緬羊第一胃及びラット消化管内容のウレアーゼ活性とアンモニア濃度に及ぼすカプリロヒドロキサム酸の影響

誌名	日本獣醫學雜誌 = The Japanese journal of veterinary science
ISSN	00215295
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巻/号	35巻5号
掲載ページ	p. 425-432
発行年月	1973年10月

Jap. J. vet. Sci., **35**, 425～432 (1973)

EFFECT OF CAPRYLOHYDROXAMIC ACID ON RUMEN CONTENT OF SHEEP AND INTESTINAL CONTENT OF RAT

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(Received for Publication January 10, 1973)

Some properties of ruminal urease and the effect of caprylohydroxamic acid (CHA) on the ureolytic activity of ruminal urease have been reported in the previous paper[11]. It was thought that ruminal urease was so strong that urea poured into the rumen might be decomposed rapidly in to ammonia. To elevate the safty and efficiency of urea added to the diet, many attempts have been made to control the production of ammonia in the rumen[1,2,7,13]. In one of them, STREETER et al.[13] found that acetohydroxamic acid had improved the nitrogen retention in sheep fed a urea-containing diet. Recently, urease immunity has been considered to be effective for lowering the ammonia level in the blood[3,4,5,8]. It seems to be based on the inhibitory effect on urease in the rumen and intestine.

The experiments described herein were conducted for the purpose of determining the effect of CHA, which is considered to be a much stronger urease inhibitor than acetohydroxamic acid, on the rumen content of sheep fed diets containing urea and on the intestinal content of rat.

MATERIALS AND METHODS

Rumen studies

Animals and diet: Four rumen-fistulated sheep weighing 40 kg on the average were fed 400 g of such formula feed as indicated in Tables 1-a and 1-b at 9:00 a.m. and 400 g at 4:30 p.m. The crude protein content, including no urea, of the feed was 7.4%. Approximately 66% of the total dietary nitrogen was found to have been supplied as urea nitrogen. Sheep received CHA at a level of 5, 10, 15, or 20 mg per kg of body weight per day. A daily dose of CHA was mixtured with a daily amount of formula feed. A half of the mixture was administered orally twice a day. Rumen fluid samples were collected via the rumen fistula prior to the morning administration of the diet and 0, 1, 3, and 6 hours after the feeding.

Assay of urease activity in rumen fluid: Rumen fluid was filtered through two layers of gauze. The filtered rumen fluid was homogenized by a glass homogenizer in an ice bath for 3 minutes. The homogenate was used for the determination of urease activity and ammonia. The urease activity was assayed by essentially the same procedure as described in the previous paper[11]. One milliliter of 0.6 M phosphate buffer at pH 7.0, 0.5 ml of homogenate, and 0.5 ml of 1 M urea were placed in a SELIGSON's diffusion flask.

Table 1-a. Ingredient Composition of Diet

Ingredient	%
Corn	18.94
Molasses	7.58
Beet pulp	47.36
Corn starch	18.94
Soybean oil	1.89
Mineral mixture (a)	3.78
$NaHCO_3$	0.95
$KHCO_3$	0.47
Vitamin E supplement (b)	0.06
Vitamin A and D mixture (c)	0.02

(a) Consisting of 660 mg of Fe, 132 mg of Cu, 132 mg of Co, 132 mg of Zn, 3300 mg of Mg, 330 mg of Mn, 469 mg of S, 761 mg of Ca, 225 mg of P, and 965 g of NaCl per 100 kg of mixture.
(b) Containing 100 I. U. of vitamin E per gram of supplement.
(c) Containing 1,000 I. U. of vitamin A and 2,000 I. U. of vitamin D per gram of mixture.

Table 1-b. Chemical Composition of Diet

Composition	%
Moisture	15.1
Crude protein	7.4
Crude fat	2.9
Crude ash	3.6
Crude fiber	6.3
N-free extract	59.7
TDN	69
DCP	3.2

Table 2. Chemical Composition of Diets Used

Composition	Control diet	Experimental diet
	%	
Moisture	11.5	6.6
Crude fat	4.53	4.36
Crude protein	23.7	23.9
Crude ash	6.76	6.02
Crude fiber	3.9	3.6
N-free extract	49.61	55.52

The flask was incubated at 37°C for 30 minutes. The reaction was terminated by the addition of 1 ml of 1 M perchloric acid. The amounts of ammonia before and after the incubation were determined by the method of SELIGSON and HIRAHARA[10]. The urease activity was determined from the difference in amount of ammonia between before and after the incubation and expressed as μmoles of ammonia nitrogen per ml of rumen fluid per hour.

