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CORRELATION OF WHOLE BLOOD CONCENTRATIONS OF ACETOACETATE, β-HYDROXYBUTYRATE, GLUCOSE AND MILK YIELD IN DAIRY COWS AS STUDIED UNDER FIELD CONDITIONS

By

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KAUPPINEN, KAUKO: *Correlation of whole blood concentrations of acetoacetate, β-hydroxybutyrate, glucose and milk yield in dairy cows as studied under field conditions.* Acta vet. scand. 1983, 24, 337—348. — The whole blood concentrations of acetoacetate (AA concn), β-hydroxybutyrate (BHB concn) and glucose (gluc concn) of 662 Ayrshire and Friesian dairy cows were measured and their milk yield during the indoor-housing period was recorded. Simple correlations among these parameters were evaluated. The correlation between the AA concn and the BHB concn ($r = 0.869$) was statistically highly significant ($P < 0.001$), as were the correlations of the logarithmic value of the AA concn with the gluc concn ($r = —0.471$) and with the milk yield ($r = 0.259$), and the correlation between the BHB concn and the gluc concn ($r = —0.288$). The milk yield was found also to be associated with the BHB concn and the gluc concn ($P < 0.001$). The associations between each pair of blood parameters were highly significant, too ($P < 0.001$). The AA concn was taken to be at least as good an indicator of the energy status of dairy cows as the BHB concn and the gluc concn. The AA concn is not diet-derived like the BHB concn and not as stress-sensitive as the blood concentration of free fatty acids and its diurnal variation is not as wide as the BHB concn and the gluc concn in subclinically ketotic cows. Thus the AA concn may be used as a proxy of the energy status and the ketotic stage of dairy cows under field conditions.

acetoacetate; β-hydroxybutyrate; glucose; energy status; dairy cattle.

Finnish dairy cattle are kept under extreme environmental conditions. Finland is one of the northernmost agricultural countries where the dairy industry is still of great importance. The management system differs from that in most other countries. Particularly typical of Finnish commercial dairy farming is the long indoor-housing period. Due to the climatic conditions, the highest frequency of calvings of Finnish dairy cows falls within the period from March to June.

Finnish dairy cattle are mostly fed with domestic fodder, i.e. silage, oats, barley and dry hay. Only small amounts of imported soybean are used in commercial feed as a protein source. The milk yield of Finnish dairy cattle, however, is relatively high, an average of 5 891 kg, 4 % fat-corrected milk (*Anon.* 1980). Consequently the energy balance of Finnish dairy cows is easily jeopardized, and bovine ketosis is a common metabolic disorder.

In order to follow the nutritional status of dairy cows, various blood parameters have been monitored (*Hewett* 1974, *Parker & Blowey* 1976, *Lee et al.* 1978). *Henricson et al.* (1977) have determined ketone body and glucose content and the so-called "lipid pattern" in the blood of cattle in order to monitor variations with age, with stage of lactation and between groups of half-sisters. In particular the determination of acetoacetate under field conditions has been problematic, due to its instability in the blood sample (*Lindsay* 1977). A standardized method for the treatment of blood samples to prevent acetoacetate loss was therefore developed by *Työppönen & Kauppinen* (1980).

The purpose of the present study was to compare the usefulness of the whole blood concentration of acetoacetate (AA concn), β-hydroxbutyrate (BHB concn) and glucose (gluc concn) in indicating the energy status of dairy cow. The values of each parameter were therefore statistically analyzed in order to determine the correlations among them and between each parameter and milk yield. The dependences of the milk yield on the AA concn, the BHB concn and the gluc concn were evaluated as were the pairwise dependences of the blood parameters.

MATERIALS AND METHODS

Blood samples were obtained during the indoor-housing period from 662 lactating and non-lactating Ayrshire and Friesian dairy cows. The blood was drained from the mammary vein, and

0.5 ml of the heparinized whole blood was immediately pipetted into 2.0 ml of 0.6 mmol/l perchloric acid. The precipitated samples were frozen within 2 h. The concentrations of AA and BHB in the samples were determined using a Gilford 3500 automatic analyzer (Gilford 3500, Computer Directed Analyzer, Gilford Instrument, Oberlin, Ohio, U.S.A.), according to the method of *Työppönen & Kauppinen* (1980).

