

From the Department of Biochemistry, College of Veterinary Medicine, Helsinki, Finland, and the Department of Animal Hygiene and Clinical Nutrition, Faculty of Veterinary Medicine, University of Agricultural Sciences, Uppsala, Sweden.

## PREVALENCE OF BOVINE KETOSIS IN RELATION TO NUMBER AND STAGE OF LACTATION

By

*Kauko Kauppinen*

KAUPPINEN, KAUKO: *Prevalence of bovine ketosis in relation to number and stage of lactation.* Acta vet. scand. 1983, 24, 349—361. — The prevalence of bovine ketosis as distributed among various selected subgroups of 504 Ayrshire and Friesian cows was studied during the indoor-housing season 1978—1979 in western and eastern parts of Finland. Special attention was paid to variation by lactation number. Also the differences in prevalence at various phases of lactation and at the end of pregnancy were investigated. The whole blood concentration of acetoacetate (AA concn) was analysed and used as a measure of subclinical and clinical bovine ketosis. AA concns below 0.35 mmol/l, between 0.36 mmol/l and 1.05 mmol/l and over 1.05 mmol/l were considered to indicate normal, subclinical and clinical ketotic stages, respectively. The prevalence of clinical ketosis in cows during 3 post-calving months was 13 %. The percentage of subclinical ketosis was 34. The prevalence of clinical ketosis was 3 %, 7 %, 20 %, 22 % and 13 % at the first, second, third, fourth and fifth, or sixth and later calving, respectively, and the frequency of subclinical ketosis 31 %, 41 %, 35 %, 32 % and 32 %, respectively. No case of ketosis was found in 54 cows at the end phase of pregnancy and only one during the first postcalving week. Only 2 cows (4 %) had the AA concn at a clinically ketotic level during the 65th—92nd postcalving days, 16 %, 17 % and 16 % during the 8th—21st, 22nd—42nd and 43rd—64th post-calving days, respectively.

acetoacetate blood concentration; prevalence of bovine ketosis; lactation number; stage of lactation.

In 1978 Finnish veterinarians recorded a total of 54 319 cases of bovine ketosis. The total number of dairy cows in the same year

was 742 000 (Anon. 1979). The incidence of clinical bovine ketosis was thus 7.3 %. For 1979, the corresponding data (Anon. 1980) yielded an incidence figure of 7.7 %.

The actual incidence of bovine ketosis may be considered to be higher than that recorded in the official statistics. A considerable number of bovine ketosis cases is probably treated by farmers themselves with glucogenic substances, especially with propylene glycol. If the cows recover, these cases are not recorded in the official statistics. On the other hand, bovine ketosis can be considered to be a man-made disease arising from the increasing milk production requirements placed on dairy cows, and a certain degree of ketosis may thus be regarded as normal at an early stage of lactation (Pehrson 1966). Baird *et al.* (1974), in their extensive studies of bovine ketosis, have even drawn the conclusion that "a certain degree of ketosis is a natural state in the ruminant, and the ketotic animal may only represent the extreme of a normal metabolic range".

During clinical bovine ketosis the blood concentration of ketone bodies is many times higher and the glucose level clearly lower than during food deprivation (Baird *et al.* 1968). The prevalence of clinical and subclinical ketosis can be estimated by analysing the concentrations of ketone bodies and glucose in blood samples obtained randomly under field conditions (Kauppinen 1983). The clinical signs of ketosis should of course be significantly related to values interpreted as clinically ketotic. Use of the acetoacetate concentration in the whole blood, rather than that of  $\beta$ -hydroxybutyrate or glucose, to indicate the ketotic stage and the energy status of the dairy cow has been discussed by Kauppinen (1983). Consequently in the present study the whole blood concentration of acetoacetate (AA concn) was used in categorizing the cows as normal or as subclinically or clinically ketotic.

The purpose of the study was to determine the prevalence of clinical and subclinical bovine ketosis especially at the early lactation stage by means of the AA concn. The study was designed to clarify the distribution of the prevalence among various subgroups determined by lactation number as well as the time interval preceding and following parturition. As Pehrson (1966) has shown, bovine ketosis is more common during the indoor-housing period than during the grazing period. This study was carried out during a stable-feeding period.

