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BASAL SERUM CORTISOL CONCENTRATIONS IN CATTLE

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Glucocorticoids play an important role in many metabolic pathaways concerned with development and control and their serum concentrations reflect the overall balance between synthesis/secretion and elimination/degradation. This paper summarises the results obtained for serum concentrations of cortisol, the predominant glucocorticoid molecule in calves, bulls and cows of different breeds and ages. Thus, a total of 484 blood samples taken from 329 beef or dairy animals was examined using either liquid-phase or solid-phase radioimmunoassay (RIA) procedures. Values obtained with the easier to perform solid-phase RIA tended to be higher than those with the more sensitive liquid-phase RIA but the difference was not statistically significant. Although there were large individual variations between animals in most of the studies, mean values of the order of 20 nmol/L were observed in all nonstressful situations. Increased values were found around the time of parturition in cows, and in the abattoir for bulls. Cows exhibiting ketosis tended to have below average serum concentrations of cortisol. Cortisol levels increased with age in young cattle kept under extensive conditions.

Key words: beef cattle, dairy cows, calves, cortisol, radioimmunoassay

INTRODUCTION

In species in wich cortisol is the major glucocorticoid, basal circulating concentrations have been reported to vary from 10 - 50 nmol/L in cows to 1500 - 5500 nmol/L in some New World primates (Gayrard et al., 1996). Within the range for cattle, differences have ben associated with breed, age, nutrition and various stresses (Anderson et al., 1988; Hristov et al., 1994; Sartin et al., 1988; Smith et al., 1973; Stahringer et al., 1994). In view of the importance of glucocorticoids in

many metabolic pathways concerned with development and control, the low levels found in cattle are surprising and, together with the presence of specific and non-specific binding proteins, contribute to the difficulties associated with accurate determination of basal cortisol concentrations in bovine species. Thus, Smith and coworkers (1973) used a complicated procedure involving two extractions before a competitive protein binding reaction.

Many methods/kits based on imunoassay principles have been developed, primarily for use in human medicine where the range of cortisol concentrations and the properties of the binding proteins differ from bovine material (McConway and Chapman, 1986; Seal and Doe, 1963). Breves and coworkers (1980) established that cortisol may account for total glucocorticoid activity in cow plasma, while several authors have described modifications to commercial cortisol assay systems to validate them for bovine materials (Sartin et al., 1988; Verkerk et al., 1994). Definitive methods based on isotope dilution gas chromatography-mass spectrometry have also been developed for reference (De Brabandere et al., 1995). The requirement for specific, highly sensitive, easy to perform assays has led to continual improvement of radioimmunoassays (RIA) for cortisol. Thus, solid phase RIAs involve adsortption of the specific antibody reagent to surfaces in the vessel used for analysis, thereby eliminating the need for precipitation and centrifugation, so that the assay procedure is simplified and shortened.

Upto 1996 a sensitive liquid-phase system including a radioidinated cortisol labed and a specific antibody with minimal cross-reactivity with other steroids was used in our investigations. Cortisol was displaced from endogenous binding proteins by performing the assay at low pH and the method was adapted for use with bovine sera. Recently an improved solid-phase RIA for determination of cortisol in human serum was introduced in our laboratory (Nikolić et al., 1996b). The present paper summarises the results obtained for serum cortisol concentrations in various breeds and categories of healthy cattle using both assay systems.

MATERIALS AND METHODS

Animals and sample treatment. Blood samples were obtained from: a) 22 female and 22 male Holstein calves on a large intensive dairy farm (Farm A) on the day of birth and from the same animals at 4.5 months of age (Nikolić et al., 1996a);b) 15-day-old female calves of the Holstein (n=14) and Charolais-Holstein (n= 14) breeds as well as 7 male Charolais-Holstein calves on the same farm before or 3th after consumption of the morning portion of milk (Nikolić et al., 1996c); c) Limousine and Charolais cattle of both sexes at 7 days,6 months and 12 months of age kept under extensive conditions on pasture with their dams (Farm B); d) Simmental type domestic pied (n= 20) and Limousine (n = 6) bulls the day before slaughter (Farm B); e) two groups of 7 lactating Holstein cows kept on Farms X and Y under different conditions (Šamanc et al., 1993a); f) periparturient Holstein cows on a large dairy farm (Farm C) over several years (n = 182). Namely, a total of 484 blood samples was obtained from 329 animals. After coagulation, the serum was separated by centrifugation and the samples stored at -20^{0}C until required for analysis. Samples collected under c) and d)

were assayed successively in small groups whereas the remainder were determined on single occassions for one complete experiment.

