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RELATIONSHIP BETWEEN CONCENTRATIONS OF NEFA AND CHOLESTEROL IN BLOOD SERUM OF COWS WITH PUERPERAL DISEASES

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Puerperal diseases of cows are one of the dominent factors with a negative influence on reproductive performance of dairy cows. The energic balance of the organisms is reflected in nonesterified fatty acid (NEFA) concentrations in the blood serum. Cholesterol, being a component of serum lipopotens, reflects the overall lipoprotein concentrations in blood serum of the cows. Energy deficiency causes increased metabilism of fats and lipoproteins.

Significantly most apparent uterus retraction was recorded in dairy cows in group A (with physiological development of the puerperium) on 15th-17th day post partum when the thickness of cervix uteri was 46 ± 4.55 mm (P < 0.001), and of corpus uteri 42±2.36 mm. In group B (with clinical symptoms of primary endommetritides) and C (with retained placenta) uterus retraction was completed on 25th-27th day post partum when the thickness of cervix uteri was 45 ± 0.47 mm in group B and 50 ± 0.65 mm in group C (P < 0.01), and of corpus uteri 43 ± 1.41 mm in group B and 46 ± 1.41 mm in group C. NEFA concentrations in dairy cows blood serum in group A before partus, as well as durring the next three weeks post partum, exceeded the levels of reference values (0.45 ± 0.30 mmol/l, 0.48 ± 0.28 mmol/l, 0.38 ± 0.21 mmol/l and 0.37 ± 0.10 mmol/l). They indicated increased metabolism of fat depots. The cholesterol levels at that time were below reference values (2.44 ± 0.54 mmol/l in group A, up to 2.54 ± 0.87 mmol/l in group C). Their concentrations are a effected by esterification of cholesterol, unsaturated fatty acids, or rising lactation. Since fine third week post partum, we recorded rising concentrations of cholesterol (3.03 ± 0.87 mmol/l and 3.80 ± 0.31 mmol/l in dairy cow blood serum in group B and C.This also occurred in group A from the fourth week post partumm (3.79 ± 0,74 mmol/l) The rise in concentrations is probably related to the decrease of NEFA and their decreased esterification, or with increased forage intake with predominance of the protein component.

Key words: dairy cows, puerperal diseases, ultrasonography, nonesterified fatty acids, cholesterol

INTRODUCTION

Puerperal diseases are one of the dominant factors which negatively influence the reproductive performance of dairy cows. This can cause large economic losses to the breeders. The organism, weakened by partus, loaded with increased milk yeilding post partum, has difficulties with resistance to stress factors (agents of infectious diseases, poor zoohygienic conditions, as well as quantitative or qualitative faults in feeding (Bireš et al., 1996). The puerperium course in cows is also influenced by season, parity, length of gestation, previous diseases, and management of breeding (Markusfield, 1984, 1987; Grohn et al., 1990). The result of this is activation or inhibition of lutenising hormone secretion (LH) and dominant follicle growth. Finally, it can cause retardation of involutional processes of the genital complex of dairy cows and late onset of ovarian activity. Though opinions on the efects of negative energy balance on LH secretion and its influence on follicle growth differ (Savio et al., 1990;Murphy et al., 1990), practical experiences indicate slowing-down of involution and more frequent occurrence of puerperal diseases in animals with negative energy balance. Among metabolic diseases the most frequent are post partum paresis, ketosis, acidosis or metabolic diseases, endometritides, metritides, and retained placentas. Early diagnosis of negative energy balance in cows and its subsequent compensation by appropriate adjustment of the feeding, enables us to lower the risk of these diseases. By adherence to further technological measures we can optimize the reproductive indices of dairy cows and make the economy of breeding more effective. Possible indices of negative energy balance are the concentrations of nonesterified fatty acids (NEFA), glucose, insulin, ketone substances and urea (Russel and Wright, 1983, Kovač et al. 1996). An indicator of negative energy balance of dairy cows is also the mutual ratio and concentrations of NEFA and cholesterol in blood serumm (Grummer, 1993; Vasques-Anon et al., 1994).

