Daily Profiles of Blood Insulin, Insulin-Like Growth Factor-I, Thyroxine and Triiodothyronine in Ewes Under Three Levels of Feed Intake

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ABSTRACT : Daily variation in the serum concentrations of insulin and insulin-like growth factor-I and in the plasma concentrations of thyroxine and triiodothyronine were evaluated in ewes fed 30%, 100% and 200% of theoretical maintenance energy requirements. The single daily meal has had significant effects ($p<0.05$) on almost all profiles. In general, serum or plasma hormone concentrations have increased after the meal, in particular at the two higher levels of energy intake. In the group submitted to the lowest level of energy intake, the consequences of the meal on circulating levels were almost imperceptible. The effects of feeding levels on serum or plasma concentrations have widely varied among hormones, not showing any objective pattern or relationship. Because these variations may affect the interpretation of these blood indicators, knowledge of daily profiles and of the effect of feed level must be considered. In order to maximize the diagnostic value of those indicators, the most suitable times for blood collection seem to be 16 h after the meal and (or) just before the meal. The collection 16 h after the meal apparently allows the characterization of a relatively steady metabolic state, intermediate between the close effects of food intake and the final phase of the intensification of body reserves mobilization. The collection just before the meal will give a good indication of the level of activity of those mobilization mechanisms. (*Asian-Aus. J. Anim. Sci. 2000. Vol. 13, No. 8 : 1121-1126*)

Key Words : Blood, Insulin, Insulin-Like Growth Factor-I, Thyroxine, Triiodothyronine, Sheep

INTRODUCTION

Blood profiles have been studied for a long time with the purpose of developing adequate methodology to monitor animal metabolic status in different physiological stages (Blowey et al., 1973; Lee et al., 1978; Payne and Payne, 1987; Caldeira, 1995). However, the effects of feeding level and of time of blood collection on serum or plasma concentrations are not well established. Knowledge of daily profiles of these biological indicators is important for a better understanding of the effects of circadian biological cycles and of meals on the metabolism of animals. It can also allow the assessment of the best time in the 24-h period to collect blood in order to get the most accurate picture of metabolic status. Furthermore, in animals fed with a single daily meal, the variation of those indicators in this period can be seen as a simulation of the metabolic response to consecutive phases of food availability and deprivation, a typical cycle in Mediterranean areas. On the other hand, the development of blood analysis techniques, with simpler and more automatic procedures and moderate costs allows their utilization in a more extensive way, especially in dairy cows which genetic enormous improvement lead them to very unstable metabolic conditions.

This experiment was designed specifically to evaluate 24-h variation in some hormones and to determine the most suitable time for blood collection that maximize their diagnostic value in ewes exposed to different levels of feeding in a single daily meal.

MATERIALS AND METHODS

Nine dry and non-pregnant Serra da Estrela ewes, the top Portuguese milk breed of sheep, were assigned randomly to three groups (n=3) and lodged in individual pens. Ewes had the same age (three years) and body condition score (2.25, scale of Russel et al., 1969) and similar liveweights (46.78 ± 1.81 kg).

Diet was composed of grass silage supplemented with barley grain and was offered once daily at 18:00 h. The amount of this diet offered to each animal was calculated to provide 30, 100 and 200% of estimated maintenance energy requirements (Ministry of Agriculture, Fisheries and Food, 1975) respectively in groups 1, 2 and 3. The nutritive values of feed were: grass silage - 22.6% dry matter (DM), 10.8% crude protein (CP) and 6.26 MJ metabolizable energy (ME)/kg DM; barley - 88% DM, 11% CP and 13.5 MJ ME/kg^{-1} DM. The N/MJ ME and rumen degradable protein/MJ ME ratios were, respectively, 1.92 g and 8.4 g. The silage to grain ratio on a DM basis was 1.58 and was constant for all levels of feeding. Water and a vitamin-mineral mixture were provided to animals during the entire trial. Ewes were

