Acta vet. scand. 1990, *31*, 5-15.

# Plasma Glucose, Ketone Bodies, Insulin, Glucagon and Enteroglucagon in Cows: Diurnal Variations Related to Ketone Levels Before Feeding and to the Ketogenic Effects of Feeds

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**Borrebaek, B., K. Halse, B. Tveit, H. K. Dahle and L. Ceh: Plasma glucose, ketone bodies, insulin, glucagon and enteroglucagon in cows: Diurnal variations related to ketone levels before feeding and to the ketogenic effects of feeds. Acta vet. scand. 1990, 31, 5-15.** - Ingestions of a moderately ketogenic silage twice daily were followed by transient increments in plasma insulin and ketone bodies and decreases in plasma glucose. Ketone bodies and glucose were negatively correlated throughout the day, but the insulin elevations culminated before the maximal effects on ketone bodies and glucose were established.

Cows with varying glucose levels before morning feeding reacted to a highly ketogenic silage by decreasing their glucose level uniformly to about 3 mmol/l, in spite of a widely varying feeding-induced insulin increment.

Hay-feeding caused insulin increments of the same magnitude as silage-feeding, but the glucose decrease and the ketone increment was much smaller. The results indicate some direct action of ketone bodies on blood sugar regulation, in addition to effects mediated by insulin. The role of ketone bodies as the insulinotropic factor was not confirmed. The insulin level after feeding seems to be determined by the carbohydrate status of the animal before feeding.

No significant changes in plasma glucagon were observed after feeding, and no consistent differences in plasma levels of this hormone were found when non-ketonemic, ketonemic, and clinically ketotic cows were compared. The plasma level of enteroglucagon (GLI) was positively correlated to the relative amount of concentrates consumed, but no relation to plasma glucose was found.

ketosis; ketonemia; hypoglycemia; silage.

## Introduction

Feed ingestion can lead to decreases in plasma glucose and increments in ketone body levels in the blood plasma of cows. Related to such changes in blood composition are after-feeding increments in plasma insulin (*Hove & Blom* 1971, *McAtee & Trenkle* 1971, *Hove & Blom* 1973). The ketonemic-hypoglycemic effects of feeding seem to vary widely with the nature of the feeds ingested. In feeding experiments with heifers (*Malmstrøm et al.* 1987) the diurnal variations in ketone bodies have been found to be very large after feeding with special silages on which the animals become susceptible to clinical ketosis. Apparently, feeding on such silages leads to a high rate of ketogenesis from buryrate in the rumen epithelium. The natu-

re of the mechanisms responsible for the severity of the accompanying hypoglycemia remains unexplained.

We therefore initiated the present study of variations in plasma insulin in relation to plasma metabolite levels. We also investigated the possible involvements of glucagon and enteroglucagon (GLI). Glucagon has a ketogenic effect in rats (*Heimberg et al.* 1969, *McGarry et al.* 1975a, *McGarry et al.* 1975b, *Christiansen* 1977), while GLI has been suggested to compete with pancreatic glucagon at the receptor level, and could thus possibly be a hypoglycemic factor (*Rehfeld et al.* 1973, *Bataille et al.* 1973, *Gutman et al.* 1973).

## Materials and methods

### *Animals and feeding*

Dairy cows of the Norwegian Red Cattle breed, belonging to 2 different herds, were used for the study. In herd I, the experimental herd of the Norwegian Veterinary College, multiparous cows were sampled 3-10 weeks post partum while receiving 8-12 kg of a concentrate mixture per day and grass silage ad libitum, unless otherwise stated. The feeds were given in equal allotments twice per day, immediately after blood sampling at 0530 and at 1400 hours. Herd II consisted exclusively of individually fed heifers which were tested for productivity for 1 indoor season in connection with breeding experiments (*Malmstrøm et al.* 1987) by the Department of Animal Science, Agricultural University of Norway. Feed rations consisted of 6 (group A) or 3 kg (group B) of concentrates per day and grass silage ad libitum. Feeds were given in equal allotments at 0600 and 1500 hours. At the times of sampling the animals were between 20 and 35 weeks post partum. The quality of the silage used in this herd seemed to vary considerably according to harvesting conditions. Batches with pH $>4$ and containing traces of butyrate produced strong after-feeding increments in plasma ketone bodies (ketogenic silage).

In a special experiment the reproducibility of differences in ketogenic property between silages was tested in herd II. Two types of silage previously found to be different in this respect were fed alternatingly to the same animals after freeze-storage (*Tveit et al.* 1987). As a third alternative a period of hay-feeding was interposed.

The adequacy of feed rations was estimated approximately in fattening feed units (FFU). The amounts of silage and concentrates providing 1 FFU were assumed to be respectively 6.25 and 1 kg. Daily FFU requirements were arrived at by the formula: $0.06 \times$ body weight $+ 1 + 0.4 \times$ FCM (fat corrected milk in kg). Percentage of concentrates of dry matter in feed rations was (kg concentrates $\times 0.86 \times 100$)/(kg concentrates $\times 0.86 +$ kg silage $\times 0.2$).

