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CLINICAL AND BIOCHEMICAL STUDIES ON THE BASIS OF KETONE BODIES IN CATTLE

II. URINE KETONE BODIES IN NORMAL DAIRY CATTLE

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INTRODUCTION

In the previous paper,³⁾ the authors had reported the results of their study on blood ketone bodies in clinically healthy cows and clarified the actual state of them in the field. In this instance, there were two groups, viz., "normal and sub-normal groups" in the clinically healthy cows and the characters of them were discussed. In the present paper, the results of studies on the urine ketone bodies conducted in the same way as that on blood ketone bodies are described.

MATERIALS AND METHODS

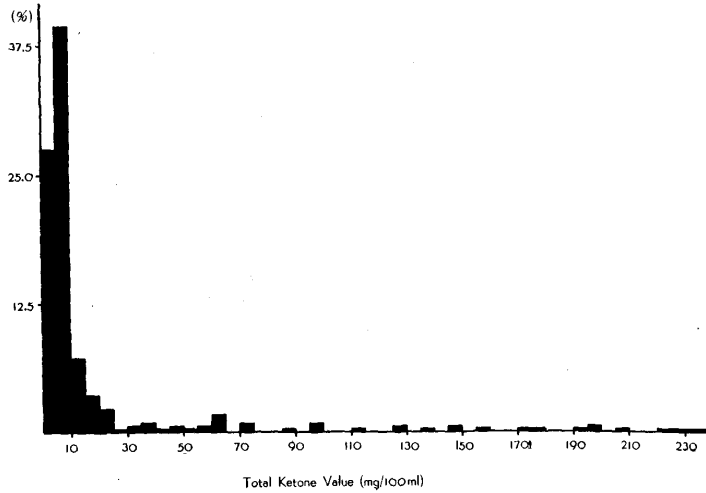
The cases and methods were just the same as those described in the previous report.

RESULTS OF THE INVESTIGATIONS

1. The Values for Total Ketone Bodies in the Urine

The distribution of frequencies of the values for ketones in the urine of 368 clinically healthy cows is shown in chart 1. This is an exponential distribution in which the range of 5~10 mg/100 ml of urine was highest; the mean value was 8.24 mg/100 ml, and its range was 2.12~258.00 mg/100 ml. The under and upper limits of this distribution under 5% level of significance were 1.56 and 43.41 mg/100 ml, respectively; the mean value of the 257 cases between the rejection limits was 6.19 mg/100 ml. This value may be considered as the mean value for ketones of the normal cows, excepting special cases, from the clinically healthy cows in the field. There were 37 cases over the upper limit of the rejection limits; their appearance rate was 10.0% and their mean value was 122.50 (47.97~258.00) mg/100 ml. In the cases of the urine, also the group of cases above the upper limit are called the "sub-normal group" and the cases between the rejection limits are grouped as the "normal group".

CHART 1. *The Distribution of the Values for Total Ketones in Urine*



2. The Values for Free Acetone in the Urine

The distribution of frequencies of the values for acetone in the urine of 215 clinically healthy cows is indicated in chart 2. This also is an exponential distribution in which the range of 0.5~1.0 mg/100 ml was highest, and the mean value was 1.59 (0.18~123.00) mg/100 ml. By statistical methods as in the ketones, the rejection limits were calculated

CHART 2. *The Distribution of the Values for Free Acetone in Urin*



as 0.10 mg/100 ml in the under limit and as 24.95 mg/100 ml in the upper limit. The mean value of the 199 cases (normal group) between the two limits was 1.25 (0.18~24.95) mg/100 ml and the mean value of the 16 cases over the upper limit (sub-normal group) was 54.50 (25.83~123.00) mg/100 ml; their appearance rate was 7.5%.

