J. Fac. Appl. Biol. Sci.,
Hiroshima Univ. (1979), 18 : 43 – 53

Occurences of Bovine Ketosis by Administration of Ammonium-phosphate

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Received April 10, 1979
(Figs. 1–3, Tables 1–6)

INTRODUCTION

The main sign of bovine ketosis is the elevation of ketone bodies in the body fluids, urine, milk and breath. The biochemical changes were pointed out that there were a reduction in available carbohydrate and/or an increase in the quantities of fat and ketone bodies being metabolized.

Now the author could point out another hypothesis which is the disturbances in the oxidative decarboxylation reaction from α-ketoglutarate into succinyl-CoA in TCA cycle, that might bring on the shortage of succinyl-CoA[6]. When succinyl-CoA is depressed, the acetoacetate accumulates and converts itself into acetone and β-hydroxybutyrate. This reaction from α-ketoglutarate into succinyl-CoA requires the presence of a considerable number of cofactors as a whole, including Thiaminediphosphate, Lipoate, Coenzyme A, Nicotinamide adenine dinucleotide, Flavin adenine dinucleotide, and Magnesium ion[2].

If the cow falls into deficiency of one of these cofactors, the cow must be attached by ketosis. This present paper describes the experimental results of bovine ketosis produced by artificial magnesium deficiency in lactating dairy cows. The chronic magnesium deficiency is caused by shortage of magnesium intake in poor herbage or by the reduction of magnesium availablility in the gastrointestinal tract from restricted roughage and high concentrate intake which is rich in phosphate or phytate and protein. Under certain conditions of the rumen, i.e., an excess of phosphate, ammonia, alkaline pH, a most insoluble compound in the magnesium salt may be build up which can not be utilized in the digestive tract.

EXPERIMENTS AND RESULTS

Experiment I

The cow used in this experiment is a lactating Holstein, 550 kg in body weight and 10.5 kg/day in milk yield, three months postpartum of the first calving.

Before this experiment the cow was quite healthy and her nutritional conditions, brightness of hair, appetite, rumination, peristalsis of stomach and intestine were completely normal. A blood sample had been taken from the jugular vein, the rumen fluid too had been taken.

The 1st day; 500g of $NaHCO_3$ dissolved in 5 liter of water were added into the rumen compulsorily using a flexible tube for the purpose of change of pH into the rumen.
The 2nd day – the 8th day; 132 g of $(NH_4)_2HPO_4$ and 200 g of $NaHCO_3$ were taken into the rumen with 3 liter of water every day.

The rumen fluid, urine and blood samples were collected from the cow every day. pH value of rumen fluid was changed from pH 6.7 to pH 8.0 by the administration of sodium bicarbonate and ammonium phosphate. Her appetite declined by half and the concentrate intake also were reduced. The cow become scurby and the brightness of the hair disappeared. The milk yield also was reduced. The cow showed same symptoms of bovine ketosis but ketone bodies had not increased in the urine. It seemed that the experimenting should be stopped after seven days administration of phosphate and sodium bicarbonate for reason of the severe exhaustion of ketosis-like symptoms. An injection containing 10 g of $MgSO_4 \cdot 7H_2O$ and 200 g of glucose in 500 ml was given intravenously once a day from the 9th day to the 11th day. After this magnesium-treatment, the cow recovered from illness to good health, got back her good appetite and the rumen fluid returned to pH 6.5.

Blood serum phosphate levels decreased inspite of the phosphate dosing but were recovered by magnesium treatment. Blood serum magnesium level was also lowered by phosphate dosing and then increased by magnesium treatment, as shown in Table 2. From these results, it seems that pH changes of rumen fluid into alkaline side and phosphate dosing must bring about the decrease of the blood serum magnesium level, as caused by the decrease of the magnesium availability in the digestive tracts. The results were shown in Table 1 and Fig. 1.

Table 1. Details of Experiment I in the administration of ammonium phosphate and the treatment by magnesium injections.

