Relationship between Ruminal Endotoxin Level and Number of Bacteria in Goats Engorged High-Concentrate Ration

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Abstract The present study aimed to investigate the relationship between ruminal endotoxin (ET) level and number of bacteria in goats engorged a high-concentrate ration. Three hay-fed goats fitted with ruminal fistula were voluntarily fed with a 90% concentrate ration in order to induce acidosis. Clinical signs such as anorexia or depression and significant decline of ruminal pH accompanied with an elevation of VFA level were observed in all goats soon after the change of diet. Molar percentages of acetate and iso-valerianate decreased and that of propionate increased from 6 to 48 h. There were no changes in ruminal L(+)- and D(-)-lactate levels. Ruminal ET level, however, gradually elevated from 6 h and, after reaching a maximum level of 167.7 ng/ml at 24 h, declined to the initial level at 168 h. Both numbers of gram-positive and -negative bacteria tended to slightly increase at 24 h. The proportion of gram-positive cocci tended to increase, and that of gram-negative cocci significantly decreased at 6 and 12 h. The significant decrease in total number of protozoa was observed from 6 to 24 h and 72 h. It was clarified that ruminal ET production increased without a decrease in number of gram-negative bacteria, when ruminal fermentation was activated.

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Beef cattle is usually given an easily fermentable grain feed in order to promote the growth and to improve the feed efficiency. An accumulation of lactate in rumen, however, often occurred by the ingestion of an excess amount of feed and resulted in acute ruminal acidosis. A high-toxic substance was detected in the ruminal fluids of acidosis cattle²⁾ and was thought to be endotoxin (ET) released from dead gram-negative bacterial bodies³⁾. It was also reported that an increase of ruminal ET level was observed after a decrease in the number of gram-negative bacteria in steers⁹⁾

and goats¹⁰⁾, but this matter still remains unclear. Furthermore, Andersen *et al.*¹⁾ assessed the influence of concentrate diets on ruminal ET level and pH, but the information was not enough. The present study aimed to investigate the relationship between ruminal ET level and number of bacteria in goats engorged a high-concentrate diet.

Materials and Methods

Three female Tokara goats (body weight 20-35 kg) fitted with ruminal fistula were given 400 g of an alfalfa haycube (TDN 60.2%) twice a

day (8:00 and 17:00) for more than two weeks before the beginning of the experiment. The diet was changed to a 90% concentrate (TDN > 71.0%, Tokai Kumiiai Feed Mill Co., Ltd.) and 10% alfalfa haycube ration from 8:00 on a test day (0 h) and it was given *ad libitum* throughout the experiment. The goats had free access to fresh water and a mineral salt block (Kouen, Nippon Zenyaku Kogyo). Ruminal fluids through the fistula from all goats were collected at -24, 0, 6, 12, 24, 48, 72 and 168 h after the change of diet.

The ruminal fluids were filtered through two layers of gauze and, after measuring the pH (HM-5 ES, TOA), a part of the filtrate was mixed at the equivalent rate in 20% formalin solution for bacterial analysis and in 10% methylgreen-formalin solution for protozoal analysis. The remaining filtrates were centrifuged at $30,000 \times g$ for 20 min and the supernatant fluids were obtained. The fluids were stored in a freezer at -20°C for the later assays of VFA by gas-chromatography (GC-6 A, Shimadzu), L(+)- and D(-)-lactate by the enzymatic method⁹⁾ (826-UV, Sigma) and ET by the method of turbidimetric-kinetic assay⁵⁾ (Limulus ES-II, Wako). Numbers of bacteria identified on a stained smear according to Minato's method⁹⁾ and those of protozoa

morphologically identified according to Ogimoto and Imai's method⁸⁾ were enumerated.

Analysis of Variance in each obtained data was used for statistical analysis and t-test was carried out where the difference was significant.