## MATERIALS AND METHODS

During a period from November of 1978 to June 1979 blood samples were obtained from a total of 504 Ayrshire and Friesian cows in 24 herds in the western and eastern parts of Finland. Fiftyfour cows were sampled during their 20 last precalving days in order to evaluate the significance of bovine ketosis in late pregnancy. The prevalence of bovine ketosis in a high risk subpopulation was determined in the samples obtained from 450 cows within 92 days following parturition. The cows were clinically examined to exclude the differential diagnosis of secondary ketosis. The material did not include any case of secondary ketosis.

The fodder of dairy cows sampled consisted of silage (15—30 kg/day), oats and barley, alone or combined, (2—10 kg/day) and dry hay. The amounts of fodder given were adjusted to milk yield and weight of the cow.

The blood was drained from the mammary vein, 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 as soon as possible. The AA concn was determined using a Gilford 3500 automatic analyzer (Gilford 3500 Computer Directed Analyzer, Gilford Instrument Inc. Oberlin, Ohio, U.S.A.), according to the method of *Työppönen & Kauppinen* (1980). The milk yield was recorded monthly according to the recording system of the Finnish Breeding Association.

The material was divided into normal, subclinically and clinically ketotic groups according to the AA concn. An AA concn lower than 0.35 mmol/l was regarded as normal, a concentration higher than 1.05 mmol/l as clinically ketotic and a concentration between 0.36 and 1.05 mmol/l as subclinically ketotic.

The normal distribution approximation of the binomial distribution was used in testing the equality of proportions. The t-test was applied to find out the differences between the means of the milk yield and the AA concn of the lactation groups. The chi-square test was applied to evaluate the independence of the clinical symptoms of the AA concn.

## RESULTS AND DISCUSSION

Table 1 shows the two-dimensional distribution of lactation number and time as related to parturition in all the cows sampled. It appears that the two factors occur independently of each other

Table 1. Two-dimensional distribution of calving number and time as related to parturition among cows sampled.

Time as related to parturition (in days)	Calving number					Total cows
	1	2	3	4—5	< 5	
—20—0	11	6	7	15	15	54
1—7	10	17	11	13	11	62
8—21	23	12	17	28	22	102
22—24	34	20	19	30	25	128
43—64	26	17	18	27	25	113
65—92	16	4	14	9	2	45
Total cows	120	76	86	122	100	504

$\chi^2 = 26.787$  with  $df = 20$ .

Table 2. Two-dimensional distribution of clinical signs detected and whole blood concentration of acetoacetate (AA concn) among cows sampled.

AA concn mmol/l	Clinical signs detected			total cows
	none	temporary inappetence	clinical signs of ketosis	
0—0.35	281	5	0	286
0.35—1.05	40	119	0	159
1.05	0	7	52	59
Total cows	321	131	52	504

$\chi^2 = 734.87^{***}$  with  $df = 4$ .

in the data material. Table 2 shows the two-dimensional distribution of the clinical signs detected and the AA concn. The clinical signs of ketosis were highly significantly associated with the AA concn ( $P < 0.001$ ).

The classification of the 450 cows sampled post partum into three groups by the AA concn is presented in Table 3. According to this classification, 59 cows (13.1 %) fell into the clinically ketotic group and 152 (33.8 %) into the subclinical one.

Table 3. Distribution of cows after parturition according to acetoacetate (A) blood concentration and calving number. AA blood concentration up to 0.35 mmol/l is regarded as normal. Concentrations between 0.35 and 1.05 mmol/l, and > 1.05 mmol/l indicate subclinical and clinical ketosis, respectively.

AA blood concentration mmol/l	1		2		Calving number						Total	
	n	%	n	%	3		4-5		>5		n	%
0—0.35	72	66.1	36	51.5	35	44.3	49	45.8	47	55.3	239	53.1
0.36—1.05	34	31.2	29	41.4	28	35.4	34	31.8	27	31.8	152	33.8
>1.05	3	2.7	5	7.1	16	20.3	24	22.4	11	12.9	59	13.1
	109	100.0	70	100.0	79	100.0	107	100.0	85	100.0	450	100.0

The divisions are partly theoretical, especially as far as the limit between the subclinical ketosis and the normal state is concerned. *Baird et al.* (1968) measured an AA concn of  $0.019 \pm 0.011$  mmol/l (mean  $\pm$  s) for normal lactating cows. *Työppönen & Kauppinen* (1980) found the corresponding figure to be  $0.22 \pm 0.07$  mmol/l. *Kauppinen* (1983) measured an AA concn below 0.35 mmol/l for the majority of cows (67.1 %) which were eating normally and showed no symptoms of ketosis.