The aim of our work was to evaluate involutional processes in the genital complex of cows during different puerperium courses and to compare their mutual relation with concentrations of NEFA and cholesterol and subsequent reproductive index.

MATERIALS AND METHODS

In Slovak crossbreed spotted dairy cows and their crossbreeds, aged 4 to 11 years, in the usual breeding conditions, from January to April, we performed clinical and gynecological examinations on 1 st - 2 nd day post partum, on 5^{th}-7^{th} day on 15^{th}-17^{th} day, on 20^{th} - $22t^{h}$ day and on $25t^{h}$ -27^{th} day. We diagnosed involutional changes in the genital complex and detected reproductive disorders. Involutional changes were also evaluated by ultrasonographic observation (Figure 1),using an Aloka SSD 500 apparatus. (Tokyo MURE HITAKA - SH Co, LTD. Japan) and a 5 MHz linear probe (UST-588-U). We divided the examined dairy cows into three groups based on the puerperium development. Dairy cows with physiological development of the puerperium

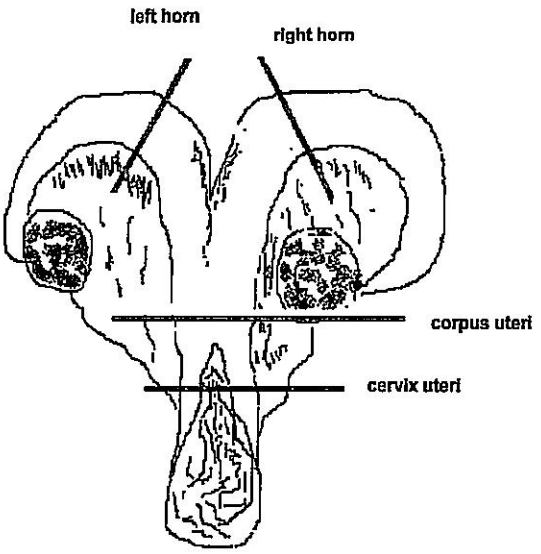


fig 1. Schematic visualisation of cows genital complex with the USG lines evaluating the thickness of cervix, corpus and cornua uteri

(n=34) formed the first group (A), dairy cows with clinical symptoms of primary endometritides (n=29) were placed the second one (B), and the third one (C) consisted of dairy cows with retained placenta and different stages of secondary endometritides (n=10). We evaluated selected metabolic indices (NEFA and cholesterol) from their blood serum, obtained from v. jugularis and processed as usual beginning from the 10^{th} to 10^{th} to 7^{th} day pre partum on 1^{st} - 2^{nd} day post partum, and subsequently each 7^{th} day during six weeks post partum in 22 dairy cows from group A, in 17 dairy cows from group B and 10 dairy cows from group C. We performed the analysis of cholesterol by Bio-La tests (Lachema Brno) and analysis of NEFA by a photometric method (Curtis, H. CH., 1974: Man Roth Clinical Biochemistry II. De Grytes, 1034).

Statistical significance was tested by Student's t-test, dynamics of changes in the uterus was expressed by a polynomic curve using the formula $y = b+c_1x +c_2 x^2 +C_3 x^3+ ... + c_6x^6$

RESULTS

Evaluating the involutional changes in the cows, genital complex on 1^{st} - 2^{nd} day post partum we recorded the thickenss of cervix uteri in dairy cows in group A to be 95 ± 7.66 mm (Figure 2), in dairy cows in group B