### *Blood sampling*

Blood was drawn from the jugular vein with heparinized vacutainers. In the case of herd I they contained aprotinin (1 mg/15 ml blood) to inhibit glucagon degradation. The samples were stored in ice water and centrifuged within 1 h. Plasma was kept at -80°C to minimize losses of acetoacetate in storage. In herd I a large number of blood samples were taken before morning feeding and subsequently at intervals of 1-2 h throughout the day. Three cows which got clinical ketosis were sampled immediately before treatment. One of these was followed with frequent samples before feeding during the development of and recovery from the disease in the course of 4 weeks.

In herd II samples were routinely taken before feeding at 0600 hours and at 1800, i.e., 3 h after feeding in the afternoon.

*Hormone assays*

Insulin, glucagon and enteroglucagon (GLI) were measured with radioimmunoassay kits purchased from NOVO BIOLABS, Denmark. The sum of glucagon and GLI was determined with the unspecific antibody (NOVO K 4023) while glucagon alone was measured with the antibody which is specific for pancreatic glucagon (NOVO K 5563). GLI was calculated by subtraction.

Relatively steep standard curves for glucagon were obtained when the standard glucagon was diluted in a glucagon-poor plasma extract in stead of the recommended solution. In our opinion, the most correct values for the glucagon levels are obtained when the standards are measured in the same environment as the samples. However, we have then obtained lower average plasma glucagon levels than those reported previously (*Basset* 1975, *Berzins & Manns* 1979, *De-Boer et al.* 1985).

*Metabolite analysis*

Plasma sugar was estimated by the reduction of ferricyanide and acetoacetate by the nitroprusside reaction in plasma dialysates (*Blom & Halse* 1975). NEFA were determined by a test kit (Wako Pure Chemical Industries, LTD., Osaka, Japan). β-Hydroxybutyrate was measured enzymatically (*Williamson & Mellanby* 1974). In part of the present work measurements of acetoacetate alone were used for the quantitative estimation of ketonemic conditions. The reliability of this parameter as a metabolic indicator is evidenced by the strength of the correlation to β-hydroxybutyrate. ($r = 0.89$, $n = 96$).

*Statistical evaluation*

The statistical evaluations were carried out according to *Fisher* (1970). Students t-test was used for calculation of probabilities.

## Results

*Diurnal variations in metabolites and hormones of cow plasma*

Fig. 1 shows the diurnal variations in plasma metabolites and insulin in cows of herd I. The animals were fed concentrates and silage with a moderate ketonemic effect. Glucose and acetoacetate varied inversely throughout the day. Initially insulin increments were synchronous with increases in acetoacetate and decreases in glucose, but the hormone level culminated before the flattening out of the metabolite curves. A delayed effect of in-

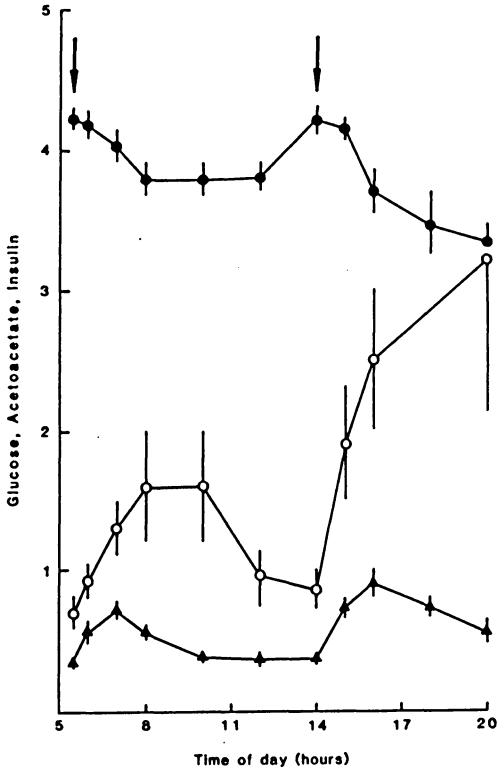


Figure 1. Variations in plasma glucose (●), acetoacetate (○) and insulin (▲) during the diurnal feeding cycle. Cows of herd I fed at 0530 and 1400 hours as indicated by arrows. Glucose is given as mmol/l, acetoacetate as mmol/10 l and insulin as ng/ml. Data are means of 5-13 observations ±S.E.M.