The higher AA concn measured by *Työppönen & Kauppinen* compared to the results of *Baird et al.* arises probably from the way in which the blood samples were treated by *Työppönen & Kauppinen* in order to maintain the stability of the AA concentration and from the differences in the methods of analysis. On the other hand *Baird et al.* determined the AA concn only for 5 normal lactating cows. According to the 2 studies mentioned above, the AA concn of 0.35 mmol/l was defined as the upper limit of normal cows. *Baird et al.* measured an AA concn for ketotic cows of  $0.85 \pm 0.32$  mmol/l and *Työppönen & Kauppinen* one of  $2.14 \pm 0.89$  mmol/l. The borderline AA concn between subclinical and clinical values are considered to be empirically justified, since the clinical symptoms were highly significantly associated with the AA concn measured (Table 2). Additionally *Kauppinen* (1983) measured an AA concn below 1.05 mmol/l for 87.8 % of all the cows sampled.

The lactation number seemed to have a considerable effect on frequency of bovine ketosis. Only 3 cases of clinical ketosis were demonstrated among 109 primiparae (Table 3). The percentage

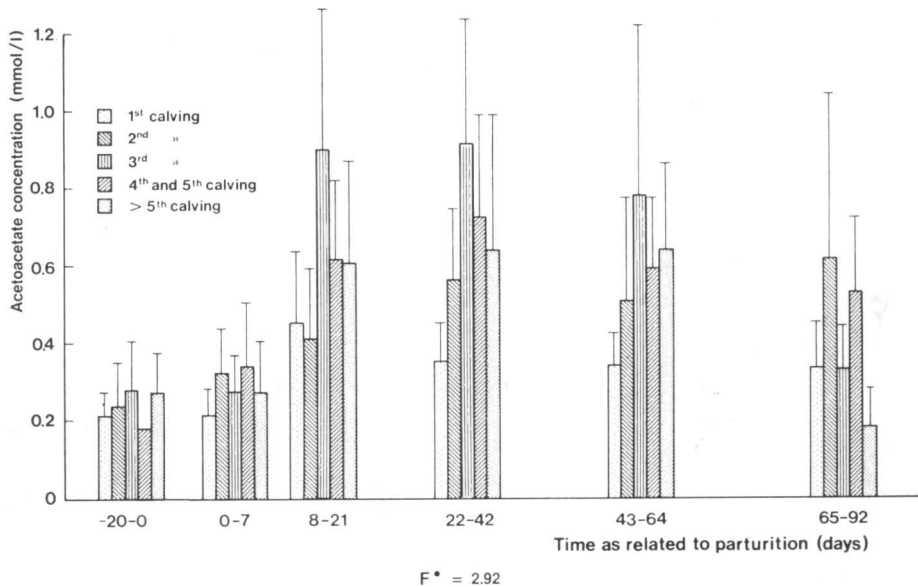


Figure 1. The whole blood acetoacetate concentration of cows at different calving times as related to parturition (means with 95 % confidence intervals for population means).

of subclinical cases of ketosis in this group did not differ from that in other groups. No statistical difference in prevalence of clinical ketosis was found between cows lactating for the first and those lactating for the second time. The figure of 20.3 % for clinical ketosis among dairy cows in the third year of lactation was highly significantly ( $P < 0.001$ ) and almost significantly ( $P < 0.05$ ) higher than that for cows in the first and the second lactation year, respectively. The 22.4 % of the fourth group differed highly significantly from the first group ( $P < 0.001$ ), significantly from the second ( $P < 0.01$ ), and not significantly from the third. The percentage of clinical cases of ketosis, 12.9 %, measured for the older cows differed only from that of primiparae ( $P < 0.05$ ). It seems that cows after their third, fourth and fifth calving more often than younger cows go through a period of negative energy balance, which leads to clinical ketosis. Since there were no significant differences in percentages of subclinical ketosis between various lactation numbers, the negative nutritional balance is also evident among primiparae and cows calving for the second time. However, it leads relatively seldom to so high

a level of the AA concn, which could be interpreted as clinically ketotic. Among cows calving for more than the fifth time the AA concn reached the subclinically ketotic level as often as among other cows, but the AA concn of these older cows did not seem to develop to the clinically ketotic level as often as among cows calving for the third, fourth and fifth time.

