Journal of Animal and Veterinary Advances 7 (11): 1383-1386, 2008
ISSN: 1680-5593

The Effects of Dietary Protein Level on Serum Growth Hormone, Insulin-Like Growth Factors and Performance in Kivircik Lambs

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Abstract: Due to studies on the effect of dietary protein on serum Growth Hormone (GH) and insulin-like growth factor-1 (IGF-1) concentrations in lambs fed at different levels of dietary protein are limited, this study was conducted to determine the effect of dietary protein level on GH and IGF-1 concentrations and performance in lambs. A total of 50 Kivircik male lambs were randomly selected for this experiment. Lambs were randomly assigned ten lambs to each of the five treatment diets containing 10, 12, 14, 16 and 18% CP to determine the effects of dietary CP level on performance, serum GH and IGF-1 concentrations. The initial body weight was similar for all lambs and averaged 26.20±1.10 kg. Final body weight of lambs were 37.31±1.46, 39.60±1.18, 39.85±1.24, 42.04±1.25 and 41.44±1.13 kg for groups fed 10, 12, 14, 16 and 18% CP, respectively and lambs fed 16 and 18% CP diet had significantly higher body weight than those of 10% CP ($p<0.05$). Mean concentrations of GH and IGF-1 in blood serum of lambs were not different significantly between groups.

Key words: Dietary protein, growth hormone, insulin-like growth factor, Kivircik lamb

INTRODUCTION

Growing is a complex event that controlled by the somatotropic line functioning with a number of hormones such as somatotropin (Growth Hormone [GH]), insulin, catecholamine, thyroid gland hormones, glucocorticoids and steroids. This somatotropic line is mainly controlled by somatotropin (Serpek and Haliloglu, 2000). Nutritional status is known to regulate the endocrine GH/IGF-1 system in humans and other species (Thissen *et al*., 1994). During low food intake, somatotropin is lipolytic and thus, provides energy for maintenance (Claus and Weiler, 1994). Increased plasma GH concentrations are associated with dietary protein and (or) energy restriction in various species (Buonomo and Baile, 1991; Thomas *et al*., 1990). Most of the effects of GH on hard and soft tissues, occur through induction of insulin-like growth factors (IGF-1) (Rosen and Wuster, 1996). The neuroendocrine mechanism by which intake of nutrients alters hypophysial GH secretion and the response of IGF-I on dietary manipulations in long-term conditions is less known. IGF-I mediates the anabolic actions of GH in peripheral tissues (Daughaday and Rotwein, 1989) and appears to be an important factor in the regulation of protein metabolism (McGuire *et al*., 1992). However, IGF-I is believed to be a good hormonal indicator of nutritional status in many species (Jahreis, 1993). Nutritional studies conducted in normal growing rats fed a low energy and/or low protein diets have provided some insights into the molecular mechanisms involved in regulation of GH/IGF-1 system (Meija-Naranjo *et al*., 2003).

Due to little, research has been done to evaluate the role of dietary factors in regulating endocrine system and research on the effect of dietary protein on serum GH and IGF-I concentrations in lambs fed at different levels of dietary protein is limited, this study was conducted to determine the effect of dietary protein level on GH and IGF-I concentrations and performance in lambs.

MATERIALS AND METHODS

Animals and housing: The experiment was conducted at the Department of Animal Nutrition and Nutritional Diseases of Istanbul University. A total of 50 Kivircik male lambs were randomly selected for this experiment. Ages ranged between 120-135 days and average body weight was 26.20±1.10 kg. Animals were vaccinated against enterotoxemia and treated against external parasites before the beginning of the experiment. Lambs were randomly assigned ten lambs to each of the five treatment diets containing 10, 12, 14, 16 and 18% CP to determine the effects of dietary CP level on performance, serum GH and IGF-I concentrations.

