Acta Veterinaria (Beograd), Vol. 56, No. 1, 25-36, 2006.

UDK 619:612.018.2

ADRENOCORTICAL AND THYROID FUNCTION, HORMONE AND METABOLITE PROFILES AND THE ONSET OF OVARIAN CYCLICITY IN DAIRY COWS SUFFERING FROM VARIOUS FORMS OF KETOSIS

HUSZENICZA GY*, KULCSÁR M*, KÓRÓDI P*, BARTYIK J*, RUDAS P*, RIBICZEI-SZABÓ P*, NIKOLIĆ JUDITH ANA**, ŠAMANC H***, IVANOV I*** and GVOZDIĆ D***

**University of Veterinary Science, Budapest, Hungary*
***Institute for the Application of Nuclear Energy, Zemun*
****Faculty of Veterinary Medicine, Beograd*

(Received 27 May 2005)

The involvement of adrenocortical and thyroid hormones in the pathogenesis of ketosis, as well as the ovarian consequences of this metabolic disorder, were studied in ≥2 parity cows (n=199) in 3 large scale dairy herds. To compare the plasma/serum concentrations of certain hormones [cortisol, thyroxin (T_4), triiodothyronine (T_3), insulin, insulin-like growth factor-1 (IGF-1)] and metabolites [glucose (G), acetoacetic acid (ACAC), βOH-butyrate (BHB), non-esterified fatty acid (NEFA), trigliceride (TG), total cholesterol (TCh)], and the activity of aspartate aminotransferase (AST), blood samples were taken 1 to 3 days after calving and again 4 times 7 days apart. The ACTH-challenged cortisol responsiveness and the TRH-induced T_4/T_3 increase were determined between days 1 to 3 and again between days 28 to 35. The resumption of ovarian cyclicity was followed up by individual progesterone (P4) profiles based on milk samples taken 3 times a week for about 80 to 85 days. BHB level of 1 mmol/L was estimated as a border line between hyper- (>1 mmol/L) and normoketonaemic (<1 mmol/L) conditions. Five different ketone patterns were distinguished: (1) non-ketotic (n=98; normoketonaemia in all samples), (2) early type ketosis (n=45; hyperketonemia was detected only in the first week after calving), (3) late type (lactational) ketosis (n=11; after a normoketonaemic period increasing hyperketonaemia was detected in the 5th, or in the 4th and 5th weeks), (4) temporary ketosis (n=11; hyperketonaemia was detected for 1-2 weeks in the 2nd and 3rd or in the 3rd and 4th weeks); (5) long-lasting ketosis (n=34; hyperketonaemia has been detected since calving for 4 to 5 weeks or until dying / emergency slaughtering). Simultaneously with the hyperketonaemic stage increased NEFA, ACAC, depressed TCh, glucose and decreased insulin, IGF-1, T_4 and T_3 concentrations were detected in almost all the cases. Obvious metabolic and endocrine alterations were found, however, only in long-lasting ketosis. The TRH-stimulated T_4 and T_3 responses remained almost unaffected proving intact thyroid function in early and late type as well as in temporary ketosis. Depressed thyroid

response and delayed onset of cyclic ovarian function were detected only in cases of long-lasting ketosis. The cows characterized by lower than normal (<mean-SD of non-ketotic cows) ACTH-stimulated cortisol response on days 1-3 after calving showed poorer chance for spontaneous recovery. There was a significant negative correlation between the IGF-1 level in the 1st week after calving and the duration of the postpartum acyclic period. In late type (lactational) ketosis the cessation of ovarian cyclicity was the most characteristic genital malfunction.

Key words: ketosis, dairy cows, adrenal, thyroid, ovary

INTRODUCTION

Ketosis is the known consequence of the unbalanced energy supply to high-producing postpartum dairy cows (Duffield *et al*., 1998). Several endocrine aspects of this metabolic disorder have already been reported (Nikolić *et al*., 1997). The lower than normal rate of insulin to glucagon and growth hormone is proved as its main endocrine predisposing factor (Sartin *et al*., 1988). The involvement of thyroid hormones in the postpartum adaptation of energy metabolism is a generally accepted idea, and glucocorticoid containing preparations have been widely used in the therapy of bovine ketosis for many years (Baird, 1982). The possible role of these hormones, as well as of the (mal)function of the thyroid gland and/or the adrenal cortex, in the pathogenesis of ketosis was supposed a long time ago (Hill *et al*., 1950; Robertson *et al*., 1957). These ideas, however, have remained poorly documented. In most of the trials only the circulating total thyroxine (T_4), 3,3',5-triiodo-thyronine (T_3), and cortisol levels were determined (Tiirats, 1997; Đurđević *et al*., 1980). The postpartum changes in blood level of the inactivated thyroid hormone formation (3,3',5'-triiodothyronine or reverse-triiodothyronine, rT_3) were followed-up only in cows with various degrees of energy imbalance (Tiirats, 1997; Kunz and Blum, 1985; Pethes *et al*., 1985), but not in those affected by ketosis. There are only limited data on the determination of standard low dose adrenocorticotrophin hormone (ACTH)-induced cortisol response (Kolk, 1991; Andriaens *et al*., 1995; Lay *et al*., 1996), as well as of the thyrotrophin releasing hormone (TRH) challenged T_4/T_3 increases (Lapierre *et al*., 1990; Tveit *et al*., 1990a; Tveit *et al*., 1990b; Andriaens *et al*., 1995), which can inform us on the functional reserve capacity of these endocrine glands. In addition, the degree of energy imbalance and/or the presence of hyperketonemia were identified in few of these studies (Nikolić *et al*., 1977). The actual levels of the biologically active, protein-unbound T_4, T_3, and cortisol levels remained unknown in healthy and different level of hyperketonaemic postpartum cows. The low concentration of insulin-like growth factor-1 (IGF-1) was proved to reflect the degree of energy imbalance in cows early in their lactation (Chelikani *et al*., 2004; Zulu *et al*., 2002), however, limited data are available on hyperketonaemic individuals. Recently a stimulatory role has been attributed to both insulin and IGF-1 in follicular growth and maturation

