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Effect of dietary energy and protein restriction followed by realimentation on pituitary mRNA expression of growth hormone and related genes in lambs

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ABSTRACT

The objective of the current study was to evaluate the influence of dietary energy and protein restriction followed by realimentation on pituitary growth hormone (*GH*), growth hormone-releasing hormone receptor (*GHRHR*), GH secretagogue receptor (*GHSR*), somatostatin receptor 1 (*SSTR1*), somatostatin receptor 2 (*SSTR2*), insulin-like growth factor 1 receptor (*IGF-1R*), and *IGF-2* mRNA expression in lambs. Forty 3-month-old native Mongolian wether lambs were selected, and randomly assigned to control group (CON), moderately energy-restricted group (MR1), moderately protein-restricted group (MR2), or severely energy- and protein-restricted group (SR). The feed restriction stage lasted for 60 days and then all groups were fed the same diet for 90 days (realimentation stage). Pituitaries were collected from four lambs in each group at the end of the two stages. Pituitary gene expression was determined by quantitative real-time reverse transcription PCR. At the end of the restriction stage, the body weight of lambs in all restricted groups was significantly lower than that in the CON group. Pituitary *GH*, *GHRHR*, *GHSR* and *IGF-2* expression in lambs in the SR group was greater than that in the CON group ($P < 0.05$), whereas the mRNA expression of *SSTR1*, *SSTR2* and *IGF-1R* did not change ($P \geq 0.05$). At the end of the realimentation stage, the body weight of lambs in the MR1 and MR2 groups was not different from that of lambs in the CON group ($P > 0.05$). However, the body weight of lambs in the SR group was still lower than that in the CON group ($P < 0.05$). The pituitary *GH* expression of lambs in the SR group was still greater ($P < 0.05$) than that of the CON group, whereas expression of the other genes had returned to control levels. No differences were found in pituitary *GH* and related gene expression in lambs in the MR1 and MR2 groups compared with those in the CON group throughout the entire experiment ($P > 0.05$). Our results indicated that moderate dietary energy and protein restriction did not influence pituitary *GH* and related gene expression. Only severe energy and protein restriction caused a significant increase in pituitary *GH* transcription, which lasted throughout the realimentation stage.

1. Introduction

Growth hormone (GH) is one of the most important hormones coordinating carbohydrate, lipid and protein metabolism changes during feed restriction and realimentation. In most animal species, feed restriction causes an increase in plasma GH concentration (Hornick et al., 2000). It has been suggested that increased expression of hypothalamic growth hormone-releasing hormone (GHRH), accompanied by reduced

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Table 1
Composition (DM basis) of diets fed to lambs in the feed restriction and realimentation experiments.

	Restriction stage[a]				Realimentation stage
Item	CON	MR1	MR2	SR	
Ingredients, %DM					
Grass hey	38	14	38	25	61.1
Corn straw	–	44	–	53	–
Corn	36	3	57.8	15.7	21
Soybean meal	22	17.7	–	2	14
Sunflower meal	–	17.4	–	–	–
Limestone	1.1	1	0.9	0.58	1
$CaHPO_4$	–	–	0.4	0.82	–
NaCl	0.4	0.4	0.4	0.4	0.4
Premix[b]	0.5	0.5	0.5	0.5	0.5
Bentonite	2	2	2	2	2
Chemical composition[c]					
ME, MJ/kg DM	10.63	8.37	10.51	8.12	9.80
Crude protein, % DM	15.08	15.01	9.24	6.27	12.48
NDF, % DM	33.3	48.0	32.6	54.0	46.1
Calcium, % DM	0.43	0.44	0.42	0.47	0.40
Phosphorus, % DM	0.29	0.28	0.278	0.34	0.27

[a] CON = control group; MR1 = moderately energy-restricted group; MR2 = moderately protein-restricted group; SR = severely energy- and protein-restricted group.
[b] Contained per kilogram of basal diet: Fe 25 mg; Zn 35 mg; Cu 9 mg; Co 0.1 mg; I 0.9 mg; Se 0.25 mg; Mn 19.5 mg; nicotinic acid 60 mg; vitamin E 15 IU; vitamin A 3000 IU; vitamin D3 1000 IU (Zhengda, Inner Mongolia, China).
[c] Calculated Zhang and Zhang (1998).

somatostatin (SST), are responsible for the elevated circulating GH in chronic feed-restricted sheep (Henry et al., 2001). Receptors of these hormones in the pituitary also participate in the regulation of GH secretion (Luque et al., 2006, 2011).

