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Effect of glycerol on GHR and IGF-1 gene expression in breast muscle and on growth of Japanese meat quails

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ABSTRACT. We evaluated messenger RNA (mRNA) expression of the growth-hormone (GHR) and insulin-like growth factor (IGF-1) genes in 28-day-old Japanese meat quails fed diets containing 0, 8, or 12% dietary glycerol in substitution of corn. Total RNA was extracted from the breast muscle and the DNA was amplified with specific primers using real-time PCR. Feed conversion ratio and feed intake were evaluated. The birds fed 8 and 12% glycerol presented higher IGF-1 mRNA expression [0.059 and 0.049 arbitrary units (AU), respectively] relative to those not fed with glycerol (0.029 AU), while 12% glycerol reduced GHR mRNA expression (0.022 AU). Dietary inclusion of 8% glycerol promoted similar performance results (feed conversion) as the diet with no glycerol. We conclude that inclusion of glycerol in the diet affects GHR and IGF-1 gene expression in Japanese meat quails. However, considering the performance results

and the expression of the GHR and IGF-1 genes, 8% glycerol may be safely included in the diet of meat quails.

Key words: Body growth; Japanese quail production; Nutrigenomics

INTRODUCTION

The use of byproducts in animal feed may reduce production costs and consequently increase profitability. At affordable prices, glycerol, a biodiesel byproduct, can partially replace corn in animal feeds (Cerrate et al., 2006) as this feedstuff has similar energy levels (3434 kcal/kg) (Dozier et al., 2008). Glycerol is passively absorbed (Guyton, 1991), and it has a sweet flavor and low molecular weight (Rivaldi et al., 2008). The dietary inclusion of glycerol may affect the performance of Japanese quails (Raber et al., 2009), and in addition, it may influence the mRNA expression of some hormones and receptors related to growth, such as GH (growth hormone), IGF-1 (insulin-like growth factor), and GHR (growth hormone receptor) (Gasparino et al., 2012a). Changes in the expression of these genes may affect nutrient utilization and basal metabolism, changing energy expenditure and, consequently, influencing heat increment (Johnson et al., 2003; Bottje and Carstens, 2009).

GH shows both metabolic and anabolic action, stimulating tissue growth and changing the metabolism of different nutrients, such as an insulin-like initial effect on carbohydrate metabolism (Strobl and Thomas, 1994). GH modes of action can be divided into direct and indirect actions: the direct actions are triggered by the recognition and specific binding to its membrane receptor (GHR) in the target cells, whereas the indirect actions are mediated mainly by the regulation of the synthesis of insulin-like growth factors, which are important mediators of GH anabolic effects, particularly those related to growth (Cruzat et al., 2008). Thus, the aim of the present study was to evaluate the effect of dietary glycerol inclusion on the mRNA expression of GHR and IGF-1 in the breast muscle of Japanese meat quails up to 28 days of age.

MATERIAL AND METHODS

The present study was submitted and approved by the Committee of Ethics and conducted at the poultry sector and molecular genetics laboratory of the State University of Maringá. Accordingly, 450 one-day-old meat quails were distributed according to a completely randomized experimental design consisting of three treatments (dietary inclusion of 0, 8 or 12% glycerol) with five replicates of 30 birds each. Birds were housed in a conventional poultry house divided into 2.5-m^2 pens with rice husk litter. A continuous light lighting program was used during the entire experimental period. The control diet (0% glycerol) and test diets containing 8 and 12% glycerol were formulated on a corn and soybean meal basis according to the nutritional recommendations of Rostagno et al. (2005) and NRC (1994), except for glycerol inclusion (Table 1).

Birds were weighed at one and 28 days of age to determine body weight gain. The amounts of experimental feeds offered and of feed residues were weighed to calculate feed intake. Feed conversion ratio was determined as the ratio between weight gain and feed intake, and was adjusted for mortality. Ten birds per treatment were sacrificed by neck dislocation at 28 days of age. A sample of their breast muscle (pectoralis superficialis) was collected, properly packed in an RNA Holder® (BioAgency) and stored at -20°C until RNA extraction.

Table 1. Ingredients and composition of the experimental feeds.