	Administration or medical treatment	Clinical symptoms	(Rumen) pH	(Blood serum) Ca meq/l	Mg meq/l	Pi mg%	GOT	ALP	Globulin %	Albumin %
	Before adminstration		6.7	3.88	2.46	5.58	50.5	10.23	5.18	2.99
1st day	500g of $NaHCO_3$ dosing		–	–	–	–	–	–	–	–
2	200g $NaHCO_3$+132g $(NH_4)_2HPO_4$ dosing		–	3.71	1.70	4.77	39	7.73	5.18	2.76
3	"	lose of appetite, weakness of peristalsis of stomach and intestine	7.3	3.91	1.72	4.77	44	6.86	5.26	2.91
4	"		–	4.08	1.68	4.17	42	7.79	5.43	2.81
5	"	decrease of milk yield	7.4	4.18	1.91	5.34	47	7.03	5.54	2.87
6	"		–	3.84	2.08	3.75	40	7.29	5.71	2.99
7	"	stop of rumination	–	3.84	1.99	5.22	50.5	6.14	5.26	2.72
8	"		8.0	4.07	1.93	4.54	49	7.98	5.45	2.75
9	No-treatment		–	–	–	–	–	–	–	–
10	10g $MgSO_4 \cdot 7H_2O$+200g Glucose injection		–	4.22	1.88	4.17	50	8.32	5.71	2.92
11	"		7.3	4.36	1.84	5.33	51	9.01	5.56	2.89
12	"	recover of appetite and rumination	–	4.22	1.81	7.04	56	9.35	5.70	2.86
27	After experiment		6.8	4.69	2.02	3.84	48	5.70	6.33	3.17

Table 2. Changes of the blood serum Ca, Mg and Pi in the administration of ammonium phosphate and the treatment by magnesium injections on the Experiment I.

	Before experiment	Average in phosphate administration	Average in magnesium treatment
Serum Ca(meq/l)	3.88	3.98	4.42
Serum Mg(meq/l)	2.46	1.63	1.89
Serum Pi(mg%)	5.58	4.59	5.40

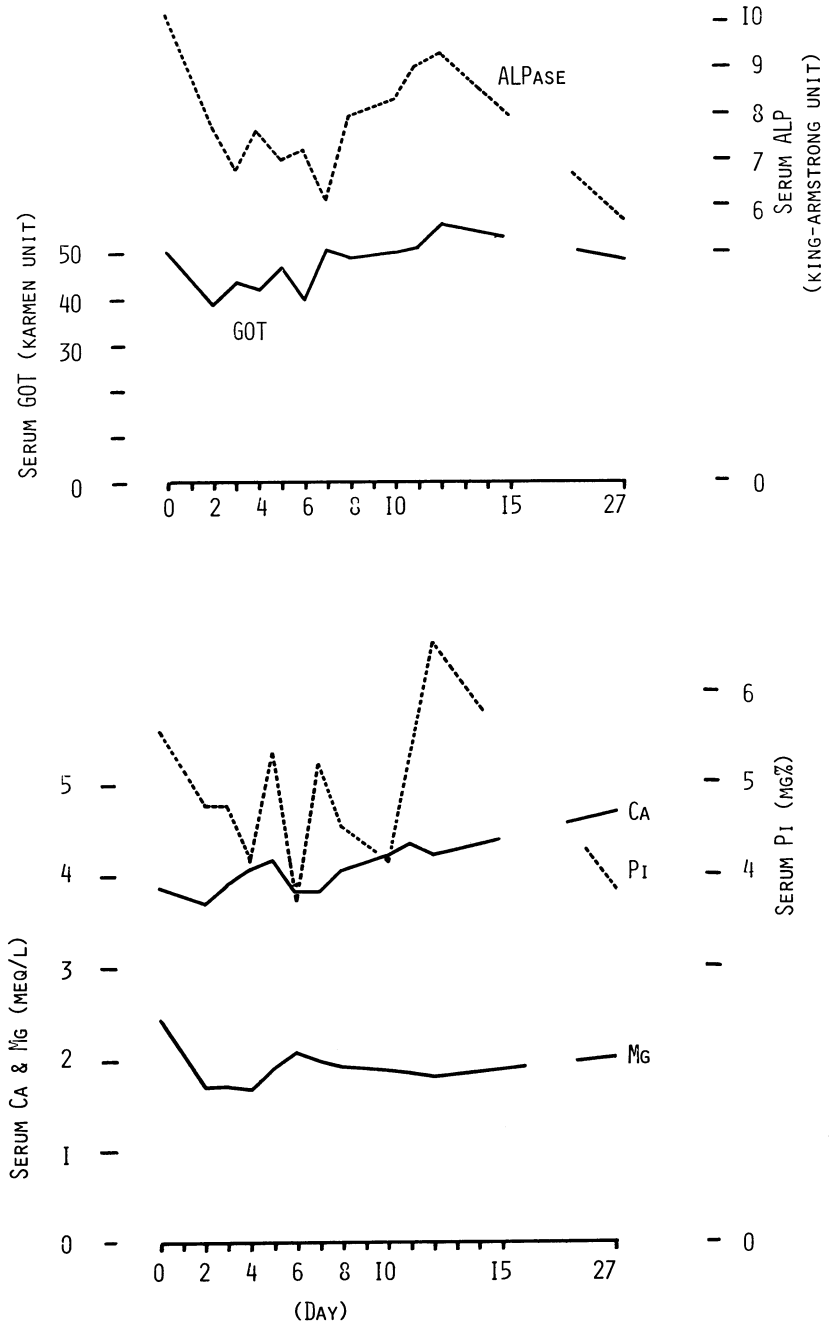

Fig. 1. Serum chemical and biochemical changes in Experiment I in the administration of ammonium phosphate and the treatment by magnesium injections.

