

## BONE TISSUE METABOLISM IN CATTLE

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### ABSTRACT

Bone metabolism is closely connected to calcium metabolism. Biochemical markers of bone metabolism in blood serum and/or urine are indicators of bone metabolic activity at real time. Disorders of bone metabolism represent a significant scope of health problems in cattle. The objective of the study was the evaluation of bone-alkaline phosphatase (BALP) a marker of bone formation and C-terminal telopeptide (CTX), a marker of bone resorption in blood serum in dairy cattle of different ages and different productive stages. The following groups of Slovenian Black and White breed cattle were investigated: calves, primiparous cows and cows in the fourth or higher lactation kept in tie-stalls type and cows at early dry off period after the fourth or higher lactation in tie-stalls type and on pasture. Calves have statistically significantly higher BALP values in blood serum than cows ( $P < 0.05$ ). Statistically significantly ( $P < 0.05$ ) higher values for BALP and CTX were obtained also in primiparous cows at the pick of lactation than in the fourth or higher lactation cows at the same phase of lactation. We found statistically significantly lower ( $P < 0.05$ ) mean BALP blood serum activity in cows at early dry off that were housed in tie-stall type than in those on pasture. The difference in mean BALP between cows in the fourth or higher lactation at pick lactation and those at early dry off period was not statistically significant. Biomarkers of bone metabolism can be implemented in monitoring cattle bone metabolism and calcium metabolism.

Key words: cattle / bone tissue / metabolism / biomarkers

## PRESNOVA KOSTNEGA TKIVA PRI GOVEDU

### IZVLEČEK

Motnje v presnovi kostnega tkiva predstavljajo pomemben delež zdravstvenih motenj pri govedu. Povezane so z metabolizmom kalcija. Biokemijski kazalci presnove kosti v krvnem serumu in / ali urinu ob času odvzema omenjenih vzorcev so pokazatelji metabolične aktivnosti (anabolične / katabolične) kostnega tkiva. Namen raziskave je bil preučevanje kostno specifične alkalne fosfataze (BALP), ki je kazalec izgrajevanja kostnine in C-terminalnih telopeptidov (CTX) kot kazalca razgrajevanja kostnine v krvnem serumu goveda mlečne pasme različnih starosti in v različnih fazah proizvodnega ciklusa. Preučevali smo sledeče skupine goveda slovenske črno-bele pasme: teleta, krave v prvi in četrti ali višji laktaciji v vezani reji ter krave na začetku presušitve po četrti ali višji laktaciji v vezani reji in na paši. Teleta so imela v krvnem serumu statistično značilno ( $p < 0,05$ ) višje vrednosti BALP kot krave. Statistično značilno ( $p < 0,05$ ) višje vrednosti BALP in CTX v krvnem serumu smo ugotovili pri kravah v prvi laktaciji na višku laktacije kot pri kravah v četrti ali višji laktaciji v isti fazi proizvodnega ciklusa. Krave na začetku presušitve, ki so bile na paši, so imele statistično značilno ( $p < 0,05$ ) višje vrednosti BALP v krvnem serumu od tistih v vezani reji. Razlika med vrednostmi BALP v krvnem serumu pri kravah na višku laktacije in tistih, ki so bile na začetku presušitve, ni bila statistično značilna. Biokemijski kazalci metabolizma kostnega tkiva v krvnem serumu so lahko v pomoč pri spremljanju metabolizma kostnega tkiva in kalcija.

Ključne besede: govedo / kostno tkivo / presnova / biomarkerji

## INTRODUCTION

Bone metabolism is a continuous process of bone modelling (in young growing individuals) and remodelling (in grown-up individuals) which is tightly coupled absorption of old bone tissue and synthesis of new bone tissue. The purpose of remodelling process is primarily to maintain high quality of bones (strength and flexibility) and especially in high yielding dairy cows also to supply calcium (Ca) when demand for it is too high to be covered from ration (Goff, 2000). In this case, resorption of bone tissue is more pronounced than synthesis. Any disturbances in this process (reduced or increased resorption or synthesis or both) lead to substantial changes in functional integrity of bone over time (Christenson, 1997). Bioindicators of bone metabolism at near real time of sample collection (blood and/or urine) are biochemical markers of bone metabolism. Markers of bone resorption (for example tartrat resistant acid phosphatase (TRAP) and CTX) indicate osteoclast activity (catabolic) and markers of bone synthesis (for example BALP and osteocalcin (OCN)) indicate osteoblast (anabolic) activity in bones (Starič, 2005). They can be used as early indicators of bone and Ca metabolism disturbances. Because of subclinical nature of metabolic bone diseases especially in early stages, many diseases go unnoticed until animals are seriously ill. Diseases of bone and Ca metabolism, especially puerperal hypocalcaemia in high producing cows are still very common in cattle (Goff, 2000). In high producing dairy cows we expect increased catabolic bone metabolism at the beginning of lactation as absorbable calcium demand suddenly increase from about 22 g per day to more than 50 g or even 60 g per day in high yielding animals because of colostrums and milk production (NRC, 2001). Cortical bone calcium concentration decreases as milk production increases as was shown in a study conducted by Beighle (1999). When milk production starts to decline body stores of Ca have to replenish and bone metabolism is more anabolic (Underwood and Suttle, 2001). Bone diseases and calcium metabolism disorders that are closely connected to bone metabolism are also related to suboptimal production in cattle. The objective of this study was the evaluation of different biochemical biomarkers of bone metabolism in cattle of different ages, different phase of lactation and different physical activities.