3. The Values for Each of the Individual Ketone Bodies in the Urine

The urine samples of 111 cases were used for fractionations into 3 kinds of ketone bodies. These values were arranged into 9 sub-groups in accordance with the respective total ketone values. As shown in table 2, the contents of any one of the individual ketone bodies increased in parallel with the increase of total ketones, but in the ratio to the values for total ketones, free acetone showed a decreasing tendency antagonistically to the increase of the total ketones. Between acetoacetic acid and total ketones, there were no special relations observed. But in the ratio of β -hydroxybutyric

TABLE 1. *The Values for Ketone Bodies in Urine*

GROUP	TOTAL KETONE			FREE ACETONE		
	No. of Cases	Mean (mg/100 ml)	Range (mg/100 ml)	No. of Cases	Mean (mg/100ml)	Range (mg/100 ml)
Clinically Healthy Cows	368	8.24	2.12~258.00	215	1.59	0.18~123.00
Normal Group	331	6.19	2.12~ 43.41	199	1.25	0.18~ 24.95
Sub-normal Group	37	122.50	47.97~258.00	16	54.50	25.83~123.00

TABLE 2. *The Values for Each of the Individual Ketone Bodies in Urine*

LEVEL FOR TOTAL KETONE (mg/100ml)	NO. OF CASES (111)	FREE ACETONE		ACETOACETIC ACID		β-HYDROXYBUTYRIC ACID		MEAN VALUE FOR TOTAL KETONE (mg/100ml)
		Mean (mg/100ml)	Ratio to Total Ketone (%)	Mean (mg/100ml)	Ratio to Total Ketone (%)	Mean (mg/100ml)	Ratio to Total Ketone (%)	
Under 4	22	0.76	22.9	1.32	39.9	1.23	37.2	3.31
4~7	28	0.98	19.0	1.81	35.5	2.31	45.5	5.10
7~10	35	1.34	16.0	3.16	37.9	3.83	46.1	8.33
10~13	4	1.37	12.0	4.37	38.0	5.75	50.0	11.49
13~16	4	1.88	12.7	5.12	34.7	7.77	52.6	14.77
16~21	4	3.00	16.9	6.00	33.8	8.77	49.3	17.77
21~31	3	7.07	29.7	7.59	31.9	9.12	38.4	23.78
31~44	2	3.23	9.3	13.38	39.0	17.83	51.7	34.44
Over 44	9	16.55	14.3	20.73	17.3	78.28	68.4	115.56

acid to the total ketones, there appeared an increasing tendency in parallel with the increase of the total ketones.

4. The Relations Among the Values for Ketones and Some External and Internal Factors

1) The relation between age and ketone values: As indicated in table 3, it may be seen that the mean value for ketones in normal group showed in general an increasing tendency in parallel with the advance of the age. The rate of appearance of sub-normal cases increased also in parallel with the former, but with some exceptions.

2) The relation between the period of pregnancy and ketone values: In comparison with that of the non-pregnant group, any of the mean ketone values of pregnant sub-group were higher from 9.8~56.7%, respectively. Also the rate of appearance of sub-normal group was higher in pregnant group than that of non-pregnant group except 9 months' sub-group. On the other hand, only in pregnant group, the mean values of sub-groups were higher in the initial stage than those in the terminal stage and also the same tendency was observed in the appearance rate of sub-normal cases.

TABLE 3. *The Relation between Age and Ketone Values*

AGE	NO. OF CASES (354)	NORMAL GROUP		SUB-NORMAL GROUP	
		No. of Cases (317)	Mean (mg/100 ml)	No. of Cases (37)	Appearance Rate (%)
Under 1	5	5	3.47	0	0
1	8	8	2.73	0	0
2	43	42	4.95	1	2.3
3	60	57	6.27	3	5.0
4	35	31	6.03	4	11.4
5	21	17	5.66	4	19.0
6	31	25	5.74	6	19.4
7	21	19	4.95	2	10.5
8	27	23	7.16	4	14.8
9	29	26	6.20	3	10.3
10	9	9	10.30	0	0
11~12	20	14	4.95	6	30.0
13~14	19	18	5.95	1	5.3
Over 15	26	23	7.36	3	11.5