Experiment II

The cow used in this experiment is a lactating Holstein, 506 kg in body weight and 12.3 kg/day in milk yield, nine months postpartum of her 3rd claving.

The cow is in good conditions as for nutrition, appetite, rumination and peristalsis of the stomach and intestines before the experiment. Her medical record was ketosis, retention of the placenta and the Utrecht abnormality of milk. Before this experiment her urine showed negative in the ketone test and the pH value of the rumen fluid was pH 7.0. After the sampling of the blood sample from the jugular vein, the experiment began.
The 1st day; 500 g of $NaHCO_3$ dissolved in 5 liter of water were taken into the rumen compulsorily.
The 2nd day – the 8th day; 132 g of $(NH_4)_2HPO_4$ and 200 g of $NaHCO_3$ were taken into the rumen with 3 liter of water every day.
The rumen fluid and blood samples were taken every day. There were no marked changes in appetite nor in the milk yield but her rumen pH had kept at pH7.4 to pH7.5. The cow became scurby and the brightness of the hair disappeared.

After the 9th day to the 16th day, the cow was held under observation without administration of phosphate and sodium bicarbonate nor treatment of magnesium sulphate. At the 11th day, the appetite suddenly dropped and her milk yield too decreased about 10 per cent of the previous day value. Her peristalsis of stomach and intestines were weak and her feces became slushy. Rumen fluid showed pH7.4. 100mg/d*l* acetone was detected in her urine by nitroprusside sodium reaction. From these clinical symptoms and urinary acetone excretion, it could be concluded that the cow has fallen in ketosis. Some veterinary surgeons who had no foreknowledge of this experiment also diagnosed that the cow suffered from ketosis certainly. The cow was observed then during six days without any administration of phosphate yet she continued still excreting ketone bodies with ketotic symptoms and at last the cow fell into a condition of suffering from recumbency.

Table 3. Details of Experiment II in the administration of ammonium phosphate and the treatment by magnesium injections.

	Administration or medical treatment	Clinical symptom		(Rumen) pH	Ca meq/*l*	Mg meq/*l*	Pi mg%	Glucose mg%	GOT	ALP	Globulin %	Albumin %
	Before adminstration			7.0	3.85	2.02	4.66	63.0	47	5.58	4.95	3.42
1st day	500g of $NaHCO_3$ dosing			–	–	–	–	–	–	–	–	–
2	200g $NaHCO_3$+132g $(NH_4)_2HPO_4$ dosing			–	4.17	1.66	4.64	32.3	66.5	5.64	4.46	3.39
3	"			7.4	3.96	1.55	4.04	66.7	46	4.36	4.76	3.28
4	"			7.4	4.06	1.86	5.08	65.1	44	3.95	5.21	3.32
5	"			7.5	4.06	1.78	4.33	68.7	52.5	4.07	5.20	3.20
6	"			7.5	–	1.70	3.87	56.6	67	5.29	5.58	3.26
7	"			7.5	–	–	–	–	–	–	–	–
8	"			–	–	–	–	–	–	–	–	–
9	observation			–	–	–	–	–	–	–	–	–
10	"			–	–	–	–	–	–	–	–	–
11	"	decrease of appetite, weakness of peristalsis of intestine, dyspepsia	Urinary acetone (100mg/dl)	7.4	4.16	2.06	4.27	67.0	56	5.12	5.05	3.39
12	"		"	–	4.13	2.13	3.73	67.0	53	5.52	5.17	3.56
13–16	"		"	–	–	–	–	–	–	–	–	–
17	10g $MgSO_4 \cdot 7H_2O$ injection		"	–	4.26	1.85	3.25	72.9	66.5	5.47	5.00	3.59
18	"		"	–	–	–	–	–	–	–	–	–
19	"		"	–	4.16	1.60	5.41	52.8	52.5	6.57	4.35	3.32
30	after experiment			–	4.24	1.57	5.90	48.2	74.5	6.40	5.10	3.16

After the confirmation of the experimental ketosis occurence by ammonium phosphate dosing, the recovery experiment were treated using magnesium injections. In this manner the cow recovered from illness.