The concentrations of glucose in the samples were determined according to a Gilford standard method (GOD-PAP), Trinder method with the reagents of the Boehringer Corp. Ltd. Milk yields were recorded monthly according to the milk production recording system of the Finnish Breeding Association. Standard statistical methods were used in order to determine the level of significance of the correlation coefficient. The chi-square test of independence was used in order to evaluate associations among parameter levels formed.

RESULTS

Table 1 shows the two-dimensional distribution of lactation number and days after calving in the whole material. These parameters were found to be independent of each other. Fig. 1 shows the AA concn plotted against the BHB concn, the correlation coefficient being r = 0.869. In Fig. 2 the logarithmic value of the AA concn is plotted against the gluc concn with a correlation

Table 1. Two-dimensional distribution of lactation number and days after calving in the whole material.

	Time as related to parturition (days)							
Lactation number	-20-(-)1	0-20	21-40	41-60	61-80	81-100	101-(-)21	Total cows
1	13	29	43	38	23	10	10	166
2	15	19	30	23	7	5	6	105
3	14	24	23	20	10	11	7	109
4—5	20	34	41	35	19	6	5	160
> 5	15	33	32	28	7	5	2	122
Total cows	77	139	169	144	66	37	30	662

$\chi^2 = 26.293$ with df = 24.

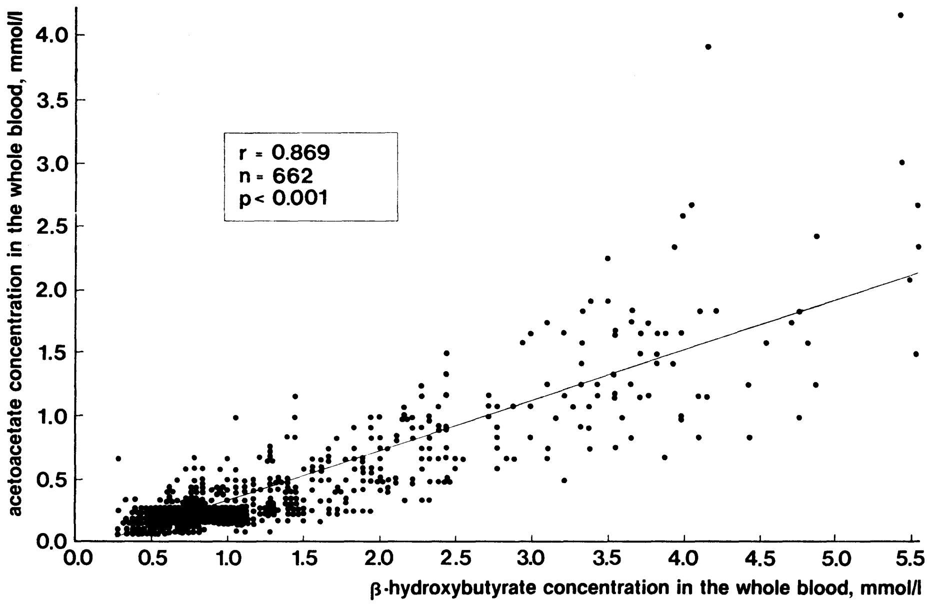

Figure 1. The correlation between acetoacetate and β-hydroybutyrate concentration.

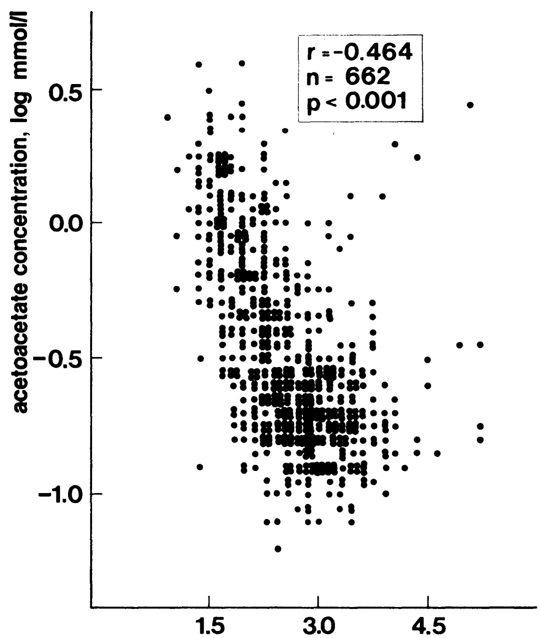

Figure 2. Logarithmic value of whole blood concentration of acetoacetate plotted against whole blood concentration of glucose.