TABLE 4. *The Relation between the Period of Pregnancy and Ketone Values*

MONTH OF PREGNANCY	NO. OF CASES (297)	NORMAL GROUP		SUB-NORMAL GROUP	
		No. of Cases (264)	Mean (mg/100 ml)	No. of Cases (33)	Appearance Rate (%)
Non-Pregnancy	153	137	6.11	16	10.5
1~2	21	18	8.24	3	14.3
3~4	50	44	9.58	6	12.0
5~6	26	23	6.96	3	11.5
7~8	24	21	6.73	3	12.5
9	23	21	6.71	2	8.7

From the above results, the ketone values of the pregnant group may be stated to be higher than that of the non-pregnant group; especially in the initial stage a marked increase appeared.

3) The relation between calving frequency and ketone values: The relation between these factors are shown in table 5. The mean values for ketones in each sub-group ranges from 3.79~7.92 mg/100ml, respectively. In comparison with the mean value of the normal group, those of the sub-groups, viz., 0, 1, 4~8 and 10 were all higher; they showed increase by 0.2~27.9%. The appearance rate of sub-normal cases showed in general an increasing tendency in parallel with the advance of calving frequencies. Therefore, it may be said

that the ketone values increased in 4~8 calvings' sub-group and there was a tendency for the appearance rate of the sub-normal cases to become higher in accordance with the advance of the calving frequencies, but there were some exceptions.

TABLE 5. *The Relation between the Calving Frequency and Ketone Values*

CALVING FREQUENCY	NO. OF CASES (225)	NORMAL GROUP		SUB-NORMAL GROUP	
		No. of Cases (198)	Mean (mg/100 ml)	No. of Cases (27)	Appearance Rate (%)
0	36	34	6.20	2	5.6
1	36	32	6.47	4	11.1
2	26	22	4.13	4	15.4
3	22	18	4.80	4	18.2
4	17	16	7.21	1	5.9
5	30	28	6.47	2	6.7
6	14	10	7.92	4	28.6
7	5	4	7.01	1	20.0
8	16	16	7.14	0	0
9	11	7	5.31	4	36.4
10	8	7	7.45	1	12.5
Over 11	4	4	3.79	0	0

TABLE 6. *The Relation between the Time after Calving and Ketone Values*

MONTH AFTER CALVING	NO. OF CASES (197)	NORMAL GROUP		SUB-NORMAL GROUP	
		No. of Cases (169)	Mean (mg/100 ml)	No. of Cases (28)	Appearance Rate (%)
1~ 2	41	31	5.85	10	24.4
3~ 4	26	24	8.12	2	7.7
5~ 6	30	25	7.46	5	16.7
7~ 8	16	13	5.07	3	18.8
9~10	25	20	6.24	5	20.0
11~12	22	21	7.26	1	4.6
13~14	6	5	4.17	1	16.7
15~16	7	7	3.99	0	0
17~18	8	8	5.37	0	0
19~20	6	6	5.51	0	0
21~22	4	3	4.04	1	25.0
23~24	4	4	5.49	0	0
25~26	2	2	3.36	0	0

4) The relation between the time after calving and ketone values: In the normal group, the mean ketone values showed generally higher values in the sub-groups during one year after calving, especially the value was highest in 3~4 months. The appearance rate of sub-normal cases markedly increased in the sub-groups in the period less than 14 months; they appeared at the rate of 4.6~20.0%.

From the above findings, it may be said that the mean ketone values of sub-groups in the period less than 12 months showed the increasing tendency and the appearance rate of sub-normal cases increased in sub-groups for a shorter time after calving.

