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THYROID HORMONES, INSULIN, BODY FAT, AND BLOOD BIOCHEMISTRY INDICES IN DAIRY COWS DURING THE REPRODUCTION/PRODUCTION CYCLE

Paulíková, I.[1], Seidel, H.[2], Nagy, O.[1], Tóthová, Cs.[1] Konvičná, J.[1], Kadaši, M.[1], Kováč, G.[1]

[1]Clinic for Ruminants
[2]Clinic for Swine, University of Veterinary Medicine and Pharmacy, Košice
Slovakia

iveta.paulikova@uvlf.sk

ABSTRACT

This study investigated the changes in: thyroid hormones, amount of subcutaneous fat, and selected indices of blood biochemistry in dairy cows in relation to the reproduction/production cycle. The blood samples were collected both *ante-* and *post-partum* every two weeks. When evaluating the mean values of the investigated indices, the major changes were recorded in dairy cows 3 to 14 days after calving. During this period, we observed a significant decrease in the mean serum levels of T_3 ($P < 0.05$), T_4 ($P < 0.01$), and triglycerides ($P < 0.01$). An opposite trend was observed with a significant increase after calving in the: mean serum levels of β-hydroxybutyrate ($P < 0.05$), urea ($P < 0.01$), and mean AST activities ($P < 0.05$). A significant increase over the normal range was recorded in the average levels of non-esterified fatty acids ($P < 0.01$) and total bilirubin ($P < 0.01$). From the next sampling (28 days after calving) onwards we recorded a significant increase in the blood serum levels of cholesterol ($P < 0.01$), total lipids ($P < 0.01$), total protein ($P < 0.01$), as well as a significant decrease in the insulin levels ($P < 0.05$) and a reduced layer of subcutaneous fat ($P < 0.01$). The blood serum iodine concentration showed only slight significant changes ($P < 0.05$) during the observation. Blood serum levels of glucose did not show any significant changes during the whole observation period. Within the whole observation period we found a negative correlation between T_3 levels and the layer of subcutaneous fat ($r = -0.2606$; $P < 0.05$). This correlation was much more marked in cows 3 to 14 days after calving ($r = -0.5077$; $P < 0.05$), which may indicate a possible relationships between the thyroid status, body condition, and *post partum* negative energy balance.

Key words: body fat volume; dairy cows; insulin; negative energy balance; thyroid

INTRODUCTION

The transitional period in dairy cows includes 3 weeks before and 3 weeks after calving, when the metabolic pro-

cesses are adapting to provide energy and nutrients for the synthesis of milk compounds [15, 39].

During this period, the dairy cows undergo dramatic changes in lipid metabolism during the transition from gestation to lactation [8, 14]. Even during ongoing lactation, homeostatic control of the metabolism varies markedly depending on the stage of lactation [8]. The *post partum* feeding pattern of nutrient intake does not keep up with the requirements. The peak milk production, at about 8 to 10 weeks *post partum*, occurs earlier than the maximum energy intake. Therefore, a negative energy balance (NEB) develops, most severely after parturition [14]. As a consequence, the body fat reserves are mobilized, resulting in elevated non-esterified fatty acids (NEFA) concentrations in the plasma [8]. These are taken up by the liver where they are either processed by the β-oxidation pathway or re-esterified to triglycerides (TG) and exported as very low-density lipoproteins (VLDL) [16]. If the TG synthesis exceeds the TG export capabilities as VLDL, a fatty liver develops [16]. A fatty liver usually provokes other metabolic diseases and reproductive disorders that are initially derived from NEB during early lactation.

A negative energy balance in the transition period is the key factor determining the adaptation of a dairy cow's metabolism [17], including adaptation of the endocrine system, which is crucial to maintaining the metabolic balance [2]. The changes in the endocrine system affects predominately the glucose and lipid metabolism, to ensure the homeorhetic nutrient partitioning towards the prioritized mammary gland despite a catabolic state [14]. This selectivity in directing nutrients coincides with the reduced responsiveness and sensitivity of extrahepatic tissues to insulin, i. e. insulin resistance is thought to be markedly involved in developing ketosis and hepatic lipidosis [19].

Hormonal changes during the transition period are characterized by an increase in growth hormone and a decrease in insulin, thyroid hormones and insulin like growth factor (IGF-I) [30]. A positive correlation between circulating thyroid hormones and energy balance is well known in many species including cattle [3, 4, 23, 33, 34, 36].