(Diskin *et al*., 2003; Whates *et al*., 2003), directing the attention of gynaecologists to the postpartum changes of these hormones nowadays. Butler *et al*., (1981) and many others clearly demonstrated that the first postpartum ovulation takes place about 10 days after the nadir of the energy imbalance in the non-suckled dairy cows. Thereafter the cow becomes cyclic. The negative influence of the energy-imbalance on reproductive performance has become widely accepted (Lucy, 2003; Taylor *et al*., 2002). However, its detrimental effect on the resumption of cyclic ovarian function is only poorly documented and the possible involvement of insulin and IGF-1 levels in the supposed (Zulu *et al*., 2002), hyperketonaemia-induced delay of the onset of ovarian cyclicity has not been clearly demonstrated yet. This trial was conducted to study whether there are any interrelations of circulating ketone body (e.g. acetoacetic acid, ACAC and OH-butyrate, BHB) levels with (1) andrenocortical and/or thyroid gland functions, (2) circulating insulin and IGF-1 concentrations and (3) the onset of cyclic ovarian activity.

MATERIALS AND METHODS

Experimental animals

Interrelationships of circulating ketone body (e.g. acetoacetic acid, ACAC and OH-butyrate, BHB) levels with (1) andrenocortical and/or thyroid gland functions, (2) circulating insulin and IGF-1 concentrations and (3) the onset of cyclic ovarian activity were studied in ≥2 parity cows in 3 large-scale dairy herds with about 1850, 500 and 950 animals. All individuals calving within a pre-selected period in October-November (Farm A and B) or in May-June (Farm C) were involved in the study unless they needed veterinary intervention at calving, calved twins and/or showed clinical symptoms of parturient paresis just after calving. All cows on these farms including those enrolled in this study were kept and fed in groups of 80 to 100 individuals under free housing conditions with no possibility for individual feeding or pasturing. These groups of cows were formed in accordance with their monthly checked daily milk yield. Their daily ration was made up from ensilaged maize and alfalfa products, alfalfa and grass hay and cereals completed with vitamins and minerals, in accordance with the NRC (1989) recommendations.

In total, data of 199 cows were evaluated (milk production in previous lactation: 5069 to 16 291 kg FCM). Using 1 mmol/L of BHB threshold as a cut off value 5 different ketone patterns could be distinguished: (1) *Non-hyperketonemic* (n=98): normoketonemia in all samples; (2) *Early type hyperketonemia* (n=45): hyperketonemia was detected only in the first week after calving (3) *Late type (lactational) hyperketonemia* (n=11): after a normoketonemic period increasing hyperketonemia was detected in the 5th (or 4th and 5th) week(s); (4) *Temporary hyperketonemia* (n=11): (usually mild) hyperketonemia was detected for 1-2 weeks in the 2nd and 3rd or 3rd and 4th weeks; (5) *Long-lasting hyperketonemia* (n=34): (usually severe) hyperketonemia has been detected since calving for 4 to 5 weeks or until dying / emergency slaughtering.

Blood sampling, endocrine and metabolic parameters determination

To assay the concentrations of cortisol, T_4, T_3, insulin, IGF-1, and glucose, ACAC, BHB, non-esterified fatty acid (NEFA), trigliceride (TG) and total cholesterol (TCh), as well as the activity of aspartate aminotransferase (AST) enzyme, blood samples were taken 1 to 3 days after calving and again 4 times 7 days apart. All these samples were taken before the morning milking. We have used 1 mmol/L of BHB level as a border line between *hyperketonemic* (>1 mmol/l) and *normoketonemic* (<1 mmol/L) conditions.

The ACTH-challenged cortisol responsiveness and the TRH-induced T_4/T_3 increase were estimated on days 1 to 3 and again on days 28 to 35. For this reason 60 minutes after the morning milking blood samples were collected to determine the baseline cortisol, T_4 and T_3 levels. Just after sampling 60 g $_{1\text{-}24}$ACTH (*Cortrosin inj.*®, Organon, Oss, The Netherlands) and 400 g TRH (*pGLU-HIS-PRO amide acetate salt*, Sigma, St. Louis, USA) dissolved in saline were administered in the jugular vein simultaneously. The endocrine response was determined 60 (cortisol), 240 and 360 (T_4 and T_3) minutes later.