Ingredients (%)	Levels of glycerol inclusion		
	0%	8%	12%
Corn grain	54.67	46.49	42.44
Soybean meal 45%	37.84	39.37	40.13
Crude glycerol	-	8.00	12.00
Soybean oil	2.96	1.44	0.73
Dicalcium phosphate	1.62	1.63	1.64
L-lysine HCl	0.65	0.84	0.82
DL-methionine	0.66	0.66	0.67
L-threonine	0.44	0.43	0.43
Salt	0.40	0.40	0.40
Vitamin supplement[1]	0.40	0.40	0.40
Limestone	0.28	0.26	0.26
Mineral supplement[1]	0.07	0.07	0.07
Antioxidant[2]	0.01	0.01	0.01

[1]Vitamin/mineral supplementation (guaranteed levels/kg product); Vit. A - 4,500,000 IU; Vit. D3 - 1,250,000 IU; Vit. E - 4000 mg; Vit. B1 - 278 mg; Vit. B2 - 2000 mg; Vit. B6 - 525 mg; Vit. B12 - 5000 µ; Vit. K3 - 1007 mg; calcium pantothenate - 4000 mg; niacin - 10,000 mg; choline - 140,000 mg; antioxidant - 5000 mg; zinc - 31,500 mg; iron - 24,500 mg; manganese - 38,750 mg; copper - 7656 mg; cobalt - 100 mg; iodine - 484 mg; selenium - 127 mg; [2]BHT (butyl-hydroxy-toluene).

Total RNA was extracted using the Trizol® reagent (Invitrogen; Carlsbad, CA, USA) in a proportion of 1 mL per 100 mg tissue. The muscle tissue was ground using an electric homogenizer until its complete dissociation, after which 200 µL chloroform were added and manually stirred for one minute. The tissue was then centrifuged for 15 min at 12,000 rpm at 4°C, and the liquid phase was then collected and transferred to a test tube, to which 500 µL isopropanol were added. The material was then homogenized and centrifuged for 15 min at 12,000 rpm at 4°C. The supernatant was discarded and the precipitate was washed with 1 mL 75% ethanol. The material was again centrifuged at 12,000 rpm for 5 min, and the supernatant was discarded. The precipitate was dried for 15 min and homogenized in RNAse-free distilled and demineralized water.

Total RNA concentration was determined with the aid of a spectrophotometer at 260 nm. RNA integrity was evaluated in 1% agarose gel and visualized under ultraviolet light. The SuperScript™ III First-Strand Synthesis SuperMix kit (Invitrogen Corporation, Brazil) was used to synthesize the complementary DNA (cDNA). Volumes of 6 µL total RNA and 1 µL oligo (dT) (50 µM oligo dT) and 1 µL annealing buffer were added. The reaction was incubated for five minutes at 65°C and then placed on ice for one minute. Subsequently, 10 µL 2X First-Strand Reaction Mix and 2 µL solution of SuperScript III reverse transcriptase enzyme plus RNAse inhibitor were added. The solution was then incubated for 50 min at 50°C for the synthesis of cDNA. The reaction was then again incubated for five minutes at 85ºC and immediately placed on ice. Samples were then stored at -20°C until subsequent analyses.

The fluorescent compound SYBR GREEN (SYBR® GREEN PCR Master Mix (Applied Biosystems, USA) was used for real-time PCR. The primers of the GHR, IGF-1 and β-actin genes were designed according to the sequences deposited at GenBank (NM001001293.1, FJ977570.1 and L08165) through the website www.idtdna.com. All analyses were carried out in a final volume of 25 µL and in duplicate. Data were analyzed using the GLM procedures of SAS statistical package (2000). The univariate procedure was used to assess the normality of the residuals of in the gene expression (expressed as $2^{-\Delta Ct}$) and performance data. Data were

submitted to analysis of variance (ANOVA) with three treatments and ten replicates per treatment (diets with 0, 8 or 12% glycerol). Means were compared by the Tukey test ($P < 0.05$).

RESULTS

The performance of one- to 28-day-old Japanese quails fed different glycerol levels is shown in Table 2. There was no statistical effect of the treatments on weight gain, but feed intake and feed conversion ratio significantly increased. Feed intake was significantly higher in birds fed 12% glycerol, whereas it was not statistically different between those fed 0 and 8% glycerol. The group fed 12% glycerol showed a worse feed conversion ratio (2.34) relative to those fed the diet with no glycerol (1.99), representing a 14.59% lower feed conversion ratio in quails during the rearing phase studied. Diet composition also strongly influenced the expression of the genes of the GH-IGF axis. Studies have shown that mRNA expression of IGF-1 and GHR in the liver and muscle of animals may be affected by dietary energy and protein levels, as well as by ingredient replacement (Katsumata et al., 2002).

Table 2. Effect of glycerol levels on meat quail performance and mRNA gene expression.

