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BUFFERING COMPONENTS IN RUMEN LIQUOR AND BLOOD OF BUFFALO HEIFERS

Neama A. Ashmawy

Department of Animal Production, Faculty of Agriculture, Cairo University

SUMMARY

Five buffalo heifers aging 18-30 months were used to study buffering components in rumen liquor and blood. Samples of rumen liquor were taken by stomach tube, to determine the buffering components in the rumen fluid, mainly, total volatile fatty acids (TVFA's), bicarbonate (HCO_3), phosphates and ammonia (NH_3) concentrations with simultaneously measuring the pH, and buffering capacity value. Blood samples were withdrawn from the jugular vein to determine the relevant buffering components and pH value. Two hours postfeeding, rumen bicarbonate concentration showed high concentration, inorganic phosphorus concentration varied widely among animals, that may be due to different rate of saliva secretion and rumination rate of each animal. At nearly neutral pH, ammonia concentrations showed positive relationship between rumen and blood ammonia concentration. Total volatile fatty acids in rumen were appreciably high, that is attributed to the rapid fermentation of the ingested diets (concentrate, hay and rice straw). Rumen buffering capacity was approximately equal in all animals.

Keywords: Buffalo, buffers, bicarbonate, blood, rumen, ammonia, inorganic phosphorus, total volatile fatty acids

INTRODUCTION

The buffering components of the reticulo-rumen contents results directly from the complex interrelationships among the amount and rate of saliva production, saliva composition and rate of microbial metabolism within reticulo-rumen (Kay, 1960 and Baily & Balch, 1961). Many factors affect buffering value of the rumen content such as the concentration of the end-products of fermentation, particularly, VFAs, carbon dioxide, bicarbonate (HCO_3), phosphate and ammonia concentrations (Turner & Hodgetts, 1955). Within the

normal range of ruminal pH (5.5 - 7.5), bicarbonate concentration, phosphate and the volatile fatty acids are all important buffers. At pH levels below 5.5, the VFAs become the major components for preventing further reduction in pH. These authors concluded that, during fasting, rumen bicarbonate concentration was relatively more important than phosphate; when ruminal fermentation progresses, VFAs accumulate and pH decreases, bicarbonate levels decline and phosphate becomes relatively more important. During active fermentation, phosphate is relatively more important. With high concentrations of VFAs and pH below 6.0, VFAs contributed the major buffering action against further additions of acids. Briggs *et al.* (1957) stated that, rumen pH was closely related to VFA levels in the rumen.

This study was conducted to determine buffering components in rumen liquor and at the same time in blood serum, plus haemoglobin as functional factor in the stability of blood pH.

This work was designed as a preliminary investigation for the coincidentally relative concentrations of buffering components in rumen liquor and blood serum as related to general acid-base balance. The study is considered to provide parameters for subsequent studies of dynamic changes, in this components, in reaction to different thermal conditions and nutritional treatments.

MATERIALS AND METHODS

Animals:

This work was carried out during summer (August). Ambient air temperature and relative humidity means were 28.4±1.21 °C & 68.0 ± 0.58% in the Animal Physiology Laboratory, Animal Production Department, Faculty of Agriculture, Cairo University, Egypt.

Five buffalo-heifers 2.5 years old, weighing 240 - 380 kg were used in this study. The heifers were offered daily ration of hay (5 kg/head/day) plus concentrate (5 kg/head/day) and rice straw. The concentrate ration was composed of, undecorticated cotton seed cake, rice bran, yellow corn, limestone and common salt plus molasses. The nutrients contents were appoximalty 16% crude protein, 15% crude fiber and 3% crude fat.

The animals were deprived of food and water for 20 hours before feeding at 08:00 h. Samples of the rumen liquor and blood serum were collected simultaneously at 2 hours post feeding.

Sampling of rumen liquor:

Samples of rumen liquor were obtained by a stomach tube at two hours postfeeding under a layer of liquid paraffin. The sample was centrifuged, the supernatant rumen liquid was transferred to another tube under liquid paraffin to determine rumen bicarbonate and buffering capacity value. Another rumen

liquid sample was drawn at the same time to determine pH value, TVFAs, ammonia concentration and inorganic phosphorus.

Blood sampling :

Blood samples were taken from jugular vien, one sample under a layer of liquid paraffin to determine serum bicarbonate concentration. Other heparinized blood samples were obtained to determine blood pH value, ammonia concentration, TVFAs, inorganic phosphorus and haemoglobin concentration.