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Table 1. Kits used and intra and interassay coefficients of variation obtained

Variables and references of kits	Intraassay CV (%)	Interassay CV (%)
Insulin (Diagnostic Products Corporation, TKIN2)	4.12	9.34
Insulin-like growth factor-I (Nichols, 40-2100)	8.05	11.27
Thyroxine (Bio-Mérieux, 66960)	4.67	8.42
Triiodothyronine (Bio-Mérieux, 66780)	6.52	9.97

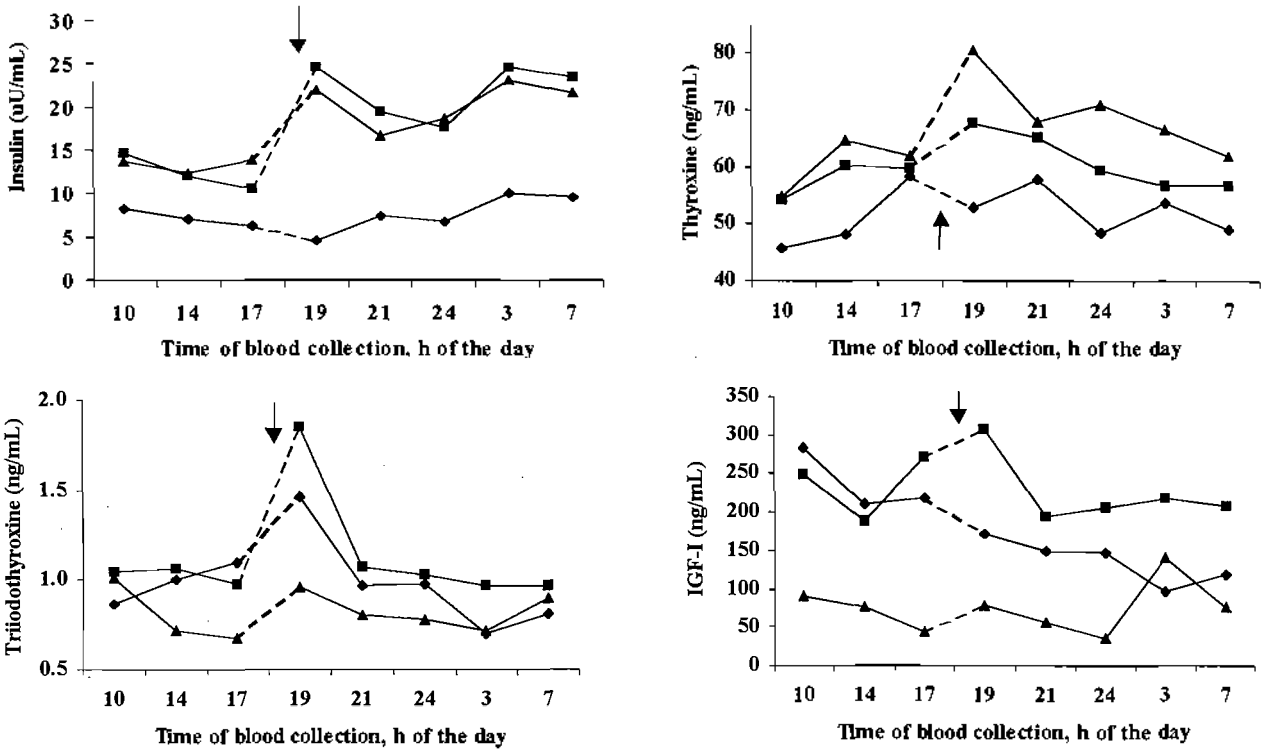

Figure 1. Daily profiles of insulin, thyroxine, triiodothyronine and insulin-like growth factor-I (IGF-I) in the serum or plasma of three groups of ewes fed 30% (Group 1 ◆), 100% (Group 2 ■) and 200% (Group 3 ▲) of maintenance energy requirements in a single daily meal (↓ or ↑)

adapted to this diet over two weeks at a level of feed intake similar to that used later in group 2. They were then subjected to their assigned level of feeding during two more weeks, prior to initiation of blood sampling. In group 3, some leftover feed was occasionally detected. Leftover feed was not weighed but was certainly not sufficient to bring the level of intake of this group closer to that of group 2.

