BLOOD METABOLIC HORMONES AND LEPTIN IN GROWING LAMBS

Antunović, Zvonko; Novoselec, Josip; Sauerwein, Helga; Vegara, Mensur; Šperanda, Marcela

Source / Izvornik: **Poljoprivreda, 2010, 16, 29 - 34**

Journal article, Published version
Rad u časopisu, Objavljena verzija rada (izdavačev PDF)

Permanent link / Trajna poveznica: https://urn.nsk.hr/urn:nbn:hr:151:762227

ISSN 1330-7142
UDK = 636.37.1:577.1

BLOOD METABOLIC HORMONES AND LEPTIN IN GROWING LAMBS

Z. Antunović [1], J. Novoselec [1], Helga Sauerwein [2], M. Vegara [3], Marcela Šperanda [1]

Original scientific paper
Izvorni znanstveni članak

SUMMARY

The aim of this paper is to determine the concentration of blood metabolic hormones and leptin levels in growing lambs. The research was carried out on Tsigai lambs in two periods (suckling and fattening) during the winter feeding season. Lambs were suckling and ate a food mixture and alfalfa hay ad libitum, while during the fattening period they were fed only with the above mentioned mixture and alfalfa hay ad libitum. Their blood was analyzed on 35th and 75th day of age. Concentrations of insulin, leptin and thyroid hormones were determined in the blood serum of lambs during both periods. In the blood of fattening lambs significantly higher (P<0.01) leptin concentrations (4.83 and 3.45 ng/mL) and slightly higher (P>0.05) insulin concentrations (1.05 and 0.54 μU/mL), were determined, compared to suckling lambs. A significant strong positive correlation between serum leptin and insulin (r = 0.85, P<0.01) was also determined in the blood of fattening lambs and a positive correlation of above mentioned indicators in the blood of suckling lambs, but with no significant difference (r = 0.25, P>0.05). The concentration of thyroid hormones did not significantly differ depending on the period of measurement. These changes indicate that the measurement concentrations of metabolic hormones and leptin in blood are very important in order to understand the changes of metabolism and nutrient supply in growing lambs.

Key-words: lambs, suckling period, fattening period, blood, leptin, insulin, thyroid hormones

INTRODUCTION

Metabolic hormones such as insulin and thyroid hormones play an important role in animal metabolism. Insulin is a 5.8-kDa protein synthesized in the pancreatic β-cells and secreted in response to evaluation of plasma glucose level (Magistrelli et al., 2008). Insulin has an important role in glucose transport and modulates peripheral satiety signals and directly targets the central nervous system to inhibit food intake (Gale et al., 2004). Also, insulin has an important role in lipid metabolism, stimulating lipogenesis and inhibiting lipolysis (Ban-Tokuda et al., 2008).

The thyroid hormones maintain the homeostasis of energy and protein metabolism, thermoregulation, growth and productivity parameters (Huszenicza et al., 2002). Changes of blood thyroid hormone concentrations are an indirect measure changes in thyroid gland activity and circulating thyroid hormones. They can be considered as indicators of the metabolic and nutritional status of the animals (Todini et. al, 2007). Thyroxin (T_4) and triiodothyronin (T_3) are also factors that regulate leptin expression or its plasma concentration to some extent (Chilliard et al., 2005). Leptin is an "adipokine" peptide hormone produced by fat cells, particularly in white adipose tissue (Erhardt et al., 2003). There are many roles of leptin in the animal organism. Production of leptin is modulated by insulin, glucocorticoids and sexual steroids and affects on the central nervous system inhibiting hypothalamic neuropeptide production, mainly neuropeptide Y, which stimulate appetite (Stephens et al., 1995). The nutritional state is closely

