Distribution of Rumen Ciliate Protozoa, Anaerobic Bacteria and their Products, Volatile Fatty Acids and Lactic Acid, in the Gut of Cattle

Soichi IMAI, Noriaki YANAGIDA**,
Katsunori KATAGIRI†† and Keiji OGIMOTO*

Department of Parasitology, Nippon Veterinary and
Zootechnical College, Musashino-shi 180

*Department of Animal Science, Tohoku University,
Sendai-shi 980

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Abstract Distribution of rumen ciliates, bacteria and their products in the various parts of the gut of cattle was investigated. The concentration of volatile fatty acids was high in the rumen and caecum and that of lactic acid high in the jejunum and ileum. Gram-negative bacteria, such as *Bacteroides*, *Veillonella* and *Selenomonas*, were mainly isolated from the rumen and caecum, and Gram-positive bacteria, such as *Streptococcus* and *Lactobacillus*, from the jejunum and ileum. No difference was recognized between the numbers of the rumen ciliates in the rumen and reticulum. However, the number in the omasum was about 13.4% of that in the rumen. About 69% of the survival ratio of the ciliates was recognized in the omasum. It was assumed that the ciliates in the rumen and reticulum had made their escape from the outflow toward the omasum for certain reasons. Very few ciliates were detected from the parts of the gut lower than the abomasum. However, the ciliates appearing in complete form were hardly observed in these parts. From the results, it seemed that the degree of designation of such region as nitrogen sources should be decreased more than formerly thought.

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Previously, OGIMOTO¹⁾ suggested from the investigation on the antigenic structure of *Veillonella alcalescens* isolated from the rumen and the caecum of the ruminants, that the ecological niche of the microbial flora and fauna in the guts of cattle was generally separated into two groups, the foregut group including the rumen, and hindgut group. Subsequently, the microbial distribution in the gut has been made clear with the increase of knowledge on the gut microbes of the ruminants^{2,3)}. It has been pointed out that microbial digestion has actively occurred not only in the rumen but also in the hindgut such as the caecum⁴⁾. However, little is known about the detailed distribution of microorganisms in the gut and the relation between them and their products.

In this paper, the distribution of anaerobic bacteria and rumen ciliates, and the change of lactic acid and volatile fatty acids (VFAs) in the various parts of the gut

†: Present address: Shioyama Health Center, Miyagi Prefecture, Shioyama-shi 985

††: Present address: Nagano Mutual Aid of Association of Agriculture, Nagano-shi 380

of cattle were investigated.

Materials and Methods

1. Materials.

The contents of the gut were collected from 20 cattle fed on concentrates and hays, slaughtered in the abattoirs at Sendai City in Miyagi Pref. and Omiya City in Saitama Pref. in the period from January to December, 1978. The contents of 9 parts of the gut, rumen, reticulum, omasum, abomasum, jejunum, ileum, caecum, colon and rectum, were collected at the time of dissection. Some samples collected were fixed with MFS solution⁶⁾ or 10% formalin for the counting of the ciliates and the bacteria. Other samples were not fixed and were used for the examinations on life and death of the ciliates, viable bacteria counting⁷ and for the determination of concentration of VFAs and lactic acid.

2. Counting of ciliates.

The procedure conformed with that of the previous paper⁶⁾.

3. Total counting of bacteria.

The procedure was in conformity with the method of MOORE and HOLDMAN⁷⁾.

4. Counting of viable bacteria.

Anaerobic culture techniques employed were similar to those described by HUNGATE⁸⁾ for rumen bacteria, with the modification of BRYANT⁹⁾. Strict anaerobic techniques were maintained throughout all procedures involving the dilution of the gut contents samples and preparation and inoculation of media.

