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COMPARATIVE STUDIES OF RUMINAL FLUID COLLECTED BY ORAL TUBE OR BY PUNCTURE OF THE CAUDOVENTRAL RUMINAL SAC

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Abstract

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Samples of ruminal fluid were collected in four groups of high-producing dairy cows simultaneously by an oral probe and by percutaneous puncture of the caudoventral ruminal sac. The samples were tested for basic indicators of the metabolic profile including pH, total acidity, ammonia, infusoria counts, and total and relative amounts of volatile fatty acids. Significant differences ($P < 0.05$) between the samples collected by oral probe (o) and by puncture (p) were observed for pH (o - 6.74 *vs*. p - 6.23), total acidity (o - 17.1 *vs*. p - 24.7), and total VFA (o - 101.18 *vs*. p - 131.70 mmol/l) in the group of cows with mean daily production of 18 l of milk. Highly significant ($P < 0.01$) differences between the samples collected by oral probe and by puncture were observed in the groups yielding daily 24 and 38 l of milk for pH (o -6.95 *vs.* p - 6.24 and o - 7.00 *vs*. 6.21, respectively), total acidity (o - 13.2 *vs*. p - 22.2 and o - 12.6 *vs*. p - 25.2, respectively), and total VFA (o - 99.23 *vs.* p - 136.25 mmol/l and o - 89.66 *vs.* p - 140.14 mmol/l, respectively). Buffering of the ration for the cows yielding 38 l of milk per day with 200 g of sodium hydrogen carbonate increased the above differences in pH, total acidity, and total VFA. Ruminal fluid samples collected by percutaneous puncture of the caudoventral ruminal sac better indicate the intensity of ruminal fermentation processes apparently because their composition is not influenced by the amount of saliva or buffer which is variable in the content of the cranial ruminal sacs where the oral samples are collected from. Although the sampling of ruminal fluid by percutaneous puncture is an easy method, it can be associated with some complications. Their risk can be minimised by strict adherence to the recommended procedure and observation of rules of aseptic work.

Cattle, sampling methods, pH, total acidity, ammonia, infusoria, VFA

Samples of ruminal fluid have been collected for diagnostic, therapeutic, and scientific purposes since the mid of the past century. Their examination has become a part of routine diagnostics of clinical and particularly subclinical forestomachal disorders (Pounden 1954; Hofírek et al. 1976; Dirksen 1981; Dvořák et al. 1997).

In the recent years, ruminal fluid tests have been used also for the control of nutrition and checks of ruminal fermentation in high-yielding dairy cows to obtain data for the elaboration of preventive measures against metabolic disorders and production diseases. Many factors must be considered in the calculation of daily rations and attempts to attain the optimal intensity of ruminal fermentation are not always fully successful owing to very complex interaction among the individual ration constituents.

The composition of the ruminal fluid is very variable, depending on the sampling site and method and the intensity of ruminal fermentation. The latter factor is particularly important in herds where total mixture rations (TMR) are fed. To assess the results of the metabolic profile test of ruminal fluid correctly it is necessary to obtain standard samples even on repeated collections. This necessity has been encouraging attempts to improve the existing ruminal fluid sampling techniques. An alternative to the conventional oral tube method

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(Serensen and Schambye 1955; Slanina 1967; Hofírek 1970; Dirksen 1975; Lebeda et al. 1981; Dvořák et al. 1997) combined with the use of various appliances is direct collection from the caudal ruminal sac using a needle run through the abdominal and ruminal walls (Hollberg 1984; Polack and Perrin 1998; Brugère et al. 1990).
The objective of this study was to compare the methods of oral tube collection and direct collection of ruminal fluid from the caudoventral ruminal sac.

Materials and Methods

Animals

The samples were collected in four herds differing in average milk yields always three to four hours after feeding. Group 1 (Table 1).

Five Bohemian Red Pied dairy cows of a herd with average daily milk yield of 18 l. The cows were fed conventionally twice a day with hay and silage; concentrates were dosed according to individual milk yield. Group 2 (Table 2).

Five Holstein dairy cows of a herd with average daily milk yield of 18 l. The cows received the total mixture ration (TMR) twice a day. Group 3 (Table 3).

Eight Holstein dairy cows of a herd with average daily milk yield of 24 l. The cows received TMR buffered with sodium bicarbonate (139 g/animal/day) twice a day. Group 4 (Table 4).

