

## INFLUENCE OF THE STAGE OF DAIRY COW REPRODUCTION CYCLE ON SOME CLINICAL AND BIOCHEMICAL PARAMETERS

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### Abstract

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The influence of the reproduction cycle stage on selected parameters of the mineral metabolism levels has been investigated on 55 clinically healthy dairy cows of the Bohemian Spotted breed, 3–8 years old, the nutrition of which met standard requirements (starch units, digestible nitrogen compounds, Ca and P). The animals were divided into three groups according to the reproduction stage (daily performance) and were followed during the winter feeding period (December - April).

The selected clinico-biochemical characteristics of blood (pH, base excess, pCO<sub>2</sub>), blood plasma (Ca, inorg. P) and urine (Ca, inorg. P) have not been found to differ from reference values. The values obtained by chemical analysis of tuber coxae biopsy samples have shown a significant decrease in the concentration of P/g of fat-free dry matter in post parturient dairy cows when compared with the dry ones. Otherwise, the reproduction cycle stage has not significantly influenced the ash content and Ca weight per 1 g of fat-free dry matter and the ash, Ca and P weight expressed per 1 cm<sup>3</sup> of cancellous bone tissue.

*Dairy cows, reproduction stage, blood, blood plasma, urine, cancellous bone tissue, fat-free dry matter, ash, calcium, inorganic phosphorus, acid-base balance.*

The requirements for maintaining homeostasis of calcium and phosphorus increase with the augmented milk production and with the advancement of pregnancy. Dämrich (1970), Šiškov and Zelenskaja (1978) rank the milk efficiency and pregnancy among endogenous factors connected with the induction of diseases of skeleton. Later stage of lactation plays a more significant role in the development of skeletal diseases than that of gravidity (Stöber 1970). In the period of late lactation the calcium balance is negative, this being connected with bone demineralization (Duckworth and Hill 1953). The skeleton demineralization occurs particularly at the onset of lactation (Illek et al. 1977). If the Ca and P losses are not adequately compensated for, hypocalcaemia and hypophosphataemia develop, inducing thus the parathyroid hypersecretion. The increased level of parathormone exerts its effect in its target organs (bone, intestine, kidney), the result being the adjustment of calcaemia.

In contrast to Ca, the mechanism of phosphate homeostasis has not been fully elucidated. Rasmussen and Bordier (1974) have repeatedly shown that the phosphate depression leads to the changes in bone, intestine and kidney cells which stimulate an increased transfer of phosphates into extracellular fluid. With the decrease of serum phosphates the tubular reabsorption in kidney increases together with phosphate absorption in intestine. The shortage of phosphates leads to the stimulation of vitamin D conversion in kidney to 1,25 dihydroxycholecalciferol, promoting the enteral absorption of Ca and P.

The aim of the present paper was to evaluate the influence of the reproduction stage on chemical composition of skeleton of clinically healthy and optimally fed cows.

## Materials and Methods

Clinico-biochemical investigation has been carried out during winter feeding season (December - April) on 55 cows of the Bohemian Spotted breed, 3-8 years old; they came from five farms located in the sugar-beet production area.

The farms were selected on the basis of the evaluation of feed rations, performance and health condition. Feed analyses (quality of a fermentation process in silages, starch unit content, digestible nitrogen substances, Ca and P were examined) and the efficiency level were used for the evaluation of feed rations. The average mild yield efficiency was 4 000 kg of milk. Two years' health analysis, particularly with regard to the incidence of skeletal diseases, has been carried out.

For the evaluation of the reproduction stage influence (daily performance) upon the chemical composition of the skeleton, 10-15 cows, representatives of a herd (dairy cows 2-6 weeks post partum, 4-5 months pregnant and dry cows), were selected. The milk yield of the post partum dairy cows (group 1,  $n = 16$ ) and those in the 4th-5th month of pregnancy (group 2,  $n = 18$ ) was  $16-25 \text{ l. d}^{-1}$  and  $12-18 \text{ l. d}^{-1}$ , resp. The dry cows were included in the 3rd group.