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Table 1: Ingredient composition and proximate analysis of the five diets used in the experiment (%)

	Dietary protein concentration (%)				
Ingredients	10	12	14	16	18
Alfaalfa hay	15.00	15.00	15.00	15.00	15.00
Wheat bran	10.00	10.00	10.00	12.00	13.00
Barley	11.50	69.50	50.00	50.00	47.50
Soybean meal	-	2.00	10.00	15.00	21.00
Sugar beet bulb	60.00	-	11.50	4.50	--
NaCl	1.00	1.00	1.00	1.00	1.00
Sodium bicarbonate	0.50	0.50	0.50	0.50	0.50
Limestone	1.50	1.50	1.50	1.50	1.50
Vit. and min. premix*	0.50	0.50	0.50	0.50	0.50
Analysis (DM basis)					
Dry matter (%)	90.09	89.75	90.44	89.95	90.06
CP (%)	10.30	12.05	13.92	15.81	17.94
Calculation					
ME (Mcal kg^{-1})	2.53	2.54	2.54	2.52	2.52

*Composition of premix kg^{-1}: vitamin A, 20,000,000 IU; vitamin D_3, 3,000,000 IU, vitamin E, 25,000 mg, cobalt, 200 mg; manganese, 45,000 mg, zinc, 40,000 mg, iodine, 300 mg, iron, 50,000 mg and copper, 10,000 mg, selenium, 300 mg, magnesium, 100 mg

Table 2: Performance of lambs fed different protein levels, mean±SE (n = 10)

	Dietary protein concentration (%)				
	10	12	14	16	18
Body weight (kg)					
Initial	26.21±1.13	26.32±1.08	25.03±0.88	26.99±1.47	26.47±0.98
30 days	30.87±1.17^{b}	32.50±1.08^{ab}	31.50±0.91^{b}	35.60±1.31^{a}	34.60±1.48^{ab}
60 days	37.31±1.46^{b}	39.60±1.18^{ab}	39.85±1.24^{ab}	42.04±1.25^{a}	41.44±1.13^{a}
Feed intake (g)					
30 days	1095	1098	1100	1110	1105
60 days	1216	1220	1218	1230	1232

$^{a-b}$, values for each body weight with superscripts are different ($p<0.05$)

Table 3: Serum GH and IGF-1 concentrations in lambs, mean±SE (n = 10)

	Dietary protein concentration (%)				
	10	12	14	16	18
Growth hormone (GH), ng mL^{-1}					
Initial	15.04±3.75	11.88±1.67	14.08±3.34	14.13±2.98	10.89±1.38
30 days	10.66±3.45	10.37±4.11	8.84±1.45	8.03±1.30	10.50±2.48
60 days	6.90±0.98	6.90±2.58	5.06±0.76	5.83±0.55	6.18±2.33
Insulin like growth factor-1 (IGF-1) (ng mL^{-1})					
Initial	46.86±3.12	48.65±2.71	44.55±3.28	46.71±3.15	45.82±2.25
30 days	47.15±1.86	47.86±3.12	48.72±2.47	46.57±1.85	49.12±3.11
60 days	49.23±2.41	48.63±3.25	50.05±3.02	49.94±2.45	51.32±2.63

Feeds and feeding procedures: The study was consisted of 2 periods, adaptation period (15 days) and experimental period (60 days), respectively. The adaptation period consisted of a 65:35 forage to concentrate ratio for a 15 day period. This ratio was then gradually decreased until it reached the ratio of 15:85. During the adaptation period, lambs were fed at maintenance level. In experimental period, lambs were randomly assigned to 5 diets contained five dietary crude protein levels (10, 12, 14, 16 and 18% CP). During the experiment, lambs were fed twice a day at 09:00 and 16:00 h. Water was offered *ad libitum* in buckets and was changed daily. Ingredients and crude protein percentages of diets are shown in Table 1.

Analytical procedures: Amounts of offered feeds and refused feeds were recorded daily and feed consumption was determined at the end of the experiment.

Lambs were fasted overnight and weighed individually at the beginning of the study then 4 weeks intervals and data were recorded for statistical performance analysis during the experiment.