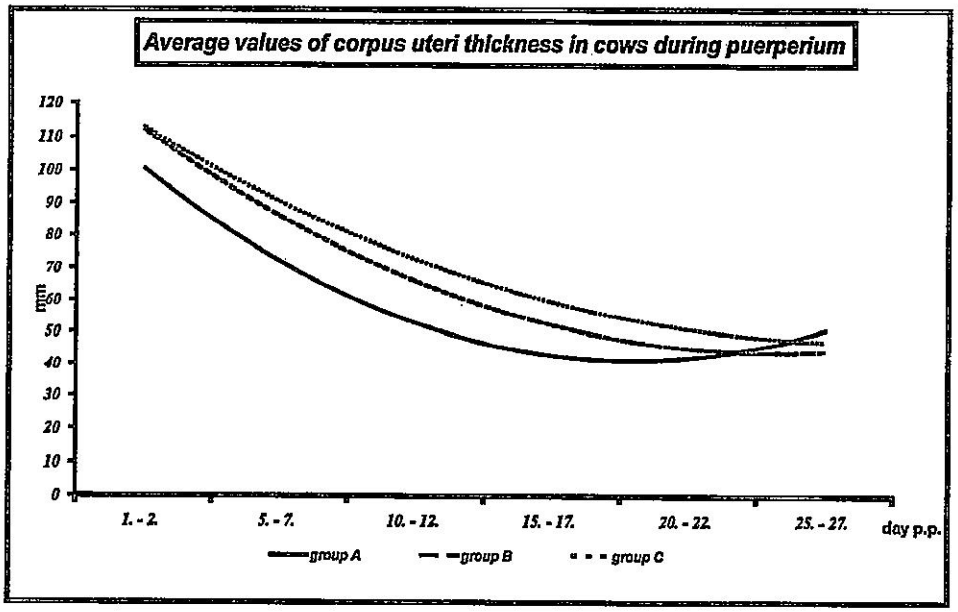
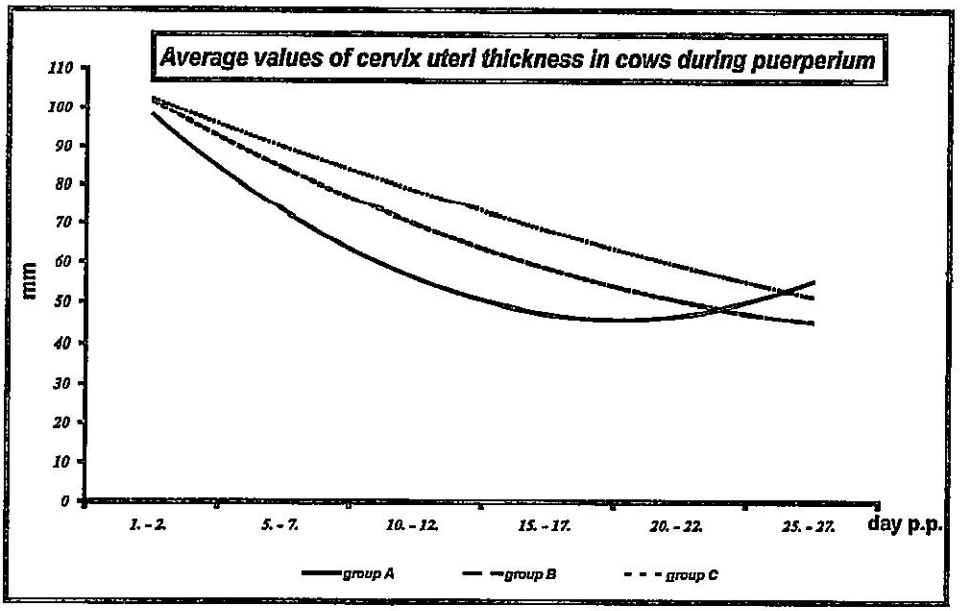


fig 2.

