and could be considered in diet formulation, however, the protein and gene activity levels should be ascertained since this study primarily focused on mRNA abundance.

ACKNOWLEDGMENT

Naiara S. Fagundes was supported by The Sao Paulo Research Foundation-FAPESP (Proc. FAPESP n.2015/00656-9). We thank Lindsey Rachett of the Poultry Research Center, University of Georgia for her assistance. We also thank Ijeoma Okoye and Ashley Turner for their assistance in collecting the data.

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