The thyroid gland function and hormonal changes in the different reproductive periods in cows have been investigated by many authors [1, 37, 48]. The concentrations of thyroid hormones change significantly during the reproduction cycle. In accordance with the nutritional and metabolic processes during advanced pregnancy, dried cows showed high concentrations of thyroid hormones followed by a significant decrease in the *peri partum* period. Blood levels of thyroid hormones in *peri partum* cows decrease, particularly in early lactation, when the body reserves are mobilized for high milk production [21, 22, 25, 28, 44, 47, 48].

During *post partum* negative energy balance (NEB), dairy cows respond by lowering T_3 and T_4 and increasing rT_3 concentrations [35, 42]. Dairy cows in the first third of lactation showed low concentration of T_3 and T_4 [40], despite recovery of ß-hydroxybutyrate (BHB) and non-esterified fatty acids (NEFA) [12]. The concentrations of T_3 and T_4 correlate negatively with milk yield [48].

This study was aimed at the evaluation of energy metabolism, thyroid hormones, insulin, and body fat thickness in *pre partum* and *post partum* dairy cows.

MATERIALS AND METHODS

The experiments were carried out in accordance with the established standards for animal care and use on a farm near Košice. Dairy cows (n = 21) were in certain phases of *ante partum* (a. p.) and *post partum* (p. p.). The mean production age was 2.5 lactations (3—5 years of age). The milk yield during the previous lactation was 6 668.5 kg milk during a 305-day lactation. The animals were fed a total mix ration (TMR) twice daily, nutrient composition of the TMR varied with the stage of pregnancy and lactation (Table 1). The dairy cows had free access to drinking water.

Blood samples were collected by direct puncture of *vena jugularis*, 3 h after feeding, every two weeks (from the 6th week before expected calving until the 12th week after calving). In the blood serum we analysed the concentrations of: triiodothyronine (T_3), thyroxin (T_4), insulin, iodine (I), aspartate aminotransferase (AST), glucose (Glu), total protein (TP), urea (U), cholesterol (Chol), triglycerides (TG), total lipids (TL), non-esterified fatty acids (NEFA), β-hydroxybutyrate (BHB), and total bilirubin (TBil). The hormones T_4 and T_3 were determined by the ELISA method with the use of commercial ELISA kits (Human, Germany) and microtitration plates. The readings of absorbancies and calculations of the concentrations were done by automatic photometer Opsys MR (Dynex Technologies). The insulin ($IU.ml^{-1}$) was determined by an ELISA method using a commercial assay (Cusabio, China) according to the manufacturer's instructions. The iodine

Table 1. Components of *pre partum* (a. p.) and *post partum* (p. p.) diets [kg.head^{-1}day^{-1}]

	Weeks 6–1 a. p.	Week 1 p. p.	Week 3 p. p.	Week 6 p. p.	Week 9 p. p.
Meadow hay	5.5	1.5	1.5	1.5	1.5
R-24*	0.3	0.25	0.3	0.25	0.25
Haylage	4	4	6	6	6
Alfalfa silage	13	24	22	22	22
Green fodder		25	25	25	25
Soybean meal		0.8	0.8		
Rape meal		2.5	2.5	2.5	2.5
Wheat meal		3	4	2.5	
Limestone		0.2	0.2	0.2	0.2
Flaxseed meal			0.5	1	1
Maize meal				1	
Triticale					3.5

*R-24 — mineral supplement (10.4% Ca; 9% P; 11% Na; 4% Mg; 7000 mg Cu; 3000 mg inorganic Mn; 6000 mg inorganic Zn; 40 mg Se; 100 mg I; 20 mg Co; 1 000 000 IU vitamin A; 100 000 IU vitamin D3; 2 000 IU vitamin E)

concentrations were estimated by a photometric method using a catalytic reaction NO_2^-/SCN^- [49]. The concentrations of: glucose, AST, TP, urea, Chol, TG, and BHB were determined by using commercial diagnostic kits (Randox, UK) on an automatic biochemical analyser Alizé (Lisabio, France). The concentrations of NEFA and TL (Randox, UK) were assessed by the spectrophotometric method Specord 210 Plus (Analytic Jena, Germany). The concentrations of total bilirubin were determined by a classic photometric method according to Jendrassik and Grof [24]. The backfat thickness (BFT) measurements were obtained by using a 3.5 MHz linear transducer and were assessed according to Staufenbiel [45]. The examination site was located in the sacral region between the caudal one-quarter and one-fifth connection line going from the dorsal part of the tuber ischia (pins) to the tuber coxae (hooks). This site corresponds to the area between the end of the crista sacralis and the end of the os sacrum (i. e. beginning of the first coccygeal vertebra). Animals were scored for BFT on the day of their blood collection.