(1) Prof.DSc Zvonko Antunović (zantunovic@pfos.hr), Josip Novoselec, BAgr, Prof.DSc Marcela Šperanda – Department of Animal Husbandry, Faculty of Agriculture in Osijek, J. J. Strossmayer University of Osijek, Trg Sv. Trojstva 3, 31000 Osijek, Croatia; (2) Prof.DSc Helga Sauerwein - Institute for Animal Science, Physiology & Hygiene, Rheinische Friedrich-Wilhelms-Universität Bonn, Katzenburgweg 7 – 9, 53115 Bonn, Germany; (3) Prof.DSc Mensur Vegara - Norwegian University of Life Sciences (UMB), Noragric, P.O.Box: 5003, N-1432, Ås, Norway

regulated by neuroendocrine and hormonal cues. Energy restriction produces harmful consequences in growth, reproduction and other metabolic processes, throught the effect of some neuroendocrine hormones such as leptin having high effect in food intake (Legardi et al., 1997). Serum leptin sensitivity to energy balance is reduced during periods of negative energy balance in sheep (Tokuda et al., 2001). The transition from the pre-ruminant to ruminant state of lambs contributes to many changes in circulating concentration of insulin, thyroid hormones and leptin (Tokuda et al., 2001). Lambs in the suckling, weaning and initial period of fattening are very sensitive and susceptible to different stressors that can significantly affect their growth and health condition. The aim of this paper is to show the concentration of metabolic hormones and leptin level during suckling and fattening period.

Material and methods

This study was conducted during 2009 on the family farm Ursic (Croatia, located 35 km south-east of Osijek, TX (42.1500 N; long 52.6470 E). This area is located within the Baranya region at an altitude of about 91 meters. The researches were carried out on the same 9 Tsigai growing lambs in two periods (suckling and fattening period) during winter feeding season. Lambs in suckling period were placed together with their mothers. Lambs were weaned at the age of 60 days and placed in fattening. In the suckling period, lambs were aged 35 days, and 75 days in fattening period. Lambs were healthy and in good condition. The body weight and body condition score of lambs in suckling period were 16.2 kg and 2.82 while the body weight and body condition score of fattening lambs were 27.2 kg and 3.14. The body condition scores (BCS) of lambs (1= emaciated to 5 = obese) were evaluated by two trained technicians according to Russel (1991). Lambs were suckling and ate food mixture and alfalfa hay *ad libitum*, while during the fattening they were fed only with the above mixture and alfalfa hay *ad libitum*. The raw composition of food mixture is shown in Table 1. The chemical composition of food mixtures and alfalfa hay samples taken on the day of blood sampling were determined according to AOAC (1995) and is shown in Table 2. Metabolic energy was determined according to DLG (1993).

Table 1. Raw composition of food mixture in lambs during suckling and fattening period

Tablica 1. Sirovinski sastav krmne smjese janjadi tijekom razdoblja sisanja i tova

Ingredient – *Krmivo*	Share, % - *Udio*, %
Corn – *Kukuruz*	50.00
Sunflower meal – *Suncokretova sačma*	10.00
Soybean meal – *Sojina sačma*	10.00
Wheat forage meal – *Stočno brašno*	14.00
Molasses – *Melasa*	4.00
Yeast – *Stočni kvasac*	3.00
Milk replacement – *Mliječni nadomjestak*	2.50
Dehydrated alfalfa meal – *Dehidrirano brašno lucerne*	2.00
Salt – *Sol*	0.50
Limestone – *Stočna kreda*	2.00
Phosphonal – *Fosfonal*	1.00
Premix*	1.00
Total – *Ukupno*	100.00

* Contents in 1 k g- Sadržaj u 1 kg= Vitamin A 1000000 IU/g; Vitamin D_3 150000 IU/g; vitamin E 1500 mg; vitamin K_3 50 mg; vitamin B_1 100 mg; vitamin B_2 200 mg; nicotin acid 1000 mg; pantotenic acid 500 mg; vitamin B_6 200 mg; vitamin B_{12} 1.0 mg; cholin chloride 20000 mg; $FeSO_4$ 4000 mg; $CuSO_4$ 800 mg; MnO_2 3500 mg; $ZnSO_4$ 5000 mg; KI 80 mg; $CoCl_2$ 20 mg; $NaSeO_3$ 15 mg; $MgSO_4$ 10000 mg; antioxidanse 10000 mg.