Figure 1 shows the distribution of the mean AA concn of these lactation number groups by lactation stage, with 95 % confidence limits for population means. The mean AA concn was at a low level prior to parturition and during the first postparturition week.

Among the primiparae in this material, the mean AA concn did not later increase significantly from the level of the first postparturition week (Fig. 1). In the case of third calving, during the 8th—21st postparturition days the mean AA concn was almost significantly ( $P < 0.05$ ) higher than during the first postcalving week. Among cows calving for the second, fourth and fifth time the mean AA concn increased almost significantly ( $P < 0.05$ ) during the 22nd—42nd postparturition days; and for those calving more than the fifth time, the relevant period covered the 43rd—64th postparturition days ( $P < 0.05$ ). There was an almost significant difference between various lactation number groups ( $F = 2.92$ ,  $P < 0.05$ ) only during the period of the 22nd—42nd postcalving days but not during any other period. These findings showed that cows were driven to negative nutritional balance during the second and third postcalving weeks. A tendency toward the energy equilibrium was found only two months after calving.

The mean milk yields of the corresponding lactation number groups, with 95 % confidence intervals for population means are presented in Fig. 2, with the monthly milk recording figures related to the time of parturition. During the 75-day period following parturition there was difference between various groups at a high level of significance ( $P < 0.001$ ). Primiparae yielded significantly ( $P < 0.01$ ) less than other cows during this whole period, as during the first 15 postcalving days did cows calving for the second time. There were no significant differences between the milk yields of the older groups.

Table 4 shows the percentages of subclinically and clinically ketotic cows and the number of cows sampled at various times in relation to parturition. There were no significant differences

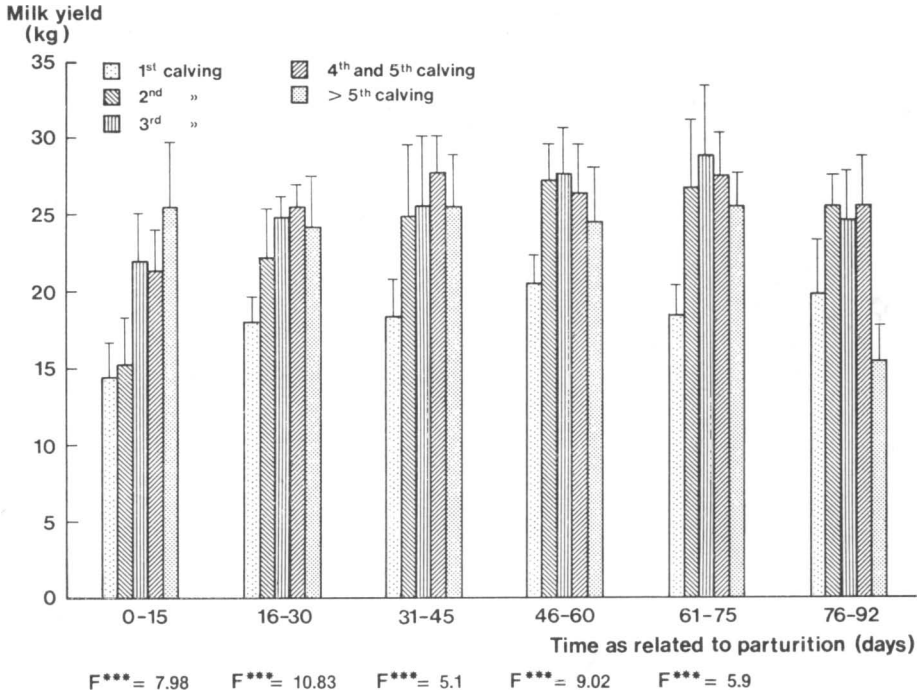


Figure 2. Milk yields of dairy cows at different calving times as ketosis during different periods as related to parturition. The percentages are presented in parentheses.