## MATERIAL AND METHODS

Biochemical markers of bone metabolism, BALP and CTX, were evaluated in blood serum of Slovenian Black and White breed cattle.

Table 1. Groups of cattle with intensive dairy production from the same herd included in the study

GROUP	N	DESCRIPTION
I	3	5 to 6 months old calves
II	14	primiparous cows at the peak of lactation in tie-stall type
III	7	cows in fourth or higher lactation at the peak of lactation in tie-stall type
IV	4	early dry cows (within a week after drying off) after fourth or higher lactation in tie-stall type
V	5	early dry cows (within a week after drying off) after fourth or higher lactation on pasture

The study was carried out in early spring when the animals were still on their according to NRC (2001) recommendations calculated winter ration served as a complete mix for pick

lactation cows and dry cows, except cows in group V which also got a small amount of pasture and calves which receive 3 kg of concentrate with 21% crude protein and hay ad libitum.

Venous blood samples were collected from *v. jugularis* in calves and *v. caudalis mediana* in adult cattle in evacuated tubes without any additives (Venoject, Terumo Europe, Leuven, Belgium). All samples were collected between 9 a.m. and 11 a.m.. Blood samples were kept at room temperature until they spontaneously coagulated (about 4 hours) when they were decanted and centrifuged at 1 800 G for 10 minutes. The serum samples were then stored at  $-20^{\circ}\text{C}$  until analysis, which was performed within two weeks of sample collection.

All tests were performed automatically. Catalytic activity of BALP was measured via enzymatic immunoanalysis by Alkphase-B, Metra, Biosystems, California, USA test kit. Concentration of CTX was measured by electrochemiluminescence immunoanalysis ECLIA using ELECYCIS 3 – CrossLaps test kit.

The obtained data were statistically analysed with SPSS version 12 (SPSS Inc., USA). Arithmetic means and standard deviations were calculated and analysis of variance (ANOVA) performed. Differences at  $P < 0.05$  were considered statistically significant.

## RESULTS

Mean BALP and CTX values in different groups of cattle are presented in Table 2.

Table 2. Mean BALP activity and CTX concentration with standard deviation in venous blood serum samples of examined groups of cattle

GROUP	n	BALP $\pm$ SD (U/L)	CTX $\pm$ SD (ng/mL)
I	3	78.7 $\pm$ 18.2	/
II	14	21.75 $\pm$ 4.1	1.014 $\pm$ 0.252
III	7	15.01 $\pm$ 1.7	0.784 $\pm$ 0.061
IV	4	14.2 $\pm$ 5.7	/
V	5	26.1 $\pm$ 4.6	/

Mean BALP and CTX values in group II and group III were statistically significantly different at  $P < 0.05$ . Interestingly we found significant difference ( $P < 0.05$ ) in mean BALP between cows in group IV and group V. The difference of mean BALP activity between group III and IV was not statistically significant.

Calves (group I) had statistically significantly ( $P < 0.05$ ) higher BALP values than cows (group II, III, IV, V).

## DISCUSSION

Mean values for BALP were the highest in calves and higher in primiparous cows than in 4th lactation cows, due to growing of skeleton (modelling) which means also more intensive anabolic bone metabolism in these animals. Higher BALP activity in younger animals was already observed in studies in foals (Hank *et al.*, 1993) and cattle (Sato *et al.*, 2002). CTX values were higher in group II than in group III probably due to the same reason and the ability of younger cows to more effectively absorb bone to cover Ca demand. This is probably the reason why primiparous cows usually do not get puerperal hypocalcaemic paresis (Goff, 2000). Bone metabolism is more active in cows on pasture, which had higher BALP activities than in cows housed in tie-stall type. Physical activity intensifies bone metabolism (Price *et al.*, 1995). Interestingly we did not observe any statistical difference in BALP activity in cows at peak

lactation and those at early dry off even though we anticipated one. We assume that one of the reasons could be  $\alpha$  error, because the sample groups were small. The other possibility for this can be also that Ca reserves were already replenished during the second half of lactation, so Ca and bone metabolism were already in equilibrium concerning anabolic and catabolic activity at the early dry off. Similar results were obtained by Holtenius and Ekelund (2005) when they measured OCN thorough lactation and dry off, when OCN as bone formation marker had similar values at the pick lactation and early dry off.

## CONCLUSION

Biomarkers of bone metabolism in blood serum can be implemented in monitoring cattle and also other productive animals bone and Ca metabolism. Examples are poultry and swine, which are also especially sensitive to bone and Ca metabolism disorders, due to intensive growth and egg production. The effect of age and physical activity on bone markers is significant and has to be considered when interpreting these results. According to BALP activity, cows that are less physically active replenish bone mineral reserves less effectively than those that are more physically active. This information might be useful for preparing preventive measures for milk fever.

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