Sampling procedure: The gut samples from each animal were placed in a sterile, stoppered tube and flushed with oxygen-free CO₂. The gut contents were weighed and processed through serial 10-fold dilutions in tubes of the anaerobic mineral solution of BRYANT and BURKEY. Fractions of a dilution of up to 10⁻⁹ were added to pre-reduced and melted agar tubes of the 98-5 agar media maintained at 50°C. Within 10 minutes after inoculation, roll tubes were made by rapidly rotating tubes in a spinner and simultaneously cooling with ice water. Solidified agar roll tubes were incubated for up to 2 weeks at 37°C. In the preparation of 98-5 agar media for roll tube media, components, with the exception of Na₂CO₃ buffer, reducing agent in the form of cysteine-sulfide and sugars, were diluted in the round-bottom flasks, equilibrated with CO₂ gas by gentle boiling, fitted with butyl rubber stoppers, and sterilized by autoclaving on 121°C for 15 minutes. After autoclaving, the sterile reducing agent and Na₂CO₃ buffer were added to media held at 50°C and then dispensed anaerobically into sterile, rubber-stoppered tubes. Colony counts from three roll tubes were determined using the colony-counting criteria of BRYANT and ROBINSON¹⁰⁾.

Isolation and presumptive identification of bacterial strains: Fifty isolated colonies were picked from one roll tube of 98-5 agar medium inoculated with 10⁻⁹ dilution of gut contents and incubated for 14 days. About 100 colonies were isolated from one part of the samples of different cattle. Isolates were subcultured onto maintenance 98-5 slant agar medium. Wet mounts of each isolated strain, prepared from the

water of syneresis of slant media for 48-hours cultures, were observed for morphology and motility. Pure cultures of strains were grouped and presumptively identified by using media and methods described by OGIMOTO and IMAI¹¹⁾. Oxygen relations of isolated strains were determined on aerobic plate of Tryptosoy agar.

5. Quantitative analysis of VFAs and lactic acid.

The procedure conformed with that of the previous paper¹²⁾.

Results and Discussion

Figure 1 shows the concentration of VFAs and lactic acid in various parts of the guts of cattle. The prosperity and decay of these two materials were opposed to each other. The VFAs concentration was the highest in the rumen and the caecum, followed by the omasum, colon and rectum, and was the lowest in the small intestine. On the other hand, the concentration of lactic acid was low in the rumen and caecum, and high in other parts. The concentrations of VFAs in the caecum and the rumen were almost the same. BRÜGGEMAN and GIESECKE¹³⁾ have reported the same results. It is, however, reported that the volume ratio of caecum to rumen in the ruminants is about 1/10 at 24 hours after feeding¹⁴⁾. Total amounts of the products may be larger than those obtained in this experiment, because the values obtained represent the difference between the amounts produced and absorbed.

Figure 2 shows the prosperity and decay of each composition of VFAs. The

Fig. 1. Distribution of lactic acid and VFAs in the gut of cattle.

Fig. 2. Proportion of VFAs in the gut of cattle.

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Fig. 3. Total counts of bacteria in the gut of cattle.
 ■: Total bacteria. □: Anaerobic bacteria

compositions of acetic acid, propionic acid and butyric acid in the rumen were similar to those in the caecum. BRÜGGEMAN and GIESECKE¹³⁾, HOOVER¹⁴⁾ and WILLIAMS¹⁵⁾ have reported similar results. It is known that the lactic acid and the VFAs are mainly produced by the physiological activities of the microorganisms, especially by those of the anaerobic ones¹⁶⁾. Therefore, in the next examination, total bacterial counts and anaerobic bacterial counts were performed (Fig. 3). As shown in the figure, both counts were high in the rumen, caecum, rectum and reticulum.

When the total bacteria were stained with Gram's staining by MOORE and HOLDMAN⁷⁾ and grouped with their staining condition, the compositions of the bacteria in the rumen, caecum, rectum and colon were similar to each other, i.e. the numbers of Gram-negative rods and cocci were high; in contrast, Gram-positive cocci were high in number in the jejunum and ileum (Table 1).

