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Effects of Plasma Insulin, Glucose and NEFA Concentrations of Feeding Frequency During Long Term in Lambs

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ABSTRACT

In ruminants, very little is known about the physiological background of the mechanisms involved in feed intake regulation. Mechanisms that control feed intake exist in ruminants but the exact nature of the control systems are not well understood. For this reason, the effects of different feeding regimens, and the initiation/termination of feeding on blood glucose, insulin, and NEFA were measured in lambs fed once, two times daily and fed ad libitum. To determine insulin hormone influence on feed intake, blood was sampled before 30 minutes of feeding (08:30) and after 1 hour of feeding (10:00) in the morning, and collected 14:00, before 30 minutes of feeding (15:30), after 1 hour feeding (17:00). In the different times fed lambs, plasma insulin levels had a significant change ($P<0.05$) between groups and between sampling times($P<0.05$) but glucose and NEFA levels had no significant changes among groups. We have demonstrated for the first time that modification of feeding regimen could affect plasma insulin levels.

Key words: Feeding frequence, insulin, glucose, NEFA and lambs.

INTRODUCTION

Central nervous system plays a significant role in the control on food intake and metabolic adaptation mechanisms in mammals. The information concerning the body's energy sources and endocrine status is known to be communicated by long term signals. Despite substantial fluctuations in daily food intake, animals maintain a remarkably stable body weight, because overall caloric ingestion and expenditure are exquisitely matched over long periods of time, through the process of energy homeostasis. In ruminants, very little is known about the physiological back ground of the mechanisms involved in feed intake regulation. Mechanisms that control feed intake exist in ruminants but the exact nature of the control systems are not well understood. There is evidence that circulating hormones such as insulin may act in the control of voluntary feed intake and meal termination in ruminants (Baile 1975; Chase 1977; Deet 1980).

Blood glucose concentrations in ruminants are considerably lower than those of nonruminants, and ruminants are relatively insensitive to insulin. These major differences in carbohydrate metabolism have led to considerable speculation on the role of glucose in ruminant metabolism (Annison 1961). Glucose metabolism is expected to increase with feeding, because propionate, the major precursor for gluconeogenesis, is produced in the rumen and absorbed after feeding (Bergman 1975; Sano et al 1999). Moreover, insulin secretion is accelerated by feeding (Bassett 1975; Sano et al 1999). Information is lacking on the combined effects of feed restriction on blood glucose metabolism in response to feeding. Also, Mears and Mendel (1970) found plasma NEFA levels to be inversely related to long-term food intake in sheep. They suggest that the different NEFA values apparently were not simply the result of the level of food intake by the sheep but might be involved in regulating food intake. Various physiological models, including chronic food-restriction or photoperiodically driven changes in voluntary food intake, add further perspective to the issue but a few studies on insulin, glucose, NEFA and their role in feed intake in sheep and ruminants have been performed. In this regard, sheep provide an innovative model whereby long-term changes in body weight or extended feeding rhythms can be investigated.

The study reported here was designed to investigate the relationship between pre prandial jugular plasma glucose, NEFA and pre-post prandial the plasma insulin levels in sheep fed different feeding frequence.

MATERIALS AND METHODS

This study was conducted at the Animal Welfare and Animal Production Research and Application Center in Uludag University, Faculty of Veterinary Medicine. Fifteen male lambs were applied to homogenity test according to similar weight and age. The animals approximtely body weight of 26 kg were used and they were put into individuals cages.

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The male lambs were assigned randomly to each of the following three dietary treatment groups: Group I were fed ad libitum, group II were fed once a day (09:00), group III were fed twice a day (09:00 and 16:00) and in this sudy was used totally fifteen animals as five lambs in each groups. The daily food allowance was adjusted to metabolic body weight in a day and the feeding was maintained until approximately body weight of 43 kg. The diets were identical in whole groups prior to the study and the time of sampling was the same. The animals were fed trefoil as dry matter. Concentrate feed ingredients were showed table 1 and 2.