Eleven Holstein dairy cows of a herd with average daily milk yield of 38 l. The cows received TMR buffered with sodium bicarbonate (200 g/animal/day) three times a day.

Ruminal fluid sampling

First the direct sampling method was used. The puncture site on a horizontal line connecting the pregenual skin fold with the last rib was clipped and disinfected. Then abdominal and ruminal walls were punctured with a 90-mm-long 1.5 mm gauge epidural needle with mandrin (Hofírek and Haas, 2000) and a sample was taken. Oral tube was run and another sample was taken immediately thereafter (Dvořák et al. 1997) to minimise possible error due to lag. The technique described by Sorensen and Schambye (1955) was used. Altogether 29 + 29 samples of ruminal fluid were collected. The sample volume required for the analyses done in our experiments was 20 to 30 ml.

The following characteristics of the ruminal fluid metabolic profile were tested: pH, total acidity, infusoria count, and concentrations of ammonia and total and individual volatile fatty acids (VFA). Significance of differences was assessed by the paired *t*-test. The puncture site and the general state of health were monitored for several weeks after the sampling.

Table 1
Characteristics of ruminal fluid collected by oral tube and by ruminal puncture.
Group 1, Bohemian Red Pied, n = 5, average milk yield 18 l, fed hay, silage, and concentrates.

Characteristic	a		b		*P*
	Mean	± S. D.	Mean	± S. D.	
pH	6.74	± 0.188	6.23	± 0.400	*
Total acidity [arbitrary units]	17.1	± 2.15	24.7	± 6.79	*
Ammonia [mmol/l]	6.5	± 2.01	7.4	± 2.96	N.S.
Infusoria [103/ml]	302 400	± 176 218	241.600	± 129 293	N.S.
VFA [mmol/l]	101.18	± 13.36	131.70	± 17.73	*
Acetic acid [%]	63.2	± 1.18	63.0	± 1.45	N.S.
Propionic acid [%]	20.5	± 3.97	20.7	± 4.30	N.S.
Isobutyric acid [%]	0.6	± 0.16	0.6	± 0.08	N.S.
Butyric acid [%]	13.4	± 2.78	13.4	± 2.78	N.S.
Isovaleric acid [%]	1.2	± 0.63	1.1	± 0.56	N.S.
Valeric acid [%]	1.1	± 0.26	1.2	± 0.24	N.S.

Legends: a - oral probe
b – puncture
* $P \leq 0.05$
** $P \leq 0.01$

Analyses

Immediately after collection, the samples were filtered through a sieve to remove large particles and divided into portions. The analyses were done instantly or the portions were frozen (except for those intended for protozoa enumeration). The following agents were used for sample preservation: mercury chloride (approx. 2 drops per tube) for the determination of pH, total acidity, and lactic acid and ammonia concentrations; chloroform (2 drops per tube) for analyses of VFA; 10% formaldehyde solution (l ml per 4 ml of sample) for enumeration of protozoa.

pH

pH was measured with the PHM 64 Research pH Meter (Radiometer Copenhagen) using the pH 7.00 ± 0.01 buffer supplied by the manufacturer as the calibration standard.

Total acidity

The method described by Jonov et al. (1957) was used. Ten millilitres of sample was titrated with 0.1 M sodium

Table 2
Characteristics of ruminal fluid collected by oral tube and by ruminal puncture.
Group 2, Holstein dairy cows, n = 5, average milk yield 18 l, fed unbuffered TMR.

Characteristic	a		b		*P*
	Mean	± S. D.	Mean	± S. D.	
pH	6.89	± 0.187	6.08	± 0.268	*
Total acidity [arbitrary units]	17.1	± 3.73	28.2	± 10.37	N.S.
Ammonia [mmol/l]	6.9	± 1.14	7.8	± 1.43	N.S.
Infusoria [103/ml]	276 800	± 107 420	251 200	± 48 530	N.S.
VFA [mmol/l]	88.48	± 7.38	138.16	± 20.96	**
Acetic acid [%]	58.9	± 4.08	58.9	± 4.29	N.S.
Propionic acid [%]	21.7	± 5.20	21.8	± 5.07	N.S.
Isobutyric acid [%]	0.8	± 0.11	0.6	± 0.11	N.S.
Butyric acid [%]	16.3	± 2.42	16.4	± 2.31	N.S.
Isovaleric acid [%]	1.1	± 0.17	1.1	± 0.17	N.S.
Valeric acid [%]	1.2	± 0.32	1.3	± 0.43	N.S.