Table 2. Chemical composition of diet in lambs during suckling and fattening period

Tablica 2. Kemijski sastav hrane janjadi tijekom razdoblja sisanja i tova

Indicator – *Pokazatelj*	Food mixture-*Krmna smjesa*	Alfaalfa -*Sijeno lucerne*
Moisture – *Vlaga*	8.3	9.0
Crude proteins- *Sirove bjelančevine*	17.26	15.34
Crude fiber – *Sirova vlakna*	8.14	32.60
Crude fat – *Sirove masti*	3.9	1.2
Crude ash – *Pepeo*	6.91	7.71
Metabolic energy, MJ ME/kg–*Metabolička energija*	11.26	7.47

Blood was collected from the jugular vein of the same lambs into sterile serum Vacutainer tubes (Venoject®, Sterile Terumo Europe, Leuven, Belgium) on 35th and 75th days. After that, the serum was separated by centrifugal force (10 min) at 3000 revolutions/min. Serum insulin concentrations were analyzed by MEIA (Microparticle Enzyme Immunoassay) with AxSYM Insulin Reagent Pack (2D01-21) on Abbott AxSYM Systems (Axis-Shield Diagnostic, Ltd, Dundee, UK). The sensitivity of AxSYM Insulin assay was calculated to be better than or equal to 1.0 μU/mL. Mean recovery rates were 97.6%. Concentrations of total T_3 (triiodothyronine) and T_4 (thyroxin) in blood serum were determined by means of duplicate determinations using commercial kits for clinical use in humans (Abbott Laboratories, USA) by Imx-Abbott immunoanalyser (Abbott Laboratories, IL, USA). Methods for determination of T_3 and T_4 were MEIA (Microparticle Enzyme Immunoassay) and FPAI (Fluorescence Polarization Immunoassay). Sensitivity of the assay was less than 0.4 nmol/L (T_3) and 12.8 nmol/L (T_4). Mean recovery rates were 98.6%. Serum leptin concentrations were determined by a specific enzyme immunoassay that has been validated for ovine samples (Sauerwein *et al.,* 2004). The intra-assay coefficient of variation was 6.3%, the inter-assay coefficient of variation was 13.9%, and the limit of detection was 0.3 ng/mL. All analyses using commercial kits were performed according to the manufacturer's instructions. Lambs used in this study were housed in facilities approved by the Croatian Association for Accreditation of Laboratory Animal Care, and in accordance with current regulations and standards issued by the Croatian Ministry of Agriculture, Forestry and Water Management.

Differences between the suckling and fattening lambs were statistically tested using repeated measurement model with Statistica (2008). Correlation coefficients between concentration of leptin and insulin were calculated for each period of measures. Differences were considered as significant at the level of 0.05 or less.

RESULTS AND DISSCUSION

Figure 1 shows the concentration of leptin and insulin levels in suckling and fattening lambs. Significantly higher ($P<0.01$) leptin concentrations and slightly higher concentrations of insulin ($P>0.05$) were determined in the blood of fattening lambs, compared to suckling lambs. A strong positive correlation between serum leptin and insulin ($r = 0.85$; $P<0.01$)in the blood of fattening lambs (Table 3) and a positive correlation between these parameters in the blood of suckling lambs were also determined but with no significant difference ($r = 0.25$; $P>0.05$). The above is supported by the regression equation and curve (Figure 2 and 3, Table 3).

In this research, the increase in concentration of serum leptin after weaning during fattening period could have been due to the increase live weight and consequently increase adipose cells volume (Tokuda et al., 2001). These findings may be associated with increase in size of adipocyte where leptin is secreted and fat proportion. In this research, a better BSC is determined in fattening lambs compared to lambs in suckling period. The increase serum leptin concentration may have been affected by several factors, particularly nutritional level, serum metabolites and low temperatures. In ani-

