

Evaluation of a Routine Testing for Ketonuria and Aciduria in the Detection of Sub and Clinical Ketosis Associated with Overfeeding in Dairy Cattle

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Summary

1162 adult cows were examined for urine ketone concentration and reaction 7 to 14 days postpartum and 165 were further examined for SGOT and for ketonaemia.

The rate of clinical ketosis, that of SGOT over 40 u/l and that of aciduria were all found to rise with the urine acetoacetate concentration.

The rate of clinical ketosis and the mean SGOT of cows with aciduria were higher than of those with basic reaction of the urine in all concentrations of urine acetoacetate.

Ketonuria was found to be better correlated to clinical ketosis than either ketonaemia and SGOT. Ketonaemia was highly correlated to ketonuria.

Using routine urine testing, the rate of discovery of clinical ketosis has been rising from 4.3% to 15.6% in all herds examined. It is concluded that the combined testing for ketonuria and aciduria is a simple and reliable diagnostic aid which greatly minimizes the high rate of false discoveries associated with ketonuria and when used routinely can contribute to the health status of the large dairy herds.

Introduction

Ketosis is a metabolic disease associated with an elevated concentration of acetoacetic and betahydroxybutiric acids in body fluids. Subclinical ketosis refers to ketonuria in cows showing no clinical signs (14).

While many claim that subclinical ketosis has both an imminent and future harmful effects on the ketotic cows, (1,3,6,19), the separation of the clinical from the subclinical forms of the disease is often difficult in practice because of the absence of a clear cut separating line and in view of the fact that the disease is often a self limiting one.

Field tests for the detection of ketonuria had long been used as a diagnostic aid for ketosis. These tests had been criticized as being oversensitive, giving too many false positive results (17,19), as depending upon the concentration of the urine and reflecting the mere presence of acetoacetate (7), and had been recommended therefore to

be interpreted with caution, a positive result being a "warning rather than a sign of diseases" (14).

The association between ketonuria and overfattening before calving in the population described in the present work had been established previously. (15).

The following work is proposed to examine the use of routine urine testing for ketones and reaction carried out on all adult calving cows between 7-14 days postpartum in detecting both sub and clinical ketosis.

Materials and Methods

Management and feeding regimes.

The present work is based on the first author's routine practice in seven Israeli-Friesian herds in the period from July 1983 to May 1984. The herds, which consist of 200-350 milking cows each, are characterized by a high yield (7000-9000 Kg. average annual yield per cow). The cows are kept in groups according to their milk yield, the feeding is in complete rations, concentrates being the main constituent of the ration and the roughage is limited. The rations conform generally with the American NRC recommendations.

Routine of clinical examination.

A calving cow had been presented for a routine examination 7 to 14 days postpartum on a regular day for each farm during the week. The examination was carried out in the following order: 1. Determination of the state of the uterus from the discharge removed from the cervical region by a hand inserted into the vagina. 2. Examination of the urine for the presence of acetoacetate and reaction after removing a urine sample through a metal catheter; a drop of urine was placed on the reagent strip, ("Ketostix"-Ames), and the test area was compared to the colour chart and read 15 sec. after wetting.

The reaction of the urine was determined by a universal indicator paper, (Macherey Nagel), with a range of pH 6.4 to 8.0 (.2 intervals). 3. Examination of the faeces for colour, quantity and consistency. 4. Additional clinical examination as indicated.

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Routine of laboratory examination.

Blood had been collected at the end of the clinical examination from the coccygeal vein by a vacu-container and brought to the laboratory the same or the following day.

The blood was examined in the laboratory by author No. 2 in the following manner:

1. The sample was centrifuged and the sera examined for acetoacetate in the manner described before.
2. SGOT values were determined with a Compur M 2000 S/SC instrument (Compur Electronic), by the method recommended by the Deutsche Gesellschaft fur Klinische Chemie, (12).

Definitions and statistical analysis.

1162 adult cows, (2nd calvers and upward), were examined for urine acetoacetate concentration and reaction. The last 165 of these were examined for SGOT and for ketonaemia as well and had been used for a relative evaluation of the urine tests.

A cow was defined as having clinical ketosis if the test was positive for ketonuria or ketonaemia and had yielded less than 25 KG milk on the day following the clinical examination.

Data was analysed by routine statistical methods of Chi Square tests, Fisher T test and Pearson's cosine method for correlation of attributes, (2).

Results

1. The rate of ketonuria and distribution of acetoacetate concentrations.

The overall rate of ketonuria in 1162 adult cows examined was 32.2%. The distribution of acetoacetate concentrations is described in Tab. 1.

TABLE 1. Distribution of Urine Acetoacetate Concentrations.