For legends see Table 1

Table 3
Characteristics of ruminal fluid collected with oral tube and by ruminal puncture.
Group 3, Holstein, n = 8, average milk yield 24 l, fed TMR buffered with sodium bicarbonate (139g/animal/day).

Characteristic	A		b		*P*
	Mean	± S. D.	Mean	± S. D.	
pH	6.95	± 0.168	6.24	± 0.271	**
Total acidity [arbitrary units]	13.2	± 3.13	22.2	± 4.72	**
Ammonia [mmol/l]	7.8	± 1.35	7.6	± 1.69	N.S.
Infusoria [103/ml]	432 000	± 11 .056	387 000	± 87 629	N.S.
VFA [mmol/l]	99.23	± 18.40	136.25	± 8.95	**
Acetic acid [%]	62.1	± 3.83	62.7	± 0.94	N.S.
Propionic acid [%]	20.7	± 4.06	19.8	± 1.76	N.S.
Isobutyric acid [%]	0.7	± 0.14	0.7	± 0.11	N.S.
Butyric acid [%]	14.3	± 1.44	14.3	± 1.78	N.S.
Isovaleric acid [%]	1.0	± 0.23	1.1	± 0.34	N.S.
Valeric acid [%]	1.2	± 0.20	1.4	± 0.20	**

For legends see Table 1

Table 4
Characteristics of ruminal fluid collected by oral tube and by ruminal puncture.
Group 4, Holstein, n = 11, average milk yield 38 l, fed TMR buffered with sodium bicarbonate (200 g/animal/day).

Characteristic	a		b		*P*
	Mean	± S. D.	Mean	± S. D.	
PH	7.00	± 0.63	6.21	± 0.276	**
Total acidity [arbitrary units]	12.6	± 3.40	25.2	± 6.71	**
Ammonia [mmol/l]	11.3	± 6.57	10.0	± 3.57	N.S.
Infusoria [103/ml]	394 909	± 75 833	408 727	± 52 271	N.S.
VFA [mmol/l]	89.66	± 14.54	140.14	± 11.97	**
Acetic acid [%]	59.8	± 2.88	59.3	± 3.01	*
Propionic acid [%]	23.1	± 4.03	23.9	± 4.67	*
Isobutyric acid [%]	0.8	± 0.15	0.6	± 0.13	**
Butyric acid [%]	13.9	± 1.92	13.7	± 2.09	N.S.
Isovaleric acid [%]	1.2	± 0.15	1.2	± 0.18	N.S.
Valeric acid [%]	1.2	± 0.18	1.3	± 0.20	**

For legends see Table 1

Table 5
pH of ruminal fluid samples collected by ruminal puncture or by oral tube

Author	Ruminal puncture	Oral tube
Hollberg (1984)	6.53 ± 0.59	6.89 ± 0.46
Rousseau et al. (1989)	6.27 ± 0.70	7.31 ± 0.47
Brugère et al. (1990)	6.20 ± 0.52	7.17 ± 0.51
Hofírek and Haas (2000)	6.18 ± 0.38	6.90 ± 0.20

hydroxide with pH 8.5 as the endpoint using a pH meter or an automatic titrator. The amount of sodium hydroxide necessary for this pH shift was proportional to the total acidity value.

Ammonia

Ammonia concentration was determined by the method described in detail by Zapletal and Hofírek (1971); the method is based on diffusion of ammonia in Conway dishes induced with saturated potassium carbonate solution, and subsequent titration with 0.01 M hydrochloric acid using methyl red as the indicator.

Ruminal protozoa enumertation

After filtration, the sample was thoroughly mixed and preserved by adding 1 ml of 10% formaldehyde to 4 ml of sample. The preserved samples were stored at 4 °C. The total protozoa number was established using the Fuchs-Rosenthal chamber. After mixing with 0.1% solution of methylene blue at the ratio of 1 : 20, the sample was applied into the chamber and all protozoa in all fields were enumerated. The obtained value was corrected to 1 ml of sample by multiplying the reading by 320 (chamber index) and 20 (dilution index).

Volatile fatty acids

VFA were determined by GC (Cottyn et al. 1968). Proteins present in the sample were precipitated by addition of 25% metaphosphoric acid and the "ghost effect" was eliminated by addition of 5% formic acid. Clear sample supernatant obtained by centrifugation was injected into the column directly. Standard solutions were used to calculate the concentrations of the individual VFA.