Results

All goats consumed about 80% of the total amount of the high-concentrate ration on the first day of the experiment. The goats showed clinical signs of acidosis such as anorexia or depression soon after the change of diet, and almost recovered from such damages at 168 h. As shown in Table 1, ruminal pH was significantly depressed at 6 h in comparison with the initial value (0 h, $P < 0.01$) and the depression continued throughout the experiment. A significant increase in total VFA level was observed from 6 to 168 h except 72 h to compare with 0 h (6-48 h; $P < 0.01$, 168 h; $P < 0.05$). However, there were not any changes in L(+)- and D(-)-lactate levels after the change of diet. The ET level gradually increased from 6 h and, after reaching a maximum value of 167.7 ng/ml ($P < 0.01$) at 24 h, it declined to the initial value at 168 h (Table 1). In VFA molar percentages, significant decreases in acetate and iso-valerianate and increase in propionate

Table 1. Changes of ruminal characteristics in goats engorged a high-concentrate ration ($n=3$, Mean $\pm$ SD)

Hour ¹⁾	pH	VFA mM/dl	L(+)-lactate mg/dl	D(-)-lactate mg/dl	Endotoxin ng/dl
-24	7.38 $\pm$ 0.07	6.42 $\pm$ 0.41	84.5 $\pm$ 40.8	134.6 $\pm$ 62.4	39.7 $\pm$ 20.5
0	7.25 $\pm$ 0.06	7.52 $\pm$ 0.72	85.5 $\pm$ 28.6	134.8 $\pm$ 42.4	19.2 $\pm$ 9.8
6	6.27 $\pm$ 0.06**	17.50 $\pm$ 1.38**	83.7 $\pm$ 18.9	135.0 $\pm$ 31.5	62.8 $\pm$ 15.6
12	5.75 $\pm$ 0.13**	22.52 $\pm$ 3.26**	77.7 $\pm$ 11.0	125.9 $\pm$ 17.1	69.5 $\pm$ 6.0
24	5.92 $\pm$ 0.50**	18.48 $\pm$ 3.39**	62.9 $\pm$ 11.7	102.4 $\pm$ 18.5	167.7 $\pm$ 34.0**
48	6.81 $\pm$ 0.15**	9.66 $\pm$ 1.12**	66.6 $\pm$ 21.5	108.8 $\pm$ 31.6	120.3 $\pm$ 66.4
72	6.54 $\pm$ 0.49*	11.27 $\pm$ 4.12	56.4 $\pm$ 25.6	92.7 $\pm$ 40.5	112.7 $\pm$ 97.3
168	6.51 $\pm$ 0.51*	15.92 $\pm$ 5.77*	84.8 $\pm$ 40.6	134.0 $\pm$ 64.5	42.6 $\pm$ 18.7

¹⁾ Time after change of diet.

*, **; Values having asterisks are significantly different from the initial values (0 hour) at $p < 0.05$ or 0.01 , respectively.

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Table 2. Changes of total numbers of bacteria and protozoa in ruminal fluids of goats engorged a high-concentrate ration ($n=3$, Mean $\pm$ SD)

Hour ¹⁾	Bacteria, log/ml			Protozoa	
	Total	G(-)-	G(+)-	Total, log/ml	<i>Entodinium</i> , %
-24	10.37 $\pm$ 0.13	10.31 $\pm$ 0.11	9.42 $\pm$ 0.27	5.54 $\pm$ 0.04	95.3 $\pm$ 1.02
0	10.32 $\pm$ 0.13	10.26 $\pm$ 0.11	9.36 $\pm$ 0.24	5.61 $\pm$ 0.04	95.1 $\pm$ 2.27
6	10.43 $\pm$ 0.07	10.38 $\pm$ 0.06	9.29 $\pm$ 0.28	5.28 $\pm$ 0.08**	93.2 $\pm$ 1.20
12	10.43 $\pm$ 0.03	10.36 $\pm$ 0.07	9.47 $\pm$ 0.35	4.88 $\pm$ 0.06**	91.3 $\pm$ 3.50
24	10.59 $\pm$ 0.14	10.49 $\pm$ 0.11	9.75 $\pm$ 0.38	4.88 $\pm$ 0.18**	84.7 $\pm$ 8.90
48	10.37 $\pm$ 0.16	10.26 $\pm$ 0.19	9.64 $\pm$ 0.17	5.61 $\pm$ 0.39	89.4 $\pm$ 6.67
72	10.34 $\pm$ 0.10	10.26 $\pm$ 0.09	9.56 $\pm$ 0.16	5.90 $\pm$ 0.06**	91.5 $\pm$ 3.09
168	10.44 $\pm$ 0.20	10.31 $\pm$ 0.20	9.80 $\pm$ 0.25	6.10 $\pm$ 0.58	87.2 $\pm$ 9.90

¹⁾ Time after change of diet.