Traits[1]	Levels of glycerol inclusion[2]		
	0%	8%	12%
WG (g)	177.65 ± 15.34	179.51 ± 10.18	182.56 ± 21.31
FI (g)	345.32 ± 31.85^{b*}	385.83 ± 24.42^{b*}	437.73 ± 31.82^{a*}
FC	1.99 ± 0.03^{ab*}	2.11 ± 0.16^{b*}	2.35 ± 0.28^{a*}
IGF-1 mRNA (AU)	0.03 ± 0.007^{b**}	0.059 ± 0.018^{a**}	0.049 ± 0.008^{a**}
GHR mRNA (AU)	0.029 ± 0.003^{ab**}	0.034 ± 0.003^{a**}	0.022 ± 0.007^{b**}

[1]WG = weight gain; FI = food intake; FC = feed conversion; AU = arbitrary units; IGF-1 mRNA = insulin like growth factor mRNA gene expression; GHR mRNA = growth hormone receptor mRNA gene expression. [2]Comparison between different glycerol levels. *$P < 0.05$, **$P < 0.01$. Mean values with the same letters in the line do not differ among themselves by the Tukey test at the 5% level of probability.

The mRNA expression of GHR and IGF-1 in the breast muscle (pectoralis superficialis) of 28-day-old Japanese quails observed in the present study is shown in Table 2. The breast muscle of Japanese quails fed the diet with 12% glycerol exhibited lower GHR mRNA expression relative to those fed 8% glycerol (0.021 *vs* 0.033); however, there was no difference when compared to the birds fed no glycerol (0.028). Dietary glycerol levels of 8 and 12% glycerol influenced IGF-1 mRNA expression, which increased by 34% relative to the treatment with no glycerol. The IGF-1 mRNA expression of birds fed 8% glycerol was similar to that of birds fed the diet with 12% glycerol. In general, the group of Japanese quails fed 8% glycerol had higher absolute values of mRNA expression of both GHR and IGF-1.

DISCUSSION

The dietary inclusion of the glycerol levels evaluated in the present study had different influences on the expression of the genes studied, showing that glycerol may influence the expression of the genes of these growth hormones. The changes in the expression of these genes may partially explain the performance results obtained. According to the literature, the maximum value of dietary glycerol inclusion for 15- to 35-day-old meat quails is 15% (Pasquetti, 2011). However, the inclusion of 12% glycerol may exceed the birds' metabolic

capacity, thereby limiting its absorption (Min et al., 2010), which suggests the need of further studies to determine maximum dietary glycerol inclusion levels for this age range. Moreover, the inclusion of glycerol at either 8 or 12% results in an apparent increase in litter moisture, which is suggestive of increased feed passage rate and, hence, worse nutrient utilization (Le Goff et al., 2002).

Diet components change the circulating levels of GH and IGF-1 (Bernal et al., 2002). The birds fed the diets containing glycerol showed higher IGF-1 mRNA expression. Glycerol has high intestinal absorption rates - reaching 89% absorption in rats - due to its low molecular weight and to its passive absorption, which does not require micelle formation, such as medium- and long-chain fatty acids (Guyton, 1991). This high absorption rate apparently resulted in high energy availability for the birds fed glycerol, promoting an increase in IGF-1 mRNA expression in an attempt to convert this energy into growth, as growth stimulation in response to increased energy intake is regulated by an increase in IGF-1 (Weller et al., 1994). Nevertheless, such growth was not observed probably due to the observed increase in digesta passage rate, which may have determined lower utilization of other dietary components. Gasparino et al. (2012a) observed reduced IGF-1 expression in 14-day-old Japanese quails fed 12% glycerol, but this was not observed in the present study, suggesting that 28-day-old quails may have developed a mechanism of adaptation to the diet with this glycerol level.

The capacity of target cells to respond to GH depends mainly on the expression of GHR (Talamantes and Ortiz, 2002). Therefore, the higher IGF-1 mRNA levels observed in birds fed 12% glycerol in the present study may have reduced GH levels by negative feedback, consequently reducing the need and the levels of GHR mRNA in these birds. Such is observed when there is malnutrition, fasting or deficient absorption of nutrients, where GHR mRNA expression is reduced (Straus and Takemoto, 1990). Considering quail performance and the expression of the genes for GH and IGF-I, Gasparino et al., 2012b considered that 4% glycerol could be used in quail feed without any harmful effects. On the other hand, we found that birds fed 8% glycerol had higher GHR mRNA expression relative to those fed 12% glycerol, suggesting that it is possible to include 8% glycerol in Japanese quail diets with no reduction in GHR mRNA expression.

The analysis of the results obtained allows us to infer that the level of a nutrient or the nutrient itself – such as in the case of glycerol inclusion at the expense of corn - is capable of increasing or reducing gene expression and influencing live performance. Considering the recommendations for glycerol utilization in the literature, the results of the present study indicate that this feedstuff can be used in the diets of meat quails, but that levels higher than 8% are not recommended.

CONCLUSION

The inclusion of glycerol in the diet of Japanese meat quails up to 28 days of age causes changes in the mRNA expression of GHR and IGF-1 in breast muscle.

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