100 ± 4.11 mm and in dairy cows in group C 103+12.47 mm. The thickenss of corpus uteri was at that time 93±3.28 mm in group A, 113±17.50 mm in group B and 113±20.55 mm in group C cours. (Figure 3.) The most apparent



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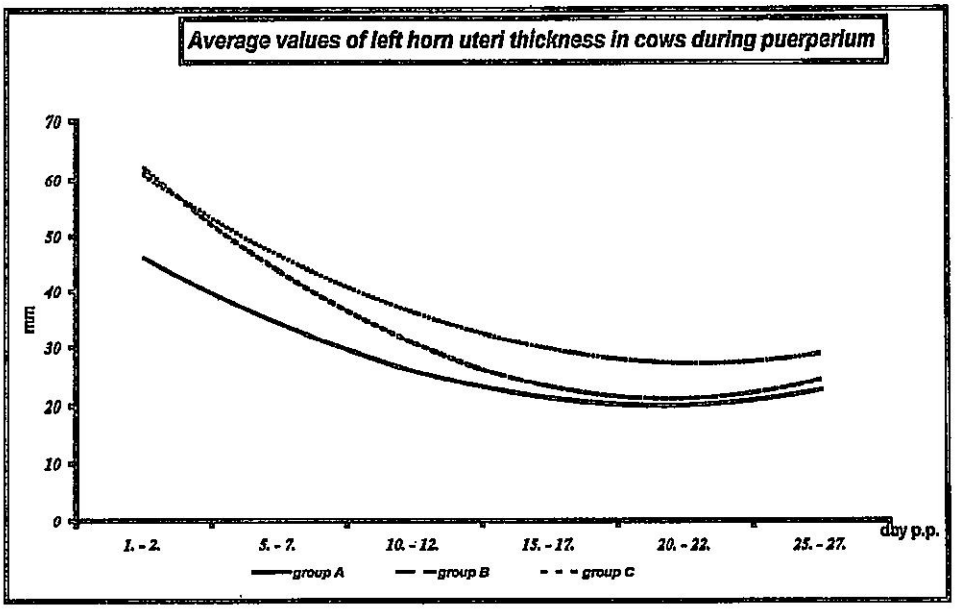

fig 4

uterus retraction was recorded in dairy cows in group A on 15 th 1-7 th day post partum, when the thickness of cervix uteri was 46 $\pm$ 4.55 mm ($P < 0.001$), of corpus uteri 42 $\pm$ 2.36 mm, of left horn uteri 19 $\pm$ 0.94 mm (Figure 4) and

