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GLYCEMIA, GLUCOCORTICOIDS AND ADRENOCORTICAL RESERVE IN POSTPARTAL DAIRY COWS

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The useful life of dairy cows on many modern farms is short. With the aim of detecting possible differences in metabolism in relation to parity, blood glucose, serum cortisol and adrenocortical reserve were measured in five groups of ten healthy Holstein-Friesian cows during the second week after calving. The groups of cows represented the populations in the first five lactations on the farm. Mean daily milk production from day 7 to day 14 showed steady increases in each group. The average for the whole week was significantly higher for lactation 2 or 3 than for lactaction 5 ($P<0.05$), while the heifers gave the lowest average yeld. Conversely, the average blood glucose concentration was higher in the groups of heifers and cows in lactation 5 than for those in lactations 2 or 3. Thus, daily milk yield was inversely correlated with blood glucose concentration ($r = -0.482$; $P < 0.0001$; $n = 400$). The overall temporal profile of changes in blood glucose level showed a minimum on day 10, although there were marked differences in the pattern between different lactations. Moreover, individual variability between cows within a group decreased with parity. Basal serum cortisol concentrations were significantly lower in the group of heifers than for cows in lactation 3 or 4. The mean response to stimulation by adrenocorticotropin tended to diminsh with increasing parity as determined by the cortisol increment at 60 min after intravenous injection. This may reflect a lower secretion rate or an increased rate of utilisation of cortisol. Further work is necessary to show whether the slightly lower milk yield and serum cortisol parameters, together with higher blood glucose concentrations found for cows in lactation 5 are inherent characteristics of the result of adaption.

Key words: dairy cows, parity, glucose, cortisol, milk yield

INTRODUCTION

The load on the metabolic capabilities of the modern dairy cow is such that many do not survive more than two or three lactations. The most critical time is during the periparturient period when the organism is attempting to maintain itself and suddenly produce large quantities of carbohydrate and protein-rich fluid. Glucocorticoids may play an important role at this time by stimulating gluconeogenesis. Thus, Breves and coworkers (1980) found a positive correlation between blood glucose and plasma cortisol and a negative correlation between blood ketone bodies and plasma cortisol for the period 2 weeks before to 7 weeks after parturition in fourteen German Schwarzbuhte cows. Sartin and coworkers (1987) showed that Holstein cows producing 41 kg milk per day had lower mean cortisol concentrations over a 6 h period than cows producing 32 kg milk per day on day 30 of lactation. This was due to a reduced pulse amplitude.

Basal serum cortisol concentrations reflect only the overall balance between synthesis/secretion and elimination/degradation at any one time, so the above associations may reflect reduced production and/or increased use of cortisol. Therefore, tests to assess the adrenocortical reserve have been developed involving intramuscular injection of adrenocorticotropic hormone (ACTH) and monitoring of the cortisol response (Alam et al., 1986; Šamanc et al., 1993). Individual variability in the response and the necessity of multiple sampling can be diminshed by using the intravenous rout and a carefully chosen time and dose (Verkerk et al., 1994).

In this work, in an attempt to detect possible differences between cows that were in their fifth lactation and their younger sisters, an ACTH challenge was performed on representative healthy cows during the second week after calving. Daily milk production and blood glucose levels were also monitored.

MATERIALS AND METHODS

Animals and procedure

A total of 50 Holstein Friesian cows kept tethered in the maternity parlour on a large dairy farm were included in the investigation. Ten representative healthy cows in each of the first five lactations were selected and the yield of milk from the twice daily milkings recorded from day 7 to day 14 after calving inclusive.

Blood samples were obtained by jugular puncture at about 10.00 am approximately 3 h after offering the morning portion of concentrate on each of these days. During this period, usually on day 8 or 9, each cow received a single intravenous dose of tetracosactide (0.06 mg (48 IU) ACTH 1-24; Cotrosyn depot suspension;N. V. Organon Oss Holland) per cow. Blood samples were taken 30 minutes before, immediately before and 60 minutes after the injection.

Glucose was determined in all samples using Dextrostix tracks which were read on an Eyeton Refraktans colorimeter.

Blood samples taken before and after ACTH injection were allowed to clot spontaneously at room temperature. The serum was decanted, centrifuged at 3000 rpm and preserved at -18 °C until analysed for cortisol concentration. Cortisol was determined using a sensitive liquid phase radioimmunoassay.

Statistical analysis

The results were subjected to analysis of variance (ANOVA) using a two factor model in which factor A (lactation number) was regarded as completely randomized and factor B (time after calving or time in relation to ACTH injection)as a split plot. The statistical significance of differences between individual mean values was estimated by the least significant difference (LSD) test. Single and two-factor ANOVA programmes were also used where appropriate. Associations between the different parameters were sought by correlation analysis.