Table 4. The numbers of subclinical and clinical cases of bovine ketosis during different periods as related to parturition. The percentages are presented in parantheses.

Time as related to parturition (in days)	Number of subclinical cases of ketosis	Number of clinical cases of ketosis	Total number of cows
-20— 0	7 (13 %)	0	54 (100 %)
0— 7	14 (22.6 %)	1 (1.6 %)	62 (100 %)
8—21	37 (36.3 %)	16 (15.7 %)	102 (100 %)
22—42	46 (35.9 %)	22 (17.2 %)	128 (100 %)
43—64	37 (32.7 %)	18 (15.9 %)	113 (100 %)
65—92	18 (40 %)	2 (4.4 %)	45 (100 %)
	159	59	504



between the figures for subclinical and clinical ketosis during the 8th—64th postcalving days. Thus it seems evident, according to present findings, that the occurrence of bovine ketosis is equal during this period but lower before and after it. This finding does not completely agree with the results of *Halse & Mogstad* (1975), who found the frequency of clinical ketosis to be highest during the postparturition period from 20 to 35 days. It has also been suggested that bovine ketosis is a risk at the end of pregnancy (*Pehrson* 1966). In this material no clinically ketotic AA concns and only 7 subclinical ones (13 %) were detected during the last 20 preparturition days. Only 1 clinical case of ketosis was encountered during the first lactation week, and 2 during the third month.

According to the findings mentioned above a negative energy balance was evident as early as in the second week after calving. This means a decrease in the glucose concentration in the blood, and the mobilization of the body fat deposits. *Sato* (1978) carefully monitored the blood concentrations of free fatty acids (FFA) and reported the highest value ( $0.38 \pm 0.06$  mEq/l) as occurring during the first postcalving week. According to *Athanasiou & Phillips* (1978 b), the FFA reached their peak concentration in the blood during the postfasting period 12 h earlier than the ketone bodies. *Phillips & Athanasiou* (1978) analysed the steadily falling plasma concentration of FFA for cows during the 10 first postparturition days. It also seems to drop due to a higher hepatic uptake of FFA in bovine ketosis (*Kronfeld* 1965). *Bassett* (1970) has furthermore shown the blood concentration of FFA to be extremely stress-sensitive. Thus the AA concn seems to be a better measure of the ketotic stage and the energy status of a dairy cow than the blood concentration of FFA. *Kauppinen* (1983) has shown the AA concn to be correlated with the whole blood concentrations of  $\beta$ -hydroxybutyrate and glucose and to indicate the energy status of dairy cows at least as validly as these.

As can be seen in Figs. 1 and 2, the AA concn and milk yield increased during the first two months of lactation much in the same way. These have been shown to be somewhat correlated in the individual cow (*Kauppinen* 1983). Milk production increased significantly ( $P < 0.01$ ) from its level of the 15 postparturition days in cases of the first, second, fourth and fifth calving, but not in cases of the third or more than the fifth calving time (Fig. 2).

In the present study the milk yield of primiparae was found to be lower than that of others ( $P < 0.01$ ), as was the prevalence of ketosis. During the first 15 postcalving days the mean milk yield of cows calving for the second time was lower than that of older ones ( $P < 0.01$ ). Likewise no case of ketosis was detected among these cows during this time. Older cows yielded roughly equal amounts and showed a corresponding prevalence of ketosis. The lower prevalence of ketosis among younger cows found in this study may partly be explained as a consequence of the lower milk yield. Since the prevalence of subclinical ketosis of these cows did not differ significantly from that of the older cows, there might be some factors which caused the clinical symptoms of ketosis in the older cows but not in the younger ones. The nutritional status of these younger animals may not be disturbed by milk production to the same extent as that of older ones. The composition and concentration of the feed is naturally of importance in this respect. The fodder fed to cows sampled was approximately the same.