The effect of feed restrictions on the expression of pituitary *GH* and related genes is different among animal species (Luque et al., 2011). Forty percent dietary caloric restriction in aging rats positively regulated hypothalamic *GHRH* mRNA translation and stimulated the synthesis of pituitary GHRH receptor (*GHRHR*) (Bedard et al., 2010). However, feed deprivation reduced the pituitary response to ghrelin and GHRH in sheep (Takahashi et al., 2009). Information regarding how prolonged dietary energy and protein restriction influences GH synthesis in the pituitary through related genes in ruminants is scarce. To provide some insight into these questions, we evaluated the influence of dietary energy and protein restriction followed by realimentation on pituitary *GH*, *GHRHR*, GH secretagogue receptor (*GHSR*), somatostatin receptors 1 (*SSTR1*), somatostatin receptors 2 (*SSTR2*), IGF-1 receptor (*IGF-1R*), and *IGF-2* mRNA expression in lambs.

2. Materials and methods

This work was approved by the Animal Experimentation Ethics Committees of Inner Mongolia Agriculture University.

2.1. Animal handling and sample collection

Forty native wether lambs (3 months old; 14.72 ± 1.10 kg body weight) were selected from flocks in the Xilinhaote pasture. Lambs were randomly assigned to one of the four groups (10 per group), including moderately energy-restricted group (MR1), moderately protein-restricted group (MR2), severely energy- and protein-restricted group (SR), and control group (CON). The nutrition level of the CON group during the restriction stage and the diet offered to all sheep in the realimentation stage were designed according to the China Animal Nutrition Parameters and the Breed Standard (Zhang and Zhang, 1998). Based on the energy and protein level of the CON group, the level of energy and/or protein was reduced in the MR1, MR2 and SR groups. The composition and nutrient content of the diets fed to the lambs are presented in Table 1. The dietary restriction stage lasted 60 days and then all groups were fed the same diet for 90 days of realimentation. Four lambs, whose weight was similar to their group average weight, were selected from each group and slaughtered for pituitary collection at the end of the two experiment stages. Lambs were fed *ad libitum* in individual pens, and feed was provided at 08:00 and 18:00 daily. Feed that was not consumed was collected and weighed daily before the morning feeding. Animals were weighed weekly and the amount of feed was adjusted as body weight increased. Water and salt licking blocks were freely available.

Water and feed were removed from the animals 12 h before slaughter. The entire pituitary gland was removed immediately after slaughter, weighed and stored in RNA storage solution (Tiangen, Nanjing, China) at a 1:10 (w/v) ratio. Following 12-h incubation at 4 °C, the RNA storage solution was discarded and the samples were stored at −70 °C.

2.2. RNA isolation and cDNA synthesis

RNA was isolated from the pituitary glands using RNApure lipid tissue kits (CWBIO, Beijing, China). The yield and the purity of the RNA were measured spectrophotometrically at 260 and 280 nm. RNA (500 ng) was reverse transcribed in a final volume of 15 μl using PrimeScript RTase (Takara, Dalian, China) at 37 °C for 15 min and inactivated at 85 °C for 5 s.

2.3. Quantitative real-time reverse transcription PCR (qRT-PCR)

The pituitary *GH* and related gene expression were measured by qRT-PCR using the ABI StepOne Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions. The primers (Table 2) have previously been validated in other sheep trials (French et al., 2006; MacLaughlin et al., 2007). Each qRT-PCR well contained 5 μl SYBR PreMix DimerEraser (2×) (Takara), 0.6 μl each of forward and reverse primers (Takara), 2.6 μl water, 0.2 μl internal passive reference dye ROX, and 1 μl cDNA to give a total volume of 10 μl. PCR conditions were as follows: one cycle at 95 °C for 35 s, followed by 40 cycles at 95 °C for 5 s, 55 °C for 30 s, and 72 °C for 30 s, plate-reading; holding stage: 72 °C for 5 min. Control containing no cDNA was used to test for contamination. Melt curve analysis was performed to ensure quality of amplification. qRT-PCR results were analyzed using the $2^{-\Delta\Delta CT}$ method. All samples were measured in triplicate for each gene. $\Delta\Delta$CT values were obtained by calculating the degree of the Ct differences between the target gene and reference gene (*GAPDH*) in treatment versus control samples.

2.4. Statistical analysis

Data for nutrients intake, body weight and average daily gain were analyzed using the PROC MIXED procedure (SAS Inst. Inc., Cary, NC, USA). The model included the fixed effect of diet and diet × day, where day was considered a repeated effect. Pituitary weight and gene expression were