Dry matter content of of dietary samples was determined by drying at 105^{o}C for 12 h, and crude protein was determined by the KJELDAHL method (AOAC, 1990). Ash was determined by combustion at 550^{o}C for 6 h. The NDF (Neutral Detergent Fiber) contents were determined using the methods described by Van Soest et al (1991) with heat-stable amylase (Sigma No: A-3306, Sigma Chemical Co., St Louise, MO, USA) and sodium sulfite used in the NDF procedure.

Table 1. Composition of Concentrate feed

Ingredient	%
Corn grain	50.0
Sunflower meal	16.5
Soybean meal	13.2
Limestone	1.2
Salt (NaCl)	0.5
Vitamin-Mineral Premix*	0.1

* Supplied per kilogmale lambs of premix (Kavimix VM, Kartal Kimya A.S., Gebze, Turkey) : Vitamin A 12000000 IU, Vitamin D_3 3000000 IU, Vitamin E 30 g, Mn 50 g, Fe 50 g, Zn g, Cu 10 g, I 0.8g, Co 0.1 g, Se 0.15 g, Antioxidant 10 g.

Table 2. Chemical composition of feed ingredients

Chemical Composition	Concentrate feed (%)	Alfalfa Hay (%)
Dry Matter	88.30	90.30
Ash	4.67	9.93
NDF*	15.09	39.40
Crude Protein	15.80	15.5
Ether Ekstract	2.56	2.26
Calcium	0.59	1.33
Phosphorus	0.41	0.25

* NDF: Neutral Detergent Fiber

Collection of Blood Samples and Laboratory Analyses:

Blood samples were collected by jugular venipuncture. Collect blood samples into the vacutaner tubes which contain EDTA, at the beginning of experiment and before slaughter. To determine measurment of insulin hormone, sampling blood was collected before 30 minutes of feeding (08:30) and after 1 hour of feeding (10:00) in the morning, and collected 14:00, before 30 minutes of feeding (15:30), after 1 hour feeding (17:00). For the other biochemical parameters as glucose and NEFA, sampling blood was collected once a day (08:30) with 15 days intervals. Blood samples were collected at 15 days intervals as total tree period for insulin and as total four periods for glucose and NEFA. Plasma kept at -20 $^{\circ}$C until analyses.

Plasma Insulin Levels Assay

Consentrations of insulin in plasma were determined by ELISA (Sheep Insulin, Mercodia Elisa).

Biochemical Parameters Measurment

Glucose was measured by the glucose oxidase enzymatic method (BIOLABO, Glucose GOD-PAP, Cat. No 87109) and NEFA-C (WAKO Chemicals, 999-75406) concentrations were measured spectrophotometrically (Schimadzu UV-1601).

Statistical Analysis

The Statistical Package for the Social Sciences version 14.0 (SPSS, Chicago, IL, USA) was used for data analysis. Values are expressed as aritmetic means ± SDEV Within-group and group interactions with time were analysed using ANOVA for repeated measures. Where violations in parametric assumptions were found within the data set, the within group and group interactions were measured using a ANOVA . Tukey Honestly Sigificant Test were considered significant when $p < 0.05$.

RESULTS

Changes in plasma insulin levels in male lambs subjected to the three different feeding regimens are presented in Table 3 and 4. Mean plasma insulin levels of the series 300 samples obtained from each of the groups were analysed. Concentrations of insulin, as a feeding regimens, mean (± SD) insulin showed no significant changes between periods in whole goups. In the different-fed lambs, plasma insulin levels had a significant change ($P<0.05$) between groups and between sampling times ($P<0.05$). Insulin levels in the highest peak values reached III. period of fed twice a day lambs. In twice fed animals showed the highest peak values as the other groups and, this values decreased second period but again increased in third period.