The lower mean AA concn measured in this study during the first 7 postcalving days is in accordance with the findings by *Athanasίου & Phillips* (1978 a). It may be partly due to cautious milking during that time. In Finland cows are not usually milked completely during the first 2—5 days after parturition. On the other hand it is known that the blood glucose level is higher than normal during the day preceding and following calving (*Athanasίου & Phillips* 1978 a). Feeding, too, is increased progressively during the first 10—14 days post partum, in order to help the cow reach the peak of milk production smoothly.

The prevalence of clinical bovine ketosis found in this work was 13.1 %. An incidence of 7.3 % or 7.7 % has been recorded by the official Finnish statistic. The incidence based on official statistics was in Norway 11.7 %, in Sweden 5.0 % (*Halse et al.* 1978), in Great Britain 1—5 % (*Baird et al.* 1974) and in the U.S.A. 4 % (*Schultz* 1968). The comparability of the prevalence and incidence figures given above, weak as such, is additionally weakened with differences both in the method and material of the measurements. Additionally the category of "clinical ketosis" in the AA concn classification includes 7 cases, which would not be recognised as sick by the ordinary clinical investigation (Table 2).

As far as subclinical ketosis is concerned, comparable figures first 7 postcalving days is in accordance with the findings of by

from other countries are few. The prevalence of subclinical ketosis in cows during the first 3 postcalving months found in this study was 33.8 %. The definition of subclinical bovine ketosis by means of the value of the AA concn as has been pointed out, is to a certain extent theoretical. In particular the differentiation between normal and subclinical values is arbitrary. Even with this reservation in mind, the extent of subclinical ketosis seems to be remarkable. Subclinical ketosis may be described as a state of increased concentration of ketone bodies and of FFA and decreased one of glucose in the blood without clinical symptoms of ketosis. A large proportion of these cases may recover spontaneously, while others develop into clinical ketosis (*Kronfeld & Emery 1970*). Subclinical ketosis can only be overcome by balancing the energy status of the cow. On the other hand, long lasting subclinical ketosis is evidently a stress on the liver of the cow and causes infiltration of fats into the cells of the liver (*Roberts et al. 1981*). In this way subclinical ketosis may also disturb the normal reproductive performance of the cow (*Reid 1980*). Subclinical bovine ketosis additionally can be expected to reduce milk yield and cause loss in the body weight of a cow. The economical significance of subclinical ketosis should preferably be estimated on the basis of all the parameters affected. This is a strong incentive for further study.

In conclusion it may be stated that bovine ketosis affected cows calving for the third, fourth and fifth time more likely than the younger cows. The cows calving for more than the fifth time showed a ketotic AA concn more often than primiparae. The relative frequency of ketosis was equal from the second week till the end of the second month after calving. The overall prevalence of clinical ketosis was 13.1 % and that of subclinical ketosis 33.8 %. The negative energy status indicated by the subclinically increased AA concn was equally common in cows of various age.

#### ACKNOWLEDGEMENTS

This work was subsidized by a grant from the Ministry of Agriculture and Forestry, Veterinary Department.

#### REFERENCES

- Anonymous*: Veterinary Service Report. Official Statistics of Finland XXXIV, 1979, 49.  
*Anonymous*: Veterinary Service Report. Official Statistics of Finland XXXIV, 1980, 50.