Blood serum phosphate decreased by phosphate dosing and recovered by magnesium treatment. Blood serum calcium increased and it's magnesium decreased by phosphate dosing. Blood serum glucose also decreased by phosphate dosing and recovered to the previous level by magnesium treatment. These result are shwon in Table 3 and Fig. 2.

Table 4. Changes of the blood serum Ca, Mg and Pi in the administration of ammonium phosphate and the treatment by magnesium injections on the Experiment II.

	Before experiment	Average in phosphate administration	Average in magnesium treatment
Serum Ca*meq/*l*)	3.85	4.07	4.18
Serum Mg*meq/*l*)	2.02	1.71	1.91
Serum Pi(mg%)	4.66	4.47	4.17
Serum Glucose(mg%)	63.0	57.9	64.9

Experiment III

The cow used in this experiment was a lactating Holstein, 550 kg in body weight and 16kg/day in milk yield, six months postpartum of her 3rd calving.

Her physical condition was medium, nutritional condition and state of health, appetite, rumination, peristalsis of stomach and intestines were normal. Before the experiment, blood smaple and rumen fluid were taken.

The 1st day; 500 g of $NaHCO_3$ were dissolved in 5 liter of water and dosed into the rumen compulsorily.

The 2nd day – the 10th day; 132 g of $(NH_4)_2HPO_4$ and 200 g of $NaHCO_3$ were dissolved in 3 liter of water and dosed into the rumen once a day.

The pH value of rumen fluid was pH6.9 before the experiment and changed to pH7.4 but there were no marked changes in appearance except the scurby and the disappearance of hair brightness. At the 8th day 30mg/d*l* of urinary acetone were detected by nitroprusside reaction and the acetone excretion lasted unto the 15th day. Dyspepsia started from the 9th day. At the 11th day, the cow lost her appetite and rumination, and her milk yield reduced. Her peristalsis of stomach and intestines was weak and her hair was rough and hard. She was unsteady on her hind legs from 14th day.

From these clinical ketotic symptoms and urinary acetone excretion, it could be concluded that the cow had fallen in ketosis, and veterinarians also diagnosed that the cow certainly had ketosis. 10 g of $MgSO_4 \cdot 7H_2O$ in 50m*l* of water, contain 1g of net magnesium, was given by intravenous injection to this experimental ketotic cow every day from the 16th day to the 20th day. From the next day of the magnesium treatment, her appetite recovered and her peristalsis of stomach and intestine returned to normal. After