Samples were taken only once in sampling time as total four periods for glucose and NEFA from different fed lambs. Plasma glucose concentrations (mg/100 ml) during a four times at 15 day intervals are presented Table 5 and 6. We found significant changes among the periods ($p< 0.01$), the groups showed same values and no significant changes. Plasma glucose concentrations were not different between animals but in twice fed lambs were only slightly higher than other groups. Plasma NEFA concentrations (mg/dl) during a four periods with 15 day intervals in a scheduled meal fed lambs are presented Table 7 and 8. Plasma NEFA concentrations had significant changes among periods ($p<0.01$) but the groups had no significant changes for glucose. NEFA levels showed the highest peak values in first period (8.23±9.82 mg/dl), also once fed lambs had higher values than other groups, furthermore this levels showed the lowest plasma concentrations in third period in whole groups.

DISCUSSION

In this study, plasma insulin, glucose and NEFA changes have been shown in male lambs under the different feeding regimens during the periods of the experimet. Physiological mechanisms involving blood biochemical metabolites and hormones may limit feed intake in ruminants in situations where physical limitation in gut capacity is not the major factor limiting feed intake (Baumgardt 1974). For this reason, a planned feeding was applied to the sheep, the groups were formed as ad libitum fed-lambs, once a day fed lambs and twice a day fed lambs and we have tested if changes of the insulin levels were occured before and after feeding in this study. No differences was observed in insulin levels between the groups during the course of the trial but plasma insulin levels were permenantly high during the periods in all groups. In restricted feeding trials, mean peaks of insulin surge in the fed-twice daily group were lower and higher than those of the fed-once time daily group, before and after every feeding, respectively ($P < 0.05$), even though the two groups were provided with the same amount of feed daily on a daily basis. It has been reported that the plasma insulin concentration is increased after feeding in ruminant animals (Bassett 1975; Hart et al 1980; Chase et al 1977), as in other mammalian species, reflecting an augmented release of insulin by the pancreas. Insulin is known to be at the centre of metabolic regulation in ruminants, as in other species (Bassett 1975). In rats, Strubbe et al (1977) showed that insulin is low up to 5 min before a meal but increases upon the ingestion of food. Insulin is a critical regulator of energy metabolism, and evidence suggests a close relationship between circulating glucose levels and insulin secretion. Glucose, insulin and NEFA data are consistent with those reported in the literature for cattle in the fed or fasted state (Wretz-Lutz et al 2006). However, Chelikani et al (2004) demonstrated that responsiveness of plasma glucose, insulin and NEFA concentrations during fasting was diffent depending on physiological state of the animal. Blood glucose and insulin concentrations fluctuate reciprocally before and after feeding (Cummings et al 2001). Lofgren and Warner (1972) reported a sustained increase in plasma insulin concentrations which peaked at 30 minutes in sheep fed with a high concentrate diet. All the findings presented here might possibly be explained as being a consequence of insulin secretion or inhibition in response to feeding and fasting. However, these results indicate that the mechanism(s) causing meal termination could involve normal, endogenous insulin secretion during feeding. The short-term effect(s) of insulin on facilitating satiety may

depend upon the availability of energy substrates (Lovett et al 1970) and the rate of change in concentrations of blood hormones and metabolites (Thye et al 1970).

Table 3. Plasma insulin concentrations during pre-feeding and post-feeding and periods (15 day intervals) in male lambs subjected to the three different feeding regimens (ng/ml).