** ; Values having asterisks are significantly different from the initial values (0 hour) at $p < 0.01$.

in comparison with 0 h were observed from 6 to 48 h ($p < 0.05-0.01$; Fig. 1).

The big changes in both numbers of gram-positive and -negative bacteria were not observed, but they slightly increased at 24 h. In protozoa, *Entodinium* was predominant and the total number significantly decreased at 6-24 and 72 h ($P < 0.01$; Table 2). In bacterial proportions, an increasing tendency in both gram-positive cocci at 6-168 h and gram-negative small rods at 6-12 h was obtained. Although

the proportion of gram-negative cocci significantly decreased at 6-12 h ($P < 0.05$), it recovered to the initial level at 72 h (Fig. 2).

Discussion

An increase in ruminal ET level was observed in steers⁹⁾ and goats¹⁰⁾ induced acidosis by ruminal sucrose administration. The ET increase is usually accompanied with ruminal pH depression and lactate accumulation. In this study, however, the pH depression

Fig. 2. Changes of bacterial proportion in ruminal fluids of goats engorged a high-concentrate ration (*, **; $P < 0.05, 0.01$ vs 0 h).

▨ G(+)cocci ▤ G(+)rods ▧ G(-)cocci ▩ G(-)small rods
 ▨ G(-)large rods ▪ G(-)curved rods ▦ G(±)cocci

followed by ET increase was caused by the elevation in VFA level, but not in lactate. Therefore, the ruminal fermentation after the change of diet is considered to be activated. Andersen *et al.*¹⁾ also observed similar changes in ruminal ET and pH of cattle fed on concentrate diets. These results suggest that ruminal ET production is stimulated not only in acidosis but also in normal feeding.

According to several researches^{1,4,5,9,10)}, ruminal ET level seems to be widely varied from 112 to 4,497 ng/ml depending on animal species or rations. The ET level is generally thought to be caused by the decrease in the number of gram-negative bacteria in rumen. But, in this study, the number of gram-negative bacteria did not decrease in spite of increasing ruminal ET. Nagaraja *et al.*⁷⁾ also reported the same results with this study *in vitro* culture of ruminal fluids and suggested that the decrease was dependent on bacterial multiplication and not on bacterial lysis. We conclude that the shift among the species of gram-negative bacteria was enhanced and the death in certain species of gram-negative bacteria would be reflected in ruminal ET production.

ET absorbed from rumen in acidosis animals has a lot of physiological activity and relates to several diseases through various cytokines⁹⁾. Suda *et al.*⁹⁾ reported the death in steers induced acidosis after the extreme elevations of both ruminal and serum ET which were more than 10-fold. In the present study, the ruminal ET increase was less 10-fold and severe clinical signs were not observed. Therefore, if ruminal ET level could be maintained at a certain range, even if a large amount of ET is produced in rumen, the ET related diseases would not occur.

In conclusion, it was clarified that ET production increased without a decrease in the number of gram-negative bacteria, when ruminal fermentation was activated.

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濃厚飼料を飽食させたヤギのルーメン内エンドトキシン濃度 と細菌数との関係

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濃厚飼料を飽食させた時のヤギのルーメン内エンドトキシン (ET) 濃度と細菌数との関係を検討することを目的とした。粗飼料で飼育したルーメンフィステル装着ヤギ3頭を供試し、実験当日から90%濃厚飼料多給に切り換えて不断給与した。いずれのヤギも飼料切換後すぐに食欲不振や元気消失などのアシドーシス様の臨床症状を示した。ルーメン内ではVFA濃度の有意な上昇を伴ったpHの有意な低下がみられ、VFAのモル比では、6-48時間後にプロピオン酸の増加やイソ吉草酸の低下がみられた。L(+)型およびD(-)型乳酸濃度に飼料切換による影響はみられなかった。ET濃度は6時間後から徐々に上昇し、24時間後に最大値の167.7 ng/mlに達した後、168時間後には初期値に回復した。グラム陽性、陰性菌数とも24時間後にわずかに増加する傾向がみられた。その割合では、6-12時間後にグラム陽性球菌が増加傾向を示し、グラム陰性球菌が有意に減少した。プロトゾア数は6-24および72時間後に有意に減少した。以上より、濃厚飼料飽食に切り換えたヤギでは、ルーメン内グラム陰性菌数が減少することなく、ET濃度が高まることが判明した。

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