Indwelled catheters (Abbocath-T® venisystems 14G ×51 mm) were inserted into the right external jugular vein of all animals 48 h before blood collection and filled with heparinized isotonic saline. Blood was collected from each ewe at 10:00, 14:00, 17:00, 19:00, 21:00, 24:00, 03:00 and 07:00 h. After centrifugation, the serum and plasma (EDTA) from the blood were frozen under -20℃ until analyses were performed.

Hormone concentrations were determined with commercial kits. The identification of these kits as well as the intra and inter-assay coefficients of variation obtained for each assay method are shown in table 1. Concentrations of insulin (INS) and insulin-like growth factor-I (IGF-I) were determined in serum, whereas concentrations of triiodothyronine (T3) and thyroxine (T4) were determined in plasma.

The effects of treatment and sampling time were determined by ANOVA. The model was based on a "split-plot in time" design (Gill and Hafs, 1971; Steel and Torrie, 1980), with treatment as whole plot variable and sampling time as subplot variable. The error term for the whole plot was ewe (treatment) and the residual error was used for the subplot. When the F-test was significant ($p<0.05$), differences between means were determined by the least square means test.

RESULTS AND DISCUSSION

Insulin

Serum concentrations of INS (figure 1, table 2) clearly show that overall mean of each treatment (mean of values from the eight blood collection times

Table 2. Means and SE of serum or plasma concentrations of hormones in each of eight blood collections during a 24 h period in ewes fed with 30% (Group 1), 100% (Group 2) and 200% (Group 3) of maintenance energy requirements

Hour	10:00	14:00	17:00	19:00	21:00	24:00	3:00	7:00	overall mean
Serum insulin (μU/mL)									
	B a	B ab	B ab	B b	B ab	B ab	B a	B a	B
Group 1	8.16	7.03	6.31	4.69	7.34	6.84	10.10	9.54	7.50
SE	1.51	1.07	1.17	0.01	0.52	0.75	2.44	0.76	0.50
	A bcd	A cd	AB d	A ab	A ab	A abc	A a	A ab	A
Group 2	14.75	12.03	10.47	24.54	19.52	17.60	24.54	23.47	18.36
SE	0.69	2.43	2.55	9.47	1.96	3.18	2.01	2.89	1.61
	AB cd	A d	A cd	A a	A abcd	A abc	A a	A ab	A
Group 3	13.74	12.42	13.79	21.97	16.73	18.56	23.09	21.60	17.74
SE	4.97	3.84	2.04	0.54	3.39	2.47	1.41	0.85	1.17
plasma triiodothyronine (ng/mL)									
	A bc	A abc	A ab	AB a	A bc	A abc	A c	A bc	A
Group 1	0.86	1.00	1.10	1.46	0.97	0.98	0.70	0.81	0.986
SE	0.19	0.13	0.10	0.19	0.21	0.15	0.26	0.15	0.069
	A b	A b	AB b	A a	A b	A b	A b	A b	A
Group 2	1.05	1.06	0.98	1.85	1.07	1.03	0.97	0.97	1.123
SE	0.02	0.10	0.02	0.45	0.12	0.10	0.08	0.11	0.078
	A a	A a	B a	B a	A a	A a	A a	A a	A
Group 3	1.01	0.72	0.67	0.96	0.80	0.78	0.72	0.90	0.820
SE	0.21	0.26	0.09	0.05	0.06	0.14	0.11	0.44	0.066
plasma thyroxine (ng/mL)									
	A a	B a	A a	B a	A a	B a	B a	B a	A
Group 1	45.69	48.15	58.26	52.77	57.67	48.44	53.59	49.10	51.71
SE	6.05	7.21	8.39	6.09	10.09	8.01	8.99	9.68	2.58
	A b	AB ab	A ab	A a	A ab	AB ab	AB ab	AB ab	A
Group 2	54.18	60.12	59.67	67.62	65.01	59.46	56.56	56.62	59.91
SE	5.85	10.83	2.99	8.52	7.15	3.76	2.96	3.92	2.07
	A c	A bc	A bc	A a	A ab	A ab	A bc	A bc	A
Group 3	54.76	64.61	61.91	80.24	67.91	70.83	66.56	61.86	66.08
SE	14.44	13.37	9.60	8.84	6.36	9.92	6.90	13.32	3.48
serum insulin-like growth factor-I (ng/mL)									
	A a	A ab	A ab	A bc	A abc	A abc	B c	AB bc	A
Group 1	282.96	210.78	217.96	171.15	149.99	147.41	96.38	119.35	174.50
SE	74.36	35.26	52.16	13.04	14.49	35.92	2.11	31.08	16.44
	A a	A a	A a	A a	A a	A a	A a	A a	A
Group 2	248.48	187.28	270.56	307.18	193.80	206.48	219.04	208.57	230.17
SE	84.40	42.67	46.81	119.82	64.79	82.08	33.83	42.94	22.24
	B ab	B bc	B c	B ab	B bc	B c	AB a	B bc	B
Group 3	90.71	76.57	45.39	78.68	56.74	37.40	141.40	77.39	75.53
SE	26.19	37.51	15.88	2.32	5.99	7.56	7.26	28.67	8.70