The resumption of ovarian cyclicity was followed up by individual progesterone (P4) profiles based on ELISA determination of P4 from defatted milk samples taken 3 times a week for about 80 to 85 days after calving. The laboratory procedures used for all these determinations were validated for bovine samples, as described elsewhere (Huszenicza et al., 1998; Nikolić et al., 2003; Meikle et al., 2004). The milk production was checked every 7 days for 10 weeks and once a month thereafter. Body weight was measured on days 1-3, 28-35 and 63-70. Clinical events and data on reproduction were followed for 150 days after calving.

Statistical analysis

Data were analyzed using Students t-test, Chi-square test, Pearson correlation coefficients, regression analysis, or by one way analysis of variance. If the between-group differences were significant ($P<0.05$ or more), the least significant difference (LSD) was given on 5 % level for pair-wise comparison of group means (Kleinbaum and Kupper, 1978; Juvancz and Paksy, 1982; Snedecor and Cochran, 1982).

RESULTS AND DISCUSSION

Values of insulin, IGF-1, T_4 and T_3 concentrations in cows with different ketone bodies pattern are given in the Table 1. Simultaneously, with the hyperketonemic stage we have recorded increased NEFA and ACAC concentrations, depressed TCh and glucose levels, elevated AST activity (*details are not given*), as well as decreased insulin, IGF-1, T_4 and T_3 concentrations in almost all the cases. There is evidence that peripheral plasma concentrations of insulin, T_4 and T_3 are positively correlated with energy balance in dairy cows in the period from 5-70 days after parturition (Kunz *et al*., 1985). It also seemed possible to increase milk production by feeding a iodinated casein containing 1% of

Table 1. Base-line levels (mean ± SD) of *insulin*, *IGF-1* and *thyroid hormones* in cows showing various ketone patterns

		Day 1-3	Week 2	Week 3	Week 4	Week 5	Day 1-3	Week 2	Week 3	Week 4	Week 5
		Insulin (IU/l)					Insulin-like growth factor 1 (g/l)				
Non-hyper-ketonemic (n=98)	mean	4.67	2.95	3.28	4.17	5.02	26.82	21.89	29.79	35.14	41.61
	SD	3.10	1.87	2.14	2.79	3.24	15.52	13.36	15.28	17.25	18.34
Early type hyper-ketonemia (n=45)	mean	1.32	1.22	2.40	3.27	5.27	15.18	13.44	26.50	32.82	46.53
	SD	0.97	0.74	1.18	1.41	3.15	5.23	4.36	13.66	13.90	21.83
Late type hyper-ketonemia (n=11)	mean	3.23	2.51	2.07	1.44	1.40	25.31	15.04	10.17	11.68	12.80
	SD	1.11	0.94	1.14	0.86	0.92	10.81	5.39	3.24	2.04	3.37
Temporary hyper-ketonemia (n=11)	mean	3.59	2.05	2.86	3.00	3.40	22.68	18.02	18.08	22.81	25.47
	SD	1.83	1.08	1.38	1.48	2.02	6.18	6.68	7.81	8.79	12.21
Long-lasting hyper-ketonemia (n=34	mean	2.28	1.33	1.51	1.84	2.36	15.82	10.56	13.46	17.22	26.41
	SD	2.03	0.91	0.88	1.15	1.58	7.26	5.71	4.65	8.32	12.46
F =		*16.62*	*14.47*	*6.65*	*7.98*	*8.11*	*10.09*	*10.21*	*12.88*	*12.57*	*12.66*
$LSD_{(P<0.05)}$ =		1.29	0.78	0.94	1.24	1.63	6.34	5.41	7.14	8.22	10.05
		Thyroxine (nmol/l)					Triiodothyronine (nmol/l)				
Non-hyper-ketonemic (n=98)	mean	31.27	28.25	33.09	38.10	43.28	1.31	1.22	1.38	1.53	1.77
	SD	10.21	9.52	8.48	7.97	11.05	0.29	0.29	0.36	0.34	0.40
Early type hyper-ketonemia (n=45)	mean	25.50	22.25	29.69	35.73	43.57	1.03	1.02	1.23	1.45	1.65
	SD	7.03	5.50	8.61	8.44	10.34	0.18	0.18	0.28	0.30	0.38
Late type hyper-ketonemia (n=11)	mean	32.38	29.08	28.64	28.65	27.04	1.40	1.20	1.20	1.09	1.03
	SD	6.20	4.91	7.37	4.81	7.37	0.28	0.17	0.23	0.17	0.11
Temporary hyper-ketonemia (n=11)	mean	31.08	24.50	22.30	28.90	40.57	1.28	1.04	0.89	1.05	1.54
	SD	7.73	5.47	3.81	7.68	13.06	0.18	0.17	0.14	0.23	0.39
Long-lasting hyper-ketonemia (n=34)	mean	23.31	19.63	20.05	25.26	29.97	0.95	0.74	0.81	0.97	1.15
	SD	9.30	8.06	5.69	7.71	11.86	0.22	0.18	0.26	0.24	0.28
F =		*6.94*	*9.53*	*19.46*	*19.52*	*14.00*	*20.11*	*25.53*	*20.68*	*22.05*	*20.03*
$LSD_{(P<0.05)}$ =		4.82	4.30	4.18	4.23	5.92	0.13	0.13	0.17	0.17	0.21