Intestnal and blood studies

Table 3. Effect of CHA on Ruminal Urease

Group		I	II
CHA		0	5 mg/kg
1 st	(p)	31.2±3.2	11.0±0.7
	(0)	29.8±1.7	11.0±0.7
	(1)	23.1±0.6	3.0±0.4
CHA		5 mg/kg	0
2 nd	(p)	6.3±0.7	8.8±3.9
	(0)	3.2±1.5	17.9±2.2
	(1)	3.5±0.4	5.4±2.0

All the values are expressed as NH_3–N μ moles/hour/ml of rumen fluid.

(p) Prior to morning feeding.

(0) Immediately after feeding.

(1) 1 hour after feeding.

The experimental condition used is described in the text.

Table 4. Urease Activity at Various Levels of CHA Added

Group	CHA mg/kg of body weiget	Sampling time* (p)	(0)	(1)	(3)
Control	0	30.32±0.32	7.71±0.10	26.75±0.97	48.36±0.44
CHA	10	5.70±2.47	6.36±0.74	5.07±1.40	2.38±2.24
	15	5.67±1.47	7.93±1.04	9.43±2.03	8.53±1.67
	20	9.76±3.34	8.10±2.15	8.72±4.48	9.67±3.24

All the values are expressed as NH_3–N μ moles/hour/ml of rumen fluid.

The experimental condition used is described in the text.

* See the footnote of Table 3, except that (3) indicates "3 hours after feeding."

Table 5. Concentration of Ammonia at Various Levels of CHA Added

Group	CHA mg/kg of body weight	Sampling time (p)	(0)	(1)	(3)
Control	0	28.49±7.47	61.39±2.39	47.85±1.19	42.89±9.35
CHA	10	6.50±0.73	11.77±0.61	12.41±0.67	7.45±0.71
	15	16.86±1.54	20.16±0.41	17.85±1.83	15.99±4.07
	20	13.71±2.95	15.38±0.65	13.84±0.96	13.24±1.32

All the values are expressed as NH_3–N mg/dl in the rumen.

Animals and diet: Twenty-four rats of the Wistar-Imamichi strain were used in this experiment. They were devided into two groups. An experimental diet containing 1% CHA was given to one group and a basal diet of essentially the same chemical composition, except CHA, as the experimental diet to the other group (Table 2). The animals were allowed to have feed and water *ad libitum* throughout the experiment.

Ammonia in portal and cardiac blood: Blood samples were collected from the portal vein and the heart by surgical operation under anesthesia. They were examined for the

Table 6. Effects of Urea and CHA on the Concentration of Volatile Fatty Acids (VFAs)

Group	Urea	CHA	VFAs
	%	mg/kg of body weight	m moles/l
Commercial formula feed	0	0	87.6±12.5
Experimental formula feed	0	0	62.1± 5.9
	0	5	85.6± 1.4
	5	0	103.0±19.5
	5	15	128.8±25.8
	5	20	146.7±36.1

All the samples were collected prior to the morning feeding.

Table 7. Effect of CHA on Levels of Ammonia and Urea in Portal and Cardiac Blood of Rat

Group	Blood ammonia		Blood urea	
	Portal	Cardiac	Portal	Cardiac
	mg/dl	mg/dl	mg/dl	mg/dl
CHA	0.58±0.11	0.37±0.12	22.3±1.7	23.3±2.2
Control	1.17±0.23	0.75±0.15	22.4±2.5	23.0±2.2

Table 8. Effect of CHA on Urease Activity and Ammonia Level in the Intestines of Rat

Group	Ammonia in intestine		Urease activity in intestine	
	Small	Large	Small	Large
	NH_3–N mg/g of dry matter		NH_3–N mg/g of dry matter/30 minutes	
CHA	1.09±0.44	1.73±0.93	0.45±0.35	4.73±2.22
Control	0.97±0.33	2.36±0.92	0.97±0.98	6.98±2.59

level of ammonia and urea nitrogen by the method of SELIGSON and HIRAHARA[10] and of ORMSBY[9], respectively.