Diets for each group were prepared every 2 weeks and samples of each diet were collected for analysis. Samples of each diet were analysed according to the standard procedures of the AOAC (1990). Results of chemical analysis of each diet were presented in Table 1.

Blood samples were collected from each lamb in 0, 30 and 60 days of experiment. The samples were taken by jugular venipuncture using vacuum tubes before the morning feeding. Serum samples were separated by centrifugation and stored at -20°C until the analyses were completed. Concentrations of GH were determined by an enzyme immunoassay (Serpek and Haliloglu 2000). IGF-1 concentrations were analysed by using a

modified sheep specific kit (OCTEIA IGF-1; Immunodiagnostic Systems Ltd, Boldon, UK).

Statistical analyses: All statistical analyses were carried out by using SPSS (1999) package programme. Results of performance, serum GH and IGF-1 concentrations are presented as means with their standard errors in Table 2 and 3, respectively.

RESULTS AND DISCUSSION

The initial body weight was similar for all lambs and averaged 26.20±1.10 kg (Table 2). Final body weight of lambs were 37.31±1.46, 39.60±1.18, 39.85±1.24, 42.04±1.25 and 41.44±1.13 kg for groups fed 10, 12, 14, 16 and 18% CP, respectively and lambs fed 16 and 18% CP diet had significantly higher body weight than those of 10% CP ($p<0.05$).

In this study, lambs fed 16 and 18% CP diet had significantly higher body weight than those of 10% CP. However, Titi *et al.* (2000) reported 16 and 18% CP levels was better. When performance data in this study were compared with result of recent studies conducted to investigate the effect of different protein level on lamb performance, our data were in accordance to the results of these trials (Haddad *et al.*, 2001; Titi *et al.*, 2000).

GH and IGF-1 concentrations of experimental groups were presented in Table 3. Mean concentrations of GH and IGF-1 in blood serum of lambs were not different significantly between groups.

It was reported that nutritional status did play a major role in regulating circulating levels of GH and IGF-I (Breier, 1999). Especially, prolonged fasting in growing animals is characterised by an elevation of plasma GH concentrations, while in animals submitted to limited feed restriction (Renaville *et al.*, 2002). Tannenbaum *et al.* (1979) reported that protein malnutrition in rats caused a reduction in the circulating level of GH. Similarly, Meija-Naranjo *et al.* (2003) reported that protein restriction caused a significant decrease in serum GH levels in rats fed either 0 or 4% protein diets compared to rats fed 20% protein diet. However, Lee *et al.* (2005) observed that there were no significant difference in Holstein steers fed diets with 13.8 and 6.6% crude protein. In contrast, Cheema *et al.* (1991) reported that serum GH significantly decreased and changed quadratically with increasing dietary protein level in lambs. In this study, it was observed that there were no effects of dietary protein level on GH concentration. Similarly, Lee *et al.* (2005) reported that there were no significant difference in steers fed diets with 13.8 and 6.6% CP. Also, Swanson *et al.* (2000) were observed that serum GH concentrations in sheep were not influenced by dietary protein level.

It was reported that nutritional status could depress plasma concentrations of IGF-I (McGuire *et al.*, 1992). In this study, there were no significant differences in IGF-I concentrations. Similarly, Grant *et al.* (1991) reported no response of plasma IGF-I to dietary protein level. In contrast, Kriel *et al.* (1992) reported that dietary protein supply was the limiting factor for maximal stimulation of IGF-I plasma concentrations. However, Hua *et al.* (1995) observed differences in circulating IGF-I concentrations in sheep only under severe nutritional deficiency, i.e., starvation. Also, Breier *et al.* (1986) reported IGF-I differences were not apparent in animals underfed moderately. It is possible that the level of nutrition in this study were not sufficient to observe significant differences in serum IGF-I concentration.

CONCLUSION

It is concluded from the study that dietary protein level had positive effect on performance and 16 and 18% CP level was better than 10% CP. However, serum GH and IGF-1 concentrations were not influenced by dietary protein level.

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