The evaluation of the results was performed by the assessment of the mean values (x) and standard deviations (SD) in each group of dairy cows. The significance of differences in the mean values in relation to the several monitored periods were evaluated by a one-way analysis of variance (ANOVA). The significance of differences in the mean values between groups was evaluated by Tukey's multiple comparisons test. The statistical analyses were done with the GraphPad Prism 3.0 software. The level of significance was set to $P < 0.05$, respectively.

RESULTS

The results of our investigation are presented in tables 2—16. When evaluating the mean values of investigated indices, the major changes were recorded in dairy cows 3 to 14 days after calving. During this period, we observed a significant decrease in the mean serum levels of T_3 ($P < 0.05$) and T_4 ($P < 0.01$) (Tables 2, 3), a decrease in triglycerides ($P < 0.01$) below the normal range (Table 4), and a slight insignificant decrease in the mean serum cholesterol concentrations (Table 5).

An opposite trend, i. e. a significant increase after calving was found in the mean serum levels of β-hydroxybutyrate ($P < 0.05$), urea ($P < 0.01$), and mean AST activities ($P < 0.05$) (Tables 6, 7, 8). A significant increase over the normal range was recorded in the average levels of non-esterified fatty acids ($P < 0.01$) and total bilirubin ($P < 0.01$) (Tables 9, 10).

From the next sampling (28 days after calving) onwards we recorded a significant increase in the blood serum levels of cholesterol ($P < 0.01$) (Table 5), total lipids ($P < 0.01$) and total protein ($P < 0.01$) (Tables 11, 12), as well as a significant decrease in the insulin levels ($P < 0.05$) and reduced layer of subcutaneous fat ($P < 0.01$) (Table 13, 14).

The blood serum iodine concentration showed only a slight significant change ($P < 0.05$) during the observation (Table 15). The blood serum levels of glucose did not show any significant change during whole observation period (Table 16).

Within the whole observation period we found a significant negative correlation between T_3 levels and layer of subcutaneous fat ($r = -0.2606$; $P < 0.05$). This correlation was much more marked in cows 3 to 14 days after calving ($r = -0.5077$; $P < 0.05$).

Table 2. Mean blood serum T_3 concentrations [ng.ml^{-1}] in dairy cows *ante partum* (a. p.) and *post partum* (p. p.)

Sampling	1	2	3	4	5	6	7	8	9
	Days 3—14 p.p.								
x	2.00	1.88	2.03[a]	1.55[b, c]	1.98	2.00	2.11d	1.92	1.82
± SD	0.232	0.377	0.218	0.352	0.391	0.48	0.536	0.398	0.572
ANOVA	**0.0051**								

[a, b; c, d] — values with the superscripts differ at P < 0.05

Table 3. Mean blood serum T4 concentrations [µg.dl^{-1}] in dairy cows a. p. and p. p.

Sampling	1	2	3	4	5	6	7	8	9
	Days 3—14 p. p.								
x	6.82[e]	7.01[a, g]	5.78	4.75[c, f, h]	5.13[b]	5.41	5.86	6.26[d]	5.77
± SD	0.496	1.019	0.885	1.381	1.117	0.63	1.293	1.216	1.46
ANOVA	**0.0001**								

[a, b; c, d] — values with the superscripts differ at P < 0.05; [e, f; g, h] — values with the superscripts differ at P < 0.01

Table 4. Mean blood serum triglycerides concentrations [mmol.l^{-1}] in dairy cows a. p. and p. p.

Sampling	1	2	3	4	5	6	7	8	9
	Days 3—14 p. p.								
x	0.217	0.273[a]	0.265[e]	0.135[b, f, g]	0.281[h]	0.345[c, h]	0.285[h]	0.231[d]	0.206[d]
± SD	0.074	0.085	0.084	0.055	0.169	0.109	0.091	0.031	0.037
ANOVA	**0.0001**								

[a, b; c, d] — values with the superscripts differ at P < 0.05; [e, f; g, h] — values with the superscripts differ at P < 0.01

Table 5. Mean blood serum cholesterol concentrations [mmol.l^{-1}] in dairy cows a. p. and p. p.