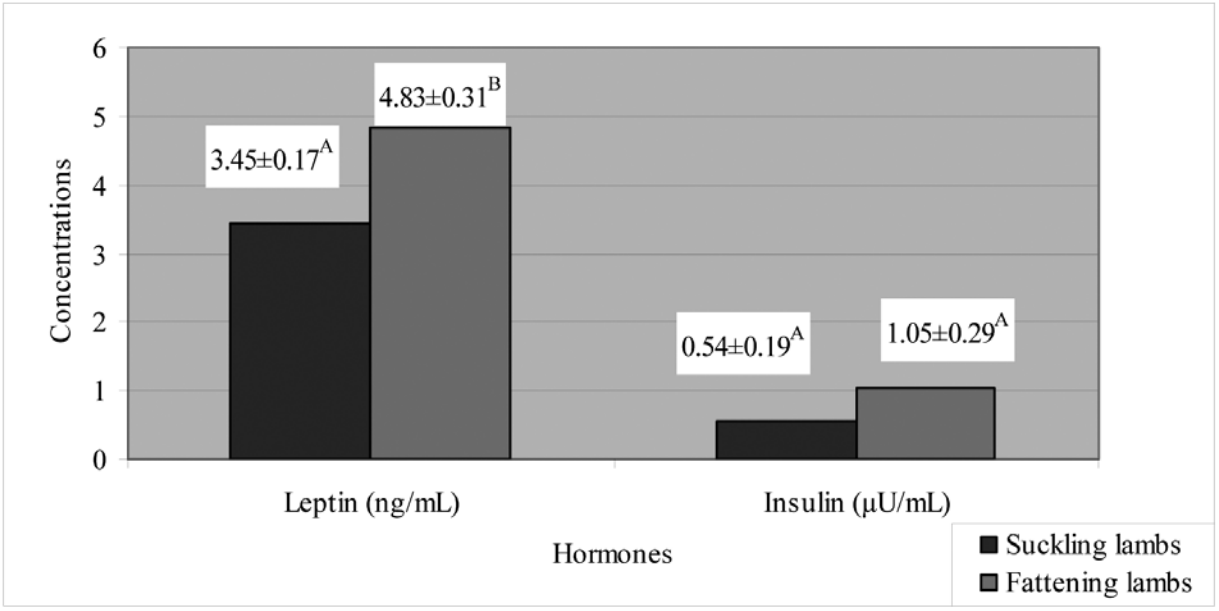

Figure 1. Leptin and insulin concentrations in blood of lambs (mean ± standard error; AB- P<0.01)

Grafikon 1. Koncentracije leptina i inzulina u krvi janjadi (srednja vrijednost **± standardna greška)**

Table 3. Correlation and regression equation between blood leptin and insulin in suckling and fattening lambs

Tablica 3. Korelacija i jednadžbe regresije koncentracija leptina i inzulina u krvi i janjadi u tovu

Indicators *Pokazatelj*	Period *Razdoblje*	Insulin	
		Suckling lambs *Sisajuća janjad*	Fattening lambs *Janjad u tovu*
Correlation – *Korelacija*			
Leptin	Suckling lambs - *Sisajuća janjad*	0.255	-
	Fattening lambs - *Janjad u tovu*	-	0.851*
Regression equation- Jednadžba *regresije*			
Suckling lambs - *Sisajuća janjad*		insulin = -0.7445 + 0.37262 x leptin	
Fattening lambs - *Janjad u tovu*		insulin = -4.131 + 1.0735 x leptin	

* (P<0.01)

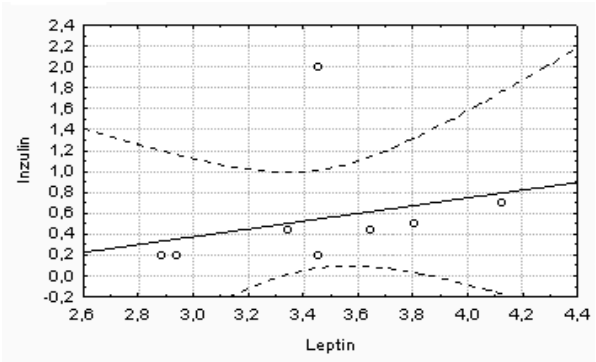

Figure 2. Regression curve for blood concentration of insulin and leptin in suckling lambs

Grafikon 2. Jednadžba regresije za koncentracije inzulina i leptina u krvi sisajuće janjadi

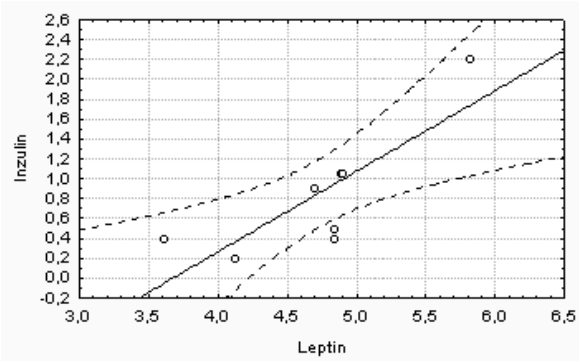

Figure 3. Regression curve for blood concentration of insulin and leptin in fattening lambs