5) The relation between milk yield and ketone values: In 338 cases, the results of examination of the relations between them are shown in table 7. The mean ketone values of 132 cases of dry cows was 6.11 mg/100 ml; it decreased by 1.3% compared with the mean value for the normal group. In comparison with values for lactating sub-groups,

TABLE 7. *The Relation between Milk Yield and Ketone Values*

VOLUME OF MILK (kg)	NO. OF CASES (338)	NORMAL GROUP		SUB-NORMAL GROUP	
		No. of Cases (303)	Mean (mg/100ml)	No. of Cases (35)	Appearance Rate (%)
0	137	132	6.11	5	3.7
1~5	20	20	6.05	0	0
5~9	50	46	8.75	4	8.0
9~13	32	28	7.46	4	12.5
13~17	32	22	6.52	10	31.3
17~21	28	22	5.62	6	21.4
21~29	33	28	8.74	5	15.1
Over 29	6	5	10.90	1	16.7

there was decrease in any of the sub-groups excepting 1~5 and 17~21 sub-groups. Viewing only lactating sub-groups, one may see that there was no marked relationship in the sub-groups under 21kg milk production, but in the 2 sub-groups over 21 kg, the mean ketone value increased markedly. The rate of appearance of sub-normal groups generally became higher in accordance with the increase of the milk yield.

6) The relation between the season and ketone values: The rise and fall of the ketone values of 5 periods of the year are shown in table 8. In comparison with the mean ketone value of the normal group, out of them only the value of the 3rd term (Sept.~Oct.) was lower; it decreased by 36.5% but amongst the other 4 terms in November to January, the values increased by 10.0~28.5%, respectively, and especially in the 5th term (Feb.~April) they were highest of all. Sub-normal cases were not found at all in the 2 sub-groups from July to October, but they appeared in the rate of 8.8~22.6% in the other 3 periods, respectively, especially the 1st term (May~June) showed the highest appearance rate of all. From the above reported results, the ketone mean value was lowest in September-October but it was highest in February-April which condition may be ascribed to the fact that this was the terminal time in stall. The appearance rate of the sub-

TABLE 8. *The Relation between the Season and Ketone Values*

SEASON (Month)	NO. OF CASES (352)	NORMAL GROUP		SUB-NORMAL GROUP	
		No. of Cases (315)	Mean (mg/100ml)	No. of Cases (37)	Appearance Rate (%)
I (5~ 6)	84	65	6.68	19	22.6
II (7~ 8)	50	50	6.82	0	0
III (9~10)	35	35	3.94	0	0
IV (11~ 1)	126	113	6.97	13	10.3
V (2~ 4)	57	52	7.95	5	8.8

normal cases was highest in the 1st term (May~June), which is ascribed to the fact that this was the time of a great deal of milk production. But in the 2nd and 3rd terms (July~Oct.), no sub-normal cases were observed.

7) The relation between feeding-management and ketone values: Studying 191 cases, the writers examined the relations between feeding-management and ketone values. The classification of the feeding-management was done as described in the previous report. The results are shown in table 9. The mean ketone values were highest in the moderate sub-group but were lowest in the inferior sub-group. Namely, in comparison with the

TABLE 9. *The Relation between Feeding-Management and Ketone Values*

QUALITY OF FEEDING- MANAGEMENT	NO. OF CASES (191)	NORMAL GROUP		SUB-NORMAL GROUP	
		No. of Cases (176)	Mean (mg/100ml)	No. of Cases (15)	Appearance Rate (%)
Superior	70	57	6.17	13	18.6
Moderate	97	96	7.36	1	1.0
Inferior	24	23	4.66	1	4.2

mean values of the normal group, the mean value of the former sub-group increased by 19.0% and that of the latter sub-group decreased by 25.0%. In the sub-normal group, the appearance rate showed a markedly high value in the superior feeding sub-group. From the above observations, it may be seen that in accordance with the improvement in quality of feeding-management, a tendency toward increase of the mean ketone values and also of the appearance rate of the sub-normal cases were recognizable.