Liquid phase cortisol RIA. This sensitive competitive binding assay employed a specific rabbit antiserum raised against cortisol-3-(O-carboxymethyl) oxime (CMO) congugated to bovine serum albumin (BSA) as the reagent. The tracer was prepared by labelling corticol-3-CMO-tyrosinemethylester(TME) on the tyrosine ring with ^{125}I using the classical chloramine-T method. The assay was carried out in citrate buffer (pH 4.5) whereby steroids bound to corticosteroid binding protein (CBS) are released without serious disturbance to the antibody-antigen reaction. The test was adapted to cover the range of concetrations from 6.9 to 690 nmol/L which is suitable for bovine serum samples.

Separation of antibody-bound from free cortisol was achieved by precipitation with polyethyleneglycol (PEG 6000) at a final concentration of 16% followed by centrifugation. Declared cross-reactivity of the polyclonal antiserum was 6.4% for cortisone and 0.1% for progesterone, dehydroepiandrosterone, testosterone and corticosterone at 50% inhibition of antibody binding.

Solid phase cortisol RIA. This assay involved the use of a murine monoclonal antibody to cortisol-3-CMO-BSA to coat the inner walls of polystyrene tubes in such a way that the antigen-binding portion projected freely from the surface. The same ^{125}I labelled tracer was used as the competitive species in a reagent containing 8-anilinonaphthalene sulphonic acid (ANS) which dissociates cortisol from its binding proteins at neutral pH. The assay was validated for human sera (Nikolić et al. 1996b) and adapted for use with bovine sera by increasing the sample size from 20μl to 50μl and including an additional standard containg 13,4 nmol/L cortisol. Declared cross-reactivity of the antibody to cortisone was 100%. Cross-reaction with progesterone was examined and found to be 9.6% at 50% inhibition which is negligible under normal circumstances wheere cortisol concentrations are 10-fold higher than progesterone levels.

Statistical analysis. The data were subjected to analysis of variance (ANOVA) using a computer programme (MSTATC, USA). When Bartlett's test indicated nonhomogeneity of variance between groups ANOVA was repeated using mathematically, transformed data (square root, logarithm). When the F-test indicated significant differences ($P < 0.05$),orthogonal contrasts were made between groups using the original and transformed data as necessary. Non-parametric methods were used when skewness, kurtosis and non-homogeneity of variance persisted after mathematical transformation.

RESULTS

Basal serum cortisol concentrations (mean ± standard deviation (SD)) found in the different breeds and categories of cattle examined are shown in Tables 1 - 7. Assay precision as estimated by the mean coefficient of variation between duplicate determinations of a series of samples was about 5% for the liquid-phase assay and 11% for the solid-phase assay. The former assay system was also more sensitive. Namely, 50% inhibition of tracer binding occurred at about 120 nmol/L cortisol compared with 300 nmol/L cortisol in the easier to

perform solid-phase assay. Nevertheless,for five samples determined in both assay systems the mean values were 34,0 and 35,1 nmol/L respectively with a correlation coefficient (r) of 0.98.

Slightly higher cortisol concentrations were observed in 1-day-old male calves than in their female counterparts (Table 1). The samples were obtained between May 11th and May 31st 1992 and again from the same animals in early October at about 4.5 months of age when they were moved to feedlots (Nikolić et al.,1993a).At the second sampling there was no difference between the sexes and mornign (10.30 - 11.25 am) basal values were around 20 nmol/L.The animals had been fed ground maize meal and sunflower oilmeal with hay and straw ad libitum in groups of 18-20. Two-factor ANOVA indicated no difference between the sexes (F = 0.82) but a significant decrease with age (F = 5.43; P = 0.025).

Table 1. Body weights and basal serum cortisol levels in male and female Holstein calves on the day of birth and at 4.5 months of age (Nikolić et al., 1996a)

Sex	No.	Age (months)	Body weight (kg)	Cortisol (nmol/1)
Female	25	0	38.1^{a}	24.8^{ab}
	22	4.5	116.9^{b}	21.1^{b}
Male	22	0	39.9^{a}	32.7^{a}
	21	4.5	123.9^{b}	20.2^{b}
	S.E.		2.3	3.5

^{a,b} Means not sharing the same superscript differ signiflantly ($P<0.05$)

A later investigation on the same farm (Nikolić et al., 1993c) showed a tendency for female Charolais cross calves to have lower basal preprandial cortisol concentrations than female Holstein calves at 15 days of age (Table 2). However, the large individual variation prevented statistical significance for the difference between the means (F = 0.63). The calves were kept in individual boxes and had been offered about 4 - 7 litres of liquid milk daily. Post-prandial values were similar in all groups, including male calves with mean values approaching 20 nmol/L. The liquid-phase assay was used for all the above determinations.