RESULTS

Profiles of the daily changes in mean milk yield and blood glucose concentrations during the second week of lactation are shown in Figs. 1 and 2 for cows in each of the first five lactations. Overall mean values for each variable are given in Table 1.

As expected, milk yield was much lower in the newly calved heifers than for the mature cows. There was a progressive trend for milk yield to increase during the whole of the second week for each lactation. This was most rapid in the group of cows calving for the third time, although the overall milk

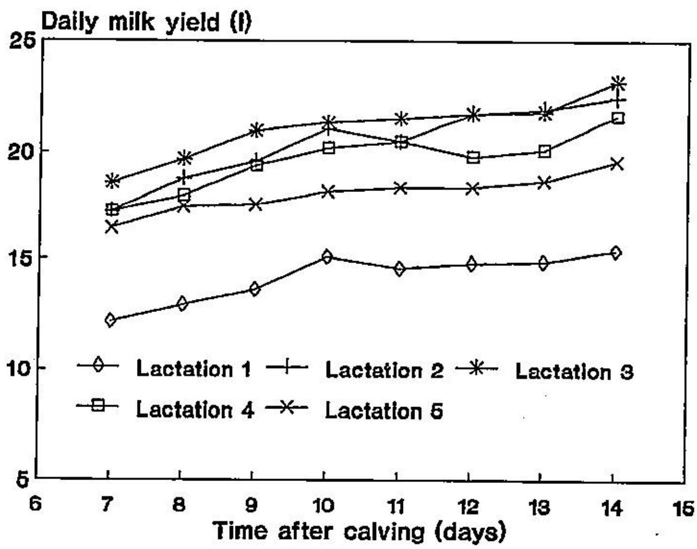

Figure 1 Mean daily milk yield during the second week after calving for groups of ten cows in each of the first five lactations.

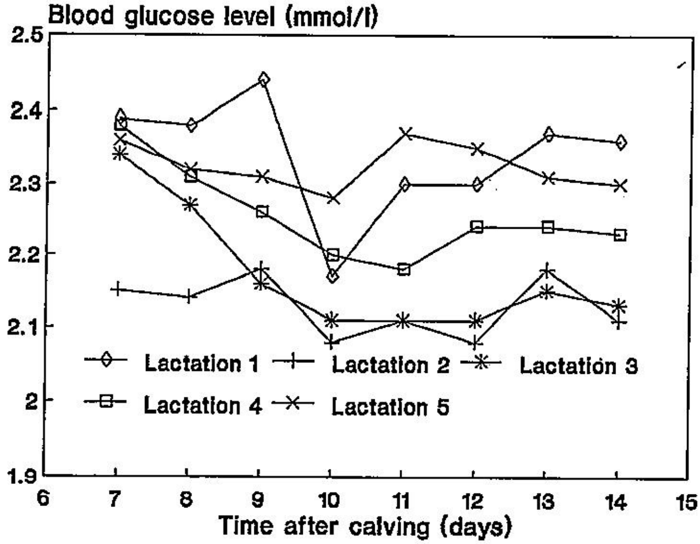

Figure 2. Mean blood glucose concentrations during the second week after calving for groups of ten cows in each of the first five lactations.

production for the standard 305 day period for the whole farm was greatest in the second lactation. However, two-way ANOVA within each lactation also showed highly significant differences between individual cows. Namely, mean milk production during the second week ranged from 11.1 to 18.0 1 per day for the heifers and 17.1 - 23.0; 17.6 - 23.3; 15.4 - 22.6 and 15.8 - 21.0 1 per day for the cows in lactations 2 - 5 respectively. In lactations 3 - 5 the effects of time and cow were additive, whereas in lactations 1 and 2 there was considerable interaction, indicating that changes of milk yield with time had different profiles in different animals. This was particularly marked among the heifers.

Blood glucose concentrations showed the reverse trend with the highest mean value registered for the heifers and the lowest in the group of cows in the second lactation (Table 1). Minimal mean glucose concentrations were recorded on day 10 after calving, when the mean value was significantly lower than those found for days 7, 8, 9 and 13 ($P < 0.05$). The cows that had survived to the fifth lactation exhibited a similar mean glucose level to that for the heifers but with much smaller daily variations (Fig. 2). Moreover, the mean blood glucose profiles differed considerably between lactations. Thus, on day 7 the mean value for cows in lactation 2 was already below 40mg/100ml and remained so throughout. The heifers showed a drop followed by a recovery, while there were continuous declines in lactations 3 and 4. The cows in lactation 5 maintained a relatively high mean blood glucose concentration during the whole period examined. However, two-way ANOVA showed that the changes with time were statistically significant only in lactation 3, while very marked differences between individual cows occurred in all except the fourth group (1.F=4.55, P = 0.0001; 2. F = 2,75, P = 0.0087; 3.F = 19.89. P < 0.0001; 4. F = 1.86, P = 0.07; 5. F = 5.36, P < 0.0001). Nevertheless, there was a continuous decrease in overall variance in glucose concentrations with lactation number as indicated by the declining SD for groups 1 to 5 (Table 1). The ten cows in each group were taken from populations of greatly different size as follows: 333, 226, 155, 95 and 40 respectively in lactations 1-5. Namely, only 12% of the heifers on the farm entered a fifth lactation.