Table 1. Correlation coefficients (r) of the material described in Fig. 1. Acetoacetate against glucose and insulin against glucose measured simultaneously and with a time delay of 1-2 h. Degrees of freedom in brackets.

| Variables | r within cows and within days | r combined material |
|---|---|---|
| Acetoacetate: glucose | -0.56[a] (90) | -0.51[a] (103) |
| Insulin: glucose | -0.35[b] (64) | -0.21 (76) |
| Insulin: delayed glucose | -0.64[a] (55) | -0.33[b] (67) |

[a] $p<0.001$
[b] $p<0.01$

sulin is indicated by the highly significant correlation between insulin and glucose measured 1-2 h after the hormone (Table 1). As shown in Table 2, the plasma level of non-esterified fatty acids (NEFA) was decreased after feeding, while no significant variations in glucagon or GLI were observed. Although feed allotments were equal at the 2 daily feedings, the amplitudes of the parameters in Fig. 1 were greater in the afternoon than during the morning hours. This indicates some cumulative effect of 2 feedings within an interval of 8-9 h.

Table 2. The effect of feeding on plasma levels of nonesterified fatty acids (NEFA), glucagon and GLI in cows of herd I (fed at 0530 and 1400). The number of observations (number of cows) are given in brackets. The data are mean values ±S.E.M.

| Hours | Glucagon (ng/l) | GLI (ng/l) | NEFA (μmol/l) |
|---|---|---|---|
| 0530 | 60.0±3.5 (12) | 114±18 (6) | 270±70 (13) |
| 0800 | 56.4±4.0 (11) | 118±17 (6) | 74±4 (12) |
| 1000 | 54.5±5.2 (11) | 135±22 (6) | 64±5 (13) |
| 1400 | 65.0±5.4 (12) | 147±15 (6) | 190±53 (13) |
| 1600 | 63.9±7.8 (9) | 126±15 (6) | 85±9 (11) |

*Ketonemia of feeding related to pre-feeding ketone levels*

Table 3 shows metabolite and insulin averages before and after feeding a highly ketogenic silage (herd II). Sampling at 0600 and 1800 hours were chosen since previous experiments had shown that 1800 is a time-point with large feeding-effects on the metabolites as well as the hormone (compare Fig. 1). There were more animals with elevated ketone levels before feeding in the low-concentrate group (category B2 and B3) than in the high-concentrate group (category A2), and 4 individuals (B3) had ketone values within the range for clinical ketosis. They were, however, not visibly ill at the time of observation.

The table demonstrates very large and practically identical after-feeding increments in ketone bodies in the 4 categories of cows with low or moderately elevated pre-feeding ketone levels. The increment was smaller in the strongly ketonemic category (B3) possibly because these animals ate less silage. In this category glucose and insulin were not significantly altered by feeding. The other categories showed decreases in glucose to approximately 3 mmol/l at 1800 hours irrespective of the glucose level before feeding at 0600.

The relationship between pre-feeding insulin and glucose at 0600 hours indicated in Table 3 was found to be statistically significant ($p<0.001$) with $r=0.44$. Insulin at 1800 hours varied widely in spite of the narrowing of the glucose range which had taken place during the day. Actually, insulin at 1800 was not significantly correlated to glucose at 1800 ($r=0.14$). It was on the other hand significantly correlated to morning glucose ($r=0.51$) and to morning insulin ($r=0.63$).

Table 3. Effects of feeding a highly ketogenic silage to heifers of herd II receiving 6 (A) or 3 kg (B) of concentrates per day. The cows were grouped in subcategories (1-3) according to prefeeding levels of ketone bodies. Blood samples were obtained at 0600 (before morning feeding) and at 1800 (3 h after feeding in the afternoon). Data are mean values ±S.E.M. Some of the cows were observed on two consequtive days.

| | A1<br>9 cows<br>(n = 12) | A2<br>7 cows<br>(n = 10) | B1<br>6 cows<br>(n = 10) | B2<br>10 cows<br>(n = 11) | B3<br>4 cows<br>(n = 5) |
|---|---|---|---|---|---|
| Feed consumption (FFU/day) | 12.0±0.2 | 12.3±0.2 | 9.5±0.1 | 9.4±0.2 | 7.5±0.5 |
| Milk production (kg/day) | 13.0±0.5 | 15.7±0.5[a] | 10.0±0.7 | 11.4±0.5 | 8.9±0.5 |
| FFU/day, balance | 3.0±0.2 | 2.3±0.2 | 2.0±0.3 | 1.2±0.2 | 0.4±0.4 |
| Glucose (mmol/l) | | | | | |
| at 0600 | 4.38±0.07 | 3.93±0.13 | 4.36±0.07 | 3.76±0.07 | 2.97±0.17 |
| at 1800 | 3.17±0.11[b] | 3.00±0.09[b] | 3.16±0.10[b] | 3.01±0.08[b] | 2.87±0.19 |
| Acac (mmol/l) | | | | | |
| at 0600 | 0.05±0.01 | 0.35±0.09 | 0.05±0.01 | 0.37±0.09 | 1.32±0.23 |
| at 1800 | 0.74±0.10[b] | 1.22±0.11[b] | 0.75±0.08[b] | 0.95±0.10[b] | 1.63±0.13 |
| βOH.B (mmol/l) | | | | | |
| at 0600 | 0.72±0.07 | 1.74±0.24 | 0.64±0.05 | 2.02±0.31 | 5.06±0.17 |
| at 1800 | 3.14±0.20[b] | 4.47±0.13[b] | 3.57±0.23[b] | 4.27±0.29[b] | 5.65±0.41 |
| Insulin (ng/l) | | | | | |
| at 0600 | 694±103 | 448±32 | 426±51 | 380±39 | 274±13 |
| at 1800 | 1277±126[d] | 903±73[b] | 750±76[d] | 595±31[c] | 396±31 |