Table 2. Serum cortisol concentrations in 15-day old calves of different breeds (mean ± SD; Nikolić et al., 1996c)

Breed	Sex	Experiment 1		Experiment 2*	
		No.	Cortisol (nmol/L)	No.	Cortisol (nmol/L)
Holstein	Female	7	20.5 ± 12.9	7	19.3 ± 19.6
Charolais/Holstein	Female	7	10.8 ± 1.9	7	18.4 ± 13.2
Charolais/Holstein	Male		–	7	19.3 ± 10.7

* 3h after morning milk (3,5 L)

A tendency for low basal cortisol concentrations to occur in young beff calves was also found in predominantly Limousine and Charolais calves kept on

pasture with their dams (Table 3). No difference was found between the sexes (F= 1,7). A highly significant effect of age was found (F = 16,0; P < 0.0001). However, the groups were not entirely independent because more than one sample was obtained from some animals. Moreover, the liquid-phase assay was used for two thirds of the samples taken at 7 days and 6 months but the solid-phase assay for the remainder. As very similar mean values were obtained for each method (18.5 ± 19 and 19.1 ± 12.4 respectively), it was concluded that differences in methodology did not confound the results. Moreover, split-plot factorial ANOVA for three Limousine and three Charolais male calves for which samples were available at each period (Table 4), showed no differences associated with breed but the increase in cortisol concentration with age was confirmed (P=0.045).

Table 3. Basal serum cortisol concentrations in beef cattle kept on pasture using both methods (mean ± SD).

Age	Sex	No. of samples*	Cortisol (nmol/L)		
			Method 1 (n)	Method 2 (n)	Combined
7 days	Male	7	8.7±12.5 (6)	8.0(1)	8.6±11.4
	Female	9	12.2± 5.0 (5)	9.8±1.2(4)	11.1± 3.8
6 month	Male	9	16.0± 4.0 (5)	24.0±13.7(4)	19.6± 9.8
	Female	10	31.3±29.5(8)	33.2± 2.2(2)	31.7±26.0
12 months	Male	8	—	36.7±13.8	36.7±13.8
	Female	7	—	55.4±19.8	55.4±19.8

F(sex) = 1.70; NS. F (age) = 16,0; P<0.0001. F(method) = 0.008 NS.
* 49 samples obtained from 17 female and 11 male animals

Table 4. Basal cortisol concentrations in six male calves of different breeds at different ages

Age	Cortisol (nmol/L)		
	Charolais	Limousine	Overall
7 days	3.5	13.8	8.7
6 months	20.9	13.0	17.0
12months	35.4	29.3	32.4

(breed) = 0.09; NS. F (age) = 4,70; P=0.045. F (interaction) = 0.81; NS.

Basal cortisol concentrations for 26 young bulls 1 day before slaughter are shown in Table 5. The mean value found in twenty domestic pied bulls of the Simmental type tended to be lower than in six Limousine bulls (P=0.061). The liquid phase assay was used for most samples (n = 18). The mean value (23.5 ± 27.0 nmol/L) tended to be lower than in the solid phase assay (37.0 ± 13.0 nmol/L) but the difference was not statistically significant (F = 1.78), although Bartlett's test showed marked non-homogeneity of variance between the groups.

The Kruskal-Wallance non-parametric test indicated a significant breed difference (P = 0.045) but no difference associated with methodology (P=0.107).

Table 5. Serum cortisol concentrations in young bulls one day before slaughter (mean ± SD)

Breed	No.	Body weight (kg)	Cortisol (nmol/L)
Simmental type	20	549 ± 46	22.8 ± 25.2
Limousine	6	600 ± 32	43.7 ± 10.4
	F-value	6.471	3.867
	P-value	0.0178	0.0609

Table 6. Basal serum cortisol concentrations in fourteen Holstein cows kept on two farms (mean and SD; Šamanc et al., 1993a).