Grafikon 3. Jednadžba regresije za koncentracije inzulina i leptina u krvi janjadi u tovu

mals, plasma leptin levels are closely correlated with body condition score and nutritional status (Chilliard et al., 2001; Delavaud et al., 2002). This thesis was supported by Considine et al. (1996) which indicates that the reduction of weight for 10% in obese humans reduce the concentration of leptin in the blood by 53%. Tokuda et al. (2001) determined an increase in leptin concentration in blood plasma of lambs was determined as fattening progressed. At the same time a positive correlation between concentrations of leptin and insulin in fattening lambs was determined. In the research of Ban-Tokuda (2008) it was out found out that leptin and insulin concentrations during fattening period in lambs changed significantly. Tokuda et al. (2003) found significant differences in leptin and insulin concentration in the blood of lambs in the period before and after weaning. Lower leptin concentrations were found in the period before weaning, compared to the weaned lambs. Blood leptin concentration increased with the growth of lambs (Tokuda et al., 2001; Cestnik et al., 2004; Čebulj-Kadunc and Cestnik, 2005).

Nutritional status had a significant influence on leptin concentrations in the serum. The leptin content is also influenced by the energy supply of lambs. In the research of Tokuda et al. (2002) it was found out that sheep fed with food rich in high energy had a higher concentration of leptin in the blood compared to sheep fed with low energy. Serum concentrations of leptin were higher in lambs fed adequately compared to lambs fed poorly (Morrison et al., 2001). In the research of Altman et al. (2004) feed deprivation in lambs reduced plasma leptin and insulin concentrations. According to

Table 4. Thyroid hormones in lambs during suckling and fattening period

Tablica 4. Hormoni štitaste žlijezde u krvi sisajuće i janjadi u tovu

Hormone	Suckling period – *Razdoblje sisanja*				Fattening period – *Razdoblje tova*				P value *P vrijednost*
	Mean	s	min	max	Mean	s	min	max	
T_3, nmol/L	1.93	0.46	1.37	2.90	1.61	0.23	1.25	2.00	0.12
T_4, nmol/L	90.34	21.87	58.90	132.40	84.92	15.83	63.95	103.1	0.42
Ration T_3/T_4 *Odnos* T_3/T_4	0.023	0.007	0.014	0.033	0.020	0.005	0.013	0.024	0.23

Mean- *srednja vrijednost*, s- standard deviation- *standardna devijacija*

NRC (2007) energy requirements for growing lambs with 30 kg body weight and 300 g body weight gain is 2.10 Mcal ME/day. In this research lambs in both periods had a meal that satisfied their nutrient needs (Table 2). The increase insulin level in fattening lambs may be related to an increase body weight and body fat accumulation. In lambs, the correlation between blood leptin and insulin concentrations increased with age (Erdhart et al., 2003). Table 4 shows the concentration of thyroid hormones and their relationship in the blood of suckling and fattening lambs.

No significant difference in concentrations of thyroid hormones (T_3 and T_4) in the blood of fattening lambs ($P>0.05$) was determined related to suckling lambs. Some lower concentrations of thyroid hormones in the blood of fattening lambs were expected since they decrease with the increasing age of lambs and sheep (Antunović et al., 2008; Novoselec et al., 2009). Neonatal lambs had higher levels of T_3 and T_4 hormones compared to growing lambs and ewes (Peeters et al., 1992).

CONCLUSION

Based on the given results it can be seen that in the blood of lambs in the fattening significantly higher ($P<0.01$) leptin concentrations and higher ($P>0.05$) insulin concentrations were determined as well as their significant correlation compared to suckling lambs. The reason for this change is likely due to increased body weight of lambs during the fattening period. These changes indicate that the measurement concentrations of metabolic hormones and leptin in blood are very important in order to understand the changes of metabolism and nutrient supply in growing lambs.

ACKNOWLEDGEMENTS

This Study forms part of the research project 079-1780469-0225 financed by the Ministry of Science, Education and Sport of the Republic of Croatia.