The nutrition corresponded to production requirements as far as the examined parameters are concerned. No defects were found in the organization of feeding rules and techniques. The milk yield in the examined animals ranged from 3 800 to 4 200 kg in the preceeding lactation. All animals were clinically healthy.

On selected animals, the clinical examination and analyses of blood, blood plasma, urine and bone tissue were conducted. Acid-base balance in blood, the Ca and inorganic P concentrations in blood plasma and urine and the ash, Ca and P mass in the bone tissue were determined. To determine the acid-base balance in venous blood the Astrup equilibration method using the BME 22 (Radiometer) instrument was applied. The Siggaard-Andersen nomogram was used for the calculation of BE and  $\text{pCO}_2$ . The Ca concentration in plasma and urine was determined by the atomic absorption spectrophotometry on the Atomspek H 1551 instrument (Hilger) and that of inorg. P in blood plasma and urine photometrically on the Eppendorf 11001 M (Eppendorf) instrument using the Bio-test (Lachema).

The biopsy samples from the tuber coxae were analysed by the following procedure: fat from the samples of cancellous bone tissue ( $v = 0.541 \text{ cm}^3$ ) was extracted by ether, then the samples were dried at  $105^\circ\text{C}$  to obtain fat-free dry matter which was mineralized at  $550^\circ\text{C}$  to yield ash. Both the extraction and mineralization were carried out to the constant weight.

The ash mass was expressed per 1 g of fat-free dry matter and per  $1 \text{ cm}^3$  of cancellous bone tissue. The ash was then diluted in a minimal amount of 3 M - HCl and after the dilution the investigated elements were determined: calcium by the AAS method on the Atomspek H 1551 and inorganic phosphorus photometrically on the Eppendorf 1101 M. The determination was carried out using standard solutions. Finally, the weight of both elements per 1 g of fat-free dry matter and per  $1 \text{ cm}^3$  of cancellous bone tissue and the Ca/P ratio were calculated.

The results of biochemical examination were computed using the Hewlett-Packard minicomputer, model 9810 A.

## Results

The results of the biochemical examination are presented in Tables 1-3. Table 1 shows the influence of the reproduction stage on the acid-base balance of blood and on the Ca and inorg. P concentrations in blood plasma and urine, Tables 2 and 3 present the chemical composition of the cancellous bone tissue of tuber coxae.

The highest value of blood pH,  $\bar{x} = 7.404 \pm 0.029$  was found in the group 1 (dairy cows post partum), the lowest one,  $\bar{x} = 7.391 \pm 0.025$ , in the group 2 (4th - 5th month of pregnancy). Also the highest and lowest values of BE ( $\bar{x} = 1.650 \pm 1.742 \text{ mmol/l}^{-1}$  and  $\bar{x} = 0.650 \pm 1.693 \text{ mmol/l}^{-1}$ ) were found in the group 1 and 2, resp. The average value of  $\text{pCO}_2$  was observed to be highest,  $5.786 \pm 0.725 \text{ kPa}$ , and lowest,  $5.731 \pm 0.552 \text{ kPa}$ , in the group 3 and 1, resp.

The highest calcaemia was observed in dairy cows in the 4th-5th month of pregnancy, i.e. in the group 2 ( $\bar{x} = 2.618 \pm 0.257 \text{ mmol.l}^{-1}$ ) and the lowest in dry cows, i.e. in the group 3 ( $\bar{x} = 2.583 \pm 0.242 \text{ mmol.l}^{-1}$ ). The highest and lowest blood plasma concentrations of inorganic P were found in group 2 ( $\bar{x} = 1.991 \pm 0.315 \text{ mmol.l}^{-1}$ ) and in the group 1 ( $\bar{x} = 1.899 \pm 0.369 \text{ mmol.l}^{-1}$ ), resp.