Time	Cortisol (nmol/L)			
	Farm X		Farm Y	
About 10 days before calving	20.3[a]	9.1	10.4[b]	4.3
About 10 days after calving	14.9	8.7	8.4	3.9
2 months lactation	14.1	4.9	13.7	4.7
5 months lactation	10.0	2.5	11.2	2.8
F (time)	2.66		2.13	

[a,b] Significant difference between farms

Prepartal basal serum cortisol concentrations were found to be higher in seven Holstein cows on farm X than in an equivalent number on Farm Y (Table 6). In the first case the diet had a roughage: concentrate dry matter ratio of 75:25 w/w, whereas the ratio was 50:50 w/w on Farm Y. During lactation no differences were detected either in relation to time or locality (Šamanc et al., 1993a). The average value was 13 nmol/L. Similar levels were found about 10 days postpartum in cows on Farm C (Table 7; Šamanc et al., 1993b; 1994). Cows exhibiting symptoms of ketosis tended to have lower concentrations of serum cortisol than healthy cows (Table 7; Šamanc et al., 1994; 1997; Nikolić et al., 1997). The difference was statistically significant in Study 2. Mean values for cortisol tended to be greater around the time of parturition. The solid phase assay was used in Studies 5 and 6 and the liquid phase assay in the earlier studies. The pattern of change in cortisol concentration in healthy cows with lean or normal conformation in three studies is shown in Figure 1. It appears that the prepartal increase commences about 5 days before parturition. The correlation coefficients (r) were 0,658 (P=0.004) and 0.471 (P = 0.020) respectively in Studies 5 and 6 but no significant correlations were observed post partum in either study. The prepartal increase may be the result of the rapid metabolic changes associated with the approaching parturition including the consequence of inadequate feed intake.

Table 7. Basal cortisol concentrations in periparturient Holstein cows on Farm C.

Study	State	Time (days)	No	Cortisol (nmol/L)		Reference
				Mean	SD	
1	Healthy	10 post partum	25	14.5	6.7	Šamanc et al., 1993b
2	Healthy	7-14 post partum	15	19.6[a]	8.1	Šamanc et al., 1994
	Ketotic	"	15	14.1[b]	6.2	
3	Lean-normal	2-12 pre partum	17	41.9	22.1	Nikolić, 1996
	Fat	"	16	67.7	89.0	
4	Healthy	2-7 post partum	12	37.5	30.6	Nikolić et al., 1997
	Ketotic	"	14	26.4	24.2	
5*	Healthy	7-14 post partum	10	41.1	32.0	Šamanc et al., 1997
	Ketotic	"	10	24.2	17.4	
6*	Lean-normal	1-7 pre partum	24	57.8	30.5	Kovačević et al., 1998
		1-7 post partum		69.8	45.5	(unpublished)
	Fat	1-7 pre partum	24	64.3	32.5	
		1-7 post partum		74.6	44.2	

* Solid phase assay used in these studies; liquid phase assay in remainder.

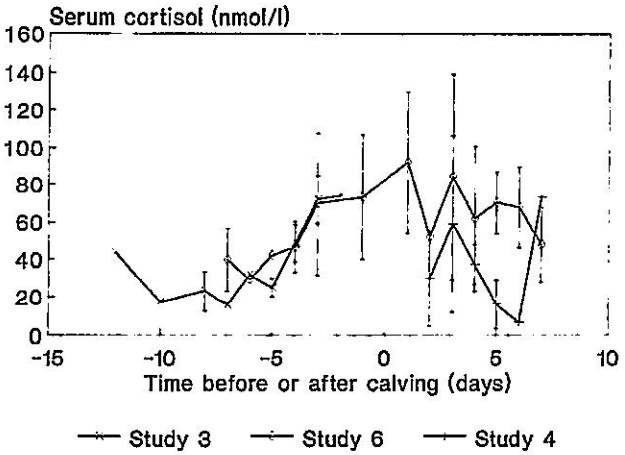

Figure 1. Cortisol concentrations before and after parturition in groups of healthy Holstein cows on Farm C (Study 3 - Nikolić, 1996; Study 4 - Nikolić et al., 1997; Study 6 - Kovačević et al., 1998).

The influence of stress was also confirmed for bulls. The mean serum cortisol concentration more than doubled when samples taken in the abattoir were compared with those obtained in the morning before transportation (28.5 ± 11.9 and 76.4 ± 26.7 respectively, $t = 3.37$; $P = 0.043$).

DISCUSSION

Considering the important role of this glucocorticoid in influencing metabolic pathways, there are relatively few data in the literature. This is probably due to methodological difficulties in determining the low levels of basal cortisol found in bovine serum accurately, as well as the wide individual differences encountered in all species which make connection of serum values for cortisol with production parameters and physiological changes difficult. Thus, in our case the two methods tended to give slightly different results each of which were were within the range of values reported by other authors. The higher values found with the simpler solid-phase assay probably reflect its lower sensitivity and the lower specificity of the reagent antibody. Namely, Huszenica and coworkers (1998) concluded that the cortisol/corticosterone ratio in bovine blood is 2.4/1, and serum corticosterone would have been partially determined in our solid phase assay.