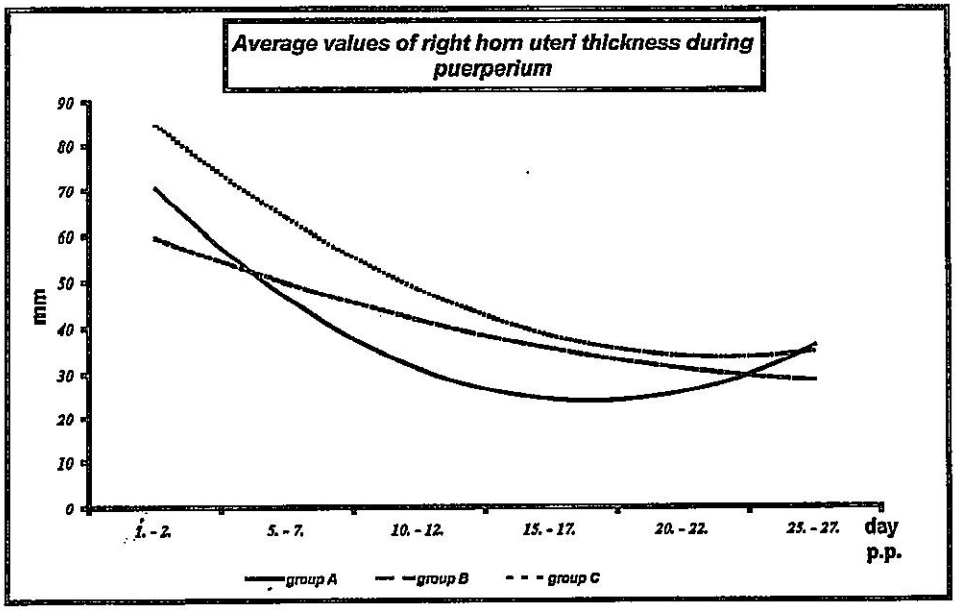

fig 5

of right horn 23 ± 1.41 mm (Figure 5.) In groups B and C uterus retraction was completed on 25 th - 27 th day post partum when the thickness of cervix uteri was 45 ± 0.47 mm in dairy cows in group B and 50 + 0.65 mm in group C ($P < 0.01$), of corpus uteri 43 ± 1.41 mm in group B and 46 ± 1.41 mmm in group C. The thickness of left horn uteri was at that time 24 ± 4.32 mm in dairy cows in group B, 30 ± 7.07 mm in group C and the thickness of right horn 29 ± 1.89 mm in group B ($P < 0.05$) ad 34 ±3.30 mm in group C. In all dairy cows of both groups the mucupurulent discharge had become mucous or mucohaemorrhagic by that time. Comparing the concentrations of nonesterified fatty acids obtained from cow blood serum on 10 th - 7 th day before partum we recorded the highest levels in dairy cows that did not show clinical symptoms of puerperal diseases post partum (Table 1.). The lowest concentrations of NEFA were at that time in dairy cows in group B 0.28 ± 0.12 mmol/l, compared with group A which showed 0.45 ±0.30 mmol/l ($P<0.05$) and with group C where they were 0.32 ± 0.15 mmol/l. Cholesterol levels were at that time highest in dairy cows in group B (2.72 + 0.59 mmol/l), while in group A they were 2.66 ± 0.63 mmol/l and in group C 2.69±0.74 mmol/l. In both groups these were at low levels of reference values. First week post partum concentrations of cholesterol in blood serum dropped below reference values in all evaluated dairy cows. In cows from group A it was 2.44 ± 0.54 mmol/l, in group B 2.44 ± 0.60 mmol/l and in group C 2.54 ± 0.87 mmol/l. Concentrations of NEFA were at that time apparently above the level of reference values in dairy cows from group A (0.48 ± 0.28 mmol/l). Concentrations of reference values in this group of cows were exceeded even in the 2nd and 3nd week post partum when we recordeded decrease of their values. However, the most significant drop of concentrations of NEFA was recorded in dairy cows from group C on the 4th - 5th week post partum. Measured values reached concentrations lower than reference values (0.17 ± 0.01 mmol/l and 0.16 ± 0.01 mmol/l, $P < 0.05$). Along with the drop of NEFA we recorded the increase of cholesterol concentrations beginning with the third week post partum. Change of mutual ratio values NEFA/cholesterol was also demonstrated. While in dairy cows from group A before partum this was 0.168 ($P< 0.01$), in the fourth week post partum the value was 0.085 ($P < 0.001$) and in dairy cows from group C 0.046 ($P < 0.05$) in the fourth week, compared with 0.120 ($P<0.01$) in the prepartum period.

Average reproductive parameters of the observed cows (Table 2) showed no pathological changes in the genital complexes in dairy cows in group A and their insemmination interval (93±51.2) compared with 104±47.3 days in group B and 107±50.5 days in group C). Despite the fastest completion of involutional processes on the uterus the service period and insemination index in these cows was the highest 151±52.1 day, compared with 120 ± 56.2 days in dairy cows from group B ($P<0.05$), and 141±59.2 days in dairy cows from group C. The insemination index for dairy cows in group A was 3+1.6 for dairy cows in group B (2+1.2) and in group C (2+0.9, $P<0.05$).

