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Effect of methionine supplementation on mitochondrial genes expression in the breast muscle and liver of broilers

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ABSTRACT

This study aimed at evaluating the expression of the mRNA of avian uncoupling protein (avUCP), of adenine nucleotide translocase (ANT), and of cytochrome c oxidase subunit III (COX III), as well as the performance of broilers fed diets containing two sources and two levels of industrial methionine. Broilers between 22 and 42 days of age were distributed into five treatments (basal diet, supplementation of 0.08% DL-methionine, 0.24% of DL-methionine, 0.11% of MHA-FA and 0.33% of MHA-FA). At the end of the experimental period, birds were sacrificed by neck dislocation and their liver and breast muscle were collected for total RNA extraction. The cDNA was amplified using primers specific for the target genes, and expression was analyzed using the real-time polymerase reaction (qRT-PCR). Methionine supplementation promoted better performance, with the second level (0.24%) of DL-methionine promoting the best results for weight gain. The mRNA avUCP concentration was significantly lower in the muscle of birds fed methionine-supplemented diets, independently of source or level. The highest mRNA avUCP expression was obtained with the basal diet, which also resulted in the worst feed efficiency. The expression of mRNA avUCP in the liver and of mRNA COX III and mRNA ANT in the liver and in the muscle was not influenced by methionine supplementation. Methionine supplementation promotes better broiler performance, and the most efficient birds were fed second level of supplementation of both sources, part of these results may be due to the lower expression of avUCP mRNA in the muscle, which was also lower in birds fed diets with higher amounts of methionine.

1. Introduction

Genetic evolution is indubitably one of the main factors responsible for the advances in broiler production, particularly in relation to feed conversion ratio. Feed cost accounts for a significant proportion of animal production costs, making feed efficiency increasingly important and object of many recent studies (Lassiter et al., 2006).

Growth rate depends on feed efficiency, as well as muscle accretion. The efficiency of an animal converting feed into muscle is related to his efficiency in producing energy. Studies have shown that broilers that produce less ATP, due to a lower efficiency of their mitochondria in producing ATP from substrates, presented worse feed efficiency (Bottje and Carstens, 2009). The efficiency in energy production depends not only on the perfect coordination among the complexes of the respiratory

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chain, but also on a potent antioxidant system that protect mitochondria against the damage by products generated during ATP production.

Considering that methionine is associated to broiler performance (Kauomar et al., 2011; Waldroup et al., 2006), and that it is also required by the synthesis of glutathione, potent mitochondrial and cell antioxidant (Griffith and Meister, 1985), further studies are needed to better understand how the supplementation of that amino acid may influence the expression of the genes involved in energy production in mitochondria. Some important proteins were involved in the process of ATP production by the mitochondria: uncoupling protein (avUCP), adenine nucleotide translocase (ANT) and cytochrome c oxidase subunit III (COX III). Several researchers demonstrated the relationship between the expression of genes encoding those proteins with feed efficiency in poultry (Bottje et al., 2009; Ojano-Dirain et al., 2007).

Another aspect that should be taken into consideration is the source of methionine that will be supplemented in the diet, because different sources present different availability and bioefficacy, resulting in different bird performance (Lemme et al., 2002; Payne et al., 2006). The two main commercial methionine sources currently used in broiler feeds are DL-methionine (99%) and methionine hydroxy analogue-free acid (MHA-FA, 88%).

The search for increasingly efficient animals clearly requires knowing how the nutrients available in the diets affect the expression pattern of genes involved in the processes of cell energy production, which are partially responsible for the feed efficiency observed. Therefore, the present study aimed at evaluating the mRNA expression of avUCP, mRNA COX III, and mRNA ANT in muscle and liver of broilers fed diets containing two sources and two levels of methionine supplementation.

2. Material and methods

Day-old male Cobb 500 slow broilers were obtained from a commercial hatchery, vaccinated against Marek's disease and infectious bursal disease, and sexed by the wing feathers. During the starter period (1–21 days of age), birds were housed in a conventional poultry houses and reared under the same experimental conditions.