Table 1. Bacterial group in the gut of cattle

	Gram-positive		Gram-negative	
	Coccus	Rod	Coccus	Rod
Rumen	15%	0%	66%	19%
Reticulum	20	5	30	45
Omasum	25	5	30	40
Abomasum	12	7	41	30
Jejunum	66	17	17	0
Ileum	73	5	22	0
Caecum	10	0	11	79
Colon	14	0	50	36
Rectum	7	0	33	60

Table 2. Number of isolated bacterial strains from the gut of cattle

Presumptive identification of bacterial species	Rumen	Jejunum	Caecum	Rectum
<i>Bacteroides</i>	54	0	24	25
<i>Fusobacterium</i>	16	0	29	15
<i>Selenomonas</i>	67	0	6	2
<i>Succinivibrio</i>	8	0	0	0
<i>Butyrivibrio</i>	12	0	7	2
<i>Lachnospira</i>	9	0	0	0
<i>Megasphaera</i>	15	0	2	0
<i>Veillonella</i>	28	0	18	0
<i>Ruminococcus</i>	19	0	7	0
<i>Streptococcus</i>	5	29	3	4

Table 2 shows the presumptive identification of the bacteria which were isolated at random from the colonies on the highly diluted RGCA medium used for the counting. From the rumen, the Gram-negative and VFAs producing bacteria, such as *Bacteroides*, *Veillonella* and *Selenomonas*, were mainly isolated. And from the jejunum and ileum, the Gram-positive and lactic acid producing bacteria, such as *Streptococcus* and *Lactobacillus*, were mainly isolated.

From the results, it is assumed that there is a relation at all times between the distributions of bacteria and of organic acids in the gut, since the lactic acid producing bacteria, such as *Streptococcus* and *Lactobacillus*, are mainly isolated from the jejunum, ileum and rectum in which the lactic acid is detected in high concentration, and the VFAs producing bacteria, such as *Bacteroides* and *Selenomonas*, from the caecum and rumen in which the VFAs are high in concentration. Reincrease of lactic acid in the colon and the rectum (Fig. 1) may be related to the increase of

Fig. 4. Counts of rumen ciliates in the gut of cattle.

Gram-positive cocci mainly composed of *Streptococcus* and of Gram-negative rods such as *Lachnospira*.

On the other hand, Fig. 4 shows the average number of the rumen ciliates per milliliter of the contents in the various parts of the gut. No difference was recognized between the number of ciliates in the rumen and that in the reticulum, in which the ciliate number was about $5.8 \times 10^5/\text{ml}$ on average, respectively. HIROSE and OTANI¹⁷⁾ have also reported that there is no difference of the ciliate number between the rumen and the reticulum. On the other hand, the ciliate number in the omasum was $7.8 \times 10^4/\text{ml}$ on average, which was about 13.4% of the number in the rumen or in the reticulum. On the ciliates in the omasum, FERBER¹⁸⁾ and HIROSE and OTANI¹⁷⁾ reported that all the ciliates were killed and destroyed. However, in the present examination, a relatively large number of ciliates survived and had motility in the omasum, the survival ratio being about 69% on average from the calculation of 5 samples.

The factor for the decrease of the ciliate number in the omasum is not clear, but it is unlikely that the ciliates were killed and destroyed rapidly in the omasum, because no secretory glands for the digestive enzymes are present in the omasum¹⁹⁾, and many ciliates survived in the omasum in the present examination. It is known that the turn-over time of rumen fluid is relatively fast^{20, 21)}, but that of ciliates is far slower²²⁻²⁵⁾. Therefore, the ciliates in the rumen and the reticulum must make their escape from the outflow toward the omasum for certain reasons. WELLER and PILGRIM²⁶⁾ have indicated that the decrease of the ciliate number in the omasum is due to sequestration of ciliates within the rumen. In the comparison of the ciliate composition between in the rumen and the reticulum and in the omasum, the proportion of the entodinid ciliates was higher in the latter than in the former, namely, the percentage distribution of those were 83.6% in the rumen and 94.3% in the omasum on average. ABE *et al.*²⁷⁾ reported that trichostomatid ciliates, such as *Dasytricha*, generally took refuge in the folds of the wall of the reticulum.