In any competitive binding assay it is very important that the standards are as similar to the unknown samples as possible in order to compensate for possible differences in the affinity of the reagent antibody for the labelled and unlabelled ligands and to equalise the influence of endogenous binding proteins. In our tests the standards were in buffer in the liquid phase assay and in steroid free bovine serum in the solid phase assay, neither of which is ideal. Assay precision was more favourable in the liquid-phase test. Both assays were standardised with internationally recognised human serum samples with known concentrations of cortisol (Biorad).

A rapid decrease in serum cortisol concentrations from about 72 nmol/L 2 h after birth to about 43 nmol/L at 24 h was reported in Holstein calves by Hristov and coworkers (1994). These results are somewhat higher than our findings but in both cases individual variation between animals was considerable. Further decreases to 27 nmol/L at 72 h and 18.5 nmol/L at 10 days old were also reported, which corresponds to our results for 15-day-old calves. Baumrucker and Blum (1994) reported values of approximately 30 nmol/L in male dairy calves up to 7 days of age (Simmental-Red x Holstein and Brown Swiss), which is in a similar range.

Henricks and coworkers (1984) showed a significant positive correlation between plasma cortisol and age or weight for beef heifers aged 7 - 12 months but not for bulls, where the concentrations remained around 7 nmol/L. Our findings for young cattle at pasture confirm this trend, although the reverse has been observed for confined Holstein cattle (Hristov et al., 1994). Breed differences may occur as indicated by the difference found for Limousine and Simmental type bulls in this investigation. Anderson and coworkers (1988) found very low cortisol concentrations in Simmental crossbred bulls aged 9-16 months (around 4 nmol/L).

Concerning cows, Wagner and Oxenreider (1972) used indwelling jugular vein cannulae to show significant differences between mean values for basal

serum cortisol in non-lactating (12.3 nmol/L) and lactating Holstein cows (milked 18.8, suckled 25.9 nmol/L) which reflects the difference in cortisol secretion rate. Diurnal variations were small, levels being 14.6 nmol/L from 1800 - 0200 h and around 20 nmol/L at all other times. Our results confirm and fill out the trends observd by Hristov and coworkers (1994) where basal cortisol concentrations were about twofold higher at mating and around partus than during gestation-lactation. Mean cortisol concentrations were lower in high producing cows at 30 days (29 nmol/L) than at 90 days postpartum (41 nmol/L) or in nonlactating cows (49 nmol/L) due to a reduced pulse amplitude (Sartin et al., 1988).

Low serum concentrations of cortisol are also associated with ketosis, although some investigations showed no differences between healthy and ketotic cows concerning basal cortisol concentrations (Đurđević et al., 1980) Even when differences appear they may not be statistically significant (Table 6), necessitating more detailed investigations of the adrenocortical reserve with stimulants such as ACTH or sodium propionate (Šamanc et al., 1993b, 1994; Verkerk et al., 1994).

From all these investigations it may be concluded that basal serum cortisol concentrations in cattle range from about 5 to30 nmol/L, higher values being found in any stressful situation such as regrouping, transport, partus etc.

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BAZIČNE KONCENTRACIJE KORTIZOLA U SERUMU GOVEDA

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SADRŽAJ

Glukokortikoidi igraju važnu ulogu u mnogim metaboličkim putevima vezanim za razvitak i kontrolu organizma. Serumska koncentracija kortizola, koji je glavni glukokortikoid kod goveda, ukazuje na trenutni bilans između sinteze/lučenja i potrošnje (degradacija, vezivanje za receptore,lučenje u mokraći itd.) U ovom radu date su vrednosti dobijene za nivo kortizola u serumu teladi, bikova i krava različitih rasa i starosti. Naime, ispitana su 484 uzorka uzeta od 329 grla mesnatih ili mlečnih rasa goveda radioimunoesejom (RIA). Vrednosti dobijene RIA-postupkom na čvrstoj fazi imale su tendenciju da budu veće od onih dobijenih RIA-postupkom u tečnoj fazi, mada razlika nije bila statistički značajna. Prvi postupak je bio jednostavniji za izvođenje dok je drugi bio osetljiviji. Iako su postojale velike individualne razlike između životinja u većem broju ogleda, srednje vrednosti su se kretale oko 20 nmol/L, osim u slučajevima stresa. Koncentracije kortizola u serumu su bile veće kod krava pred partus i kod bikova u klanici. Postojala je tendencija da nivoi kortizola u serumu budu niži kod krava koje su ispoljavale znakove ketoze. Kod teladi na paši u ekstenzivnim uslovima držanja koncentracije kortizola su se povećavale sa starošću životinja do 12 meseci.