[a] $p < 0.002$ as compared with group A1
[b] $p < 0.001$ as compared with the value at 0600 hours
[c] $p < 0.002$ as compared with the value at 0600 hours
[d] $p < 0.005$ at compared with the value at 0600 hours

Evidently, after-feeding insulin was predetermined by the metabolic condition of the animals before they were fed. The inverse correlations between glucose and ketone bodies were particularily strong (r = -0.90 in the morning and -0.60 at 1800 in spite of the narrow range for glucose in the afternoon).

*Differences in ketogenic property between roughages*

Table 4 shows that a previously observed difference in ketogenic property between 2 types of silage (highly ketogenic and moderately ketogenic) could be reproduced. The table also shows the effects of hay-feeding. The ability to produce hypoglycemia seems directly related to the ketonemia observed after feeding of the 3 roughages. No decrease in glucose was observed after hay-feeding when only a slight ketonemia appeared. However, the insulin response after hay-feeding was of the same magnitude as after silage-feeding.

*Feeding and the glucose-ketone interrelation*

Fig. 2 includes in addition to the material used in Fig. 1 samples from herd I with elevated ketone levels before feeding. Herd II is represented with observations from different series of sampling in the same season, including the measurements used in Table 3. Evidently, by plotting glucose averages corresponding to different acetoacetate ranges, similar curvilinear regressions are obtained for cows in a prefed and a fed condition. The effect of feeding can be described as a shift

Table 4. Effects of silage of different ketogenic properties versus hay. Twelve heifers of herd II were fed concentrates and alternatingly hay or 2 different types of silage ad libitum. Blood samples were obtained at 0600 (before morning feeding) and at 1800 (3 h after feeding in the afternoon). Data are mean values ±S.E.M. Some of the cows were observed on 2 consequtive days.

| | Highly ketogenic silage (n = 18) | Moderately ketogenic silage (n = 14) | Hay (n = 12) |
|---|---|---|---|
| Glucose (mmol/l) | | | |
| at 0600 | 4.28±0.10 | 4.36±0.09 | 4.15±0.6 |
| at 1800 | 3.20±0.13[a] | 3.69±0.07[a] | 4.18±0.06 |
| Acac (mmol/l) | | | |
| at 0600 | 0.045±0.011 | 0.031±0.006 | 0.042±0.007 |
| at 1800 | 0.389±0.045[a] | 0.117±0.022[a] | 0.063±0.008 |
| Insulin (ng/l) | | | |
| 0600 | 291±39 | 274±22 | 220±23 |
| at 1800 | 520±68[c] | 624±84[a] | 492±82[b] |

[a] $p < 0.001$ as compared with the value at 6 h.
[b] $p < 0.005$ as compared with the value at 6 h.
[c] $p < 0.01$ as compared with the value at 6 h.

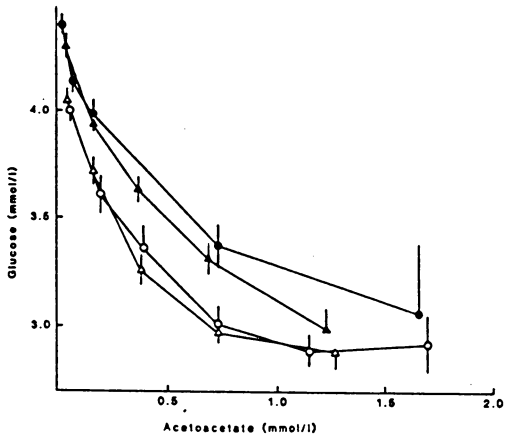


Figure 2. The inverse relationship between plasma glucose and acetoacetate before and after feeding: comparizon of herd I (circles) and herd II (triangels). Intervals for acetoacetate between 0, 0.1, 0.25, 0.5, 1.0, 1.5 and 2.0 mmol/l were arbitrarily selected and the average values plotted against the corresponding glucose averages. Filled symbols: Before feeding in the morning. Open symbols: After feeding at 0930 in herd I and at 1800 in herd II. Bars indicate S.E.M. for glucose.

of the regression curve towards lower glucose levels. The results from the 2 herds were nearly identical, even though the after-feeding samples were taken at different hours of the day.

