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Impact of feed restriction, sexual class and age on the growth, blood metabolites and endocrine responses of hair lambs in a tropical climate

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ABSTRACT

An experiment was conducted to evaluate the effects of feed restriction on the growth, blood metabolites and endocrine responses of hair lambs in a tropical environment. Thirty lambs were distributed in a completely randomized design in a 2 × 3 × 3 factorial scheme.

Experimental: treatments consisted of two sexual classes (15 intact males and 15 castrated males, 13.0 ± 1.49 kg initial BW and two months old), different levels of quantitative feed restriction (FR) (*ad libitum*, 300 and 600 g/kg) and various ages (14, 19 and 23 weeks). Lamb age influenced the blood leptin ($P < 0.001$), chloride, calcium, phosphorus and magnesium ($P < 0.05$) concentrations. The blood insulin and glucose concentrations ($P < 0.001$) were positively influenced by diet and age. The increased feed restriction resulted in a higher concentration of blood β-hydroxybutyrate (β-HB) ($P < 0.001$). The blood urea nitrogen (BUN) concentration was influenced by diet ($P < 0.001$), age ($P < 0.001$) and the interaction of sexual class and age ($P < 0.01$), with the highest concentrations in the oldest and castrated lambs. A positive correlation was observed between insulin and thyroxine in animals fed *ad libitum* ($P < 0.05$). The thyroxin is present in low concentrations in the hair lambs raised in a tropical climate. Hair lambs exhibited significant changes in their growth, blood metabolites and endocrine responses, which enabled them to adapt to the feed conditions.

1. Introduction

Seasonal fluctuations cause periodic restrictions in feed quantity and quality, but these adverse effects can be overcome by supplemental feeding. Animals in tropical conditions are able to modify their physiological processes (Costa et al., 2013) to possibly become more adapted to the hot climate (Pereira et al., 2016). The effects of dietary restrictions on hair sheep are not clearly defined (Galvani et al., 2010). The growth rate can be delayed when the energy or protein supply is limited (Lawrence and Fowler, 2002). Moreover, it has been speculated that slowing growth does not just occur by reducing energy intake below that required for maintenance (McDonald et al., 2010). If this is true, forced intake should increase lean body mass in mature animals, but it has been observed that excess nutrients are converted into lipids, excreted or catabolized (Owens et al., 1993).

The thyroid hormones maintain energy and protein metabolism homeostasis (Lawrence and Fowler, 2002), thermoregulation (Toldini, 2007), growth (Huszenicza et al., 2002) and productivity parameters. Feed restriction has been associated with variations in circulating concentrations of some hormones, such as insulin and leptin (Catunda et al., 2013), because they are affected by the nutritional condition of the animal. Thus, leptin expression and secretion are correlated with body condition, physiological status and animal age (Chilliard et al., 2005). Plasma insulin concentrations tend to be positively correlated with adiposity, and it is therefore possible that insulin may play a role in the maintenance of body weight (Lawrence and Fowler, 2002). Glucose plays a key role in animal metabolism as an essential source of energy for the maintenance of many tissues. In ruminants, absorbed propionate and amino acids are major glucose precursors, which must be converted into glucose by gluconeogenesis. Thus, this study was conducted to evaluate the effects of feed restriction, age and sexual class on the growth, blood metabolites and endocrine responses of intact and castrated hair lambs in a tropical region.

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2. Materials and methods

2.1. Site, animals, experimental diets and management

The experiment was conducted at the Federal University of Ceara in Fortaleza, Ceara State, which is located in northeastern Brazil, at 3° 45′ S and 38° 32′ W, and is 15.5 m above sea level. The average temperature inside the stalls was 25.71 °C, and the average relative humidity was 74.67%. The experimental protocol used in this study, including animal management, housing, and slaughter procedures, was approved by the Animal Care and Use Committee of the Federal University of Ceara, Brazil (Protocol n°. 98/2015).

Experimental treatments comprised two sexual classes (15 intact males and 15 castrated males, 13.0 ± 1.49 kg initial BW and 2 months old), various levels of quantitative feed restriction (FR) (*ad libitum*, 300 or 600 g/kg) and various ages (14, 19 or 23 weeks); the lambs were assigned to each group in a completely randomized design with a 2 × 3 × 3factorial scheme.

The lambs were housed in individual pens with feeding troughs that supplied the food and fresh water; they were maintained under proper hygienic conditions. Prophylactic measures were taken against sheep infectious diseases (clostridiosis) and endo and ectoparasitic infestations (haemonchosis and ticks, respectively) to ensure that the animals were healthy throughout the study. Animals were supplemented with an ADE vitamin (20,000,000 IU of vitamin A, 5,000,000 IU of vitamin D3 and 5500 IU of vitamin E per 100 mL).

The total mixed ration (TMR) was formulated to supply the nutritional requirements of late-maturity lambs with a weight gain of 150 g/day, as recommended by the NRC (2007), and the diet consisted of 60% roughage and 40% concentrate. The composition of the diet included 60% roughage (Tifton-85 hay, *Cynodon* sp.) and 40% concentrate (corn meal 20.07%, soybean meal 19.23%, limestone 0.19%, dicalcium phosphate 0.41%, salt 0.07%, premix 0.03%). Table 1 shows the chemical composition of the diet fed to the lambs.