Technical procedure:

Rumen fluid and serum bicarbonate concentration was determined by the titration method of Van Slyke (1922). Rumen buffering value was determined by the method reported by Emmanuel *et al.* (1969). Rumen fluid and blood pH values were determined instantly at sampling time by glass electrode pH meter. Total VFAs concentrations were determined by steam distillation method (Barnett & Reid, 1957). The method of Fiske and Subbarrow (1925) was used to measure the inorganic phosphorus. Ammonia concentrations in blood and rumen liquid were determined by micro-diffusion technique of Conway (1950). Haemoglobin concentration was determined colourimeterically as described by Bauer (1970).

RESULTS AND DISCUSSION

1- Bicarbonate

Two hours post-feeding, rumen bicarbonate concentration was high (Table 1), these concentrations were affected by the fairly high pH values ranging between 6.19 and 7.78. This high concentration might be due to the increase in saliva secretion rate at this time of active rumination post feeding period. Washburn and Brody (1937) observed that the concentration of carbon dioxide in the ruminal gas of normal cows varies from a maximum of 80 % at 1 - 2 hours post-feeding to a minimum of 10 % at 24 hours after feeding. Ash and Dobson (1963) concluded that hydration of CO_2 in the sheep rumen provided a source of hydrogen ions for the production of undissociated VFA. It explains the accumulation of bicarbonate within the rumen contents observed in the present work. On the other hand, Masson and Phillipson (1951) and Dobson and Phillipson (1968) reported that substantial amounts of carbonic anhydrase have been found in rumen epithelial cells, and this could be involved in subsequent release of bicarbonate into the rumen. Most of the CO_2 seems to be produced by intracellular epithelial metabolism, although CO_2 also could be absorbed from the lumen side of the epithelium to provide the carbonic acid for the donation of hydrogen and bicarbonate ions (Stevens, 1973). In most cases the animals which had high values of rumen bicarbonate, showed high serum bicarbonate and vice versa (Table 1). These results agree with those reported

Table 1. Means ± S.E. of buffering agents in rumen fluid and blood serum of buffalo heifers

Item \ Animal No.	1	2	3	4	5	Overall mean
Bicarbonate :						
Rumen bicarbonate (m.mol/l)	37.54±25.8	41.50±2.32	37.70±2.88	27.90±6.23	31.40±2.80	35.20 ± 1.79
Serum bicarbonate (m.mol/l)	31.14±1.00	29.14±2.00	28.22±1.58	25.92±2.02	27.72±2.45	28.43 ± 0.86
TVFA :						
Rumen TVFA (m.eq./100 ml)	40.26±13.17	28.06±4.25	25.62±2.99	37.21±6.13	41.48±16.86	34.53 ± 4.38
Blood TVFA (m.eq./100 ml)	7.60±0.67	6.10±1.08	6.71±1.49	4.88±0.75	5.80±0.75	6.22 ± 0.45
Inorganic phosphorous :						
Rumen inorg. phos. (mg/100ml)	30.56±11.47	31.68±7.79	17.76±3.56	39.04±14.92	28.16±4.02	29.44 ± 4.09
Serum inorg. phos. (mg/100ml)	5.60±0.66	5.38±0.48	5.94±0.32	5.73±0.45	6.37±0.56	5.80 ± 0.22
Ammonia :						
Rumen NH_3 (mg/100ml)	9.57±3.57	4.39±1.66	4.97±2.79	7.46±3.41	6.81±3.41	6.51 ± 1.30
Blood NH_3 (mg/100ml)	1.61±0.24	1.32±0.24	1.32±0.05	1.56±0.18	1.39±0.24	1.44 ± 0.09

* Each mean represents 5 observations for each animal.

by Bödeker *et al.* (1992) in sheep and Remond *et al.* (1993) in Texel wethers. The first author recorded that, bicarbonate favors NH_3 absorption across the ruminal epithelium. The second author found that, if carbonic acid was in equilibrium with CO_2 in ruminal gas, and the pH was nearly neutral, the increase in carbonic acid (H_2CO_3) may have led to an increase in bicarbonate ions (HCO_3^-).