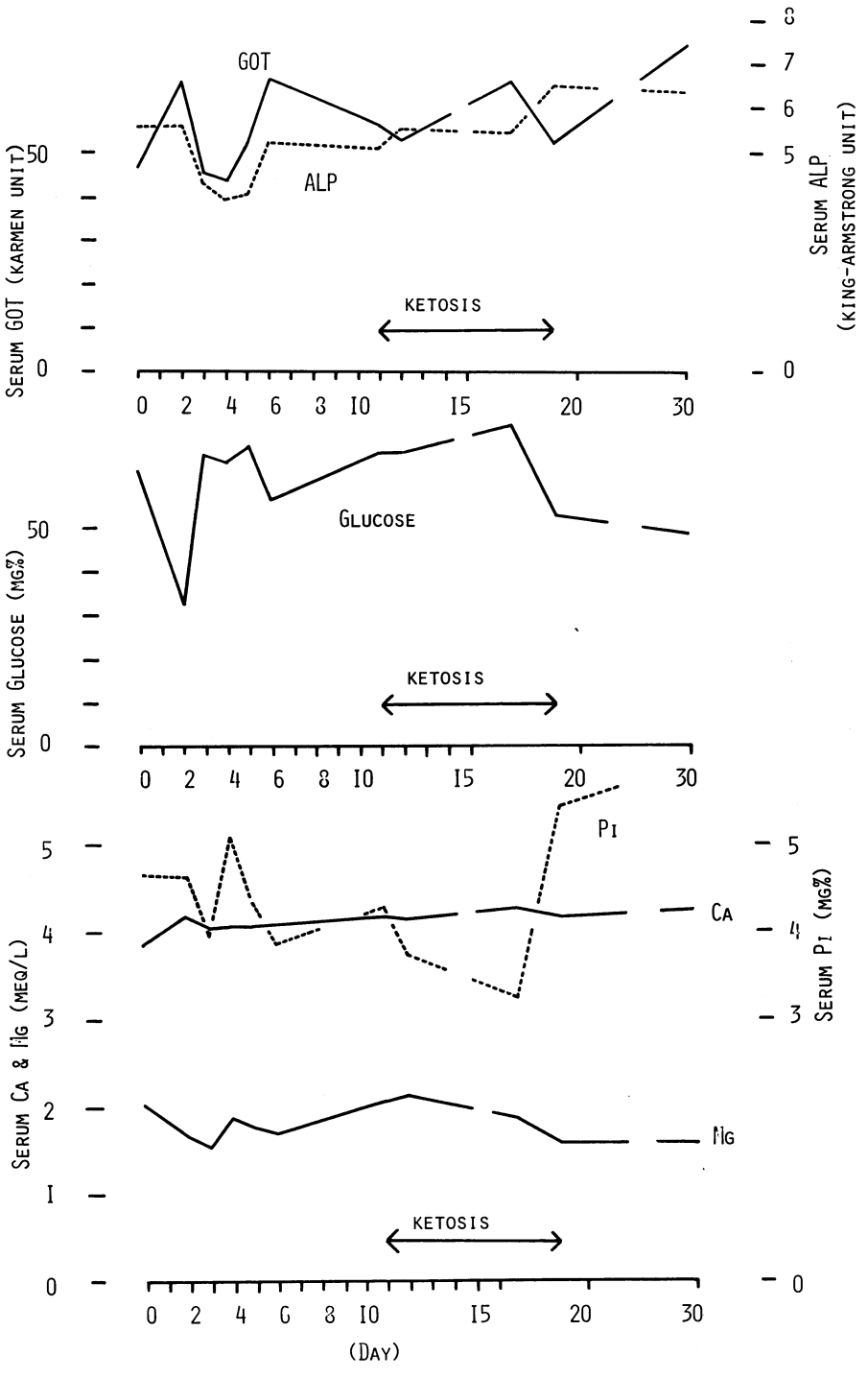

Fig. 2. Serum chemical and biochemical changes in Experiment II in the administration of ammonium phosphate and the treatment by magnesium injections.

four injections of magnesium sulphate, the ketotic cow clearly recovered to her former healthy condition, her rumen fluid was pH 6.5. Her milk yield recovered and the cow again had a strong appetite. Urinary acetone returned to negative in the ketone test.

The blood serum magnesium level remained by phosphate, dosing but increased by magnesium treatment. The blood serum calcium increased and it's phosphorus level too decreased by phosphate dosing, as shown in Table 6. The details of these results are shown in Table 5 and Fig. 3.

Table 5. Details of Experiment III in the administration of ammonium phosphate and the treatment by magnesium injections.

	Administration or medical treatment	Clinical symptom	(Rumen) pH	(Blood serum) Ca meq/l	Mg meq/l	Pi mg%	Glucose mg%	GOT	ALP	Globulin %	Albumin %	Pyruvate mg%	LDH	CPK
	Before administration		6.9	3.90	1.91	5.13	59.3	61.5	4.15	6.94	2.73	0.54	1175	6.5
1st day	500g of $NaHCO_3$ dosing		–	–	–	–	–	–	–	–	–	–	–	–
2	200g $NaHCO_3$+132g $(NH_4)_2HPO_4$ dosing		7.0	3.98	1.72	4.76	56.1	51.5	4.41	6.57	2.69	0.38	1335	5.8
3	"		–	4.25	1.82	3.70	66.3	54	4.41	7.19	2.80	0.60	1505	–
4	"		–	–	–	–	–	–	–	–	–	–	–	–
5	"		7.0	–	–	–	–	–	–	–	–	–	–	–
6	"		6.8	4.01	1.98	4.84	54.6	55	4.02	6.69	2.80	–	1440	6.8
7	"		7.1	3.84	1.81	4.70	54.6	53	4.35	6.59	2.60	0.26	1435	–
8	"		7.4	4.28	1.94	2.99	64.4	52	4.28	6.94	2.75	0.53	1420	6.3
9	"	Dyspepsia Urinary Acetone (30mg/dl)	7.2	4.38	2.15	3.48	61.0	57.5	4.87	7.62	3.09	0.27	1530	5.8
10	"	"	7.2	4.01	1.90	4.64	55.6	59	5.14	6.93	2.76	0.54	1485	3.0
11	Observation	lose of appetite " reduction of milk yield, weakness of peristalsis of stomach and intestine,	7.4	4.05	1.87	5.64	59.5	46.5	4.94	6.85	2.64	0.37	1425	4.5
12	"	rough and hard hair "	–	4.32	1.92	3.13	58.3	60	4.05	7.23	2.74	0.89	–	10.5
13	"	" "	–	4.08	1.83	4.50	49.7	50.5	5.07	6.80	2.69	0.21	1500	2.5
14	"	unsteady of hind legs "	–	4.35	1.98	4.23	62.8	58.5	4.39	7.29	2.84	0.43	–	5.5
15	"	"	–	4.01	1.89	–	59.3	45.5	4.35	6.38	2.66	0.22	–	10.0
16–19	10g $MgSO_4 \cdot 7H_2O$ injection		–	–	–	–	–	–	–	–	–	–	–	–
20	recovery from illness	Urinary	6.5	4.38	1.95	–	70.1	63	5.89	7.43	2.79	–	–	–
30	after experiment	acetone (–)		4.54	2.19	3.20	64.9	59.5	4.24	6.79	2.74	–	–	–

Table 6. Changes of blood serum Ca, Mg and Pi in the administration of ammonium phosphate and the treatment of magnesium injections on the Experiment III.