Sampling Times	FEEDING REGIMEN GROUPS									Total
	Ad Libitum			Once a Day			Twice a Day			
	Mean	±	SD	Mean	±	SD	Mean	±	SD	
	I. P E R I O D									
30BMF	0.23	±	0.13	0.25	±	0.15	0.18	±	0.13	0.22±0.13
60AMF	0.24	±	0.15	0.47	±	0.15	0.60	±	0.28	0.44±0.24
14:00	0.56	±	0.25	0.47	±	0.24	0.53	±	0.20	0.52±0.2
30BAF	0.45	±	0.47	0.50	±	0.61	0.55	±	0.54	0.50±0.50
60AAF	0.51	±	0.49	0.33	±	0.19	0.59	±	0.37	0.47±0.35
TOTAL	**0.39**	**±**	**0.33**	**0.40**	**±**	**0.31**	**0.49**	**±**	**0.34**	**0.43±0.32**
	II. P E R I O D									
30BMF	0.11	±	7.67	0.11	±	6.14	0.15	±	0.11	0.12±8.50
60AMF	9.8	±	7.66	0.80	±	0.59	0.59	±	0.50	0.49±0.51
14:00	0.31	±	0.14	0.40	±	0.33	0.22	±	0.10	0.31±0.21
30BAF	0.41	±	0.16	0.43	±	0.25	0.54	±	0.27	0.46±0.22
60AAF	0.27	±	9.57	0.34	±	0.18	0.43	±	0.30	0.35±0.21
TOTAL	**0.24**	**±**	**0.16**	**0.42**	**±**	**0.38**	**0.38**	**±**	**0.32**	**0.35±0.31**
	III. P E R I O D									
30BMF	0.35	±	0.23	0.33	±	0.14	0.43	±	0.27	0.37±0.21
60AMF	0.24	±	6.42	0.35	±	0.16	0.84	±	0.80	0.48±0.51
14:00	0.32	±	0.20	0.77	±	0.61	0.59	±	0.48	0.56±0.47
30BAF	0.54	±	0.52	0.17	±	8.35	0.18	±	4.28	0.29±0.33
60AAF	0.22	±	5.32	0.23	±	4.91	0.89	±	0.67	0.46±0.50
TOTAL	**0.34**	**±**	**0.28**	**0.37**	**±**	**0.34**	**0.59**	**±**	**0.55**	**0.43±0.42**

Feeding time: 09:00 and 16:00. **30BMF:** 30 Minutes Before Morning Feeding (08:30), **60AMF :** 60 Minutes After Morning Feeding (10:00), **30BAF:** 30 Minutes Before Afternoon Feeding (15:30), **60AAF:** 60 Minutes After Afternoon Feeding (17:00).

Table 4. Analysis of variance of different feeding regimens, periods and feeding times on for insulin concentration in male lambs.

SOURCES	df	Mean Square	Sig.
Periods	1	7.246	ns
Period x Group Interaction	2	8.450	ns
Period x Time Interaction	4	0.129	ns
Groups	2	0.490	P<0.05
Times	4	0.399	P<0.05
Eror of Periods	67	0.147	-
Eror of Groups	67	0.113	-

ns: no significant

Data were important only for the periods and sampling times especially after meal in morning. Increase and decrease in insulin levels before and after meal indicate that there was a the relationship between insulin secretion and the termination of spontaneous meals, although GH, insulin, leptin and possibly glucagon may

all be involved in the mechanisms which cause termination of feeding in sheep. In addition we have to keep in mind that satiation signals were arised from multiple sites in the GI system, including the stomach, proximal small intestine, distal small intestine, colon, and pancreas, which had effects on satiation signal. Beside these, there are number of factors sensitive to insulin, for example physiological states of the animals and duration of diets applied.

Table 5. Plasma glucose concentrations in different feeding regimens during four periods with 15 day intervals in male lambs (mg/dl)

Periods	n	I. Period Mean ± SD	II. Period Mean ± SD	III. Period Mean ± SD	IV. Period Mean ± SD
Feeding Regimens Groups					
Ad Libitum	5	91.85 ± 5.37	96.52 ± 11.25	93.33 ± 37.19	107.20 ± 16.58
Once a day	5	88.02 ± 10.3	94.78 ± 5.66	100.66 ± 5.96	112.00 ± 36.98
Twice a day	5	92.83 ± 3.39	93.91 ± 7.89	96.66 ± 8.81	113.60 ± 15.12
Total	**15**	**90.90 ± 6.85**	**95.07 ± 8.02**	**96.88 ± 20.91**	**110.93 ± 23.29**

Data are means Mean ± SD from 5 animals/group. Feeding regimens groups were assigned as fed ad-libitum, fed once a day and fed twice a day lambs.