- Athanasίου, V. N. & R. W. Phillips*: Stability of plasma metabolites and hormones in parturient dairy cows. *Amer. J. vet. Res.* 1978 a, 39, 953—956.
- Athanasίου, V. N. & R. W. Phillips*: Effect of fasting on plasma metabolites and hormones in lactating dairy cows. *Amer. J. vet. Res.* 1978 b, 39, 957—960.
- Bassett, J. M.*: Metabolic effects of catecholamines in sheep. *Aust. J. biol. Sci.* 1970, 23, 903—914.
- Baird, G. D., K. G. Hibbitt, G. D. Hunter, P. Lund, M. Stubbs & H. A. Krebs*: Biochemical aspects of bovine ketosis. *Biochem. J.* 1968, 107, 683—689.
- Baird, G. D., R. J. Heitzman, K. G. Hibbitt & G. D. Hunter*: Bovine ketosis: A review with recommendations for control and treatment. Part I. *Brit. vet. J.* 1974, 130, 214—220.
- Halse, K. & O. Mogstad*: Klinisk og subklinisk ketose hos kyr. — Tid etter kalving. (Clinical and subclinical ketosis in dairy cows. — Time after parturition). *Norsk Vet.-T.* 1975, 87, 311—319.
- Halse, K., H. Dale, A. O. Refsdal & O. Møller*: Ketoseproblemer i norsk mjølkeproduksjon. (The problems of ketosis in Norwegian milk production). *Norsk Vet.-T.* 1978, 90, 819—835.
- Kauppinen, K.*: Correlation of whole blood concentrations of acetate,  $\beta$ -hydroxybutyrate, glucose and milk yield in dairy cows as studied under field conditions. *Acta vet. scand.* 1983, 24, 337—348.
- Kronfeld, D. S.*: Plasma non-esterified fatty acids in the dairy cow: responses to nutritional and hormonal stimuli, and significance in ketosis. *Vet. Rec.* 1965, 77, 30—34.
- Kronfeld, D. S. & R. S. Emery*: Acetonemia. In *Bovine Medicine and Surgery, and Herd Health Management*. Ed. Gibbons, W. J., E. J. Catcott and J. F. Smithers, Wheaton, Ill. *Amer. vet. Publ.* 1970, p. 350—376.
- Pehrson, B.*: Studies on ketosis in dairy cows. *Acta vet. scand.* 1966. Suppl. 15, 1—59.
- Phillips, R. W. & V. N. Athanasίου*: Stability of plasma metabolites and hormones in lactating dairy cows. *Amer. J. vet. Res.* 1978, 39, 949—952.
- Reid, I. M.*: Incidence and severity of fatty liver in dairy cows. *Vet. Rec.* 1980, 107, 281—284.
- Roberts, C. J., I. M. Reid, G. J. Rowlands & A. Patterson*: A fat mobilisation syndrome in dairy cows in early lactation. *Vet. Rec.* 1981, 108, 7—9.
- Sato, H.*: Plasma glucose, lipid, mineral level and alkaline phosphatase activity in parturient dairy cows. *Japan. J. zootech. Sci.* 1978, 49, 333—338.
- Schultz, L. H.*: Ketosis in dairy cattle. *J. Dairy Sci.* 1968, 51, 1133—1140.

*Työppönen, J. & K. Kauppinen: The stability and automatic determination of ketone bodies in blood samples taken in field conditions. Acta vet. scand. 1980, 21, 55—61.*

#### SAMMANFATTNING

*Ketosförekomst hos mjölkboskap i relation till mjölkningsår och tid för kalving.*

Ketosförekomsten hos mjölkboskap undersöktes i olika djurgrupper av 504 aurshire och frisiska kor under stallutfodringsperioden 1978—79 i västra och östra Finland. Speciell vikt lades vid variationen av ketosfrekvens under olika laktationsår. Olikheterna i ketosförekomsten vid olika tidpunkter efter kalving och vid slutet av dräktigheten undersöktes. Acetoacetatkoncentrationen i blodet analyserades och användes som mått på subklinisk och klinisk ketos. AA-koncentrationer under 0.35 mmol/l, mellan 0.36 och 1.05 mmol/l och över 1.05 mmol/l ansågs vara normala, subkliniskt respektive kliniskt ketotiska värden. Förekomsten av klinisk ketos var 3 % vid första kalvningen, 7 % vid andra, 20 % vid tredje, 22 % vid fjärde och femte samt 13 % vid sjätte och senare kalvning. Förekomsten av subklinisk ketos var under motsvarande kalvningar 31 %, 41 %, 35 %, 32 % respektive 32 %. Inga fall av ketos förekom bland kor vid slutet av dräktigheten och endast ett fall under den första veckan efter kalvningen. Under 8—21, 22—42 och 43—64 dagar efter kalvning låg 16 %, 17 % respektive 16 % av korna på en AA-koncentration som indikerar klinisk ketos, medan under perioden 65—92 dagar efter kalvning låg endast 2 kor på en sådan nivå (4 %).

*(Received July 7, 1983).*

Reprints may be requested from: Kauko Kauppinen, the Department of Biochemistry, College of Veterinary Medicine, Hämeentie 57, 00550 Helsinki 55, Finland.