*Glucagon and GLI in healthy and ketotic cows*

The observations in Fig. 3 are from a case of clinical ketosis apparently induced by feed restriction. Ketonemia and hypoglycemia developed gradually until typical symptoms of clinical ketosis appeared 37 days after calving. The cow was then treated by a single intramuscular injection of prednisolone immediately after blood sampling. As expected, the ketonemia was accompanied by lowered insulin levels and reduced feed intake and milk production, but no consistent changes in glucagon were observed.

Table 5 shows the hormone levels in cows of herd I with different degrees of ketonemia. Blood samples were taken from the healthy

Table 5. Hormone and metabolite levels in dairy cows from herd I with different degrees of ketonemia. Blood samples from the healthy cows were obtained before morning feeding, while blood from the clinically ketotic cows was drawn at varying hours immediately before treatment. Some of the cows were observed repeatedly on different days. Data are means ±S.E.M.

| | No ketonemia 2 cows (n = 9) | Moderate ketonemia 4 cows (n = 20) | Marked ketonemia 2 cows (n = 4) | Clinical ketosis 3 cows (n = 3) |
|---|---|---|---|---|
| Glucose (mmol/l) | 4.25±0.13 | 3.71±0.08 | 2.93±0.12 | 2.26±0.07 |
| Acetoacetate (mmol/l) | 0.043±0.004 | 0.43±0.03 | 1.41±0.27 | 2.78±0.20 |
| Insulin (ng/l) | 241±33 | 175±18 | 130±15 | 44±11 |
| Glucagon (ng/l) | 48.4±3.9 | 50.9±1.7 | 61.5±4.6 | 52.3±5.9 |
| GLI (ng/l) | 161±26 | 76.1±7.6 | 82.4±10.1 | 77.0±22.5 |

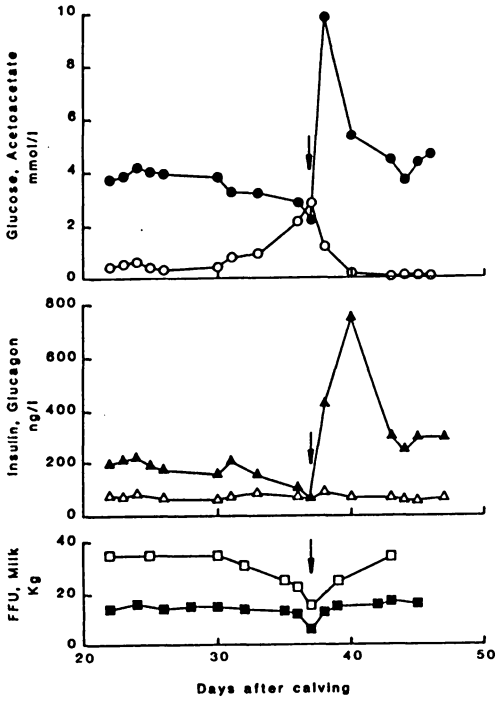


Figure 3. Clinical ketosis apparently induced by feed-restriction. A cow from herd I was underfed (feed balance about -2 FU/day) until the appearance of clinical symptoms in the morning at the 37th day after calving. Prednisolon was then given immediately after blood sampling (see arrow). Plasma levels of glucose (●), acetoacetate (○), insulin (▲), glucagon (△), feed intake (■) and milk production (□) were recorded.

cows before morning feeding, while those from the clinically ketotic cows were obtained at random hours of the day immediately before treatment. The cows with clinical ketosis had strong ketonemia and very low levels of insulin, but neither glucagon nor GLI were significantly different from values found in the healthy animals.

Table 6. Linear correlation coefficients of the total number of single observations with the healthy cows in Table 5, n = 33. Percentage concentrates in the feed rations is defined in Materials and methods.

| Variables | r | p |
|---|---|---|
| Glucose: Insulin | 0.40 | <0.02 |
| Glucose: Glucagon | -0.12 | n.s. |
| Glucose: Insulin/glucagon | 0.51 | <0.003 |
| Glucose: Acetoacetate | -0.75 | <0.001 |
| Glucose: GLI | 0.31 | n.s. |
| GLI: % Concentrates | 0.74 | <0.001 |

Linear correlation coefficients between plasma parameters of 33 samples from the healthy cows in Table 5 are shown in Table 6. The insulin/glucagon ratio, but not glucagon alone, was significantly correlated to glucose, since insulin alone gave a positive coefficient, specific glucagon effects are doubtful. GLI was significantly correlated

to the relative amount of concentrates consumed, but not to the plasma glucose level.