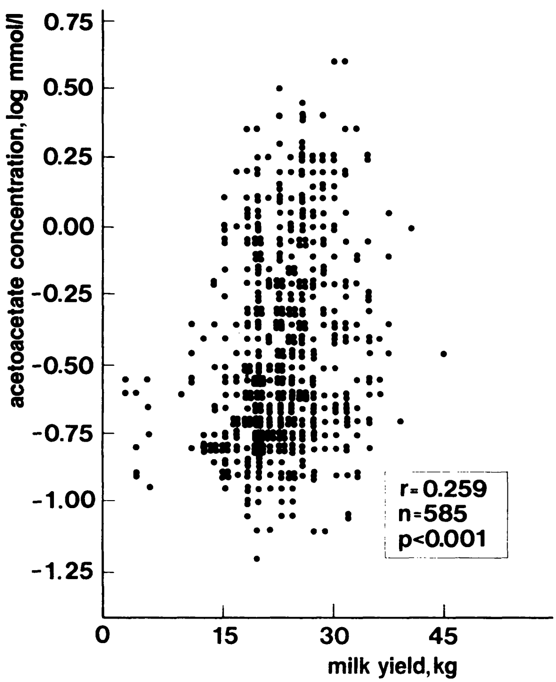

Figure 3. Logarithmic value of whole blood concentration of acetoacetate plotted against milk yield.

coefficient r = —0.471. In Fig. 3 the logarithmic value of the AA concn is plotted against the milk yield in lactating cows and correlation coefficient was r = 0.259. In lactating cows there was a positive coefficient between the BHB concn and the milk yield (r = 0.147) and a negative correlation between the gluc concn and the milk yield (r = —0.173) as the BHB concn and the gluc concn (r = —0.288).

Due to the large number of observations all above-mentioned r-values were found to be highly statistically significantly different from zero ($P < 0.001$).

In Tables 2, 3 and 4 the two-dimensional distributions of the AA conc, the BHB concn and the gluc conc on the one hand and the milk yield on the other hand in lactating cows are presented. These tables show the association of the milk yield with each blood parameter ($P < 0.001$). The direction of the dependence was positive between the milk yield and the AA concn and the BHB concn, but negative between the milk yield and the gluc

Table 2. Two-dimensional distribution of acetoacetate whole blood concentration (AA concn) and milk yield.

AA concn mmol/l	Milk yield kg/day 0.1-20.0	20.1-30.0	30.1-45	Total cows
0.1—0.35	146	140	32	318
0.36—1.05	54	108	26	188
1.06—1.75	14	36	4	54
1.76—	2	16	7	25
Total cows	216	300	69	585

$\chi^2 = 33.533^{***}$ with df = 6.

Table 3. Two-dimensional distribution of B-hydroxybutyrate whole blood concentration (BHB concn) and milk yield.

BHB concn mmol/l	Milk yield kg/day 0.1-20.0	20.1-30.0	30.1-45	Total cows
0.1—1.0	132	119	31	282
1.1—20	43	91	19	153
2.1—3.0	21	39	10	70
3.1—	20	51	9	80
Total cows	216	300	69	585

$\chi^2 = 24.465^{***}$ with df = 6.

Table 4. Two-dimensional distribution of glucose whole blood concentration (gluc concn) and milk yield.

gluc concn mmol/l	Milk yield kg/day 0.1-20.0	20.1-30.0	30.1-45	Total cows
0.1—2.0	35	109	22	166
2.1—3.0	126	158	42	326
3.1—4.0	50	30	4	84
4.1—	5	3	1	9
Total cows	216	300	69	585

$\chi^2 = 41.287^{***}$ with df = 6.

concn. The AA concn and the BHB concn increased simultaneously when the gluc concn decreased.