thyroxine to lactating cows (Shaw *et al*., 1975), but other authors did not confirm an association between milk yield and thyroid hormone concentrations (Hoshino *et al*., 1991). Investigating thyroid hormones in the blood of ketotic cows Đurđević *et al*., (1980) found significantly lower levels of T_3 and T_4 in comparison to healthy cows. More recent investigation confirmed those previous results (Nikolić *et al*., 1997), even though the authors remarqued the possibility that some of the experimental animals may have been suffering from a functional iodine deficiency. Decrease in the serum insulin and IGF-I levels in almost all ketotic cows in our experiment probably reflects the fact that the balance between anabolic and catabolic hormones due to the NEB is set to a lower point.

Functional tests of adrenal and thyroid gland in ruminants have been used in clinical endocrinology during early 1980ies (Hurley *et al*., 1981; Alan *et al*., 1981). Since that time they have been widely accepted (Tveit *et al*., 1990a; Tveit *et al*., 1990b; Kolk, 1991; Rumsey *et al*., 1999; Bage *et al*., 2000).

Obvious metabolic and endocrine alterations were found, however, only in cows with long-lasting hyperketonemia. Compared with those of non-hyperketonemic animals, in cows with early and late type, as well as with temporary hyperketonemia, the TRH-stimulated T_4 and T_3 responses were only slightly lower, proving an almost intact thyroid function. A more depressed response was detected, however, in cases of long-lasting ketosis. The peak level of T_3 seen at 240 min. after the TRH remained almost unchanged in normoketonemic condition, but decreased significantly if hyperketonemia was detected simultaneously (Fig. 1).

In dairy cows at the begining of lactation, during the period of NEB, plasma concentrations of T4 and T3 decrease, while the rT3 level increases (Pethes *et al*., 1985; Ronge *et al*., 1989; Rudas, 1990; Rudas, 1994; Tiirats, 1997; Huszenicza *et al*, 2002; Stojić *et al*., 2001). Kapp *et al*., (1979) noticed significant changes in thyroid gland histology, in cows that died because of the fatty liver syndrome. Potentialy decreased T_4-response to TRH in cases of long-lasting hyperketonemia could be induced by the functional damage of thyroid epithelial cells. Plasma levels of T_3 in cows with hyperketonemia lasting 240. and 360. minutes was significantly decreased compared to the normoketonemic animals. This could be explained by the increased activity of 5D enzyme system that inactivates T_4 and T_3 (Pethes *et al*., 1985; Rudas, 1990; Rudas, 1994), which is in accordance with significantly higher plasma level of rT_3 in cows with hyperketonemia.

Our results indicate that, on day 1-3 after calving, cows showing lower than normal ACTH-stimulated cortisol response (*hypocorticism*: <mean-SD of non-hyperketonemic cows; e.g. <44 nmol/L) could hardly tolerate the simultaneous hyperketonaemia and had poorer chance for recovery. Altogether 79 cows had elevated BHB levels at that time, and 14 of them were characterized also with hypocorticism. Eight of them were lost due to ketosis, whereas the rate of dying was only five out of 65 ($P<0.001$) among the hyperketonemic individuals with normocorticism. Application of ACTH in a dose of 25 IU/100 kg BW could effectively increase serum cortisol and glucose levels in ketotic cows (Đurđević *et al*., 1985). It seems that in the case of reduced cortisol response ketotic cows are