Chromatography conditions

Chromatograph Chrom 5. (Laboratory Instruments, Prague) flame ionisation detector; glass column 3.5mm x 1.2; packing Porapak P (Sulpeco), 80 - 100 mesh, wetting with 3% HPO; column, injector and detector temperature 170 °C carrying gas nitrogen, 1.2 kp/cm^2 hydrogen flow rate 0.3 ml/min air flow rate 600 ml/min; injection volume 0.6 µl.

Results

The results are shown in Tables I through IV.

Significant within-group differences in several characteristics were found in all the groups. pH of ruminal fluid collected by puncture was significantly ($P \leq 0.05$) or highly significantly ($P \leq 0.01$) lower in all the 29 tested samples, of all the four groups, the differences ranging from 0.51 to 0.81 units. Greater differences were found in high-yielding cows fed buffered TMR.

A similar difference was observed in titration acidity. Again, all the within-group differences were significant or highly significant, the differences ranging from 12.6 to 7.6 arbitrary units. The difference was greater in the cows fed TMR and the maximum difference was found in Group 4, i.e. in high-yielding cows fed TMR buffered with 200 g sodium hydrogen carbonate per animal per day (Table 4).

Another characteristic showing significant or highly significant within-group differences was the concentration of total VFA. The differences ranged from 30.52 to 50.48 mmol/l. The greatest difference was again found in Group 4, i.e. in high-yielding cows fed TMR buffered with 200 g sodium hydrogen carbonate per animal per day.

Differences in concentrations of the individual VFA were found rather exceptionally. Since they were observed only in some acids in Group 4, showing the highest milk yield (Table 4).

Discussion

The conventional method of ruminal fluid sampling with an oral tube has been in the recent years completed by a direct method consisting in direct puncture of the ruminal wall. The first to assess this surgical method of ruminal fluid sampling was Hollberg (1984) who described the technique, pointed out complications that may develop, and compared compositions of samples collected by the conventional method and by puncture. For the collection by puncture he used a 2.4 mm gauge 150-mm-long needle run deeply into rumen. Through this needle another thin, blunt and somewhat longer needle with side holes at its end was run and the sample was aspirated with a 200-ml syringe. The puncture site was on the horizontal line connecting stifle joint with the last rib. Inflammatory thickening of the abdominal wall was found in up to one half of the cows slaughtered within 24 h and in up to two thirds of those slaughtered within six days. Adhesive peritonitis was observed sporadically. Significant differences between the samples collected by the conventional method and by puncture were found in pH, results of the methylene blue test and concentrations of total and individual VFA. Owing to these results and the risk of complications, Hollberg was rather cautious in assessing the expedience of the puncture method.

Rousseau et al. (1989) and and Perrin (1989) used a 120-mm-long 3.5 mm gauge trocar with mandrin and a probe for direct ruminal fluid sampling. To facilitate its penetration, the trocar was run through a skin incision made with a scalpel in the left paralumbar fossa. Then the mandrin was replaced by a metallic 35-mm long probe run into the rumen down to the level of the caudoventral sac. After sample aspiration, first the probe and then the trocar are removed. The trocar was rinsed with ethanol upon removal to minimise the risk of local peritonitis. Although the authors mention possible complications, they do not emphasise this hazard too much.

Brugère et al. (1990) substantiated the use of the direct sample collection method by pragmatic reasons casting doubt on the value of orally collected samples for the assessment of the individual ruminal fermentation processes. They used a 3 mm gauge trocar, a 2.5 mm gauge probe with side holes, and a syringe. In their opinion, complications can be avoided if rules of aseptic work are adhered to.

Our experiments were done using the relatively thin 1.5 mm gauge epidural needle with a mandrin to minimise the injury to the abdominal and ruminal walls and thus the risk of

infection that may result in peritonitis. Epidural 2.2 mm gauge needles with mandrins can be used for this purpose without increasing the risk as well, but, in our opinion, the use of 3 to 3.5 mm gauge trocars can be associated with more severe complications. Needles with a smaller gauge cannot be recommended because they are prone to impaction by solid particles present in the ruminal fluid.

As shown by our results, complications as a consequence of abdominal puncture cannot be wholly precluded. However, their rise is only exceptional if rules of good surgical practice, including clipping, shaving and disinfection of the puncture site, effective sterilisation of the injection needle, and careful disinfection of the site after its removal, are strictly observed. The risk of injury to the uterus due to abdominal puncture at the level of the stifle joint in high-pregnant cows was not pointed out by any of the above authors. It can be prevented by clinical examination, balotment, and cranial shifting of the puncture site.