When all the lactating and non-lactating cows were taken into consideration exactly the same number of cows (581 or 87.8 %) fell into the categories of an AA concn below 1.05 mmol/l and of a BHB concn below 3.0 mmol/l. Of these cows the majority (390 or 67.1%) has an AA concn below 0.35 mmol/l.

DISCUSSION

In this study the diurnal time of sampling was left out of account. This is due to the study of *Phillips & Athanasiou* (1978), who have not found any diurnal pattern of variation in the bovine plasma concentrations of ketone bodies and glucose in normal lactating cows 2 months after calving and according to whom fluctuations were not correlated with milking or feeding.

Schultz (1968) has reported identical AA concentrations in blood samples drained from the mammary vein and the vena jugularis. In the present study, draining from the mammary vein was adopted in order to ensure minimal irritation of the cows. In fact it was unnecessary to restrain the animals at all during sampling. The possible effect of irritation on the blood parameters analysed could thus be ignored.

The high positive correlation between the AA concn and the BHB concn found in this study shows these two metabolites to be in equilibrium. This equilibrium is controlled by the mitochondrial ratio of NAD+ to NADH, i.e. the redox state (*Mayes* 1981). The correlation coefficient between these metabolites was numerically high, which meant a high explanatory power ($r^2 =$ 75 %). The ratio of the BHB concn to the AA concn seems to be constant at various levels. This finding conflicts with the results of *Filar* (1979), which show the AA concn as rising proportionally more than the BHB concn. *Bergman* (1971) has presented corresponding findings. *Bergman* has assumed that the alimentary output of BHB first diminishes and finally ceases at the same time that a ketotic cow loses appetite. *Baird et al.* (1979) have shown that food deprivation transforms net hepatic output of BHB and intake of AA into net output of AA in fasting non-lactating cows.

It is well established that the AA and BHB concentrations in bovine blood increase when that of gluc decreases (*Kronfeld &*

Emery 1970). Thus the negative dependences of the AA concn and the BHB concn on the gluc concn found in this study were anticipated. Since the AA concn fluctuated very widely especially at a high ketotic level, the logarithmic value of the AA concn was applied in the determination of the correlation with the gluc concn (Fig. 2). The correlation between them was statistically highly significant ($P < 0.001$). Fig. 2 shows that the logarithmic value of the AA concn had a distinct tendency toward a higher level when the gluc concn was below 3.0 mmol/l. The finding that some cows had a low gluc concn and simultaneously a low AA concn may be due to the delayed mobilization of body fat reserves, so that hepatic ketogenesis was consequently not working at total capacity. An increased AA concn was determined for some cows with a relatively high gluc concn. A possible explanation for the increased AA concn without an accompanying low gluc concn may be the fact that these cows have received a glucogenic substance, which has raised the gluc concn, while the AA concn has remained at a higher level than normal. Propylene glycol is a glucogenic substance widely fed to dairy cows at the peak stage of lactation in Finland.

The positive associations of the milk yield with the AA and BHB concns and its negative association with the gluc concn indicate that at the peak stage of lactation milk production exceeds the nutritional energy input. The high producers simply cannot eat enough to meet the energy requirement for milk production. During the peak stage of lactation, these cows thus have to go through a period of negative energy balance. This negative energy balance means a decreased gluc concn and increased blood concentrations of free fatty acids (FFA) and ketone bodies.

If these blood parameters can be interpreted to differ considerably from normal and the cows have no signs of metabolic disorder, they can be regarded as subclinically ketotic. Tables 2 and 3 show that many of the high AA and BHB concns were measured for cows producing more than 30 kg daily. These high producers are probably jeopardized by clinical ketosis. On the other hand, 87.8 % of the cows sampled had an AA concn below 1.05 mmol/l and 67.1 % of them had an AA concn below 0.35 mmol/l. The factors which induce clinical ketosis are not known. In any case inappetence, which is among the symptoms of ketosis, is decisive. Inappetence aggravates the negative energy status; the AA and BHB concns are further increased and the gluc concn is decreased.

The milk yield drops rapidly, until a nutritional equilibrium is achieved. Without adequate treatment this may nearly lead to drying off.