Means within rows with different small letters superscripts differ significantly ($p<0.05$); means within columns (within each hormone) lacking a common capital letter superscript differ significantly ($p<0.05$).

in each group) and the profiles in the 24-h period were quite similar in groups 2 and 3 and rather different from those in group 1. Values in group 1 were lower ($p<0.05$) than those observed in groups 2 and 3, including a weak and late effect of the meal. This increase in insulinemia after the meal is stimulated by the absorption from the gut of amino acids, peptides, propionate and butyrate and is responsible for the subsequent decrease of plasma glucose concentration (Katz and Bergman, 1969; Caldeira, 1995). In a later period, 4 to 7 h after the meal, the gluconeogenesis rate is high compared to

that observed in the postprandial period (Katz and Bergman, 1969). This high gluconeogenesis rate promotes a hyperglycemia, which is probably the main cause for the maintenance of the high insulinemia at this stage. INS daily profiles observed in groups 2 and 3 were similar to those found by others authors (Trenkle, 1970 and 1978; Bassett, 1972 and 1974a, b; De Boer et al., 1991). The apparent absence of a pulse after meal in group 1 was probably due to by the weak neural stimulus promoted by the small amount of food ingested by animals in this group. The inhibition of INS secretion by high serum concentrations of non-esterified fatty acids (De Boer et al., 1985) may also explain this absence of a postprandial pulse. The low level of INS observed in this group can probably be justified by the undernutrition of animals, an effect also observed by other authors in conditions of both fasting and undernutrition (Bassett, 1972; Sano et al., 1992; Ward et al., 1992). INS mean concentrations were not statistically different ($p>0.05$) between maintenance and overnutrition groups in agreement with observations in bovines (Smith et al., 1992). Therefore, insulinemia seems to be particularly sensitive to undernutrition.