## Discussion

*Insulin and postprandial glucose changes*

Logically, some causal relationship must exist between the insulin increments and postprandial decreases in plasma glucose. Apparently, some time is needed for the development of maximal effects on the blood sugar of a change in insulin secretion (Fig. 1). This may be the reason why insulin was best correlated to glucose measured 1-2 h later than the hormone (Table 1). A delay between insulin and glucose variations may be specially large in lactating cows since the mammary gland appears to be independent of insulin (*Hove* 1978b), the glucose uptake of the organ remaining practically unaffected by an after-feeding drop in glucose (*Halse et al.* 1985). The observed time lag might also be contributed to by differences in the half-life of tissue-bound and plasma insulin. Furthermore, it is possible that liver metabolism can be influenced by increments in portal blood insulin which are not mirrored peripherally (*Miles et al.* 1981). Otherwise, the relatively short duration of the insulin peaks in Fig. 1 could be due to feed-back effects from decreasing glucose levels on the secretion of the hormone (*Metzger et al.* 1973, *Goberna et al.* 1974). Insulin seems not to be influenced by feeding in cows with a sufficiently low glucose level (*Hove & Halse* 1978, *Hove* 1978a, Table 3).

According to Table 2 feeding caused an abrupt drop in NEFA. The importance of variations in insulin in the metabolism of lipids in bovines was demonstrated by *Sutton et al.* (1982), *Hart* (1983) and *Sutton* (1985). They eliminated postprandial insulin peaks in cows on high-concentrate rations by giving the same amounts of feeds in 6 instead of 2 feedings per day, which resulted in an increased yield of milk fat. Naturally, insulin increments after feeding can be expected to alter the partitioning of glucose as well as of lipids between different metabolic processes. By minimizing diurnal variations in insulin it might be possible to reduce the peripheral utilization of glucose and to prevent the waste of glycogen reserves.

*Ketone bodies and glucose*

The findings discussed above do not preclude the involvement of other factors than insulin in postprandial glucose regulation. In Table 3 glucose was depressed to nearly the same level (about 3 mmol/l) in animals with widely varying levels of the hormone. In Table 4 the decreases in glucose after feeding were related to the ketone increment but not to insulin. The strong negative glucose/ketone correlation in Fig. 2 can be explained by carbohydrate effects on lipid-metabolism (*Halse et al.* 1983). However, the postprandial hypoglycemia (Fig. 1) associated with increasing ketonemia and decreasing NEFA levels (Table 2) could be partly due to an opposite effect, of ketones on the metabolism of carbohydrates. The observation by *Mills et al.* (1986) of reduced activities of gluconeogenetic enzymes in connection with severe ketonemia is interesting in this respect. Furthermore, it has been reported that ketone bodies inhibit the monocarboxylate carrier and thereby hamper gluconeogenesis from lactate in isolated rat hepatocytes (*Metcalfe et al.* 1986). *Malmstrøm et al.* (1987) showed that cows fed on strongly ketogenic silages as in Table 3 became susceptible to clinical ketosis. A simple explanation would be a low supply of glycogen precursors, but the question remains whether or not ketone bodies can affect carbohydrate metabolism deleteriously.

*Ketone bodies and insulin*

The actual mediator of the insulin response to feeding can be propionate from the rumen (*Stern et al.* 1970, *Basset* 1978, *Bines & Heart* 1984). Reflex vagal stimulation has been suggested (*Chase et al.* 1977). Experiments with other species than bovines and with isolated pancreas preparations (*PiSunier et al.* 1970, *Hawkins et al.* 1971, *Metzger et al.* 1973, *Goberna et al.* 1974) have shown insulinotropic effects of ketone bodies. But in cows such effects may be of limited importance since hay-feeding resulted in insulin increases while acetoacetate remained practically constant (Table 4).

*Plasma glucagon and GLI*

It was believed that glucagon could be involved as a »counterregulatory factor« contributing to the limitation of the decreases in glucose shown in Table 3 and Fig. 2. Increased levels of this hormone in sheep after feeding were reported by *Basset* (1975), but not confirmed in cows by *DeBoer et al.* (1985) or by Table 2.

There was no significant difference in plasma glucagon between healthy lactating cows and those with clinical ketosis (Table 5). *DeBoer et al.* (1985) observed a moderate drop in the plasma level of glucagon when ketonemia was induced by feed-restriction. This does not seem to be confirmed by the results in Fig. 3. The significant correlation between plasma GLI and the relative amounts of concentrates consumed (Table 6) is consistent with previous reports (*Berzins & Manns* 1979). A possible explanation would be that GLI-secretion is stimulated when starch pass into the small intestine in cows on high-concentrate rations. In spite of large variations in the glucose level (Table 5) significance was not obtained for the correlation between this parameter and GLI in Table 6. Seemingly, the hormone is of minor importance in the regulation of plasma glucose in cows.

**Acknowledgements**

The present work was supported by The Norwegian Agricultural Research Council and by Torsteds Fund for Animal Welfare. The excellent technical assistance by Randi Aarsand and Berit Christophersen is gratefully acknowledged.