Regardless of whether data from the first lactation were included or not, there was a very highly significant negative correlation between milk yield and blood glucose concentration (r= -0.482; P <0.0001; n = 400) for the period examined.

Table 1. Mean daily milk yield and blood glucose concentrations in fifty Holstein-Friesian cows in the second week of lactation in relation to calving number

	Daily milk yield (l)		Glucose concentration (mmol/l)	
	Days 7 – 14	Overall*	mean	SD
Lactation 1	14.2^{c}	22.7	2.34^{a}	0.25
Lactation 2	20.5^{a}	25.1	2.13^{c}	0.24
Lactation 3	21.2^{a}	24.6	2.17^{bc}	0.18
Lactation 4	19.6^{ab}	24.8	2.26^{ab}	0.16
Lactation 5	18.1^{b}	23.5	2.33^{a}	0.14
F = 18.67; P < 0.0001				
Day 7	16.4^{e}		2.32	
Day 8	17.4^{d}		2.28	
Day 9	18.2^{c}		2.27	
Day 10	19.2^{b}		2.17	
Day 11	19.1^{b}		2.22	
Day 12	19.3^{b}		2.22	
Day 13	19.5^{b}		2.25	
Day 14	20.5^{a}		2.23	
F = 33.42; P < 0.0001				

^{a,b,c,d,e} Values in a column not sharing a superscript are significantly different (P<0.05). There was no interaction between the two factors for either variable (F =0.74 and 0.82 respectively.
*Refers to the standard 305 day lactation on the whole farm of 849 cows among which 333, 226, 155, 99 and 40 were in lactations 1 - 5 respectively.

Mean values for serum cortisol concentrations before and after Cotrosyn injection are shown in Table 2. There was a slight tendency for cortisol concentrations to increase during the half hour before but it was not statistically significant. A tenfold increase in mean serum cortisol level was recorded 60 min. after injection. Differences between the groups were detected only when ANOVA was performed for each period separately and then only for basal cortisol concentrations. Namely, mean basal serum cortisol levels were lowest in the heifers and highest in the groups of cows calving for the second or third time (Table 2). The small differences were statistically significant. However, due to the large variation between the response of individual cows, no significant differences between the group mean values were detected 60 min after ACTH stimulation despite a distinct trend for the cortisol levels to decline with increasing lactation number (r = -0.288; P = 0.042). The decline in the cortisol response increment was more closely associated with increasing lactation number (r = -0,323; P = 0.022) and amounted to an average drop of 1.9 mmol/l per lactation.

Table 2. Mean serum cortisol concentrations in five groups of ten periparturient Holstein-Friesian cows before and after Cortrosyn injection

	Cortisol (mmol/l)			
	30 mon. before	zero	60 min after	increment
Lactation 1	2.8[b]	3.1[b]	36.5	33.4
Lactation 2	3.8[ab]	4.6[a]	36.8	32.2
Lactation 3	4.5[a]	4.5[a]	37.0	32.5
Lactation 4	4.6[a]	4.7[a]	32.3	27.5
Lactation 5	3.4[ab]	3.8[ab]	30.0	26.1
SE	0.2	0.2	1.2	1.2
F	3.47	2.58	1.41	1.57
P	0.015	0.05	NS	NS

[a,b,c] Values in a column not sharing a superscript are significantly different (P < 0.05)

Basal cortisol concentrations were positively correlated with milk production only when the results for the heifers were included (r=0.353, P=0,012, n = 50; but r =0.136, P = 0.403, n = 40). No further statistically significant correlations were detected between parameters relating to glucose or cortisol in this investigation.

DISCUSSION

Basal serum concentrations of cortisol in the cows examined here, particularly in the heifers, were somewhat lower than those reported previously (Hristov et al., 1994; Nikolić et al., 1998; Smith et al., 1973; Wagner and Oxenreider 1972). The increment at 60 min in response to ACTH stimulation was also lower than increments found for healthy cows by other authors (Alam et al., 1986; Šamanc et al., 1993; Verkerk et al., 1994). Moreover, there was a marked tendency for the 60 min response to decline with age. The chosen interval should reflect the maximal increment according to response profiles given by the afore mentioned authors. Therefore, assuming that the lower value for older cows was not the consequence of slower secretion followed by a later maximum (delayed response), it is possible that either the adrenocortical reserves were smaller or the secreted cortisol was used/degraded more rapidly in the cows in the fifth lactation.