Table 1. Comparison of NEFA and cholesterol concentrations in blood serum of dairy cows with physiological and pathological course of puerperium

Sampling	Metabolic index	group A (n=22)		group B (n=17)		group C (n=10)	
		average	SD	average	SD	average	SD
(-10th.) – (-7th.) day	NEFA (mmol/l)	0.45*	0.30	0.28*	0.12	0.32	0.15
	cholest. (mmol/l)	2.66	0.63	2.72	0.59	2.69	0.74
	NEFA cholest.	0.168**		0.104		0.120**	
1st. week p.p.	NEFA (mmol/l)	0.48*	0.28	0.34	0.14	0.30*	0.18
	cholest. (mmol/l)	2.44	0.64	2.44	0.6	2.54	0.87
	NEFA cholest.	0.198		0.140**		0.119***	
2st. week p.p.	NEFA (mmol/l)	0.38	0.21	0.39	0.22	0.30	0.07
	cholest. (mmol/l)	2.53	0.48	2.52	0.85	2.28	0.23
	NEFA cholest.	0.152		0.157		0.132**	
3st. week p.p.	NEFA (mmol/l)	0.37	0.10	0.29	0.18	0.39	0.18
	cholest. (mmol/l)	2.38	0.62	3.03	0.87	3.80	0.31
	NEFA cholest.	0.153		0.096		0.104***	
4st. week p.p.	NEFA (mmol/l)	0.32	0.20	0.38*	0.16	0.17*	0.01
	cholest. (mmol/l)	3.79	0.74	3.67	0.88	3.41	0.37
	NEFA cholest.	0.085***		0.102		0.048*	
5st. week p.p.	NEFA (mmol/l)	0.33*	0.20	0.30	0.17	0.16*	0.01
	cholest. (mmol/l)	3.18	0.71	2.98	0.62	3.42	0.47
	NEFA cholest.	0.105		0.101		0.046*	
6st. week p.p.	NEFA (mmol/l)	0.15	0.03	0.22	0.06	0.20	0.03
	cholest. (mmol/l)	3.89	0.63	2.95	0.51	2.96	0.35
	NEFA cholest.	0.039		0.076		0.066**	

t-test: *O < 0,05; **P < 0,01; ***P < 0,001

Table 2. Average reproductive parameters of cows with physiological course of puerperium (n = 34), puerperal endometritides (n = 29) and retained placenta (n = 10)

Reproductive parameters	Group A ($\bar{X} \pm SD$)	Group B ($\bar{X} \pm SD$)	Group C ($\bar{X} \pm SD$)
Insemination interval (days)	93 ± 51.2	104 ± 47.3	107 ± 50.5
Service period (days)	151 ± 52.1*	120 ± 56.2*	141 ± 59.2
Insemination index	3 ± 1.6*	2 ± 1.2	2 ± 0.9*

t - test: * P < 0.05

DISCUSSION

Partus, redevelopmental changes in the genital complex of dairy cows and starting lactation require extraordinary high metabolic activity that is conditioned by a sufficient quantitative and qualitative supply of nutrients and good state of the animals. However, this activity does not start suddenly. The dairy cows begin to adapt their metabolism and utilize nutrient supplies and body depots already several days before partus. Body depot utilisation increases with increasing milk secretion since, in this period, the dairy cow is not able to cover the energy loss by ingesting nutrients from forage. Balance disorder of the internal environment caused by negative energetic balance of cows can thus cause various puerperal diseases. The most frequent are retarded ovarian activity due to inhibition of LH release, delayed involutional processes in the uterus associated with retained placenta, or endometritis or metritis. Undernourishment negatively influences the growth of dominant follicles and the onset of the first postpartal oestrus (Savio et al., 1990). However, insufficient energy intake and dietary imbalance directly do not influence negatively the reproductive ability of dairy cows. Perry and others (1991) indicate that follicular growth on ovaries was altered only in cows with low condition during calving. Individual body condition, stability of the internal environment are thus more important factors that influence reproduction than the energetic value of the fodder. Concentrations of NEFA in cows blood serum reflect the energy balance of the organism. Cholesterol, being a component of serum lipoproteins, reflects the overall lipoprotein concentrations in cow blood serum. Energy deficiency causes increased metabolism of fats and lipoproteins. Concentrations of NEFA in blood serumm of dairy cows from group A which exceeded reference values levels (0.45 $\pm$ 0.30 mmol/l) before partus, as well as during the next three weeks post partum (0.48 $\pm$ 0.28 mmol/l, 0.38 $\pm$ 0.21 mmol/l and 0.37 $\pm$ 0.10 mmol/l), indicate increased metabolism of fat depots (Grimard et al., 1995). In dairy cows in group B concentrations of NEFA exceeded the reference values only in the 2nd week post partum (0.39 $\pm$0.22 mmol/l), in dairy cows in group C only in the third week post partum (0.39 $\pm$ 0.18 mmol/l). Levels of cholesterol were at the time below reference values (2.44 $\pm$ 0.54 mmol/l in dairy cows from group A, up to 2.54 $\pm$ 0.87 mmol/l in group C). This is associated with esterification of cholesterol by nonesterified fatty acids, or with rising lactation (Kweon et al., 1985). Moreover, liver insufficiency due to fatty degeneration, or thyroid gland hyperfunction can cause decrease of cholesterol concentrations (Bekeova et al., 1988). From the third week post partum we recorded rising concentrations of cholesterol in dairy cow blood serum in groups B and C (3.03 $\pm$ 0.87 mmol/l). Similarly, this occurred blood serum in group A from the fouth week post partum (3.79 $\pm$ 0.74 mmol/l). The concentration increase is probably associated with the decrease of NEFA and lowered esterification, or with increased forage intake