It has been seriously considered that the rumen ciliates are digested and absorbed in the abomasum and the small intestine, and are utilized as a nitrogen source for the host²⁸⁾. However, WELLER and PILGRIM²⁶⁾ investigated on the protozoal counts in the rumen fluid and the rumen effluent of the sheep and the calculation of output of protozoal nitrogen from the rumen, and indicated that the concentrations of protozoa in the effluents were usually less than 20% of those in the rumen fluid and that the amount of protozoal nitrogen leaving the rumen represented less than 2% of dietary nitrogen. From the results, they suggested that the contribution of protozoa would be too small to affect significantly the composition of the total protein mixture. HARRISON *et al.*²⁹⁾ determined the quantities of total protozoal amino acid-N present in the rumen and entering the duodenum, and indicated that a substantial proportion of rumen protozoal protein was retained within the rumen.

These results including that in the present examination suggest that the degree of designation of areas as nitrogen sources should be decreased more than formerly

thought.

The ciliate number in the abomasum was $5.7 \times 10^3/\text{ml}$ on average, 0 at a minimum and $4.1 \times 10^4/\text{ml}$ at a maximum, which was nearly the same level as that of ciliate numbers in the omasum. These variations of values seem to be due to the observation of various conditions of digestion in the abomasum. No moving ciliates were observed in the examination of 4 samples of the fresh contents of the abomasum.

A few ciliates were also recognized in the parts of the gut lower than the abomasum. The number ranged from 20 to 600 per milliliter. However, the ciliates appearing in complete forms were hardly observed in these regions.

No cyst-like structures were recognized in any of the regions.

FERBER¹⁸⁾ and HIROSE and OTANI¹⁷⁾ reported that no ciliates were recognized in the abomasum. In the present examination, however, a relatively large number of ciliates was observed in the abomasum, and a few also in lower than the abomasum. But these ciliate numbers were extremely low, and ciliates with complete form were hardly observed in respective regions. Therefore, it seemed that it was impossible for the rumen ciliates to survive in the parts lower than the abomasum, that it was scarcely possible that living individual ciliates were excreted with feces, and that almost all of these ciliates were digested in the gut of the host. HARRISON *et al.*²⁹⁾ have pointed out that the mean flow of duodenal total protozoal amino acid-N was equivalent to approximately 24% of the duodenal total microbial amino acid-N, indicating that protozoal amino acids still can be an important nutritional component of the nitrogen entering the duodenum.

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牛消化管内各部位におけるルーメン内繊毛虫, 嫌気性細菌の分布および揮発性脂肪酸, 乳酸の消長

今井壮一・柳田則明*・片桐勝則・扇元敬司*

日本獣医畜産大学, 武蔵野市 180

*東北大学農学部, 仙台市 980

牛消化管内における微生物およびそれらによる生産物の消長を知る目的で, 消化管を9部位に区分し, 揮発性脂肪酸(VFA), 乳酸の消長および嫌気性細菌, ルーメン繊毛虫の分布について検討した。その結果, VFA量はルーメンおよび盲腸で, 乳酸量は空腸および回腸で高かった。細菌構成は, ルーメンおよび盲腸では *Bacteroides*, *Veillonella*, *Selenomonas* などのグラム陰性菌が主として分離され, 空腸および回腸では *Streptococcus*, *Lactobacillus* などのグラム陽性菌が優位に検出された。ルーメン繊毛虫の分布に関しては, ルーメンおよび第二胃では繊毛虫数はいずれもほぼ $6 \times 10^5/\text{ml}$ で, 両者間に差異は認められなかったが, 第三胃ではこれらの繊毛虫数のおよそ 14% に減少した。しかしながら, 第三胃においても約 69% の繊毛虫がなお運動性を保っていた。

このことは, ルーメンあるいは第二胃に生息する繊毛虫が, 何らかの要因により第三胃への流出をのがれていることを示唆するものと考えられる。極めてわずかの繊毛虫が第四胃以下の部位にも認められたが, それらの部位においては完全な形態を有する繊毛虫はほとんど認められなかった。このことは, ルーメン繊毛虫が牛の下部消化管においてそのほとんどが分解されることを示しているが, 第三胃に流出する繊毛虫数が, ルーメン内におけるそれと較べてかなり少ないことは, 繊毛虫の宿主に対する栄養的役割を考える場合, 従来考えられていた量よりかなり少ない量を見積らなければならないことを示唆している。

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