# EFFECT OF DIETARY ARGININE ON BREAST MUSCLE, DIAMETER AND PROTEIN:DNA RATIO OF BROILER MEAT

A.E. Murakami, J.I.M. Fernandes , S.R. Ferreira and E.N.Martins

*Universidade Estadual de Maringá – . Av. Colombo, 5790, 87020-900, Maringá - Paraná – Brazil, E-mail: aemurakami@uem.br*

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## Introduction

The economic importance of poultry white meat has driven extensive research efforts to increase breast yield of commercial broiler chicken. The chick emerges from the egg with a predetermined number of muscle cell fibers that does not alter during the life of the bird. Muscle growth post-hatch is the result of an increase in myofiber size and an increase in DNA content (Halevy et al., 2003). This phenomenon accounts for more than 98% of the final DNA content of muscle. Satellite cells are capable of entering the cell cycle and proliferating and either fusing into existing fibers or fusing with each other to form new fibers to maintain some finite ratio between muscle fiber size (e.g., cytoplasmic volume) and myonucleus number.

The insulin-like growth factor (IGF-I) appears to be mediating some aspects of the hypertrophy response via an increase in DNA that may be maintaining some critical DNA-to-protein ratio, possibly via the activation and incorporation of satellite cells (Adams and Haddad, 1996). The IGF-I also acts as an important regulator of muscle cell differentiation by regulating muscle-specific genes that control muscle cell mass (Fernández, et al., 2002). Arginine (Arg) is a potent stimulator of the secretion of growth hormone (GH) by the anterior pituitary gland (Flynn et al., 2002). The dietary supplementation with Arg increases plasma levels of GH.

Arg is an essential amino acid for avian species, because birds lack the enzyme carbamyl phosphate synthetase, which aids in the conversion of ornithine to citrulline and, thus, Arg (Tamir and Ratner, 1963). Diets formulated with high levels of lysine to maximize breast meat yield reponse can change the rate of arg catabolism in chicks due to the arg:lys classic dietary antagonism. This research was conducted to study the participation of Arg in activation of the satellite cell and protein miofiber accretion for evaluation of breast development, diameter skeletal muscle fiber, protein:DNA ratio of broiler chickens during the initial period (1 and 21 days of age).

## Materials and Methods

Nine hundred and ninety male obtained from the same flock of Cobb broiler breeders of 39 wk of age. The chicks were reared in a complete randomized design with five treatments and six replicates and 33 birds each. Dietary treatments consisted of one basal diet Arg not supplemented (1,390%) and four supplemental digestible Arg levels (1.490%; 1.590%; 1.690% e 1.790%). Experimental diets were based on corn and soybean meal and formulated according to the chemical composition of feedstuffs and nutritional requirements suggested by Brasilian Industrily Guidelines. The diets were isoenergetic (3,047 kcal/kg ME) isoproteic (22.4%), isocalcium (0.920%), isophosphorus (0.471%), isosodium (0.220%), isochloride (0.200%).

On days 7, 14 and 21, two birds were randomly removed from each replicate, and were sacrificed by neck dislocation and the breast was removed and weighted. The following day, the left *Pectoralis major* (breast fillets) were excised, and weights were recorded. For the evaluation of the muscle fibers diameters were collected fragments of the muscle tissue on the superficial region of right *Pectoralis major* muscle cross-sectioned perpendicular to the direction of the myofibers. The samples were stored in nitrogen liquid at - 80°C. Tissue sections of 8 $\mu$m were prepared with a cryostat at - 20°C and stained with hematoxylin and eosin. With a digital analyzer system, were measured 20 muscle fibers/image using small diameter, totalizing 200 fibers per animal (2400 fibers/treatment). The DNA and protein were extracted from *Pectoralis major* muscle segments frozen, using the commercial kit Dneasy Tissueâ of QIAGEN. For the protein determination, the samples were defrosted, ground and dissolved in a buffer solution. The sample solution (1%, w/v) was centrifuged for 15 minutes at 13,000 x g. Soluble proteins were determined by analyzing the supernatant using the Bradford method (Bradford, 1976). The degrees of freedom, referring to ARG levels had been unfolded in polynomials, using the regression analyses by SAEG software.