2- Volatile fatty acids (VFAs)

In the present results, the total VFAs concentration was appreciably high at 2 hours postfeeding (Table 1). The increase in concentration of VFAs indicates rapid fermentation of ingested diets (concentrate & hay and rice straw). This result agrees with Topps *et al.* (1968) who indicated that, total VFAs in the rumen of young Friesian steers, aged 6 months, fed concentrate diets were 55.0 m.eq./l before feeding, 186.6 m.eq./l after feeding, and when steers fed hay, TVFAs concentration was 83.8 before *vs.* 132.0 m.eq./l after feeding. In heifers No. 2 & 3 (heavier and older than other animals), rumen TVFAs and ammonia concentrations were the least while bicarbonate concentration showed the highest values. These findings indicated that TVFAs and NH_3 were absorbed from the rumen into blood and blood bicarbonate intered the rumen instead (Table 1). These results agree with Remond *et al.* (1993) who found that, at pH 6.7, NH_3 absorption increased with high NH_3 and butyrate concentrations in the rumen. These two heifers had the highest concentration, alongside heifer No. 1, in both VFAs and bicarbonat with the lowest concentration of NH_3 in heifers No. 2 & 3.

3- Inorganic phosphorous

Rumen inorganic phosphorous concentration varied widely between the studied heifers (Table 1), this may be due to the differences in the rate of saliva secretion and rumination rate of each animal. Poutiainen (1968) indicated that, daily secretion rates of saliva for non-lactating cows varied from 90 to 190 l/day. Ternouth *et al.* (1985) noted that, the salivary secretion of inorganic phosphorous, generally, increase with salivary flow rate. The daily turnover of inorganic phosphorous in the saliva of sheep was 60 - 320 mg kg^{-1} day^{-1} (Kay, 1960). Because of the relatively large addition of salivary inorganic phosphorous to the rumen contents there is no net absorption of inorganic phosphorous from this region. It is not until the small intestine that the bulk of the net absorption of Inorganic phosphorous occurs (Poppi & Ternouth, 1979) although there is also some net absorption of Inorganic phosphorous from the large intestine at higher phosphorous intake. Tomas *et al.* (1967) indicated that salivary phosphorus was the major source of phosphorus to the sheep rumen and was the principal determinant of rumen fluid inorganic phosphorus levels. The phosphorus level in blood was not affected by the phosphorus level in the rumen (Table 1). Tomas (1974 a & b) indicated that, the salivary

glands largely replace the kidneys as a means of removing excess Inorganic phosphorous from the circulation. The salivary Inorganic phosphorous helps to buffer the production of volatile fatty acids in the rumen and to satisfy the phosphorus requirements of the microbial population of the rumen. A relationship between kidney and salivary secretion of Inorganic phosphorous has been demonstrated.

4- Ammonia

Lewis (1957) demonstrated that the portal blood ammonia concentration increased as a curvilinear function of rumen ammonia content. Data in the present study agree with the finding reported by Lewis (1957) since blood ammonia concentrations were parallel to that of rumen fluid (Table 1). Hogan (1961) and Bödeker *et al.* (1992) stated that at nearly neutral pH the passage of NH_3 across the ruminal epithelium increased with rise of NH_3 concentration in the ruminal fluid. Hogan suggested that, NH_3 passage across the epithelium of the rumen mainly occurred by diffusion in the ionized form and that some NH_3 cations (NH_4^+) may accompany the absorption of VFA anions. Heifers which had minimum values of NH_3 and TVFA in the rumen, the HCO_3^- concentration in their rumen was higher. This low concentration in rumen NH_3 and TVFAs may be attributed to the release of these two buffering components from the rumen into blood, at the same time, blood bicarbonates pass across the rumen wall. The main factor that control NH3 transepithelial flux seemed to be the concentration of unionized NH_3 in the ruminal contents and blood flow to the rumen (Remond *et al.*, 1993).

Rumen buffering capacity

Rumen buffering capacity at 2 hours postfeeding (Table 2) was approximately equal in all animals (buffering capacity per 2 ml rumen liquid ranged from 0.023 to 0.029 m.eq. at pH = 4.0 and it was 0.009 to 0.013 m.eq. at pH =8.0). This denotes effective function of the buffering components in the rumen liquor.

pH values

The mean pH values in rumen liquor differed between animals within the range 6.19 - 7.98 that is 1.79 degrees of pH value (Table 2). This may be due to an increase in saliva bicarbonate concentration at the time of sampling. In spite of this wide variation of pH of rumen liquor the blood pH value was almost stable within the normal range in all animals (pH = 7.23 - 7.43). Upadhyay (1993) stated that maintenance of normal blood pH depends ultimately on the activity of blood buffers which effectively resist appreciable changes in the pH.