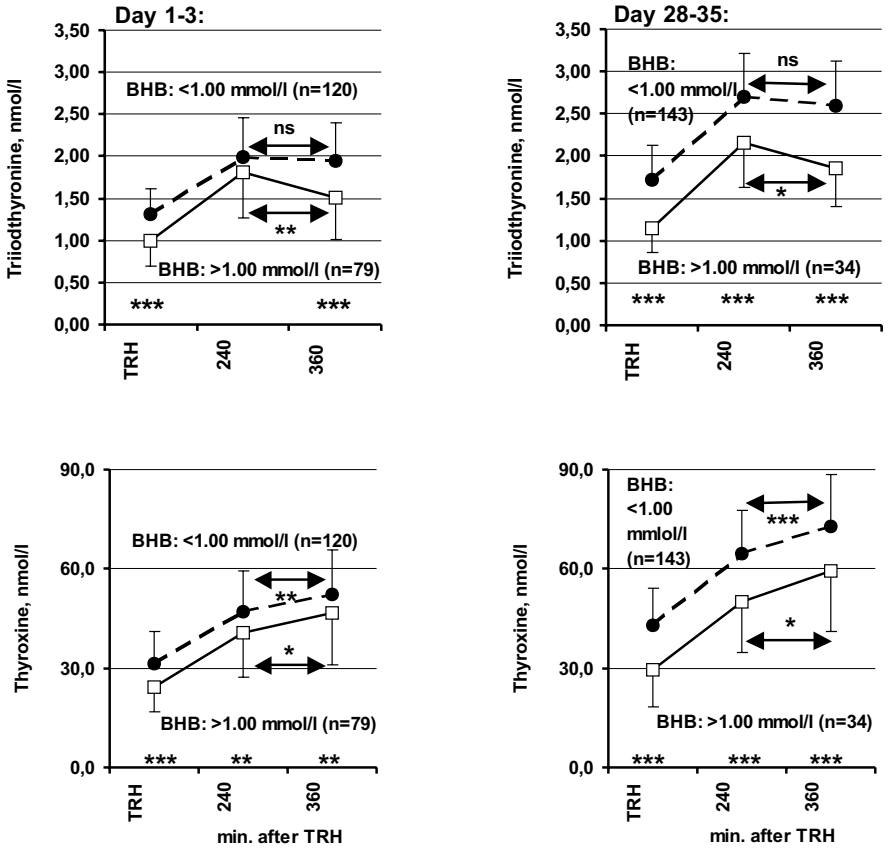

Figure 1. Changes in T_4 and T_3 levels (mean±SD) after TRH challenge in cows with *normoketonemic* (BHB level: <1.00 mmol/l) vs. *hyperketonemic* (BHB level: >1.00 mmol/l) conditions (ns: not significant difference; *P<0.05; **P<0.01; ***P<0.001)

unable to sustain metabolic stress induced by hyperketonemia. Similar situation could be found in the *E.coli* mastitis, where endotoxin initiates delayed cortisol response due to the release of TNF (Jánosi, 2002).

Only the cows with long-lasting hyperketonemia were characterized with delayed onset of cyclic ovarian function (Table 2).

There was a significant negative correlation between the insulin and IGF-1 levels in the 1st week after calving and the duration of the postpartum acyclic period. In late type (lactational) ketosis the cessation of ovarian cyclicity was the most characteristic ovarian malfunction. The first visible estrus, the first insemination and the day of re-conception was delayed by both the late type and

long-lasting hyperketonemia. The within-150-days pregnancy rate was lowered, however, only by the most severe, long-lasting form (Table 2). Blood concentrations of insulin, IGF-I, and leptin decrease shortly after calving (Butler, 2000; Lucy, 2000; Block *et al*., 2001; Meikle *et al*., 2004). Insulin and IGF-I concentrations gradually increase postpartum, while leptin concentration remains low in lactating cows. Cows with negative energy balance and cows selected for milk production have lower blood concentrations of insulin and IGF-I (Snijders *et al*., 2001; Gong, 2002). Those metabolic hormones could influence GnRH neurons in the hypothalamus or on the pituitary gonadotroph (Adam *et al*., 2000; Williams *et al*., 2002). On the other side, ovarian cells treated with insulin or IGF-I have greater numbers of gonadotrophin receptors and greater activation of second messenger pathways in response to gonadotrophins (Lucy, 2000).

Table 2. Reproductive performance of cows showing various ketone pattern (mean ± SD)

	Acyclic period	Postpartum day of			AI / pregnancies	Rate of pregnants
		1st estrus	1st insemination	re-conception		
Non-hyperketonemic (n=98)	31.2± 16.7	81.9± 29.4	83.6± 29.2	101.4± 27.2	1.86± 0.77	n=73 =74%
Early type hyperketonemia (n=45)	36.95± 14.72	88.9± 25.1	92.7± 24.3	113.3± 30.3	1.85± 0.74	n=34 =75%
Late type hyperketonemia (n=11)	25.36± 11.56	116.2± 21.8	116.2± 21.8	132.7± 21.7	1.67± 0.71	n=9 =82%
Temporary hyperketonemia (n=11)	31.27± 9.32	90.3± 29.1	90.3± 29.1	114.5± 28.9	2.00± 0.76	n=8 =73%
Long-lasting hyperketonemia (n=34)	59.0± 17.7	112.3± 28.1	114.4± 24.6	129.5± 22.7	1.62± 0.65	n=13 =38%
F =	*15.32*	*7.13*	*7.20*	*5.15*	*0.50*	p<0.01
$LSD_{(P<0.05)}$ =	8.9	16.4	16.2	17.9	ns	

It was concluded that adrenocortical and thyroid gland functions are involved in the pathogenesis of ketosis. However, only the long-lasting form of hyperketonemia is a real risk factor for subsequent reproduction. Besides the high risk of mortality it may usually result in a serious delay in the time of the first ovulation and first visible estrus. In the first few days after calving the decreased insulin and IGF-1 levels may be involved in the pathogenesis of this ovarian disorder.

ACKNOWLEDGEMENT:
The authors express their thanks the *National Found for Basic Research* (project number: OTKA T-034 435 and OTKA T-037849), and the *University of Veterinary Science* (Budapest, Hungary; *Normative Support Found of Research*) for the financial support of this study.