Sampling	1	2	3	4	5	6	7	8	9
	Days 3—14 p. p.								
x	2.6[e]	2.8[g]	2.2[i]	2.2[d, k]	3.3[b, c, m]	4.7[f, h, j, l, n]	4.8[f, h, j, l, n]	5.6[f, h, j, l, n]	4.7[d, f, h, j, l, n]
± SD	0.35	0.59	0.30	0.51	0.86	0.98	1.37	1.54	0.78
ANOVA	**0.0001**								

[a, b; c, d] — values with the superscripts differ at P < 0.05; [e, f; g, h; i, j; k, l; m, n] — values with the superscripts differ at P < 0.01

Table 6. Mean blood serum BHB concentrations [mmol.l^{-1}] in dairy cows a. p. and p. p.

Sampling	1	2	3	4	5	6	7	8	9
	Days 3—14 p. p.								
x	0.355	0.429	0.429	0.762	0.794	0.554	0.514	0.418	0.506
± SD	0.044	0.15	0.094	0.285	0.927	0.292	0.257	0.196	0.134
ANOVA	**0.035**								

Table 7. Mean blood serum urea concentrations [mmol.l^{-1}] in dairy cows a. p. and p. p.

Sampling	1	2	3	4	5	6	7	8	9
	Days 3—14 p. p.								
x	2.2a	2.3c	2.7e	4.6b,d,f	4.9b,d,f	5.7b,d,f	5.2b,d,f	4.9b,d,f	4.2b,d,f
± SD	0.73	0.63	0.84	1.87	1.27	1.24	1.19	0.97	0.90
ANOVA	**0.0011**								

[a, b; c, d; e, f] — values with the superscripts differ at P < 0.01

Table 8. Mean blood serum AST activities [µkat.l^{-1}] in dairy cows a. p. and p. p.

Sampling	1	2	3	4	5	6	7	8	9
	Days 3—14 p. p.								
x	1.35	1.19a	1.19c	1.74b,d	1.54	1.54	1.58	1.41	1.40
± SD	0.221	0.135	0.205	0.477	0.358	0.509	0.466	0.197	0.166
ANOVA	**0.002**								

[a, b] — values with the superscripts differ at P < 0.05; [c, d] — values with the superscripts differ at P < 0.01

Table 9. Mean blood serum NEFA concentrations [mmol.l^{-1}] in dairy cows a. p. and p. p.

Sampling	1	2	3	4	5	6	7	8	9
	Days 3—14 p. p.								
x	0.347[a]	0.513[c]	0.595[e]	1.566[b, d, f, g]	0.941[h]	0.815	0.702[h]	0.632[h]	0.406[h]
± SD	0.102	0.22	0.264	0.63	0.397	0.394	0.282	0.393	0.111
ANOVA	**0.0001**								

[a, b; c, d; e, f; g, h] — values with the superscripts differ at P < 0.01

Table 10. Mean blood serum total bilirubin concentrations [µmol.l^{-1}] in dairy cows a. p. and p. p.

Sampling	1	2	3	4	5	6	7	8	9
	Days 3—14 p. p.								
x	4.67[a]	5.10[c]	5.06[e]	9.85[b, d, f, g]	7.36	5.98[h]	5.94[h]	5.49[h]	4.86[h]
± SD	0.542	1.036	1.107	4.484	4.621	1.26	1.355	1.618	1.187
ANOVA	**0.0001**								

[a, b; c, d; e, f; g, h] — values with the superscripts differ at P < 0.01

Table 11. Mean blood serum total lipid concentrations [g.l^{-1}] in dairy cows a. p. and p. p.

Sampling	1	2	3	4	5	6	7	8	9
	Days 3—14 p. p.								
x	3.95$^{a, g}$	3.9$^{c, e, i}$	3.7^{k}	4.1^{m}	5.4$^{d, o}$	7.1$^{h, j, l, n, p}$	6.9$^{h, j, l, n, p}$	6.5$^{h, j, l, n, p}$	5.7$^{b, f, l, n}$
± SD	0.301	0.24	0.21	0.722	1.449	1.04	1.4	0.624	0.732
ANOVA	**0.0001**								

$^{a, b; c, d; e, f}$ — values with the superscripts differ at P < 0.05; $^{g, h; i, j; k, l; m, n; o, p}$ — values with the superscripts differ at P < 0.01

Table 12. Mean blood serum total protein concentrations [g.l^{-1}] in dairy cows a. p. and p. p.