It is clear in table (2) that their was parallel trend in the values of haemoglobin concentrations and blood serum pH values. It has been proved

Table 2. Means ± S.E. of pH values in rumen fluid and blood, buffering capacity and haemoglobin in buffalo heifers

Item \ Animal No.	1	2	3	4	5	Overall mean
pH values:						
Rumen pH	7.24± 0.29	6.88 ± 0.45	7.78 ± 0.37	6.19 ± 0.14	7.01 ± 0.22	7.02 ± 0.17
Blood pH	7.37± 0.13	7.32 ± 0.17	7.23 ± 0.25	7.39 ± 0.07	7.43 ± 0.15	7.35 ± 0.07
Rumen buffering capacity:						
at pH = 4.0	0.029± 0.01	0.025 ± 0.00	0.023 ± 0.00	0.025 ± 0.01	0.025 ± 0.01	0.025 ± 0.00
at pH = 8.0	0.011± 0.00	0.013 ±0.00	0.009 ± 0.00	0.010 ±0.00	0.009 ±0.00	0.010 ± 0.00
Blood Hb (g/100ml)	9.88 ± 0.29	8.02 ± 0.42	7.72 ± 0.36	8.91 ± 0.43	8.23 ± 0.59	8.55 ± 0.24

* Each mean represents 5 observations for each animal.

that haemoglobin has an important biological function in buffering for pH stabilization (Frandson, 1986 and Neama, 1987).

It is expected that there are integrated control on the exchange of buffering components between blood and rumen liquor to fulfill stability of the vital pH normal in blood. This concept needs extensive research work.

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منظمات الحموضة والقلوية فى سائل كرش عجلات الجاموس وعلاقتها بالمنظمات فى الدم.

نـعمة أحمد عشماوى

قسم الإنتاج الحيوانى – كلية الزراعة – جامعة القاهرة.

أجريت هذه الدراسة فى معمل فسيولوجيا الحيوان بكلية الزراعة جامعة القاهرة لدراسة مُنظمات الحموضة والقلوية فى الكرش وعلاقتها بالمنظمات الموجودة فى الدم فى خمسة عجلات جاموسى عمرها ١٨ – ٣٠ شهر.

- وقد تم سحب عينات سائل الكرش بواسطة اللى المعدى بعد ساعتين من التغذية على دريس ، عليقة مركزة ، قش أرز وقد تم تثبيت العليقة طوال فترة التجربة.
- عينات الدم تم سحبها من الوريد الوداجى جزء منها تم وضعة فى أنابيب معاملة بالهبارين وجزء أخر تحت طبقة من زيت البرافين لتقدير تركيز البيكربونات.
- وقد تم تقدير تركيز كل من الأحماض الدهنية الطيارة الكلية ، البيكربونات ، الفوسفات ، الأمونيا فى كل من عينات الدم وعينات سائل الكرش وتم تقدير قيمة pH لكل العينات عند وقت أخذ العينة، وتقدير كفاءة هذة المنظمات بالكرش.

وكان من أهم النتائج :-

- بعد ساعتين من التغذية تركيزات بيكربونات الكرش وصلت عند أعلى تركيز لها بينما تركيز الفوسفات تباينت بشدة مابين الحيوانات.
- أظهرت النتائج علاقة موجبة مابين تركيز الأمونيا فى الدم والأمونيا فى الكرش عند قيم pH القريبة من التعادل. بينما العجلات التى سجلت قيم منخفضة لتركيز الأمونيا والأحماض الدهنية الطيارة الكلية كان تركيز البيكربونات فى كرشها عالية وهذا قد يكون راجع لمرور الأمونيا والأحماض الدهنية الطيارة من الكرش للدم فى الوقت نفسة يتم عبور البيكربونات من الدم للكرش من خلال جدار الكرش.
- قيم pH سائل الكرش إختلفت مابين الحيوانات فى مدى من pH = 6.19 - 7.98 بينما pH الدم تقريبا ثابتة فى المدى الطبيعى لها pH = 7.23 - 7.43 حيث أن ثبات هذة الأخيرة يعتمد أساسا على المنظمات الموجودة بالدم.

- أما قيم كفاءة هذة المنظمات بالكرش كانت تقريبا متساوية فى كل العجلات (كفـاءة المنظـم فـى ٢ مل من سائل الكرش) وهذا قد يرجع لتغذيتها على علائق ثابتة طوال فترة التجربـة ويـدل علـى كفاءة عمل هذه المنظمات فى تنظيم درجة حموضة سائل الكرش.