While blood glucose levels were largely within the range reported earlier for healthy cows at this stage of lactation (Šamanc et al., 1994; Stamatović et al., 1983), the finding of a minimum mean blood glucose concentration on day 10 of lactation suggests that the second week is critical for the gluconeogenic pathways of the cow organism to cope with the ever increasing demands for lactose synthesis and secretion by the mammary gland. Positive correlations between blood glucose and plasma cortisol levels have been detected in lactating cows (Breves et al., 1980; Šamanc et al., 1994) indicating a role for cortisol in gluconeogenesis. However, none of the parameters related to cortisol were associated with mean glucose concentrations in the cows examined here.

Mean blood glucose levels were already low on day 7 in cows representing lactation 2. Low levels continued to day 14 in these cows and were also observed on day 14 in cows in lactation 3. The population from which the latter cows were drawn was less than half the total number of lactating heifers on the farm. Further culling proved necessary. It is interesting that the cows reaching lactation 5 maintained relatively high and stable blood glucose concentrations during the examined period. Their average milk yield was 2-3 l per day lower and their 60 min cortisol response to ACTH stimulation 6 nmol/l lower than the results for cows in lactations 2 and 3. Namely, the cows which had survived to a fifth lactation had either adapted by slightly lowering milk production and therefore the demand for glucose by the udder or they represented that part of the original population of heifers which diverted sufficient metabolites to maintain a healthy organism at the expense of a high milk yield. Further investigations of the same animals in succeeding lactations should clarify this point. If the results shows that the higher yielding cows are those that are culled early, economic issues will dictate whether it is preferable to keep a cow giving slightly less milk for five lactations (or more) rather than one giving more milk but for three lactations (or fewer) only.

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GLIKEMIJA, GLUKOKORTIKOIDI I ODGOVOR KORE NADBUBREŽNIH ŽLEZDA NA ACTH KOD MLEČNIH KRAVA U TOKU DRUGE NEDELJE LAKTACIJE

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SADRŽAJ

Ekonomski koristan deo života mlečnih krava na modernim farmama (u savremenim uslovima intenzivnog gajenja) je kratak. U ovom radu ispitivana je mogućnost potojanja rizika u metabolizmu krava u drugoj nedelji laktacije u odnosu na broj telenja. Formirano je pet grupa od po deset tipičnih zdravih krava, holštajn-frizijske, rase u rasponu od prvog do petog telenja. Određene su koncentracije glukoze u krvi i kortizola u krvnom serumu, kao i odgovor kore nadbubrežnih žlezda na stimulaciju injekcijom adrenokortikotropina (ACTH).

Nađeno je da se dnevni prinos mleka stalno povećava između 7. i 14. dana laktacije u svim grupama krava. Prosečni prinosi kod krava u laktaciji 2 i 3 su bili znatno veći nego prinosi kod junica i krava u petoj laktaciji ($P < 0.05$). Prinos mleka je bio najniži kod junica u ispitivanom intervalu. Nasuprot tome, prosečne koncentracije glukoze u krvi su bile najveće kod junica i krava u petoj laktaciji, a bitno niže u grupama krava u drugoj ili trećoj laktaciji. Naime, dnevni prinos mleka je bio u tesnoj negativnoj korelaciji sa koncentracijom glukoze u krvi ($r = 0.482$; $P < 0.0001$; $n = 400$).

U celini, najniža koncentracija glukoze je nađena 10 dana posle telenja, mada su postojale razlike između grupa krava. Pored toga, razlike između pojedinih životinja unutar grupe su bile značajne, ali su se smanjile sa povećanjem broja laktacija.

Bazične koncentracije kortizola su bile značajno niže kod junica nego kod krava u trećoj ili četvrtoj laktaciji. Međutim, povećanje nivoa kortizola, 60 minuta posle stimulacije injekcijom ACTH-a, je imalo tendenciju smanjenja sa povećanjem broja telenja. Ovo ukazuje na smanjeno lučenje i/ili povećano iskorišćavanje kortizola od strane životinja. Potreban je dalji rad da bi se odgovorilo na pitanje da li je nešto manji prinos mleka i nivo kortizola u serumu, praćen većom koncentracijom glukoze u krvi, krava u petoj laktaciji, posledica adaptacije na uslove držanja ili je to osnovna osobina krava koje su preživele do pete laktacije.