Sampling	1	2	3	4	5	6	7	8	9
	Days 3—14 p. p.								
x	72.0^{a}	72.6^{e}	71.3$^{c, g}$	75.1^{i}	79.3	79.8^{d}	81.6^{h}	84.1$^{b, f, h, j}$	81.8^{d}
± SD	11.96	5.69	4.77	6.15	6.40	5.558	6.96	7.76	7.50
ANOVA	**0.001**								

$^{a, b; c, d}$ — values with the superscripts differ at P < 0.05; $^{e, f; g, h; i, j}$ — values with the superscripts differ at P < 0.01

Table 13. Mean blood serum insulin concentrations [IU.ml^{-1}] in dairy cows a. p. and p. p.

Sampling	1	2	3	4	5	6	7	8	9
	Days 3—14 p. p.								
x	589.8	577.6	616.0	624.4	654.3	353.9	394.6	374.9	392.9
± SD	94.1	37.8	181.5	553.7	575.1	115.5	155.9	157.8	128.5
ANOVA	**0.0434**								

Table 14. Mean thickness of subcutaneous fat [cm] in dairy cows a. p. and p. p.

Sampling	1	2	3	4	5	6	7	8	9
	Days 3—14 p. p.								
x	3.30^{a}	3.39^{e}	3.45	3.02$^{c, g}$	2.50^{f}	2.35$^{b, d, f}$	2.35$^{b, f, h}$	2.39	2.41
± SD	1.025	0.555	0.092	0.491	0.513	0.404	0.318	0.226	0.253
ANOVA	**0.0001**								

$^{a, b; c, d}$ — values with the superscripts differ at P < 0.05; $^{e, f; g, h}$ — values with the superscripts differ at P < 0.01

Table 15. Mean blood serum iodine concentrations [μg.l^{-1}] in dairy cows a. p. and p. p.

Sampling	1	2	3	4	5	6	7	8	9
	Days 3—14 p. p.								
x	55.30	55.55	56.21	56.43	53.57	51.65	52.18	51.82	52.30
± SD	3.83	6.191	4.224	10.013	3.287	1.622	2.011	1.611	0.526
ANOVA	**0.0401**								

Table 16. Mean blood serum glucose concentrations [mmol.l^{-1}] in dairy cows a. p. and p. p.

Sampling	1	2	3	4	5	6	7	8	9
	Days 3—14 p. p.								
x	3.98	3.92	3.85	3.64	3.77	3.95	3.82	3.85	3.56
± SD	0.128	0.195	0.245	0.966	0.413	0.425	0.344	0.301	0.213
ANOVA	**n. s.**								

n. s. — non-significant

DISCUSSION

Within our observations, the indices of energy and lipid metabolism corresponded to the well-known findings reported in fresh cows [2, 8, 9, 13, 14, 16].

The blood serum levels of glucose, which are considered to be a direct indicator of energy balance [38], did not show any significant changes during the entire observation period. However, a more reliable indicator of the cow's energy status is the concentration of β-hydroxybutyrate [41, 46]. Within our observations, a decrease in triglycerides ($P < 0.01$) below the normal range, as well as an increase in the mean serum levels of β-hydroxybutyrate ($P < 0.05$), and non-esterified fatty acids ($P < 0.01$) in dairy cows 3 to 14 days after calving indicated some degree of negative energy balance.

The negative energy balance in the transition period is the key factor determining adaptation of a dairy cow's metabolism [17], including adaptation of the endocrine system, which is crucial in order to maintain the metabolic balance [2]. Hormonal changes in the transition period are characterized by an increase in growth hormone and a decrease in insulin, thyroid hormones and insulin like growth factor (IGF-I) [31]. A similar decrease in the mean serum insulin and thyroid hormones was observed also in our observation. The level of insulin in bovine serum strongly correlates with the body weight increase rate [11, 18, 20] and limited feed intake by the animals results in a decrease in the content of insulin in blood serum [53]. A decrease in insulin after calving is probably related to inappetence, which is typical during the periparturient period [9]. The plasma insulin is additionally known to suppress lipolysis from adipose tissues [19]. However, the plasma insulin concentration in dairy cows is decreased after parturition and enables, along with concomitant insulin resistance and the associated loss of inhibitory effects on lipolysis, the high degree of metabolic priority [14].