in which the protein component dominates. The concentration ratio between NEFA and cholesterol in dairy cows in group A, on the fourth day post partum, reached 0.085 ($P < 0.001$), and in group C 0.048 ($P < 0.05$). Dairy cows from group B did not show significant differences in the concentration ratio of NEFA and cholesterol during the observation period. In dairy cows from group A active compensation of negative energetic balance by increased metabolism occurred. The physiological course of the puerperium was characterized by completion of involutional processes of the uterus on 15^{th} - 17^{th} day post partum. (At that time retraction of uterus was complete and the uterus had the lowest values for thickness of each part. During the next period, due to oedematization caused by ovarian follicles, its thickness was increasting (Figure 2-5.). Although the reproductive functions in this group of dairy cows developed favourably, later evaluation of their reproductive indicess, chowed poorer values in comparison with cows with puerperal diseases. Hence we state that the physiological course of the puerperium is not a guarantee of active utilisation of brood-animmals in reproduction (Grafenau et al., 1993; Hajurka et al., 1990). Organizational faults by breeders only worsen the reproductive indices of dairy cows and endanger the economy of the breeding.

A c k n o w l e d g e m e n t s

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ODNOS IZMEĐU KONCENTRACIJE NEESTERIFIKOVANIH MASNIH KISELINA (NEFA) I HOLESTEROLA U KRVNOM SERUMU KRAVA SA PUERPERALNIM OBOLJENJIMA

V. MACAK, F. NOVOTNY, J. KAČMARIK i P. BALENT

SADRŽAJ

U ovom radu autori prikazuju rezultate određivanja koncentracije neesterifikovanih masnih kiselina (NEFA) i holesterola u krvnom serumu krava sa različitim puerperalnim poremećajima. Grupu A sačinjavale su životinje sa fiziološkim puerperijumom, grupu B životinje sa primarnim endometritima i grupu C krave sa retencijom placente. Kod životinja iz grupe A involucija uterusa je bila završena 15.- 17. dana postpartum a kod krava u grupama B i C posle 25-27 dana. Kod krava iz grupe A koncentracija NEFA pre teljenja i u prve tri nedelje posle njega prelazila je fiziološke vrednosti ukazujući na povećani metabolizam masti iz telesnih depoa. U tom periodu koncentracija holesterola je kod svih životinja bila ispod fizioloških vrednosti. Od treće nedelje post partumm u grupama B i C se uočava povećanje koncentracije holesterola koje postoji i u grupi A ali tek od četvrte nedelje. Autori smatraju da je ovo povećanje rezultat pada koncentracije NEFA i njihove smanjene esterifikacije ili povećanog unošenja hrane bogate proteinima.