Table 1  
Results of the blood, blood plasma and urine analysis

Group	Blood			Plasma		Urine	
	pH	BE (mmol.l <sup>-1</sup> )	pCO <sub>2</sub> (kPa)	Ca (mmol.l <sup>-1</sup> )	P	Ca (mmol.l <sup>-1</sup> )	P
1	n	16	16	16	16	16	16
	$\bar{x}$	7.404	1.650	5.731	2.606	1.899	1.344
	s	0.029	1.742	0.552	0.216	0.369	0.947
2	n	18	18	18	18	18	18
	$\bar{x}$	7.391	0.650	5.756	2.618	1.991	0.941
	s	0.025	1.693	0.515	0.257	0.315	0.574
3	n	21	21	21	21	21	21
	$\bar{x}$	7.398	1.186	5.786	2.583	1.923	0.968
	s	0.032	2.494	0.725	0.242	0.344	0.627

1 — dairy cows 2—6 weeks post partum  
2 — dairy cows 4—5 months pregnant  
3 — cows in the dry period

Table 2  
Results of chemical analysis of the cancellous bone tissue of t. coxae

Group	1 g fat-free dry matter			1 cm <sup>3</sup>			Ca/P
	Ash mg	Ca mg	P mg	Ash mg	Ca mg	P mg	
1	n	16	16	16	16	16	16
	$\bar{x}$	625.606	273.669	130.806	217.106	94.969	45.356
	s	16.286	20.591	8.168	24.489	12.733	5.410
2	n	18	18	18	18	18	18
	$\bar{x}$	637.083	280.978	135.700	231.133	101.956	49.217
	s	16.236	19.820	6.588	27.313	13.614	5.966
3	n	21	21	21	21	21	21
	$\bar{x}$	637.257	284.362	137.576	224.500	100.148	48.429
	s	19.098	22.176	7.633	20.105	11.228	4.313

For explanation see Table 1

Table 3  
Statistical evaluation of the results of chemical analysis  
of cancellous bone tissue of t. coxae

Parameter	1 : 2	1 : 3	2 : 3
Ash/g of fat-free dry matter	—	—	—
Ash/cm <sup>3</sup>	—	—	—
Ca/g of fat-free dry matter	—	—	—
Ca/cm <sup>3</sup>	—	—	—
P/g of fat-free dry matter	—	+	—
P/cm <sup>3</sup>	—	—	—
Ca/P	—	—	—

— not significant; + significant

For explanation see Table 1

The highest and lowest Ca concentrations in urine were determined in post partum dairy cows (group 1,  $\bar{x} = 1.344 \pm 0.947$  mmol.l<sup>-1</sup>) and in 4–5 months pregnant cows (group 2,  $\bar{x} = 0.941 \pm 0.574$  mmol.l<sup>-1</sup>), resp. The highest inorg. P concentrations were measured in urine of the dry cows (group 3,  $\bar{x} = 2.334 \pm 3.097$  mmol.l<sup>-1</sup>) the lowest ones in post partum dairy cows (group 1,  $\bar{x} = 1.802 \pm 1.863$  mmol.l<sup>-1</sup>).