REFERENCES

1. Altman, M., Sauerwein, H., von Borell, E. (2004): The relationship between leptin concentrations and body fat reserves in lambs are reduced by short-term fasting. J. Ani. Physiol. Anim. Nut. 90: 407-413.
2. Antunović, Z., Šperanda, M., Novoselec, J., Steiner, Z., Đidara, M. (2008): Influence of age on exterior traits, blood biochemical parameters and thyroid hormones of Tsigai sheep. 7th IBNA International Symposium - September 25-26, 2008.
3. A.O.A.C. (1995): Official Methods of Analysis, 16 Edition. Association of Official Analytical Chemists, Arlington, VA.
4. Ban-Tokuda, T., Delavaud, C., Chilliard, Y., Fujihara T. (2008): Plasma leptin, feed intake and body fat accumulation in fattening castrated male and female lambs. Anim. Sci. J. 79: 58-67.
5. Cestnik, V., Čebulj-Kadunc, N., Kosec, M. (2004): Leptin concentrations in Jezersko-Solchava lambs and growing rams. J. Anim. Feed Sci. 13, suppl. 1: 471-474.
6. Chilliard, Y., Bonnet, M., Delavaud, C., Falcounnier, Y., Leroux, C., Djinane, J., Bocquier, F. (2001): Leptin in ruminant. Gene expresion in adipose tissue and mammary gland, and regulation of plasma concentration. Dom. Anim.Endocrin. 21: 271-295.
7. Chilliard, Y., Delavaud, C., Bonnet, M. (2005): Leptin expression in ruminants: nutritional and physiological regulations in relation with energy metabolism. Dom. Anim. Endocrin. 29: 3-22.
8. Considine, R.V., Sinha, M.K., Heiman, M.L., Kriauciunas, A., Stephens, T.W., Nyce, M.R., Ohannesian, J.P., Marco, C.C., McKeen, L.J., Bauer, T.L. Caro, J.F. (1996): Serum immunoreactive-leptin concentrations in normal-weight and obese humans. N. Engl. J. med. 334: 292-295.
9. Čebulj-Kadunc, N., Cestnik, V. (2005): Circulating leptin concentrations in Lipizzan horses and Jezersko-solchava sheep. Slov Vet Res. 42, 11-14.
10. Delavaud, C., Ferlay, A., Falcounnier, Y., Bocquier, F., Kann, G., Chilliard, Y. (2002): Plasma leptin concentration in adult cattle: effects of breed, adiposity feeding level and meal intake. J. Anim. Sci. 80: 237-250.
11. DLG (1993): Futterwerttabellen für Wiederkäuer. 6. Auflage, Frankfurt am Main.
12. Erhardt, R.A., Greenwood, P.L., Bell, A.W., Boisclair, Y.R. (2003): Plasma leptin is regulated predominantly by nutrition in preruminant lambs. J. Nutr. 4:196-4201.
13. Gale, S.M., Castracane, V.D., Mantzoros, C.S. (2004): Energy homeostasis, obesity and eating disorders: recent advances in endocrinology. J. Nutr. 134: 295-298.
14. Huszenicza, G., Kulscsar, M., Rudas, P. (2002): Clinical endocrinology of thyroid gland function in ruminants. Vet. Med. Czech. 47: 199-210.
15. Legardi, G., Emerson, C.H., Ahima, R.S., Flier, J.S., Lechan, R.M. (1997): Leptin prevent fasting-induced suppresion of prothyrotrophin-relising hormone messenger ribonucleic acid in neurons of the hypotalamic paraventricular nuclei. J. Endocrin. 138: 2569-2575.
16. Magistrelli, D., Polo Dimel, G., Rosi, F. (2008): Leptin, insulin and ghrelin levels in goat milk and in plasma of suckling kids. Small Rumin. Res. 79: 38-41.
17. Morrison, C.D., Daniel, J.A., Holmberg, B.J., Djinane, J., Raver, N., Gertler, A., Keisler, D.H. (2001): Central infusion of leptin into well-fed and undernourished ewe lambs: effects on feed intake and serum concentrations of growth hormone and luteinizing hormone. J. Endocrin. 168: 317-324.
18. NRC (2007): Nutrient requirements of small ruminants (sheep, goats, cervids and new world camelides). The national academies press. Washington, D.C., p. 362.
19. Novoselec, J., Antunović, Z., Šperanda, M., Steiner, Z., Šperanda, T. (2009): Changes of thyroid hormones in blood of sheep depending on age and reproductive status. It. J. Anim. Sci., 8(3): 208-210.
20. Peeters, R., Buys, N., Vanmontfort, D., Van Isterdeal, J., Decuypere, E., Kuhn, E.R. (1992): Preferential release of triiodothyronine folowing stimulation by thyrothropin or

thyrotropin-releasing hormone in sheep of different ages. J. Endocrin. 132: 93-100.

