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THE INFLUENCE OF LOW TEMPERATURE FODDER ON THE COURSE OF RUMEN FERMENTATION AND BLOOD ACID-BASE EQUILIBRIUM IN SHEEP

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ABSTRACT

The aim of this work was to determine the influence of feeding sheep with frozen fodder on the profile of rumen fermentation and its reflection in blood acid-base equilibrium.

The research was conducted on 7 clinically healthy sheep, cross-bred of 3 to 5 years of age and body weight of 40 to 45 kilograms. The animals were fed with hay and C-J concentrates with addition of 0.5 kg of frozen beet pulp per animal, twice a day. Round-the-clock continuous measurements of the sheep pH and rumen contents temperature were conducted and directly before feeding as well as in 2 and 4 hours after feeding measurements of the chosen parameters of rumen fermentation (VFA, FE, NGGR, number of protozoa) and acid-base equilibrium (pH, pCO_2, BE, HCO_3).
It was found that feeding the animals with frozen beet pulp causes increase of isovaleric and isobutyric acids production after 2 and 4 hours from feeding. An increase of the rumen contents pH after feeding with frozen fodder was also observed. The number of protozoa also increased within the same time intervals. The obtained

NGGR factor was slightly lower which gives evidence of better VFA utilization. An increase of base excess and actual bicarbonates' concentration after 4 hours from feeding with frozen fodder was observed with regard to acid-base equilibrium.
Basing on the obtained results one might be of the opinion that feeding with frozen fodder caused changes quickly compensated by the rumen ecosystem as well as by blood buffers.

Key words: sheep, frozen fodder, rumen fermentation, acid-base equilibrium

INTRODUCTION

Among ruminants, sheep constitute, in many countries, an important group of animals supplying man with necessary meat, leather, wool and dairy products. They are important protein suppliers. A leading position of sheep among the cheap protein producers is explained by the fact that they can live everywhere where there is any vegetation and the development of this group of animals is a consequence of fodder availability. Of all the animals ruminants are the best prepared for the conditions of an extensive breeding and their bodies hold great production reserves [88, 90]. Sheep can eat and use low quality fodder (fibrous, containing protein of lower biological value), growing on poor soils and pastures. Sheep do not compete with man for organic alimentary components as it is in case of animals with stomach. Ruminants, best of all domestic animals, can use chemical compounds of plants for the benefit of themselves as well as the breeders and consumers [24].

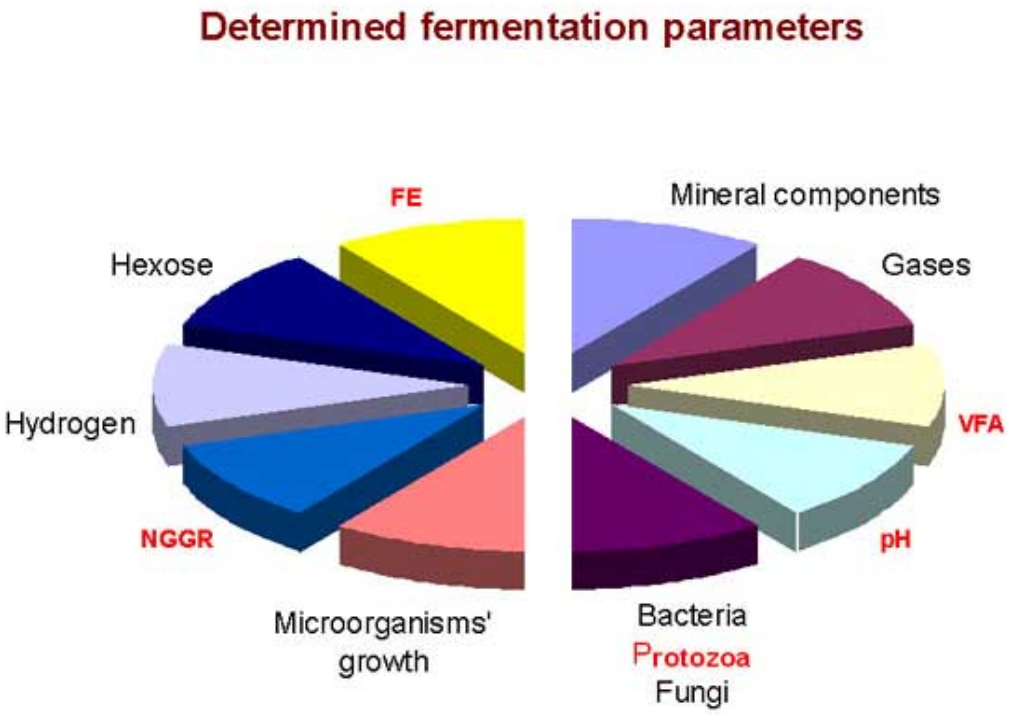
Ruminants often exist in extreme environmental conditions. This means not only those which form the environment (e.g. mountainous areas, deserts and so on) but also the fodder they are fed with. In spite of the fact that ruminants posses the ability to transform vegetable waste and substrates which are difficult to reach by the rumen microflora and microfauna, breeding them requires ensuring relative stability of the alimentary tract internal environment of which forestomachs are an important part. This ability of the ruminants concerns, among others, possibility of feeding them with various waste and by products of many branches of food processing industry (brewing, alcohol distilling, sugar and meat processing industry and others).They transform non-full value protein contained in this waste into full value one which is used by man as a final product eventually. For a long time there has been a problem of utilizing not only many kinds of seemingly useless for human nourishment products but also of fodders unsuitable for monogastric animals not only because of the lack of forestomachs but also because of their form and formula. Such is the situation in case of fodder subject to long lasting low temperature causing its freezing also in our conditions, for example, silage during winter time.