The chemical analysis of samples of cancellous bone tissue of tuber coxae revealed the highest values of ash, Ca and P per 1 g of fat-free dry matter to occur in the dry cows, i.e. group 3 ( $\bar{x}_{\text{ash}} = 637.257 \pm 19.098$  mg.g<sup>-1</sup>,  $\bar{x}_{\text{Ca}} = 284.362 \pm 22.176$  mg.g<sup>-1</sup>,  $\bar{x}_{\text{P}} = 137.576 \pm 7.633$  mg.g<sup>-1</sup>) whereas the lowest values were observed in post partum cows (group 1,  $\bar{x}_{\text{ash}} = 625.606 \pm 16.286$  mg.g<sup>-1</sup>),  $\bar{x}_{\text{Ca}} = 273.669 \pm 20.591$  mg.g<sup>-1</sup>,  $\bar{x}_{\text{P}} = 130.806 \pm 8,168$  mg.g<sup>-1</sup>). When the expression of ash, Ca and P per 1 cm<sup>3</sup> of cancellous bone tissue was used, the highest values were found in the group 2 (4–5 months pregnant cows,  $\bar{x}_{\text{ash}} = 231.133 \pm 27.313$  mg.cm<sup>-3</sup>,  $\bar{x}_{\text{Ca}} = 101.956 \pm 13.614$  mg.cm<sup>-3</sup>,  $\bar{x}_{\text{P}} = 49.217 \pm 5.966$  mg.cm<sup>-3</sup>) and the lowest ones again in post partum cows (group 1,  $\bar{x}_{\text{ash}} = 217.106 \pm 24.489$  mg.cm<sup>-3</sup>,  $\bar{x}_{\text{Ca}} = 94.969 \pm 12.733$  mg.cm<sup>-3</sup>,  $\bar{x}_{\text{P}} = 45.356 \pm 5.410$  mg.cm<sup>-3</sup>). The Ca/P ratio was found to be the highest in the group 1 (post partum dairy cows,  $\bar{x} = 2.092 \pm 0.083$ ) and the lowest in the group 3 (dry cows,  $\bar{x} = 2.065 \pm 0.091$ ). The statistical evaluation of the results obtained by chemical analysis of the cancellous bone tissue of t. coxae revealed that the differences were not significant.

## Discussion

The results obtained from the blood, blood plasma and urine examination and presented in Table 1 remain within the range of reference values (Jagoš et al. 1975a, 1981a).

The results presented in Table 2 show a certain influence of the reproduction cycle stage (daily milk efficiency) upon the chemical composition of the cancellous bone tissue of tuber coxae. In cows with the highest daily milk efficiency (group 1) the lowest average value of ash, Ca and P has been demonstrated, whereas in 4–5 months pregnant cows (group 2) and in the dry ones (group 3) these values were observed to increase.

These results that could be regarded as changes induced the the lactation stage are in accordance with the findings of Duckworth and Hill (1953), Benzie et al. (1956, 1959), Illek et al. (1977). It can be concluded from the presented observations that the onset of lactation or its late stage induces the increased demineralization of the skeleton. Also Gabel and Poppe (1971) demonstrated roentgeno-photometrically the lowest value of bone ash to occur in the 1st–3rd lactation month and the highest one in the 7th lactation month and in dry cows. The decrease of mineralization degree of the skeleton in first lactation months was reported also by Zetterholm (1978a, b). Schröter and Seidel (1980) have found that in the postparturient period not only an intensive skeleton demineralization but also organic matrix degradation takes place. Seffne et al. (1976) arrived to this conclusion even earlier.

The increase of ash, Ca and P concentration expressed per 1 g of fat-free dry matter of cancellous bone particularly in dry cows (group 3) observed in the present work might be associated with the elevation of CT level during pregnancy and thus with the reinforced effect of CT on the skeleton (Aliapoulios et al. 1966; Klotz et al. 1968).

The statistical evaluation of individual subgroups (Table 3) revealed, however, no significant differences, the only exception being significantly higher value of

P/1 g of fat-gree dry matter in dry cows (group 3) as compared with post partum cows (group 1).

In our previous report (Jagoš et al. 1981b) we reported that a higher influence upon the chemical composition of the cancellous bone tissue of tuber coxae was exerted by the reproduction stage in connection with deficiencies in mineral nutrition.

In the study of the reproduction stage influence upon the chemical composition of a cow skeleton the age of animals examined has to be taken into consideration as well. Many authors have proved the dependence of chemical composition of cattle bone tissue on the animal age (Strobino and Farr, 1949; Hammer 1962; Pannendorf and Gerhardt 1969; Gabel and Poppe 1971; Blincoe et al. 1973; Field et al. 1974; Jagoš et al. 1981b and others).