## Results and Discussion

In accordance with the results, that breast meat yield was responsive to Arg levels above that required for growth (Table 1). There was positive linear effect ($P<0.05$) of dietary ARG supplementation on breast weight (BW) and breast weight fillet (BWF) ($\hat{Y}$=8.50411+6.82833X and $\hat{Y}$=1.37943+1.59333X, respectively) to the 7 d. On day 14, the effect of Arg levels on breast weight fillet increasing quadraticlly ($P<0.05$) up to 1.560% Arg were observed ($\hat{Y}$=-56.8868+91.3004X-29.2666$X^2$) and at 21 d, was observed a cubic behavior (P<0.05) to the

BW, while that ARG levels increased linearly (*P<0.05*) the BWF ($\hat{Y}$=6891.14-13138.6X+8487.31$X^2$ - 1815.04$X^3$ and $\hat{Y}$=18.2008+10.0692X, respectively). In agreement, Corzo et al. (2003) observed that increasing dietary arginine led to increasing amounts of fillets and total breast meat that optimized at a greater level than observed with live weights. ARG levels produced significant increases (*P<0.05*) in DSMF to the 14 d, for a quadratic form up to 1.510% Arg ($\hat{Y}$= 99.6647-109.641X+36.3181$X^2$) and at 21 d, the Linear Response Plateau model better adjusted to data, indicating a effect in plateau (*P<0..05*) up to 1.490% % Arg level. There was no significant effect on Protein:DNA ratio any evaluated age suggesting that Arg levels did not activate the satellite cells via IGF-I. However, dietary ARG supplementation resulted in a significantly higher raise in protein accretion for increasing BW, BWF and DSMF. Arg increases the secretion of GH that stimulates hepatic in other tissue such as skeletal muscle production of IGF-1. The IGF-1 is known to have anabolic effects on skeletal muscle cells as the myofibrilar protein aggregation (Duclos, 2005). Still, the dietary arginine supplementation could enhance the rate of synthesis or decreased the rate of degradation of chick skeletal muscle and resulted in improvements in breast growth. It remains unclear whether this beneficial effects of ARG intake on the muscle hypertrophy occur through a direct ARG effect or through indirect IGF-I action.

**Table 1.** Effect of dietary arginine supplementation on breast muscle growth, diameter and protein:DNA ratio of the skeletal muscle fibers of broiler chickens.

| | ARG levels, % | | | | | CV (%) | effect | $R^2$ |
|---|---|---|---|---|---|---|---|---|
| | 1.390 | 1.490 | 1.590 | 1.690 | 1.790 | | | |
| | | | 7 days | | | | | |
| Breast weight, g | 18.00 | 18.68 | 19.36 | 20.04 | 20.73 | 13.78 | linear | 0.13 |
| Breast fillet weight, g | 3.59 | 3.75 | 3.91 | 4.07 | 4.23 | 14.54 | linear | 0.15 |
| Diameter skeletal muscle fiber, µm | 12.71 | 12.01 | 13.20 | 13.04 | 13.42 | 9.28 | NS | - |
| Protein:DNA ratio | 12.09 | 11.99 | 12.67 | 12.36 | 12.00 | 24.72 | NS | - |
| | | | 14 days | | | | | |
| Breast weight, g | 66.87 | 71.18 | 64.82 | 66.45 | 62.05 | 8.06 | NS | |
| Breast fillet weight, g | 13.47 | 14.18 | 14.29 | 13.82 | 12.77 | 9.63 | quadratic | 0.14 |
| Diameter skeletal muscle fiber, µm | 17.43 | 16.93 | 17.15 | 18.10 | 19.77 | 5.12 | quadratic | 0.59 |
| Protein:DNA ratio | 25.99 | 23.35 | 25.20 | 26.13 | 25.20 | 27.06 | NS | - |
| | | | 21 days | | | | | |
| Breast weight, g | 153.04 | 151.54 | 161.71 | 168.49 | 156.85 | 7.79 | cubic | 0.24 |
| Breast fillet weight, g | 32.20 | 33.20 | 34.21 | 35.22 | 36.22 | 12.91 | linear | 0.10 |
| Diameter skeletal muscle fiber, µm | 30.30 | 33.04 | 32.80 | 32.90 | 32.96 | 7.00 | LRP | 0.16 |
| Protein:DNA ratio | 32.08 | 34.84 | 31.40 | 34.90 | 35.43 | 22.13 | NS | - |

NS= Not significant (P>0.05), LRP= linear response plateau

**Conclusion**

The breast meat yield was responsive to dietary arginine supplementation Arg for increasing BW, BWF and DSMF, however did not enhance satellite cell mitotic activity via stimulatory action on IGF-I.

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