Table 6. Analysis of variance of different feeding regimens, periods and feeding times on for glucose concentration in male lambs.

SOURCES	df	Mean Square	Sig.
Periods	1	2873.742	P<0.01
Period x Group Interaction	2	78.204	ns
Groups	2	23.192	ns
Eror of Periods	12	319.111	-
Eror of Groups	12	546.292	-

ns: no significant

Table-7 Plasma NEFA concentrations in different feeding regimens during four periods with 15 day intervals in male lambs (mg/dl).

Periods	n	I. Period Mean ± SD	II. Period Mean ± SD	III. Period Mean ± SD	IV. Period Mean ± SD
Feeding Regimens Groups					
Ad Libitum	5	6.13 ± 6.02	3.15 ± 1.69	1.99 ± 0.74	4.64 ± 2.38
Once a day	5	15.5 ± 13.89	6.46 ± 4.52	2.82 ± 0.74	5.14 ± 2.30
Twice a day	5	2.98 ± 0.74	2.48 ± 2.34	3.48 ± 0.90	3.64 ± 3.54
Total	**15**	**8.23 ± 9.82**	**4.03 ± 3.38**	**2.76 ± 0.97**	**4.47 ± 2.67**

Data are means Mean ± SD from 5 animals/group. Feeding regimens groups were assigned as fed ad-libitum, fed once a day and fed twice a day lambs.

Table 8. Analysis of variance of different feeding regimens, periods and feeding times on for NEFA concentration in male lambs.

SOURCES	df	Mean Square	Sig.
Periods	1	153.342	P<0.01
Period x Group Interaction	3	66.126	ns
Groups	3	71.893	ns
Eror of Periods	16	34.421	-
Eror of Groups	16	32.493	-

ns: No significant

It is clear that there was an adverse relation between concentrations of glucose. But we observed that glucose levels in the sheep with programmed feeding in this study did not signifcantly change. Preprandial glucose levels were not significant in each groups, although glucose concentrations showed significant changes during periods (P<0.01). We did not find any relation between insulin and glucose concentration in this study. Other studies with sheep and cattle reported fluctuant results on this topic (Blache 2000; Block 2001). The reason for this different data obtainted in different studies and our work is not clear, but may be because of types of feed, energy levels in feed or feeding frequency, or differences in the physiological states of animals used in studies.

Other hormones which affect glucose metabolism may have influenced the response to feeding which we found. However, because no consistent relationship exists between blood glucose and plasma insulin levels in ruminants (Manns 1967; Horino 1968), it seems unlikely that blood glucose concentrations could be totally responsible for the changes in insulin secretion after feeding. Also, we should consider that in ruminants glucose is constantly synthesized from volatile fatty acids (VFAs), the main energy source, in the liver, and the amount of change in circulating glucose is small.

Zierler (1976) has suggested that muscle in non-ruminants relies mainly on NEFA as an energy source although the evidence for this has largely been obtained from studies in the post-absorptive state. The experiments were planned to determine the contribution of NEFA to the energy metabolisms of scheduled feeding lambs. Our results revealed that NEFA levels in the groups did not change. We could not find correlation among insulin, glucose and NEFA levels. NEFAs are well documented to promote glucose stimulated insulin secretion (Stein 1996) although no study investigating a possible interaction between NEFA, insulin and glucose concentrations during long-term different feeding on lambs has not yet been reported. Because of that, our work is the first study showing the changes of concentrations of these three substances during long term diffent feeding on lambs.

In conclusion, we measured the effects of different feeding-regimens on the concentration of insulin, glucose, NEFA, which had an influence on energy metabolism, blood glucose metabolism and insulin action of lambs in this study. We have demonstrated for the first time that modification of feeding regimen could affect plasma insulin levels. Thus, detailed mechanism between the changes in insulin levels before and after feeding and feeding frequency was estimated. by which feeding frequency influences the variations in the pre-prandial and post-prandial plasma insulin levels to be determined.

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