**References**

*Basset JM:* Dietary and gastro-intestinal control of hormones regulating carbohydrate metabolism in ruminants. In: Digestion and metabolism in the ruminant. Edited by I. W. McDonald & A. C. Warner. Proc. Univ. New England Publ. Unit., Armidale, Australia 1975, p. 383-398.

*Basset JM:* Endocrine factors in the control of nutrient utilization: ruminants. Proc. Nutr. Soc. 1978, *37*, 273-279.

*Bataille DP, Freychet P, Kitabgi PR, Rosselin GE:* »Enteroglucagon«: a common receptor site with pancreatic glucagon in rat liver plasma membranes. Diabetologia 1973, *9*, 60.

*Berzins R, Manns JG:* How concentrate feeding affects glucoregulatory hormones in ruminants: Implications in bovine ketosis. J. Dairy Sci. 1979, *62*, 1739-1745.

*Bines JA, Hart IC:* The response of plasma insulin and other hormones to intraruminal infusion of VFA mixtures in cattle. Canad. J. Anim. Sci. 1984, *64*, (Suppl.), 304-305.

*Blom AK, Halse K:* Corticosteroids in nucturnal blood plasma of cows in the field related to stage of lactation and plasma acetoacetate. Acta endocr. (Kbh.) 1975, *78*, 306-315.

*Chase LE, Wangsnes PJ, Martin RJ:* Portal blood insulin and metabolite changes with spontaneous feeding of steers. J. Dairy Sci. 1977, *60*, 410-415.

*Christiansen RZ:* Regulation of palmitate metabolism by carnitine and glucagon in hepatocytes isolated from fasted and carbohydrate refed rats. Biochim. Biophys. Acta 1977, *488*, 249-262.

*DeBoer G, Trenkle A, Young JW:* Glucagon, insulin, growth hormone, and some blood metabolites during energy restriction ketonemia of lactating cows. J. Dairy Sci. 1985, *68*, 326-337.

*Fisher RA:* Statistical Methods for Research Workers, 14. ed., Oliver and Boyd, Edinburgh 1970.

*Goberna R, Tamarit Jr J, Osorio J, Fussganger R, Tamarit J, Pfeiffer EF:* Action of β-Hydroxybu-

tyrate, acetoacetate and palmitate on the insulin release in the perfused isolated rat pancreas. Horm. Metab. Res. 1974, *6*, 256-260.

*Gutman RA, Fink G, Voyles N, Selawry H, Penhos JC, Lepp A, Recant L:* Specific biologic effects of intestinal glucagon-like materials. J. clin. Invest. 1973, *52*, 1165-1175.

*Halse K, Hove K, Ertsaas P:* A biological definition of ketonemia in cows. Proceedings of the Fifth International Conference on Production Disease in Farm Animals, Uppsala, Sweden 1983, p. 137-139.

*Halse K, Borrebaek B, Dahle HK:* Jurstoffskifte og ketose hos kyr. (Mammary metabolism and ketosis in cows). Norsk Veterinærtidsskrift 1985, *97*, 651-654.

*Hart IC:* Endocrine control of nutrient partition in lactating ruminants. Proc. Nutr. Soc. 1983, *42*, 181-193.

*Hawkins RA, Alberti KGMM, Houghton CRS, Williamson DH, Krebs HA:* The effect of acetoacetate on plasma insulin concentration. Biochem. J. 1971, *125*, 541-544.

*Heimberg M, Weinstein I, Kohout M:* The effects of glucagon, dibutyryl cyclic adenosine 3', 5'-monophosphate, and concentration of free fatty acids on hepatic lipid metabolism. J. Biol. Chem. 1969, *244*, 5131-5139.

*Hove K:* Insulin secretion in lactating cows - responses to glucose infused intravenously in normal, ketonemic and starved animals. J. Dairy Sci 1978a, *81*, 1407-1413.

*Hove K:* Maintenance of lactose secretion during acute insulin deficiency in lactating goats. Acta physiol. scand. 1978b, *103*, 173-179.

*Hove K, Blom AK:* Plasma levels of growth hormone, insulin, sugar and acetoacetate in cattle. Diurnal variations and responses to feeding. Acta vet. scand. 1971, *12*, 460-463.

*Hove K, Blom AK:* Plasma insulin and growth hormone in dairy cows; diurnal variations and realation to food intake and plasma sugar and acetoacetate levels. Acta Endocrinol. 1973, *73*, 289-303.

*Hove K, Halse K:* Absence of feeding-induced variations in plasma insulin in hypoglycemic-ketonemic cows. Acta vet. scand. 1978, *19*, 215-228.

*Malmstrøm P, Halse K, Tveit B, Skipenes P, Svendsen M:* Miljömessige og genetiske årsaker til klinisk og subklinisk ketose. (Environmental and genetic explanation factors for clinical and subclinical ketosis). Norsk Veterinærtidsskrift 1987, *99*, 95-102.