Triiodothyronine and thyroxine

The pulses after the meal in groups 1 and 2 were undoubtedly the most remarkable events in T3 plasma concentrations (figure 1, table 2), whereas values observed in group 3 were not significantly different ($p>0.05$) during the 24-h period. On the contrary, T4 plasma concentrations (figure 1) only showed a pulse in group 3, whereas in groups 1 and 2 variations were insignificant. Overall means were not significantly different ($p>0.05$) among the three groups both for T3 and T4, probably due to the small number of animals used and the great variability among animals that had also been observed by other authors (Burrin et al., 1990; Cisse et al., 1991). The pulse after the meal in T3 concentrations could have been produced by an increase in overall metabolism promoted by the intake of food, that would have improved the conversion of T4 to T3. In group 3, this effect was not so expressive possibly because of a prolonged period of food intake due to the high amount offered. However, this amount of food will have probably induced the pulse in T4 secretion in this group. Although not significant ($p>0.05$) in this trial, the direct relationship between the nutritional level and T4 concentrations has been reported previously (Ferguson and Cox, 1975; Blum et al., 1980; Tepperman and Tepperman, 1987). It is probably due to the availability of precursors to the synthesis of this hormone and (or) by the inhibition that the high concentrations of IGF-I, observed in groups 1 and 2, will have had on thyroid-stimulating hormone (TSH) release (Granner, 1988). The high concentration of T4 and the low concentration of T3 in group 3 could possibly be explained by a lack of time to convert all that T4 to T3.

Insulin-like growth factor-I

Daily variation of IGF-I serum concentration did not show any defined pattern in any group (figure 1, table 2), as already observed by others (Cisse et al., 1991; Clement et al., 1991; De Boer et al., 1991). Nevertheless, the decrease of IGF-I in group 1 and its lower overall mean in group 3 deserve some attention.

Serum concentrations of IGF-I seem to vary directly with nutritional level (Kerr et al., 1991; Nugent III et al., 1993; Yung et al., 1996), a relationship that was not observed in this trial in group 3, when compared with the other two groups. The effect of growth hormone (GH) on IGF-I secretion and the complex relationship between GH and the level of nutrition can probably explain these results and must therefore be remembered. In fact, it is well known that GH is the main stimulant of IGF-I secretion (Hodgkinson et al., 1987; Kopchick and Cioffi, 1991) and that GH secretion varies indirectly with the nutritional level (Rhind and Schanbacher, 1991; Bass et al., 1992; Enright et al., 1994). However, this stimulus of GH on IGF-I secretion changes with the availability of nutrients. Undernutrition can consequently produce a resistance to GH, because of a decrease in the number of GH receptors, with the subsequent high GH and low IGF-I serum concentrations (Bass et al., 1992; Ward et al., 1992; Burton et al., 1994). On that account, the relative short period of adaptation to the experimental diets would have possibly determined that the animals were still in a phase of metabolic adjustments to the nutritional levels, which led to different situations in each group:

Group 1 - the gradual decrease in IGF-I concentrations, probably in the presence of a simultaneous increase in GH level, suggests that the resistance to GH was in progress at that time;

Group 2 - the moderate food intake level apparently kept GH and IGF-I secretions balanced;

Group 3 - low IGF-I serum concentrations are possibly an effect of an intermediate phase in this group, where overnutrition will have presumably produced a decrease in GH secretion by this time but not the expected increase in IGF-I level.

Rhind and Schanbacher (1991) observed similar results in ewes whose diets had been changed three weeks before. Ewes with *ad libitum* access to feed showed lower serum IGF-I concentrations than these on a maintenance level.

In conclusion, the single daily meal has had significant effects ($p<0.05$) on almost all profiles. In

general, serum or plasma hormone concentrations have increased after the meal, in particular at the two higher levels of energy intake. In the group submitted to the lowest level of energy intake, the consequences of the meal on circulating levels were almost imperceptible. The effects of feeding levels on serum or plasma concentrations have widely varied among hormones, not showing any objective pattern or relationship. A daily profile was then observed in almost all hormones, which may affect the interpretation of those variables. In order to maximize the diagnostic value of those blood indicators, the most suitable time for blood collection seems to be 16 h after the meal and (or) just before the meal. Collection 16 h after the meal apparently allows the characterization of a relatively steady metabolic state, intermediate between the close effects of food intake and the final phase of the intensification of body reserves mobilization. Sample collection just before the meal will give a good picture of the level of activity of those mobilization mechanisms.

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