The gluc concn can be regarded as a measure of the energy status of dairy cows, since the excessive requirement of glucose for milk production is regarded as a primary reason for a lower gluc concn. The low gluc concn has been suggested as a factor limiting milk production (*Kronfeld & Emery* 1970). The low degree of negative correlation between these parameters in lactating cows found in this study ($r = -0.173$, $r^2 = 3\ \%$), suggests that the gluc concn is not a good indicator of the energy status of dairy cows. *Kronfeld* (1972) and *Parker & Blowey* (1976) have neither considered the gluc concn an ideal measure of the energy balance, since there is not always a constant relationship between them. *Andersson* (1982) has additionally shown that there is considerable diurnal variation of the gluc concn in subclinically ketotic cows. Thus it seems that the gluc concn has certain disadvantages as a measure of the nutritional status of dairy cows.

In this study the BHB concn was found to be as valid a measure of the energy balance as the AA concn and the gluc concn (Fig. 1 and Table 3). Exactly the same number of all the cows (581 or 87.8 %) sampled belonged to the lowest categories classified according to both the AA concn and the BHB concn. *Kelly et al.* (1979) and *Herdt et al.* (1981) have also shown the BHB concn to be a valuable measure of the energy balance of dairy cows. The AA and BHB concns are increased by hepatic ketogenesis when the negative energy status already is present. Dairy cows try to achieve a nutritional equilibrium by reducing milk production simultaneously. In practice the AA concn has certain advantages in measuring the energy status of dairy cows. According to *Lindsay* (1977) the normal AA concn of dairy cows is very low; thus even in the non-pregnant and non-lactating ruminant a rise offers a sensitive indication of an inadequate energy intake. The level of the BHB concn is normally higher than the AA concn due to absorption from the alimentary tract when the cow is eating well (*Bergman* 1971). Thus, according to *Lindsay*, diet-derived ketonemia in particular is due to an increase in the BHB concn rather than the AA concn. The AA concn of dairy cows is only elevated when they are in negative energy status. On the other hand the diurnal variation of the BHB and gluc concns in subclinically ketotic cows are significant but that of the AA concn is not (*Andersson* 1982).

The FFA blood concentration is also a well established measure of the energy status of dairy cows (*Kronfeld & Emery* 1970). The increase of the ketone body concentration in the blood follows that of the FFA concentration with a certain delay (*Athanasiou & Phillips* 1978). The FFA blood concentration, however, is far too stress-sensitive (*Basset* 1970), whereas the AA concn is only weakly stress-sensitive (*Lindsay* 1977). In this study the AA concn was found to be at least as good an indicator of the energy status of dairy cows as the BHB concn and the gluc concn in practice. The AA concn is also a well established indicator of the ketotic stage of a cow. Thus the AA concn should be included in the metabolic tests of dairy cows at least with as good a reason as the BHB concn and the gluc concn. Using the method of *Työppönen & Kauppinen* (1980) the instability of AA should no longer present any practical obstacle.

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SAMMANFATTNING

Korrelation mellan blodkoncentration av acetoacetat, β-hydroxybutyrat, glukos och mjölkproduktion hos nötkreatur, en fältstudie.

Acetoacetat- (AA konc), β-hydroxybutyrat- (BHB konc) och glukoskoncentrationen (gluk konc) i blodet samt mjölkproduktionen mättes hos 662 ayrshire och frisiska mjölkkor under stallutfodringsperioden. Korrelationen mellan dessa parametrar undersöktes. Korrelationen mellan AA konc och BHB konc ($r = 0,869$) var starkt signifikant ($P < 0.001$). Korrelationen mellan det logaritmiska värdet av AA konc och gluc konc ($r = —0,471$) och mellan det logaritmiska värdet av AA konc och mjölkproduktionen ($r = 0,259$) var starkt signifikant liksom korrelationen mellan BHB konc och gluc konc ($r = —0,288$). Mjölkproduktionen var associerat med AA konc, BHB konc och gluc konc ($P < 0.001$). AA konc kan anses vara en minst lika god indikator av mjölkkors energistatus som BHB konc och gluc konc. AA konc är inte av dietiskt ursprung som BHB konc och inte så stress-sensitivt som fria fettsyrars koncentration i blodet och dess dygnsvariation är inte så vidsträckt som BHB konc och gluk i blodet hos subkliniskt ketotiska kor. AA konc kan användas som ett mått på kons energistatus och ketosstadium i fältförhållanden.

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