Address for correspondence:
Prof. Dr Gyula Hyszenicza
University of Veterinary Science,
P. O. Box 2, H-1400
Budapest, Hungary
e-mail: huszenicza.gyula@aotk.szie.hu

REFERENCES

1. *Adam CL, Gadd TS, Findlay PA, Wathes DC,* 2000, IGF-I stimulation of luteinizing hormone secretion, IGF-binding proteins (IGFBPs) and expression of mRNAs for IGFs, IGF receptors and IGFBPs in the ovine pituitary gland, *J Endocrinol*, 166, 247-54.
2. *Adriaens FA, DL Hard, MA Miller, RH Phipps, RH Sorbet et al.,* 1995, Pituitary response to thyrotropin, corticotropin, and gonadotropin-releasing hormones in lactating cows treated with sometribove for a fourth consecutive lactation, *Domest Anim Endocrinol*, 12, 4, 301-16.
3. *Alan MGS, Dobson H, Fitzpatrick RJ*, 1981, Endocrine response to different doses of ACTH in cows, *British Vet J*, 142, 239-45.
4. *Bage R, Forsberg M, Gustafsson H, Larsson B, Rodriguez-Martinez H*, 2000, Effect of ACTH-challenged on progesterone and cortisol levels in ovariectomized repeat breeder heifers, *Anim Reprod Sci*, 63, 65-76.
5. *Baird GD*, 1980, Primary ketosis in the high-producing dairy cow: clinical and subclinical disorders, treatment, prevention and outlook, *J Dairy Sci*, 65, 1-10.
6. *Block SS, Butler WR, Ehrhardt RA, Bell AW, Van Amburgh ME et al.,* 2001, Decreased concentration of plasma leptin in periparturient dairy cows is caused by negative energy balance, *J Endocrinol*, 171, 339-48.
7. *Butler WR, Everett RW, Coppock CE*, 1981, The relationships between energy balance, milk production and ovulation in postpartum Holstein cows, *J Anim Sci*, 53, 742-8.
8. *Buttler WR*, 2000, Nutritional interactions with reproductive performance in dairy cattle, *Anim Reprod Sci*, 60-1, 449-57.
9. *Chelikani PK, Ambrose JD, Keisler DH, Kennelly JJ*, 2004. Effects of short-term fasting on plasma concentrations of leptin and other hormones and metabolites in dairy cattle, *Domest Anim Endocrinol*, 26, 1, 33-48.
10. *Diskin MG, Mackey DR, Rosche JF, Sreenan JM,* 2003, Effects of nutrition and metabolic status on circulating hormones and ovarian follicle development in cattle, Anim Rerod Sci, 78,3-4, 345-70.
11. *Duffield TF, Sandals D, Leslie KE, Lissemore K, McBride BW et al.,* 1998. Efficacy of monensin for the prevention of subclinical ketosis in lactating dairy cows. *J Dairy Sci*, 81, 2866-873.
12. *Đurđević Đ, Stojić V, Jovanović MJ, Radaković N*, 1980, Concentration of thyroxine and cortisol in the blood serum of ketotic cows, *Acta Vet Beograd,* 30,1-2, 7-12.
13. *Gong JG,* 2002. Influence of metabolic hormones and nutrition on ovarian follicle development in cattle: practical implications, *Dom Anim Endocrinol*, 23, 229-41.
14. *Hill SR, Reiss RS, Forsham PH, Thorn GW*, 1950. The effect of adrenocorticotrophin and cortisone on thyroid function: Thyroid-adrenal-cortical interrelationships, *J Clin Endocrinol,* 11, 137-8.
15. *Hurley WL, Convey EM, Leung K, Edgerton LA, Hemken RW*, 1981, Bovine prolactin, TSH, T4 and T3 concentrations as affected by tall fescue summer toxicosis and temperature, *J Anim Sci*, 51, 374-9.