Urine Concentration of Acetoacetate mmol/l	n = 1162.					
	negative	.5	1.5	3.9	7.8	15.6
%	67.8	8.3	9.0	6.0	5.4	3.5

2. The association between urine acetoacetate concentration and the rate of clinical ketosis.

The overall rate of clinical ketosis in 278 cows with urine acetoacetate concentration of more than 1.5 mmol/l was 57.2%. The rate of clinical ketosis was rising with urine acetoacetate concentration from 29.8% in concentration of 1.5 mmol/l to 95.1% in concentration of 15.6 mmol/l (Tab. 2).

3. The association between acetoacetate concentration of urine, aciduria and clinical ketosis.

The total rate of aciduria in the cows examined was 27.6%. The rate of aciduria in ketotic cows was higher than in the non ketotic ones and was rising with the concentration

of urine acetoacetate (Tab. 3).

The total rate of clinical ketosis in cows with acid reaction of the urine was significantly higher than in those with basic reaction (76.8% and 41.2% respectively). This difference was maintained in all concentrations, being statistically significant in concentrations of 1.5 mmol/l and 3.9 mmol/l.

Rates of clinical ketosis were rising in both cows with aciduria and those with basic reaction of the urine with the concentration of acetoacetate, the latter being statistically significant.

4. The association between urine acetoacetate concentration and SGOT.

The overall rate of cows with SGOT of over 40 u/l in all 165 cows examined was 59.4%. The rate was rising with the concentration of acetoacetate in the urine from 27.9% in those which were negative to 95.7% in those with acetoacetate concentration of 15.6 mmol/l (Tab. 4).

TABLE 2. The Association Between Urine Acetoacetate Concentration and Clinical Ketosis.

Urine Concentration of Acetoacetate mmol/l	Total	1.5	3.9	7.8	15.6
n Positives	278	104	70	63	41
% of Clinical Ketosis *	57.2	29.8	58.6	76.2	95.1
* $\chi^2=27.90$ p < .01					

TABLE 3. The Association Between Acetoacetate Concentration of the Urine, Clinical Ketosis and Aciduria.

Urine Concentration of Acetoacetate mmol/l	Total	Negative and				
		.5	1.5	3.9	7.8	15.6
n Examined	1162	884	104	70	63	41
% with Aciduria *	27.6	22.2	32.7	37.1	57.1	70.7
% of Clinical Ketosis in Cows with Aciduria **	76.8	52.9	80.8	80.6	96.6	
	a	a	a			
% of Clinical Ketosis in Cows without Aciduria ***	41.2	18.6	45.5	70.4	91.7	
	b	b	b			

* $\chi^2=62.87$ p < .01 ** $\chi^2=N.S.$ *** $\chi^2=26.05$ p < .01
 ab = Difference highly significant. (p < .01)

TABLE 4. The Association Between Urine Acetoacetate Concentration and SGOT.

Urine Concentration of Acetoacetate mmol/l	Total	negative	.5	1.5	3.9	7.8	15.6
n Examined	165	68	7	22	25	20	23
% of SGOT Over 40 u/l *	59.4	27.9	57.1	72.7	88.0	75.0	95.7
* $\chi^2=20.95$ p < .01							

The mean SGOT in 45 ketotic cows with aciduria was 72.6 ± 34.3 compared to $51.1 \text{ u/l} \pm 17.5$ in 51 ketotic cows with no aciduria. ($P < .01$).

5. The association between ketonuria, aciduria, ketonaemia, SGOT and clinical ketosis.

Ketonaemia was highly correlated to ketonuria ($r = .801$), and less so to SGOT over 40 u/l , ($r = .474$), both correlations being statistically significant.

Ketonuria was found to be associated to clinical ketosis, ($r = .926$) more than ketonaemia ($r = .535$) and SGOT over 40 u/l ($r = .535$). Rate of discovery of clinical cases was high with the test for ketonuria (96.7%), and for SGOT over 40 u/l (90.0%), and less so for the test for ketonaemia (66.7%).

The rate of false discovery, (positive normals), was similar for all three tests (36.3%, 42.8% and 30.8% respectively).

Combination of ketonuria and aciduria minimizes the rate of discovery from 96.7% to 65.0% but also that of false positives from 36.3% to 6.6% (Tab. 5).

6. Rate of discovery of clinical ketosis by a routine urine testing compared to a discovery by herdsmen.

The rate of clinical ketosis was compared in each of the seven farms between two successive years (Tab. 6).

The overall rate of clinical ketosis in the year when no routine urine test had been practiced was 4.3% compared to 15.6% when the urine was routinely tested, the range of improvement in diagnosis being from X1.6 to X22.6 in the various farms.

TABLE 5. The Association Between Ketonuria, Aciduria, Ketonaemia, SGOT and Clinical Ketosis.