Urease activity and ammonia in intestinal content: The intestinal content was divided into two parts by ligation at three sites, the pylorus, the bandary between the small intestine and the blind gut, and the end of the large intestine. The content of each part was freed from tissues and homogenized by a glass homogenizer in 0.02 M phosphate buffer. The content of the small and large intestines were diluted in such amount of the same buffer as to make a final volume of 25 and 50 ml, respectively. The diluted contents were filtered through two layers of gauze and used for the determination of urease activity and ammonia. The procedure of assay used was essentially the same as that for the rumen content. Since the diluted contents were variable in amount, the results obtained are indicated in the tables as values based on dry matter.

RESULTS AND DISCUSSION

Effect of CHA (5 mg/kg of body weight) on urease activity in rumen fluid: Four

sheep were divided into two groups. The groups were subjected to such treatment in a single reversal design that when one group receievd CHA, the other served as the control. Each administration period was one week. A rumen sample was collected on the last day of the week. Table 3 shows the urease activity in the rumen fluid of the control group and that of the experimental group receiving 5 mg of CHA per kg of body weight per day. As indicated in the table, urease activity was reduced significantly by the administration of CHA. In spite of the absence of CHA, a relatively low activity was shown in the control group at the end of the second week. The effect of CHA may have remained in the rumen.

Effect of CHA level on urease activity, ammonia, VFAs, and pH in rumen fluid: Animals received CHA at a level of 0, 10, 15, or 20 mg per kg of body weight at intervals of one week. They were fed a fomula feed containing urea at a 5% level as usual, unless otherwise stated. As shown in Table 4, the urease activity was reduced remarkably by the administration of CHA, although there were no differences in this activity among the levels of CHA. There may be no significant differences in the final concentration in the rumen among these levels of CHA. Then, from Table 3, it is suggested that the change in amount of microorganisms in the rumen may not be so severe that there may be no significant differences in urease activity among samples collected from the same individual at different times.

Usually, the concentration of ammonia in the rumen increases promptly after a high protein diet or a diet containing urea is fed. If ruminal ureolytic activity is reduced concurrently with microbial ability to synthesize protein from ammonia, there will be on increase in the efficiency of urea utilization. As shown in Table 5, no rapid production of ammonia was observed in the case of administration with any level of CHA, although the production of ammonia began to increase immediately after feeding in the absense of CHA.

The total content of volatile fatty acid (VFAs) was also determined. The concentration of VFAs was slightly elevated prior to the morning feeding, by the administration of urea and CHA (Table 6). STREETER et al.[13] stated that any effect of acetohydroxamine acid on VFAs production was masked by a great variation among animals. In the previous paper[11], the experimental results indicated that no effect of CHA on VFAs production had been found in the fermentation *in vitro*. Therefore, CHA might not effect VFAs production. No pH was affected in the rumen by the administration of CHA at any level.

Although I_{50} value of 8.0×10^{-2}M and 6.0×10^{-6}M were found for urease activity in the cell-free extract and the intact call of *Proteus vulgaris*[6], respectively, an I_{50} value of 10^{-4}M was indicated in the rumen fluid[11]. Since the volume of the rumen fluid was about 5 litters for the sheep used here, the concentration of CHA was considered to be in the order of 10^{-4}M in each animal. Therefore, it is thought that the urease activity in the rumen fluid may have been depressed by such level of CHA and that consequently the concentration of ammonia may trend to remain at a lower level without showing any great variation than the rumen fluid containing no CHA. STREETER et al.[13] administered three doses of acetohydroxamic acid (90, 180, and 360 mg/kg of body weight/day) into the rumen of sheep and obtained nearly the same results as the same authers.

Ammonia and urea levels of portal and cardiac blood in rats: It is well known that the concentration of ammonia is higher in the portal blood than in any other blood. The fact indicates that on one hand, ammonia produced from various nitrogenous compounds by the microflora of the digestive tract is absorbed through the intestinal mucous membrane, and that on the other hand, urea in the blood is decomposed to ammonia

in the intestinal mucous membrane by urease located in this membrane. As shown in Table 7, the concentration of ammonia was lower in the CHA-supplemented group than in the control group not only in the portal but also in the cardiac blood, especially in the former. Since CHA has no inhibitory effect on any deaminase against amino acids, such as glutaminase, asparaginase, and glutamic dehydrogenase, the lower concentration of ammonia may indicate the presence of urease in the intestinal mucous membrane or the reflux of urea into the digestive tract from the blood stream.