16. *Huszenicza Gy, Janosi Sz, Kulcsar M, Korodi P, Dieleman SJ et al.,* 1998, Gram-negative mastitis in early lactation may interfere with ovarian and certain endocrine functions and metabolism in dairy cows, *Reprod Dom Anim,* 33, 147-53.
17. *Huszenicza Gy, Kulcsár M, Rudas P*, 2002, Clinical endocrinology of thyroid gland function in ruminants: A review of literature, *Vet Med Cz*, 47, 191-202.
18. *Jánosi Sz, Kacskovics I, Veresegyházy T, Huszenicza Gy,* 2002, Tõgygyulladásra hajlamosító anyagcsere-rendellenességek és hiányállapotok szarvasmarhán: I. Az ellés körüli energiahiányos állapot. Irodalmi áttekintés, *Magy Áo Lapja*, 124,57, 643-9.
19. *Juvancz I, Paksy A,* 1982, *Medical biometry* (in Hungarian), Medicina Press, Budapest, Hungary
20. *Kapp P, Pethes Gy, Zsiros M, Schuszter Z,* 1979, Adatok a nagyhozamú tejelõ szarvasmarhák zsírmáj-szindrómájának kórfejlõdéséhez, *Magy Áo Lapja*, 34, 458-61.
21. *Kleinbaum DG, Kupper LL,* 1978, Applied regression analysis and other multivariable methods, Duxbury Press, North Scituate, MA, U.S A.
22. *Kolk JH van der*,1991, Some aspects of the bovine pituitary-adrenocortical axis with special reference to persistent udder edema, PhD Dissertation, Utrecht.
23. *Kunz PL, Blum JW*, 1985, Relationships between energy balances and blood levels of hormones and metabolites in dairy cows during late pregnancy and early lactation, *Z Tierphysiol Tierernährg Futtermittelkde,* 54, 239-48.
24. Lapierre H, Petitclerc D, Pelletier G, Delorme L, Dubreuil P et al., ????, Effect of human growth hormone-releasing factor and(or) thyrotropin-releasing factor on hormone concentrations in dairy calves, *J Anim Sci*, 68, 2436-49.
25. *Lay DC, Friend TH, Randel RD, Jenkins OC, Neuendorff DA*,1996, Adrenocorticotropic hormone dose response and some physiological effects of transportation on pregnant Brahman cattle, *J Anim Sci*, 74, 1806-11.
26. *Lucy MC,* 2000, Regulation of ovarian follicle growth by somatotrophin and insulin-like growth factors in cattle, *J Dairy Sci*, 83, 1635-47.
27. *Lucy MC*, 2003. Mechanisms linking nutrition and reproduction in postpartum cows, *Reproduction Supplement,* 61, 415-27.
28. *Meikle A, Kulcsár M, Chilliard Y, Fébel H, Delavaud C et al.,* 2004, Effects of parity and body condition score at calving on endocrine and reproductive parameters of the dairy cow under grazing conditions, *Reproduction,* 127, 727-37.
29. *Nikolić AJ, Šamanc H, Begović J, Damnjanović Z, Đoković R et al.,* 1997, Low peripheral serum thyroid hormone status indepedently affects the hormone profile of the healthy and ketotic cows during the first week postpartum, *Acta Vet Beograd*, 47,1, 3-14.
30. *Nikolic AJ, Kulcsár M, Kátai L, Nedic O, Jánosi, Sz et al.,* 2003, Periparturient endocrine and metabolic changes in healthy cows and in those affected by various forms of mastitis, *J Vet Med A*, 50, 22-9.
31. *Pethes Gy, Bokori J, Rudas P, Frenyo VL, Fekete S,* 1985, Thyroxin, triiodothyronine, reverse-triiodothyronine, and other physiological characteristics of periparturient cows fed restricted energy, *J. Dairy Sci*, 68. 1148-54.
32. *Robertson WG, Lennon HD, Bailey WW, Mixner JP*, 1957, Interrelationships among plasma 17-hydroxycorticosteroide levels, plasma protein-bound iodine levels, and ketosis in dairy cattle, *J Dairy Sci*, 40, 732-8.
33. *Ronge H, Blum JW,* 1989, Insulin-like growth factor-I responses to growth hormone in dry and lactating dairy cows, *J Anim Physiol Anim Nutr*, 62, 280-8.
34. *Rudas P,* 1990, New aspects of thyroid physiology: a review, *Acta Vet Hung*, 38, 77- 86.
35. *Rudas P,* 1994, A pajzsmirigy-hormonok metablizmusának és dejodációjának szerepe háziállatokban, Akadémiai doktori értekezés, Állatorvostudományi Egyetem, Budapest.
36. *Rumsey TS, McLeod K, Elsasser TH, Kahl S, Baldwin RL,* 1999, Effects of oral chlortetracycline and dietary protein level on plasma concentrations of growth hormone and thyroid hormones in beef steers before and after challenge with a combination of thyrotropin-releasing hormone and growth hormone releasing hormone, *J Anim Sci*, 77, 2079-87.

37. *Sartin JL, Kemppainnen RJ, Cummins KA, Williams JC,* 1988. Plasma concentrations of metabolic hormones in high and low producing dairy cows, *J Dairy Sci*, 71, 650-7.
38. *Shaw GH, EM Convey, HA Tucker, EP Reineke, JW Thomas et al.,* 1975, Bovine serum thyroxine, prolactin, growth hormone, glucocorticoid and thyroxine binding globulin response to thyroprotein, *J Dairy Sci*, 58, 703-8.
39. *Snedecor GW, Cochran WG,* 1982, Statistical methods, 7th ed. The Iowa State University Press
40. *Snijders SEM, Dillon P, O'Farrell D, Diskin M, Wylie ARG et al.,* 2001, Genetic merit for milk production and reproductive success in dairy cows, *Anim Reprod Sci*, 65, 17-31.
41. *Stojić V, Gvozdić D, Kirovski D, Nikolić AJ, Huszenicza Gy et al.*, 2001, Serum thyroxine and triiodothyronine concentrations prior to and after delivery in primiparous Holstein cows, *Acta Vet (Beograd),* 51, 3-8.
42. *Taylor VJ, Beever DE, Bryant MJ, Wathes DC,* 2003. Metabolic profiles and progesterone cycles in first lactation dairy cows, *Theriogen*, 59, 1661-77.
43. *Tiirats T,* 1997, Thyroxine, triiodothyronine and reverse-triiodothyronine concentrations in blood plasma in relation to lactational stage, milk yield, energy and dietary protein intake in Estonian dairy cows, *Acta Vet Scand*, 38,4, 339-48**.**
44. *Tiirats, T.*, 1997, Thyroxin, triiodothyronine and reverse- triiodothyronine concentrations in blood plasma in relation to lactation stage, milk yield, energy and dietary protein intake in Estonian dairy cows. *Acta Vet. Scand*., 38. 339-348.
45. *Tveit B, Lingaas F, Standal N,* 1990a, Thyroid function in heifers measured by hormone levels before and after injection of thyrotropin releasing hormone. Methods, repeatability and correlation with production traits, *Acta Agric Scand*, 40, 175-81.
46. *Tveit B, Lingaas F, Standal N,* 1990b, Thyroid function in heifers measured by hormone levels before and after injection of thyrotropin releasing hormone. Effect of parturition. *Acta Agric Scand*, 40. 183-8.
47. *Wathes DC, Taylor VJ, Cheng Z, Mann GE*, 2003, Follicle growth, corpus luteum function and their effects on embryo development in postpartum dairy cows, *Reprod Suppl,* 61, 219-37.
48. *Williams GL, Amstalden M, Garcia MR, Stanko RL, Nizielski SE et al.,* 2002. Leptin and its role in the central regulation of reproduction in cattle, *Dom Anim Endocrinol*, 23, 339-49.
49. *Zulu VC, Nakao T, Sawamukai Y,* 2002, Insulin-like growth factor-I as a possible hormonal mediator of nutritional regulation of reproduction in cattle, *J Vet Med Sci*, 64, 8, 657-65.