	Before experiment	Average in phosphate administration	Average in magnesium treatment
Serum Ca(meq/l)	3.90	4.60	4.46
Serum Mg(meq/l)	1.91	1.90	2.07
Serum Pi(mg%)	5.13	4.34	3.20
Serum Glucose(mg%)	59.3	59.0	67.5

DISCUSSION

The explanation of the growth mechanism of bovine ketosis is not yet clear. But on the basis of a new hypothesis[6] bovine ketosis might be caused by the interception of oxidative decarboxylation reactions from α-ketoglutarate into succinyl-CoA in TCA cycle. The reaction from α-ketoglutarate to succinyl-CoA as well as that from pyruvate to acetyl-CoA requires the presence of a considarable number of co-factors such as TDP, Lipoate, CoA, NAD, FAD, and magnesium ion. Inhibition of this reaction might find

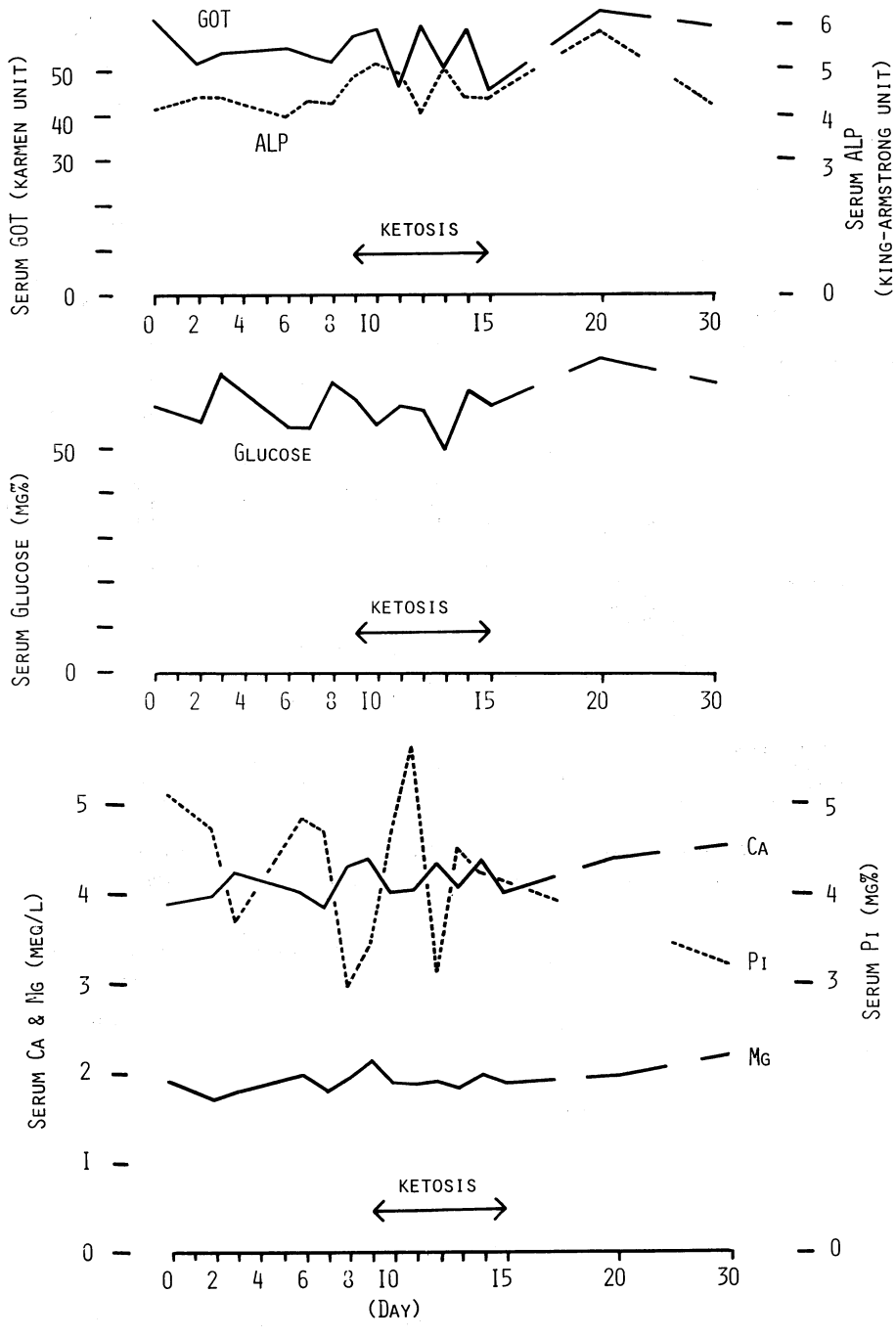

Fig. 3. Serum chemical and biochemical changes in Experiment III in the administration of ammonium phosphate and the treatment by magnesium injections.

its origin in the shortage of one or several of this cofactors. It is certain that the shortage of Vitamin B complexes is unusual in the ruminants, because the microorganism in the rumen and intestines produce the Vitamin B groups themselves. There are however some possibilities of Vitamin B complexes shortage occuring by abnormal fermentation in rumen or intestines[4]. The problem of magnesium has become very important in recent years, especially because magnesium acts as one of the cofactors in the biochemical reaction that take part in ATP, TDP etc. The oxidative decarboxylation reaction is a mechanism of energy supplying, and magnesium ion acts as a linkage between TDP and enzyme on decarboxylation reaction.

The experiment of Vitamine B group deficiency such as TDP, Lipoate, panthothenic acid is very complicated due to the duplication of Vitamin B complex intake and production in rumen and intestines. On the other hand, the experiment of mineral deficiency is much easier than that of the Vitamin B complex. Magnesium deficiency in animals is usually caused by the feeding of magnesium deficient fodder, it may also be caused by the lowering of the magnesium availability through use of some reagent that makes a combined magnesium which the animal can not absorb by digestive tracts. When the magnesium intake is depressed or the magnesium availability is lowered ketosis may appear in the dairy cow.