For the demonstration of a possible effect of reproduction stage upon the parameters examined by chemical analysis of the cancellous bone tissue of tuber coxae the cows 3–8 years old were selected. This age range is too broad to exert any influence in the case of age heterogeneity of individual groups. The cause of the observed lower average value of ash, Ca and P per 1 cm<sup>3</sup> in dry cows (groups 3) when compared with the group 2 (4–5 months pregnant) may be due to this higher age mean of dry cows.

No significant effect of daily milk yield on Ca : P ratio in ash have been found. In our previous work (Jagoš et al. 1981b) we have, however, observed lowering of the calcium: phosphorus ration in the bone ash of dairy cows in later lactation stage but these animals already suffered from clinical metabolic osteopathy.

Our results are in accordance with the findings of Rossow et al. (1976) who have shown that the actual milk production has no effect upon the value of bone ash in dairy cows if the supply of calcium, phosphorus, etc. to the organism is adequate to the efficiency reached.

### **Vliv stadia reprodukčního cyklu dojnic na některé klinicko-biochemické ukazatele**

Vliv fáze reprodukčního cyklu na vybrané ukazatele úrovně minerálního metabolismu byl sledován u 55 klinicky zdravých krav českého strakatého plemene, stáří 3–8 let, jejichž výživa odpovídala v hodnocených ukazatelích (ŠJ, SNL, Ca a P) normované potřebě. Zvířata byla rozdělena do 3 skupin podle stadia reprodukce (aktuální užítkovosti) a sledována v průběhu zimního krmného období (prosinec - duben).

Vybrané klinicko-biochemické parametry krve (pH, base excess, pCO<sub>2</sub>), krevní plazmy (Ca, anorg. P) a moči (Ca, anorg. P) nevykázaly odchylky od referenčních hodnot. Parametry stanovené chemickou analýzou bioptátů spongiózy tuber coxae prokázaly signifikantní pokles hodnoty P/g tukuprosté sušiny u dojnic po porodu ve srovnání s kravami stojícími na sucho. Jinak fáze reprodukčního cyklu již neměly signifikantní vliv na hmotnost popela a Ca vyjádřených na 1 g tukuprosté sušiny a hmotnost popela, Ca a P vyjádřených na 1 cm<sup>3</sup> spongiózy.

### **Влияние стадии цикла воспроизводства дойных коров на некоторые клинико-биохимические показатели**

Влияние фазы цикла воспроизводства на избранные показатели уровня минерального метаболизма изучалось у 55 клинически здоровых коров чешской пестрой породы, возраст 3–8 лет, питание соответствовало

в оцениваемых показателях ( $\text{Sj}$ ,  $\text{SNL}$ ,  $\text{Ca}$  и  $\text{P}$ ) установленному стандартом расходу. Животные были разделены до 3 групп по стадии воспроизводства (актуальной продуктивности) и исследования проводились в течение зимнего кормового периода (декабрь—апрель).

Избранные клинико-биохимические параметры крови ( $\text{pH}$ ,  $\text{base excess}$ ,  $\text{pCO}_2$ ) кровяной плазмы ( $\text{Ca}$ , неорг.  $\text{P}$ ) и мочи ( $\text{Ca}$ , неорг.  $\text{P}$ ) не отличались от контрольных величин. Параметры, установленные химическим анализом биоптатов спонгиоза *tuber soxae* выявили существенное понижение величины  $\text{P/t}$  безжирного сухого вещества у дойных коров после отела по сравнению с коровами на сухостое. В остальном фазы цикла воспроизводства не оказали уже существенного влияния на массу золы и  $\text{Ca}$ , выраженных к 1 г безжирного сухого вещества и массу золы,  $\text{Ca}$  и  $\text{P}$ , выраженные к 1  $\text{cm}^3$  губчатого вещества.

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