ADRENOKORTIKALNA I TIROIDNA FUNKCIJA, HORMONSKI I METABOLIČKI PROFIL I POREMEĆAJ CIKLIČNOSTI FUNKCIJA JAJNIKA U MLEČNIH KRAVA KOJE PATE OD RAZNIH OBLIKA KETOZE

HUSZENICZA GY, KULCSÁR M, KORODI P, BARTYIK J, RUDAS P, RIBICZEI-SZABO P, NIKOLIĆ JUDITH ANA, ŠAMANC H, IVANOV I i GVOZDIĆ D

SADRŽAJ

Uloga adrenokortikalnih i tiroidnih hormona u patogenezi ketoze i poremećaja funkcije jajnika, proučavana je u dve približno jednake grupe krava (n=199) u 3 velika zapata mlečnih krava. U cilju upoređivanja koncentracije određenih hormona [kortizol, tiroksin (T_4), trijodtironin (T_3), insulin, insulinu-sličan faktor rasta (IGF-1)], metabolita [glikoza (G), acetosirćetna kiselina (ACAC), βOH-butirat (BHB), neesterifikovane masne kiseline (NEFA), trigliceridi (TG), ukupni

holesterol (TCh)], i aktivnosti aspartat aminotransferaze (AST) u krvnoj plazmi/serumu, uzimani su uzorci krvi od prvog do trećeg dana posle teljenja i ponovo 4 puta u razmacima od po 7 dana. Povećanje koncentracije kortizola, posle aplikacije ACTH, i povećanje koncentracije T_4/T_3 nakon aplikacije TRH je utvrđeno između 1. do 3. dana i ponovo između 28. do 35-og dana. Ponovno uspostavljanje cikličnosti funkcije jajnika bilo je praćeno individualnim profilima progesterona (P_4) na osnovu kontrole uzoraka mleka uzetih 3 puta nedeljno tokom perioda od 80 do 85 dana. Nivo BHB od 1 mmol/l je bio utvrđen kao granica između hiperketonemičnih (>1 mmol/l) i normoketonemičnih (<1 mmol/l) stanja. Na osnovu toga krave su razvrstane u pet jasno definisanih grupa:

(1) krave koje nisu ketozne (n=98; normoketonemija u svim uzorcima), (2) rani tip ketoze (n=45; hiperketonemija utvrđena samo u prvoj nedelji posle teljenja), (3) kasni (laktacioni) tip ketoze (n=11; posle perioda normoketonemije detektovana je hiperketonemija u 4-oj i 5-oj nedelji, ili u 5-oj nedelji), (4) povremena ketoza (n=11; hiperketonemija je detektovana u trajanju od 1-2 nedelje u drugoj i trećoj ili u trećoj i četvrtoj nedelji), (5) dugotrajna ketoza (n=34; hiperketonemija je bila dijagnostikovana od teljenja tokom 4 do 5 nedelja ili posle uginuća/prinudnog klanja). Istovremeno sa stanjem hiperketonemije bilo je ustanovljeno povećanje koncentracija NEFA, ACAC, i smanjenje TCh, glikoze, IGF-1, T_4 i T_3 u skoro svim slučajevima. Ipak, očigledne metaboličke i endokrine promene su pronađene, samo kod dugotrajne ketoze. T_4 i T_3 odgovor stimulisan TRH-om ostao je skoro nepromenjen što dokazuje nepromenjenu funkciju tiroideje u ranom i kasnom kao i u prolaznom tipu ketoze. Hipofunkcija tiroideje i usporeno uspostavljanje ciklične funkcije jajnika bilo je utvrđeno samo u slučajevima dugotrajne ketoze. Krave koje su imale nižu koncentraciju kortizola, kao odgovor na aplikovan ACTH 1-3 dana posle teljenja, imale su manje šanse za spontani oporavak. Postoji značajna negativna korelacija između nivoa IGF-1 u prvoj nedelji posle teljenja i dužine trajanja postpartalnog perioda aciklije.

U kasnom (laktacionom) tipu ketoze prekid ciklične funkcije jajnika bio je najkarakterističniji genitalni poremećaj.