*McAtee JW, Trenkle A:* Effects of feeding, fasting and infusion of energy substrates on plasma growth hormone levels in cattle. J. Animal Sci. 1971, *33*, 612-616.

*McGarry JD, Wright PH, Foster DW:* Hormonal control of ketogenesis. Rapid activation of hepatic ketogenic capacity in fed rats by anti-insulin serum and glucagon. J. clin. Invest. 1975a, *55*, 1202-1209.

*McGarry JD, Robles-Valdes C, Foster DW:* Role of carnitine in hepatic ketogenesis. Proc. Natl. Acad. Sci. U.S.A. 1975b, *72*, 4385-4388.

*Metcalfe HK, Monson JP, Welch SG, Cohen RD:* Inhibition of lactate removal by ketone bodies in rat liver. Evidence for a quantitatively important role of plasma membrane lactate transporter in lactate metabolism. J. clin. Invest. 1986, *78*, 743-747.

*Metzger P, Franken P, Balasse BO:* Permissive role of glucose on the insulinotropic effect of ketone bodies in vivo. Horm. Metab. Res. 1973, *5*, 313-315.

*Miles JM, Haymond MW, Gerich JE:* Suppression of glucose production and stimulation of insulin secretion by physiological concentrations of ketone bodies in man. J. clin. Endocrin. Metab. 1981, *52*, 34-37.

*Mills SE, Beitz DC, Young JW:* Evidence of impaired metabolism in liver during induced lactation ketosis of dairy cows. J. Dairy Sci. 1986, *69*, 362-370.

*Pi-Sunyer FX, Campbell RG, Hashim SA:* Experimentally induced hyperketonemia and insulin secretion in the dog. Metabolism 1970, *19*, 263-270.

*Rehfeld JF, Heding LG, Holst JJ:* Increased gut glucagon release as pathogenic factor in reactive hypoglycemia? Lancet 1973, *1*, 116-118.

*Stern JS, Baile CA, Mayer J:* Are propionate and butyrate physiological regulators of plasma insulin in ruminants? Amer. J. Physiol. 1970, *219*, 84-91.

*Sutton JD:* Digestion and absorption of energy substrates in the lactating cow. J. Dairy Sci. 1985, *68*, 3376-3393.

*Sutton JD, Hart IC, Broster WH:* The effect of feeding frequency on energy metabolism in milking cows given low-roughage diet. Proceedings of the 9th EAAP Energy Symposium 1982, p. 26-29.

*Tveit B, Lingaas F, Sakshaug J, Halse K:* Aceton og acetoacetat-bestemmelse i blod og melk ved praktisk ketosediagnostikk. (Comparison of acetoacetate and acetone in blood and milk for epidemiological studies of ketosis). Norsk Veterinærtidsskrift 1987, *99*, 879-882.

*Williamson DH, Mellanby J:* D-(-)-3-Hydroxybutyrate. In: Methods of Enzymatic Analysis, vol. 4. Edited by H. U. Bergmayer. Academic Press, New York 1974, p. 1836-1839.

**Sammendrag**

*Plasma glukose, ketonlegemer, insulin, glukagon og enteroglukagon i kyr. Døgn-variasjoner relatert til keton-nivåer før föring og til den ketogene effekt av för-typer.*

Inntak av en moderat ketogen silo to ganger daglig medførte forbigående økninger i plasma insulin og ketonlegemer og senkninger i plasma glukose. Ketonlegemer og glukose var negativt korrelerte hele dagen igjennom, men de forbigående økningene i insulin kulminerte før de maksimale effekter på ketonlegemer og glukose ble oppnådd.

Kyr med varierende glukosenivåer før fôring reagerte uniformt på et sterkt ketogent silofôr med glukosenedgang til omtrent 3 mmol/l, til tross for stor variasjon i den fôrings-induserte insulin-økningen.

Fôring med høy ga opphav til insulin-økning av samme størrelsesorden som med silofôr, men økningen av ketonlegemer var mye mindre og glukose syntes mindre påvirket av insulin etter høy-fôring.

Resultatene indikerer en direkte effekt av ketonlegemer på reguleringen av blodsukkeret i tillegg til påvirkning via insulin. Ketonlegemenes rolle som den insulinotrope faktor ble ikke bekreftet. Insulin-nivået etter fôring ser ut til å bli bestemt av dyrets karbohydrat-status før fôring.

Det ble ikke observert noen signifikant forandring i plasmaglukagon etter fôring og det ble heller ikke funnet forskjeller i nivået av dette hormonet mellom kyr med varierende ketonemi eller med klinisk ketose.

Plasma-nivået av enteroglukagon (GLI) var positivt korrelert til den relative mengde konsumert kraftfôr, men noen relasjon til plasma glukose ble ikke observert.

*(Received September 22, 1988; accepted May 1, 1989).*

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