21. Russel, A. (1991): Body condition scoring of sheep. In: Boden, E. (Ed.), Sheep and goat practice, Bailliere Tindall, Philadelphia, p. 3.
22. Sauerwein, H., Heintges, U., Hennies, M., Selhorst, T., Daxenberger, A. (2004): Growth hormone induced alterations of leptin serum concentrations in dairy cows as measured by a novel enzyme immunoassay. Liv. Prod. Sci. 87: 189-195.
23. Statistica-Stat Soft, Inc. version 8.0, 2008. www.statsoft.com.
24. Stephens, T.W., Bashinski, M., Bristow, P.K., Bue-Valleskey, J.M., Burgett, S.G., Craft, L. (1995): The role of neuropeptide Y in the atiobesity action of obese gene product. Nature 377: 530-534.
25. Todini, L (2007): Thyroid hormones in small ruminants: effects of endogenous, environmental and nutritional factors. Animal, 1, 7: 997-1008.
26. Tokuda, T., Kimura, D., Fujihara T. (2001): The relationship between leptin and insulin in blood plasma of growing lambs. Anim. Sci. 73: 71-76.
27. Tokuda, T., Delavaud, C., Chilliard, Y. (2002): Effect of dietary levels on plasma in sheep. Anim. Sci. 73, 471-478.
28. Tokuda, T., Delavaud, C., Chilliard, Y. (2003): Plasma leptin concentration in pre- and post-weaning lambs. Anim. Sci. 76: 221-227.

LEPTIN I HORMONI KOJI REGULIRAJU METABOLIČKE AKTIVNOSTI U KRVI JANJADI U PORASTU

SAŽETAK

Cilj je istraživanja bio utvrditi koncentracije hormona koji reguliraju metaboličke aktivnosti i leptina u krvi janjadi u porastu. Istraživanje je provedeno s janjadi cigaja pasmine u sisajućem razdoblju i tovu tijekom zimske sezone hranidbe. Janjad je sisala i dobivala krmnu smjesu i sijeno lucerne po volji, dok je tijekom tova hranjena samo navedenom smjesom i sijenom lucerne po volji. Uzorkovanje krvi provedeno je 35. dana u sisajućem razdoblju i 75. dana u tovu. U krvnome serumu janjadi utvrđene su koncentracije inzulina, leptina i hormona štitaste žlijezde tijekom oba razdoblja. U krvi janjadi u tovu utvrđena je značajno viša ($P<0.01$) koncentracija leptina (4,83 i 3,45 ng/mL) te nešto viša koncentracija inzulina (1,05 and 0,54 μU/mL), ali bez značajnih razlika ($P>0.05$), u odnosu na sisajuću janjadi. Također je u krvi janjadi u tovu utvrđena jaka pozitivna korelacija između koncentracija leptina i inzulina ($r = 0,85$; $P<0,01$) te pozitivna korelacija navedenih pokazatelja u krvi sisajuće janjadi, ali bez značajnih razlika ($r = 0,25$; $P>0,05$). Koncentracija hormona štitaste žlijezde nije značajnije odstupala ovisno o razdoblju mjerenja. Navedene promjene ukazuju da je mjerenje koncentracija hormona koji reguliraju metaboličke aktivnosti (insulin i hormoni štitaste žlijezde) i leptina u krvi vrlo važno, s ciljem razumijevanja mijene tvari i hranidbene opskrbe janjadi u porastu.

Ključne riječi: janjad, sisajuće razdoblje, tov, krv, leptin, insulin, hormoni štitaste žlijezde

(Received on 1 October 2010; accepted on 15 November 2010 - *Primljeno 01. listopada 2010.; prihvaćeno 15. studenoga 2010.*)