Limited food energy content decreases also the serum levels of triiodothyronine (T_3) and thyroxin (T_4) [11]. The thyroid hormones are of importance in adapting the endocrine system during lactation, since their very low blood levels in peri-partal cows leads to a decrease in energy metabolism, mobilization of body fat reserves and their partitioning towards high milk production [21, 29, 48].

Periparturient hormonal changes including thyroid hormones and their relation to lipid metabolism and body condition has been studied by many authors [5, 6, 7, 10, 17, 26, 27, 31, 32, 50].

Kapp et al. [26] suggested an important role of an endocrine disorder, particularly thyroid, in the pathogenesis of liver steatosis in high-yielding Holstein-Friesian dairy

cows. The authors consider "fatty liver" syndrome as a consequence of hypothyreoidosis, when insufficient thyroid function (low serum levels of T_4 and T_3) leads to endocrine dysfunctions, liver disorders and frequent puerperal complications.

A decrease in circulating T_4 and T_3 hormones and functionless thyroid hypertrophy was observed in dairy cows suffering from adipose-hepatic fat syndrome. Presumably, there is protein-energy (or another) deficiency accompanied by obesity [27]. This syndrome is associated with hormonal imbalance and metabolic disorders followed by reproduction disorders (stillbirths, retained placenta, metritis, low fertility).

Durdevič et al. [10] compared T_4 and T_3 levels in the blood serum of cows with and without ketosis. In dairy cows with ketosis they found significantly lower hormone levels: T_4 0.7 ± 0.4 vs. 3.6 ± 1.1 µg.dl^{-1}; T_3 0.83 ± 0.22 vs. 1.22 ± 0.23 ng.ml^{-1}. Similarly, Djokovič et al. [7] studied the blood concentrations of thyroid hormones, lipids, glucose, and liver lipid content in dairy cows during the transitional period. In ketotic dairy cows, they suggested established a hypothyroidal status. Kostopanagiotou et al. [32] reported during acute liver failure markedly decreased serum thyroxin (T_4) and triiodothyronine (T_3) levels, whereas free-triiodothyronine and thyroxin-stimulating hormone levels did not change. T_4 and T_3 levels correlated with the degree of liver failure. Gvozdič et al. [17] reported significantly lower thyroid hormone levels in obese cows. Over conditioned dry dairy cows showed also decreased insulin sensitivity and decreased insulin responsiveness of the glucose metabolism [6], which may contribute to lipomobilisation level.

These data indicate an association between thyroid status, level of lipomobilisation and body condition, respectively. Within all of our observation period, we found a significant negative correlation between T_3 levels and the layer of subcutaneous fat ($r = -0.2606$; $P < 0.05$). This correlation was much more marked in cows 3 to 14 days after calving ($r = -0.5077$; $P < 0.05$). However, there is a question of what is primary and what is secondary — low thyroid status or over conditioning. It is well known that hypothyroidism causes a weight increase together with a decrease in basal metabolic rate [43, 52]. On the other side, obesity also contributes to thyroid dysfunction in a form of mildly elevated TSH levels [51].

CONCLUSIONS

Within our observations, the indices of energy and lipid metabolism indicate some degree of negative energy balance and corresponds to well-known findings reported in fresh cows. The analyses of thyroid hormones and back fat thickness indicate an association between thyroid status, body fat volume, degree of negative energy balance, and the level of lipomobilisation. However, the cause of lower thyroid status in cows with subcutaneous fat needs to be clarified.

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List of abbreviations

ANOVA	analysis of variance
a. p.	*ante-partum* (*pre-partum*)
AST	aspartate aminotransferase
BFT	backfat thickness
BHB	β-hydroxybutyrate
Glu	glucose
I	iodine
IGF-I	insulin like growth factor
p. p.	*post-partum*
T_3	triiodothyronine
T_4	thyroxin
TBil	total bilirubin
TG	triglycerides
TL	total lipids
TMR	total mix ration
TP	total protein
NEB	negative energy balance
NEFA	non-esterified fatty acids
SD	standard deviation
U	urea
VLDL	very low-density lipoproteins
x	mean value

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