At 22 days of age, birds were distributed to the treatments according to a completely randomized experimental design in a 2×2 factorial arrangement (two sources and two methionine levels), with a use of a basal diet (control) with no synthetic methionine addition, totaling five treatments. The treatments consisted of a basal diet, first level of (0.08%) DL-methionine supplementation (DL1), second level (0.24%) of DL-methionine supplementation (DL2), first level (0.11%) of MHA-FA supplementation (MHA-FA1), and second level (0.33%) of MHA-FA supplementation (MHA-FA2), with five replicates of 30 birds each, totaling 750 birds.

The experimental diets (Table 1) were based on corn, soybean meal, and meat meal, and formulated according to the recommendations of National Research Council (1994). The ratios of all amino acids to lysine were increased in 5% in order to prevent any limitation of the remaining essential amino acids. During the experimental period, birds were offered feed and water *ad libitum*.

In order to determine weight gain, all birds were weighed in the beginning (22 days) and end (42 days) of the experimental period. Feed intake was calculated as the difference between the amount of feed offered and the feed residue at the end of the trial. Feed conversion ratio was calculated by dividing feed intake by weight gain, and corrected for mortality.

At the end of the experimental period, five birds per treatment were sacrificed by neck dislocation and samples of the liver and breast muscle (*Pectoralis superficialis*) were collected and stored in RNA Holder® (BioAgency Biotecnologia, Brasil) at −20 °C until total RNA extraction.

Total RNA was extracted using the reagent Trizol® (Invitrogen, Carlsbad CA, USA), according to the manufacturer's instructions, at a ratio of 1 mL for every 100 mg tissue. All utilized materials were previously treated with an RNase inhibitor (RNase AWAY®, Invitrogen, Carlsbad, CA, USA). Tissue specimens with Trizol were ground using an electric homogenizer Polytron until their complete dissociation, after which 200 uL chloroform was added, and then the samples were manually homogenized for 1 min. Samples were then centrifuged for 15 min at 12,000 rpm and 4 °C, the liquid phase was collected and transferred to a clean tube. The RNA precipitated from the aqueous phase by mixing with 500 μL isopropanol. The samples were centrifuged for 15 min at 12,000 rpm and 4 °C. The supernatant was discarded, and the precipitate was washed in 1 mL ethanol at 75%. The material was again centrifuged for 5 min at 12,000 rpm, and the supernatant was discarded. The pellet was dried for 15 min and then resuspended in RNAase-free ultrapure water.

Total RNA concentration was measured using a spectrophotometer at 260 nm wave length. The integrity of the RNA was evaluated in a 1% agarose gel in the presence of 0.5 μg/mL ethidium bromide and visualized with ultraviolet light. RNA samples were treated with DNase I (Invitrogen, Carlsbad, CA, USA) for the removal of possible genomic DNA residues, according to the manufacturer's recommendations.

Complementary DNA (cDNA) was prepared using the SuperScriptTM First-Strand Synthesis Super Mix kit (Invitrogen corporation, Brazil) using the manufacturer's instructions. The cDNA mixture contained 6 μL total RNA, 1 μL oligo (dT: 50 uM oligo (dT)20) and 1 μL annealing standard. The reaction was incubated for 5 min at 65 °C and then placed on ice for 1 min. Subsequently, 10 μL of the solution 2 × First-Strand Reaction Mix and 2 μL of the solution containing the enzyme reverse transcriptase SuperScript III and RNAase inhibitor were added. The solution was incubated for 50 min at 50 °C to allow complementary DNA to be synthesized. The reaction was then incubated for 5 min at 85 °C and placed on ice immediately after. Samples were stored at −20 °C until analysis.

Real-time PCR reaction used the fluorescent dye SYBR GREEN (SYBR® GREEN PCR Master Mix, Applied Biosystems, USA). RT-PCR analyses were performed in the apparatus StepOnePlus v.2.2 (Applied Biosystems, Carlsbad, CA,