5. The Relation between Urobilinogen Reaction and Ketone Values

Shino-test No. 5 which is one of the semi-quantitative tests for urobilinogen was adopted for the examination of urine urobilinogen. The judgements were made in accordance with the attached standard color-tone-table. In this test, the signs from --~# show the degree of the reactions as follows, viz., ---0 mg/100 ml, ±... about 3 mg/100ml, +... about 10 mg/ml, ++... about 50 mg/100 ml, #... about 100 mg/100 ml, respectively. As

shown in table 10, the ketone values in the normal group increased in accordance with the increase in strength of this test, also the appearance rate of the sub-normal cases showed the same tendency as that observed in the former. In view of the above results, it may be thought that the ketone values run in parallel with the increase of urobilinogen.

TABLE 10. *The Relation between Urobilinogen Reaction and Ketone Values*

DEGREE OF REACTION	NO. OF CASES (206)	NORMAL GROUP		SUB-NORMAL GROUP	
		No. of Cases (174)	Mean (mg/100 ml)	No. of Cases (32)	Appearance Rate (%)
—	59	56	5.56	3	5.1
±	44	40	8.17	4	9.1
+	63	54	8.13	9	14.3
++	29	19	11.00	10	34.5
##	11	5	12.40	6	54.6

6. The Relation between Sugar Reaction and Ketone Values

Benedict reaction was used for the examination of urine sugar. The classification of the degree of reaction was as follows; after boiling, no change ...—, somewhat greenish but no turbidity ...±, change to greenish and slight turbidity ...+, change to stronger greenish with the appearance of a great deal of yellowish sediment ...++, change to be estaceous with appearance of a great deal of sediment ...##. All 367 cases were divided

TABLE 11. *The Relation between Sugar Reaction and Ketone Values*

DEGREE OF REACTION	NO. OF CASES (367)	NORMAL GROUP		SUB-NORMAL GROUP	
		No. of Cases (331)	Mean (mg/100 ml)	No. of Cases (36)	Appearance Rate (%)
—	128	123	5.92	5	3.9
±	22	22	6.31	0	0
+	145	127	8.74	18	12.4
++	44	35	8.40	9	20.5
##	28	24	8.04	4	14.3

into 5 sub-groups in obedience to the degree of reactions. The mean ketone values and the appearance rate of sub-normal cases in each sub-group are shown in table 11. To interpret the data in this table, in general the ketone values for the sub-groups in the positive group in sugar reaction, show markedly high values. The appearance rate of the sub-normal cases was also markedly higher in positive sub-groups than in the negative sub-group. But only in the positive group, could there not be observed any increase of the mean ketone values nor of the appearance rate of the sub-normal cases in obedience to the strength of the reaction.

7. The Relation between the Ketone Values and the Blood Sugar Levels

On the basis of the ketone values, 256 cases were divided into 6 sub-groups, in which the mean levels for blood sugar were determined, respectively. In comparison with the mean value of the normal group, the mean values for sub-group from 5~10 mg/100 ml in the normal group and the mean value for sub-normal group increased at the rate of 2.8 and 1.5%, respectively. From the above observations, it may be said that the blood sugar level for sub-normal group showed an increasing tendency.

TABLE 12. *The Relation between the Ketone Values and the Blood Sugar Levels*

GROUP	KETONE VALUE (mg/100 ml)	BLOOD SUGAR	
		No. of Cases (256)	Mean (mg/100 ml)
Normal	0~5	90	56.4
	5~10	103	62.3
	10~15	25	57.9
	15~20	10	55.3
	20~43	5	60.2
Sub-normal	Over 43	23	61.6

8. The Relation between the Ketone Values and the Number of Eosinophilic Leucocytes

The mean numbers of eosinophilic leucocytes in the 6 sub-groups were calculated in correlation with the ketone values. In the normal group, those of the 3 sub-groups between 0~15 mg/100ml for urine ketones were all over the mean number of eosinophilic leucocytes for the normal group in numbers, but the mean numbers of two sub-groups between 15 and 43mg/100 ml for blood sugar were under, respectively. Moreover, the mean number