The experimental period was 115 days, comprising 15 days of adaptation and a 100-day trial period. The diet was supplied as a total mixed ration twice daily at approximately 8:00 and 16:00 h, respectively. The dry matter intake (DMI) was calculated as the difference between the weight of the diet offered and the orts for all lambs fed *ad libitum*. Concentrate, forage, total mixed ration (TMR) and refusal samples were analyzed in triplicate for dry matter (DM, #934.01), ash (#942.05), crude protein (CP, #976.05), ether extract (EE, #920.29), and acid detergent fiber (ADF) according to the Association of Official Analytical Chemists (AOAC, 1990). The neutral detergent fiber (NDF) was determined according to Van Soest et al. (1991), and non-fibrous carbohydrates (NFC; considered NDF corrected for ash and protein) were determined according to Sniffen et al. (1992).

Table 1
Chemical composition of the diet fed to lambs.

Analytical fraction	(g/kg DM)
Dry matter (g/kg as fed)	918
Crude protein	178
Ether extract	25
Neutral detergent fiber	493
NDF_{ap} [†]	456
Acid detergent fiber	234
Total carbohydrate	734
Non-fibrous carbohydrate	276
Total digestible nutrients	558

Ca 7.5%; P 3%; Fe 16.500 ppm; Mn 9.750 ppm; Zn 35.000 ppm; Se 225 ppm; Co 1000 ppm.

[†] NDF_{ap}: Neutral detergent fiber corrected ash and crude protein.

2.2. Blood sample collection and parameters analyzed

Before feeding, blood samples were collected by puncturing the jugular veins of all lambs, using anti-coagulant (EDTA) and non-anti-coagulant vacutainers (BD Vacutainer, Franklin Lakes, NJ, USA), at three different ages: 14, 19 and 23 weeks. Samples were centrifuged at 350g/15 min, and the plasma or serum was frozen at −20 °C until it was analyzed for glucose, β-hydroxybutyrate (β-HB), urea-nitrogen, total protein, albumin and globulin concentration. These constituents were assayed in duplicate using colorimetric commercial kits from BioClin® (Bioclin, Quibasa; Belo Horizonte, Brazil). β-HB was assayed in duplicate using Precision Xtra, (Abbott Laboratories Inc., Abbott Park, Illinois, USA).

For the analyses of thyroxine (T_4), leptin and insulin, serum was stored at −80 °C. The blood leptin concentration was determined at 50 mg/mL serum by a sheep leptin (LEP) ELISA kit (Catalog No. CSB EL012870SH; Cusabio), the sensitivity limit was 0.78 ng/mL, and the intra- and inter-assay coefficients of variation were 8% and 10%, respectively. The blood insulin concentration was determined at 25 mg/mL with the Ovine Serum Insulin ELISA kit (INSOV–80–E01, ALPCO), the sensitivity limit was 0.14 ng/mL, and the intra –and inter-assay coefficients of variation were 5.69% and 5.78%, respectively. The blood thyroxine concentration was determined at 25 mg/mL sheep serum by a thyroxine (T_4) ELISA kit (Catalog No. CSB EQ027512SH; Cusabio), the sensitivity limit was 10 ng/mL, and the intra- and inter-assay coefficients of variation were lower than 15%.

2.3. Body measurements

Growth measurements were estimated beginning at the 15th day of adaptation and then once every two weeks. Parameters measured included the body condition score (BCS) according Russel et al. (1969); body weight (BW; digital scale with a 50-g sensitivity); and heart girth (HG), body length (BL), height at withers (HW), height at rump (HR), rump width (RW), rump length (RL) and chest width (CW) according to Bandeira et al. (2016) and Herrera et al. (1996). The RL and CW measurements were made using a tape measure and measuring stick.

2.4. Statistical analysis

The data analyses were performed using PROC GLM of SAS (2007), with a significance level of 5%, according to the following statistical model:

$$Y_{ijkl} = \mu + T_i + B_j + C_k + (TB)_{ij} + (TC)_{ik} + (BC)_{jk} + (TBC)_{ijk} + e_{ijkl},$$

where Y_{ijkl} = is the l^{th} observation; μ is the overall mean; Ti is the fixed effect of the feed restriction level (i = *ad libitum*, 300 or 600 g/kg); Bj is the fixed effect of age (*j* = 14 weeks, 19 weeks, or 23 weeks); Ck is the fixed effect of the sexual class (k = castrated or intact); $(TB)_{ij}$ is the interaction between the feed restriction level and age; $(TC)_{ik}$ is the interaction between the feed restriction level and sexual class; $(BC)_{JK}$ is the interaction between age and sexual class; $(TBC)_{ijk}$ is the interaction among feed restriction level, age and sexual class; and e_{ijkl} is the random residual.

The Pearson correlation coefficient was used to evaluate the degree of association between the body measurements and hormones, using the CORR procedure from SAS (2007).

3. Results

The blood thyroxine concentration was not affected ($P > 0.05$) by age, diet or sexual class or by any interaction among the factors (Table 2). Age was the only parameter that influenced blood leptin concentration, with older animals (24 weeks old) presenting the highest