From the fundamental chemical experiment we may conclude that the analytical qualitative method of magnesium or phosphate is the formation of precipitate of $MgNH_4PO_4 \cdot 6H_2O$ in ammoniac alkaline solution. This complex is the most unsoluble compound of the magnesium salt.

$$MgCl_2 + (NH_4)_2HPO_4 + NH_3 + 6H_2O = MgNH_4 \cdot 6H_2O + 2NH_4Cl$$

The intake feedstuff of all are soaked in the rumen fluid which operates as a reaction vessel for keeping warmth, and for certain materials that must be dissolved in the rumen fluid. A lactating dairy cow of 550 -- 600kg in body weight and of 15 – 18kg/day in milk yield, requires on the average 30g of magnesium per day but occasionary takes more magnesium from concentrates with phosphate or phytate. It is known that phosphate or phytate decrease the magnesium availability in digestive tracts[5]. It is estimated that the cow will fall into a condition of magnesium shortage by 1 mol of $(NH_4)_2HPO_4$ administration under alkaline pH in the rumen, because 24 g of soluble magnesium then change into insoluble magnesium.

Bovine ketosis usually occurs a few days or weeks postpartum in the rising and peak phases of lactating and also usually occurs in the fourth to sixth calving. This means at the peak of the cow's production life which agrees with the maximum nutrients requirement period not only for magnesium but also for TDP. The cow requires a maximum amount of Vitamin B_1 at the peak period of lactation and more over it is estimated that the Vitamin B complexes production must be reduced in rumen abnormally by the changes of the microorganisms. An abnormal fermentation of rumen is frequently observed in the dairy cow that takes high concentrates. This phenomenon

is also agreement with the reduction of the magnesium availability by the generation of free NH_3 from protein in ration.

It is already known that magnesium ion is necessary whenever thiamine diphosphate is required and gross magnesium deficiency leads to symptoms similar to those of Vitamin B_1 deficiency in experimental animals[2].