TABLE 13. *The Relation between the Ketone Values and the Number of Eosinophilic Leucocytes*

GROUP	KETONE VALUE (mg/100 ml)	NO. OF EOSINOPHILIC LEUCOCYTES	
		No. of Cases (232)	Mean (mg/100 ml)
Normal	0~5	51	467
	5~10	133	482
	10~15	17	506
	15~20	9	416
	20~43	7	307
Sub-normal	Over 43	15	277

of eosinophilic leucocytes in the sub-normal group decreased by 36.5% in comparison with the mean number of normal group.

DISCUSSION

The distribution of the urine ketone bodies for the clinically healthy cows ranged so widely that they were also divided into 2 groups, viz., "normal and sub-normal groups". The mean values for the ketone bodies of the normal group were 6.19 (2.12~43.41) mg/100 ml in total ketones and 1.25 (0.18~24.95) mg/100 ml in acetone. On the other hand, those of the sub-normal group were 122.50 (47.97~258.00) mg/100 ml in total ketones and 54.50 (25.83~123.00) mg/100 ml in acetone, respectively. In these cases, it may be reasonable to consider that these values of the "normal group" are the values for the normal cows, excepting certain special cases from the clinically healthy cows in the field. Now, in comparison with the results quoted by previous workers, the mean value of the total ketones for the normal group is found to be lower than the values reported by DUNCAN et al.⁴⁾, SAMPSON and BOLEY⁴⁾ and KNODT et al.⁴⁾, and it is considerably higher in comparison with those of BODDIE⁴⁾ and YOSHIDA, but it is nearest to that of SAMPSON et al. (1933). In the acetone, the value of the normal-group is slightly higher than the values reported by YOSHIDA and USHIMI. Observing the influences of the internal and external factors upon the urine ketones, any of the factors did show close relationship to the rise and fall of the ketone values, for example in the blood ketones, especially, it may be thought that among them, parturition, lactation, season and quality of management exerted marked influence upon the rise and fall of the urine ketone bodies. Subsequently, it may be interesting that are intimate relationships among the increase of the ketones and the strength of sugar and urobilinogen reactions; it has been reported that in the exhausted condition of man the reduction substances increased in the urine according to the report of SHIMIZU, and also liver damage was suggested by VIGUE in cases of ketosis.

As a result of the examinations for character of the sub-normal cases on the basis of the urine ketone bodies, it was certified that the group was a special group which showed not only the increase of the ketone bodies but also increase of the blood sugar levels and marked decrease in number of eosinophilic leucocytes. In other words, the sub-normal cases may be situated in the stage of resistance in the general adaptation syndrome reported by SELYE.

SUMMARY

These clinical and biochemical studies were made on the basis of the urine ketone bodies in 368 clinically healthy dairy cows. The results thus obtained may

be summarized as follows.

1. The values for the urine ketone bodies of the clinically healthy cows varied so widely that they were statistically divided into 2 groups, viz., "normal and sub-normal groups".

2. The mean values for the ketone bodies of the normal group were 6.19 (2.12~43.41) mg/100 ml in total ketone, and 1.25 (0.18~24.95) mg/100 ml in free acetone.

3. The mean values for the ketone bodies of the sub-normal group were 122.50 (47.97~258.00) mg/100 ml in total ketone, and 54.50 (25.83~123.00) mg/100 ml in free acetone.

4. The values for free acetone, acetoacetic acid and β -hydroxybutyric acid increased in accordance with the increase of the total ketone.

5. All of the internal and external factors exerted influence upon the rise and fall of the total ketone, especially the stress of parturition, milk yield, season and feeding-management are very important.

6. The strength of the sugar and urobilinogen reaction in urine runs in parallel with the increase of the urine ketones.

7. In the sub-normal group, not only the increase of the ketone bodies but also increase of the blood sugar level and marked decrease in number of eosinophilic leucocytes were clarified.

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