As can be seen in Table V, the differences in ruminal fluid pH due to sampling technique found in our experiments were similar to those reported by other authors. pH values may differ by 0.36 to 1.04 units and this difference is proportional to the intensity of ruminal fermentation. The difference in the concentrations of VFA (88.94 mmol/l for puncture and 73.11 mmol/l for oral tube) reported by Hollberg (1984) for cows with a low production of VFA was 15.83 mmol/l. In our experiments, the VFA concentrations in the samples collected by puncture exceeded in some animals 130 mmol/l and the differences relative to the samples collected with oral tube were 30.52, 49.68, 37.02, and 50.48 for Groups 1, 2, 3, and 4, respectively.

It is evident that, when appropriate, tests on ruminal fluid samples collected by percutaneous puncture can complete the information on ruminal fermentation processes. Sampling of ruminal fluid by puncture of the caudoventral ruminal sac is an easy and safe procedure. Complications due to puncture were observed in three cows. Two of them developed local inflammatory reactions which disappeared within a few days after topical treatment with iodine tincture. Pregnant uterus was accidentally punctured in the third cow, but no complications developed from this injury. Possible risks of this technique can be minimised by strict observation of principles of good surgical practice. Generally, this method can be recommended for individual cases in which such completion is necessary considering the results of tests of orally collected samples.

Srovnávací studie vzorků bachorové tekutiny získaných odběrem sondou per os nebo přímou punkcí kaudoventrálního vaku bachoru

U čtyř skupin vysokoužitkových dojnic byly provedeny souběžně odběry bachorové tekutiny sondou per os a přímo jehlou perkutanně z kaudoventrálního vaku bachoru. Byly sledovány základní parametry metabolického profilového testu bachorové tekutiny: pH, celková acidita, amoniak, počet nálevníků, těkavé mastné kyseliny celkově i proporcionálně. Vyšetřením bachorové tekutiny byly získány statisticky významné ($P \leq 0,05$) rozdíly u dojnic s nižší užitkovostí, denním nádojem v průměru18 l u pH 6,74 odběrem per os a 6,23 při odběru perkutánním, celkové aciditě17,1 při odběru per os a 24,7 při odběru perkutánním a celkové hodnotě TMK 101,18 mmol/l při odběru per os a 131,70 mmol/l při odběru perkutánním. Statisticky vysoce významné ($P \leq 0,01$) rozdíly v metabolickém profilu bachorové tekutiny byly zjištěny u dojnic s vysokou užitkovostí 24 l a 38 l průměrného denního nádoje, u pH 6,95 při odběru per os a 6,24 při odběru perkutánním a 7,00 při odběru per os a 6,21 při odběru perkutánním, celkové aciditě 13,2 při odběru per os a 22,2 při odběru perkutánním a 12,6 při odběru per os a 25,2 při odběru perkutánním, u celkových těkavých mastných kyselin 99,23 mmol/l u odběru per os a 136,25 mmol/l u odběru perkutánním a 89,66 mmol/l při odběru per os a 140,14 mmol/l při odběru perkutánním. U skupiny dojnic s průměrným denním nádojem 38 l a pufrací krmné dávky hydrogen uhličitanem sodným v dávce 200 g na den byly rozdíly v pH, celkové aciditě a těkavých mastných kyselinách nejvýraznější, což dokumentuje, že přítomnost

pufru v krmné dávce ovlivňuje uvedené parametry metabolického profilu bachorové tekutiny. Výsledky ukázaly, že bachorová tekutina získaná punkcí kaudoventrálního vaku bachoru lépe signalizuje úroveň fermentačních procesů v bachoru probíhajících vzhledem k tomu, že její složení není ovlivňováno množstvím slin, případně pufru, což je charakteristické pro kraniální část bachoru, odkud je odebírána bachorová tekutina sondou per os. Po metodické stránce se ukázalo že odběr bachorové tekutiny provedený punkcí bachoru přes kůži je technicky snadno proveditelný, ale může být doprovázen některými komplikacemi. Rizika je možno minimalizovat přísným dodržováním metodiky odběru a pravidel asepse. Metodu přímého odběru lze doporučit v individuálních případech, kdy metodou per os byly získány neočekávané výsledky a kdy je třeba ověřit, že v kaudoventrálním bachorovém vaku se pH a další parametry neodchýlily od fyziologické normy a že nehrozí nebezpečí zdravotních poruch.

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