The concentration of ammonia in the cardiac blood was lower in the CHA-supplemented group suggested that level of ammonia transported to each tissue was also lower in this group. VISEK[14,15] stated that the low level of ammonia was related to the stimulation of growth. Therefore, if CHA is administered continually for a long time, the growth will be expected to increase. There was no difference in the concentration of urea between the portal and cardiac blood. The fact indicates that both groups had been fed a diet containing essentially the same level of protein.

Effect of CHA on urease activity and ammonia in the digestive tract: The results obtained are summarized in Table 8. The urease activity was significantly higher in the large intestine than in the small intestine. Furthermore, the ammonia level was also higher in the former than in the latter.

There was no difference in the concentration of ammonia in the content of the small intestine between both groups. The urease activity, however, was a little lower in the CHA-supplemented group than in the control group. On the other hand, the urease activity and the level of ammonia in the large intestine were lower in the CHA-supplemented group than in the control group.

SIDHU et al.[12] reported that the urease activity in the rumen was reduced by only 8% in an immunized lamb, although it increased by 21% in a growing lamb. The urease activity in the ileum and colon was reduced by about 30%. VISEK[14,15] pointed out that if urea inhibtors were of practical importance in rumen or any farther part of the gastro- particularly active in the distal portion of the rumen or any farther part of the gastrointestinal tract. If CHA is able to each a level of the gastrointestinal tract beyond the rumen, it will efficiently act to reduce the urease activity and the level of ammonia in the content of some portions of the digestive tract, such as the small, and large intestine.

CHA was potent inhibitor for ruminal urease. In fact, it depressed the urease activity and controlled the ammonia production in the rumen when the animals were fed a diet containing urea. It is thought that the compound may be available and applicable to the ruminant for the urea utilization from the viewpoint of safety and efficacy.

SUMMARY

1. When sheep were fed a formula feed containing urea at 5% level, the urease activity in the rumen was reduced by the administration of caprylohydroxamic acid (CHA). At that time, no rapid production of ammonia in the rumen was observed in the case of administration of CHA, although the production of ammonia increased immediately after feeding in the absence of CHA.

2. The total production of volatile fatty acids and pH in the rumen were not affected by the administration of CHA.

3. When rats were fed a diet containing 1% CHA, the ammonia level was lowered significantly in both portal and cardiac blood, especially in the former.

4. Although there was no difference in the concentration of ammonia in the content of the small intestine, the urease activity was a little lower in the CHA-supplemented

group than in the control group. The urease activity and the ammonia level in the large intestine were lower in the CHA-supplemented group than in the control group.

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緬羊第一胃及びラット消化管内容のウレアーゼ活性とアンモニア濃度に及ぼすカプリロヒドロキサム酸の影響

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(昭和48年1月10日受付)

尿素を含む実験配合飼料を与えた緬羊に，カプリロヒドロキサム酸 (CHA) を経口投与して，第一胃内容におよぼす影響を調べた結果，つぎの知見を得た．

CHA 投与により，第一胃内容ウレアーゼ活性の低下がみられ，それに伴ってアンモニア産生が抑制された．CHA 無投与時にみられる食後の一過性の高いアンモニア産生はみられなかった．

第一胃内容の VFAs 産生や pH には，大きな変化が認められなかった．

つぎに，CHA を 1% 添加した固型飼料で飼育したラットを，CHA 無添加で化学組成がほとんど同じ固型飼料で飼育したラットと比較した結果，つぎの知見を得た．

CHA は門脈血のみならず，心臓血のアンモニアアンモニア濃度を有意に低下させたが，尿素濃度には影響を与えなかった．

一般に，小腸内容より大腸（盲腸内容を含む）

でアンモニア濃度が高く，ウレアーゼ活性も高かった．CHA は明らかに消化管，とくに大腸のウレアーゼ活性を抑制，かつアンモニア濃度を低下させた．