The effects of the administration of ammonium phosphate and sodium bicarbonate can be observed on the blood chemistry and clinical behaviour of the cows. The first few days of the experiment, the blood serum magnesium level suddenly lowered by the administration of 500g of sodium bicarbonate singly or 200 g of sodium bicarbonate and 132 g of ammonium phosphate that caused rapid changes of rumen pH and decrease of the magnesium availability. This period must be the alarm reaction stage against the stress of rapid reduction of magnesium absorption. The next one week, the blood serum magnesium level had recovered a little in spite of the reduction of magnesium absorption, by ammonium phosphate dosing. This period must be so-called the resistance stage against the stress removing the magnesium from the magnesium strage of bone and various soft tissues. From this stage, the cow showed certain clinical signs such as scurby, disappearance of the brightness of hair, dyspepsia, etc. About 10 days after the beginning of the experiment, the blood serum magnesium level had decreased again and the cow fell into ketosis with the usual ketotic clinical symptoms such as loss of appetite, stop of rumination, weakness of peristalsis of stomach and intestines, roughness and hardness of hair, drop in milk yield. This period is the exhaustion stage against the stress. There was somewhat a time-lag between the clinical symptoms and the acetone excretions in the urine. Some clinical symptoms can be observed before the excretion of urinary acetone by phosphate dosing and those symptoms always recovered before the disappearance of urinary acetone by phosphate dosing and those symptoms always recovered before the disappearance of urinary acetone by magnesium treatment. It must be estimated that there are some differences between the essential metabolic disturbance in bovine ketosis and the biochemical reaction of ketone bodies on the catabolism. If the essential metabolic disturbance is caused by the inhibition of oxidative decarboxylation reactions, it must be defined that bovine ketosis is an aerobic metabolic disorder of the carbohydrates in the muscles.

Some studies mentioned that bovine ketosis had relations to magnesium metabolism, but the mechanism was not clear[1,3]. The author can be brought to make the reasonable conclusion that magnesium deficiency is one of the cause of the growth mechanism on bovine ketosis.

SUMMARY

Bovine ketosis must be caused by the disturbances of oxidative decarboxylation reactions from α-ketoglutarate to succinyl-CoA. These reactions require the presence of a considarable number of cofactors including TDP, Lipoate, CoA, NAD, and magnesium ion. The administration of ammonium phosphate and sodium bicarbonate into the

rumen brought forth the reduction of magnesium absorption in digestive tract by the formation of insoluble magnesium salt in the rumen. Then the cow fell into the experimental ketosis with the lowering of blood serum magnesium level, accompanied by the ketotic clinical symptoms. After the confirmation of experimental ketosis occurences the recovery experiment was performed by using magnesium injections, this way cow recovered from illness. If the essential metabolic disturbance of the ketosis is the inhibition of oxidative decarboxylation reaction, it should be admitted that the bovine ketosis is an aerobic metabolic disorder of the carbohydrates in the muscles.

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燐酸アンモン投与による乳牛ケトージスの発病実験

吉田　繁・山足　清

乳牛のケトージスの発病機構は明らかでなかったが，著者の新しい説によると生体内の代謝系のうちTCAサイクル中のα-ケトグルタール酸からサクシニルCoA への酸化的脱炭酸反応の代謝障害こそその原因であると云う。この反応は全体としてTDP, Lipoate, CoA, NAD, FADと共にMgイオンが補酵素として必要でありMgイオンの重要性はケトージス発病のうちでも大きな位置を占めている。

乳牛をMg欠乏の状態にするとケトージスが発病するかと云う点に関して，乳牛をMg欠乏にする方法として (1) Mg欠乏飼料を給与する，(2) 摂取したMgを不溶性Mg塩に変化させて結果的にMg欠乏の状態にすると云う方法がある。乳牛に 1Molの燐酸アンモンと重曹をルーメン内に投与するとルーメン液はアルカリ性になり下記の反応が生じて不溶性の燐酸アンモニウムマグネシウムが形成され 1Molのマグネシウムを不溶化する。

$$MgCl_2 + (NH_4)_2HPO_4 + NH_3 + 6H_2O \rightarrow MgNH_4PO_4 \cdot 6H_2O + 2NH_4Cl$$

実験Ⅰにおいて健康な泌乳中の乳牛に 1Mol 燐酸アンモンを7日間投与したところ，血清中のマグネシウムは低下し，無機燐も低下した。乳牛は食欲減退，乳量低下その他ケトージス様の症状を示したが尿アセトンは増加しなかったので実験を中止し，マグネシウムとグルコースを静脈注射することにより症状は消失し健康になった。実験Ⅱ及びⅢにおいても同様に健康な乳牛に燐酸アンモンを7日間投与したのち乳牛の状態を観察したところ11日目になって尿中のアセトン 100mg／dl が検出され，不消化軟便，胃腸蠕動微弱などの症状を併いケトージスの発病が確認された。燐酸アンモン投与により血清中のマグネシウム及び無機燐は低下した。これらの症状はマグネシウム塩の投与により回復した。

燐酸アンモンの投与によりルーメン内でマグネシウム塩の不溶化が生じ，血清マグネシウムが低下し次でケトージスが発病したがこの症状はマグネシウム投与により回復したのでケトージス発病機構においてMg が重要な役割を果している事を証明できる。