Clinical Diagnosis n Examined	Negative 91	Positive 60	"r"
	(Rate of False Discovery)	(Rate of Discovery)	
SGOT over 40 u/l	42.4%	90.0%	.535 *
Ketonaemia over $.5 \text{ mmol/l}$	30.8%	66.7%	.535 *
Ketonuria over $.5 \text{ mmol/l}$	36.3%	96.7%	.926 *
Ketonuria over $.5 \text{ mmol/l}$ and Aciduria	6.6%	65.0%	.871 *

"r"—Correlation of attributes * $p < .01$

TABLE 6. Rate of Discovery of Clinical Ketosis By A Routine Urine Test Compared To Discovery By Herdsman.

Period Farm	No Routine Urine Test Oct. 82-May 83		Routine Urine Test Oct. 83-May 84	
	n Calvings	% of Clinical Ketosis	n Calvings	% of Clinical Ketosis
Total	1015	4.3	866	15.6
1	161	.6	164	13.4
2	196	2.0	160	15.0
3	170	7.6	128	18.8
4	165	6.1	150	10.0
5	119	5.9	77	19.5
6	92	7.6	80	30.0
7	112	1.8	107	10.3

Discussion

The definition by which "cows suffering from clinical diseases are those which show some abnormality of function which is harmful to the animals" (7), is difficult to apply to a disease which does not always have characteristic symptoms, in which diagnosis is based on chemical demonstration of ketones which may represent a normal physiological process, and which in most cases is complicated by a concurrent disease.

A routine urine test for the diagnosis of ketosis was evaluated in the present work against the rate of discovery of clinical cases and that of false positives, against SGOT and against ketonaemia.

As healthy adult cows should yield a week after calving more than 30 KG. milk per day in the population examined (4) a cow which yielded less than 25 KG. milk per day when examined and was positive for any of the tests applied was defined as "clinical" in the present work.

SGOT is a disputed enzyme for a specific diagnosis of fatty liver (9, 10, 19), but is agreed by most that it represents body tissue damage. Values of over 40 u/l are considered to be pathological in the postparturient cow. (19)

As ketonuria in the herds examined had been shown to be associated with overfeeding and overfattening before calving (15), and in view of the above mentioned property of SGOT, it was chosen to evaluate the urine tests.

Ketonuria proved in the present work to have a stronger association with clinical ketosis than either SGOT or ketonaemia (Tab. 5).

While the rate of discovery of clinical ketosis was high with both the urine test (96.7%), and that for SGOT (90.0%), that of the blood ketone test was lower (66.7%). The high correlation established between ketonuria and aciduria ($r = .801$) is in contrast with a previous work (10), where this correlation has been found to be low.

The relatively high rate of positive false discoveries of clinical ketosis associated with acetonaemia (30.8%), is in contrast with the claim that acetoacetate can be usually found only in the blood of sick cows (20).

Urine tests for ketonuria had been discredited in the past for the diagnosis of clinical ketosis on the ground that ketones can be found in urine of many normal cows (8), that their concentration is greatly affected by that of the urine, (14), and that they give a high rate of false positives (14, 19).

It is evident from the high association established in the present work between clinical ketosis and SGOT with the rising concentration of urine acetoacetate (Tab. 2 & 4), that the effect of urine concentration is small, otherwise that correlation could not have existed.

It should be stated, therefore, that the rising urine concentration of acetoacetate can serve as a reliable tool in the diagnosis of clinical ketosis (Tab. 2).

The relationship between ketonuria and aciduria calls for further elaboration. While it had been shown before that a negative correlation exists between the rumen pH and the

urine concentration of acetone, and a positive one between the rumen and the urine pH (18), and that acidosis due to liver damage is associated with the rise in SGOT (5), the urine reaction had not been used to minimize the false positives and so to overcome the limitations of the urine tests.

A low urine pH had been shown to be associated with starvation (10), and with catabolic changes associated with overproduction of lactic acid (11). It is expected, therefore, that clinical symptoms will be shown when a state of acidosis exists (14); Tab. 3 shows that this is indeed the case. The rate of clinical cases rises with the acid reaction of the urine and the diagnosis of clinical ketosis by the combined presence of ketonuria and aciduria had both the advantages of a high rate of discovery of clinical cases and a low rate of false positives (Tab. 5). The value of a routine screening for ketolactia had been described before (6,13). Feeding of complete diets, frequent change of milkers in the large herds milked three times a day, the proximity to parturition and the existence of concurrent diseases often mask the two classic signs of ketosis—a selective appetite for forage and a reduced milk yield. The disease being in many cases a self limiting one, it can be understood how so many cases are missed when no routine examination is practiced (Tab. 6). The resulting damage is both direct in reduced milk yield (1), and indirect in the future effect on reproduction (3, 6).

The value of a routine early postparturient uterine examination for future reproduction had been demonstrated before (16). It is suggested that a simple routine field test for urine ketones and pH, incorporated into that examination can further minimize the economic losses associated with postparturient diseases in the large heavily fed, high yielding dairy herd.

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