Normally, in order to maintain correct metabolism inside rumen, as in every ecological system, suitable conditions ensuring the so called homeostasis [3, 4, 17, 20] must be maintained. Knowledge of homeostasis allows clinicists, dieticians, physiologists and biochemists alike to shape fermentation processes otherwise called manipulation, steering or modification of digestion in the rumen [24]. Not only the necessity of suitable feeding and maintaining health demand these actions but also animal production economics [3, 4, 7, 12, 13, 14, 23, 24, 27, 35, 36, 69, 80, 83, 90, 91].

The following parameters describing rumen fermentation are presently considered as the most important:

- efficiency of volatile fatty acids fermentation - VFA (FE - *Eng. fermentation efficiency*)
- nonglucogenic : glucogenic utilization ratio VFA (NGGR - *Eng. nonglucogenic : glucogenic ratio*)

- volatile fatty acids production
- % share of the particular VFA in the general pool of these acids
- pH
- temperature
- total number of protozoa, bacteria and fungi
- hydrogen production and salvage
- level of mineral components
- quantity of the fermented hexose
- microorganisms' growth efficiency (*Eng. cell yield*)
- total gas production and participation of methane (CH_4) and carbon dioxide (CO_2) in it
- oxidation-reduction potential
- anaerobic conditions for microflora and microfauna growth.



The basic ruminants' fodder component is cellulose constituting over a half of the Earth's biomass and the main, almost inexhaustible source of alimentary, organic carbon. Bacteria and protozoa prevail among the microorganisms inside four-chambered stomach - especially inside rumen - under normal alimentary conditions. The first constitute flora, the others - fauna.

According to Kuenzle and Jenny [50] and to Yokohama and Johnson [87] we can distinguish 8-10 functional groups among the rumen bacteria. Affiliation to any of these groups is not based on the bacteriological taxonomy but on the substrates decayed by the bacteria and on the final products of this decay. Thus, we distinguish the following bacteria: cellulolytic, hemicellulo- and pectinolytic, amylolytic, saccharolytic, proteolytic and dezaminating, ureo- and biurolytic, lipolytic, acidolytic (mainly lactolytic), metanogenic and others.

Numerous species of the rumen bacteria differ from each other by their specific demands concerning an optimal environment, specific metabolized substrate and the necessary

alimentary factors; they also differ by the specific metabolism products. There are, however, no significant differences between microflora of the cattle, sheep and goats forestomachs [16, 45]. Besides, the above mentioned authors also state that bacteria population of the rumen is very stable in the presence of the "foreign bacteria" supplied with fodder and might be dominated and restrained only under the exceptional conditions. Conditions inside the forestomachs (large contents of liquid, great buffering ability of the contents, maintaining of stable pH, regular supply of the alimentary material and continuous removal of the metabolism products, anaerobic conditions, stable temperature) allowed for the development of the specific microorganism population adapted to life in the anaerobic atmosphere. They live in symbiosis with their host - ruminant for the great benefit of both partners. The rumen constitutes a complex, anaerobic ecological system (the so called ecosystem) based on numerous biochemical reactions and mutual relations between great number of the microorganisms living in the rumen environment and their relations with the host animal organism. The rumen environment might also be seen as an integrated mixed culture of mutually dependent and cooperating with each other various species of microorganisms, where for example, a change in number or metabolic activity of one species causes similar changes of the other species.

Fermentation reactions belong to the most important biochemical reactions inside rumen. Rumen fermentation means the whole of the enzymatic decay processes including metabolism of the organic substances - mainly sugars, from substrate to the final product, taking place under anaerobic conditions [3, 80]. In the course of fermentation processes microorganisms receive chemical energy from glucose and other organic compounds and, because of the lack of molecular oxygen they use it to meet their own energetic demands or accumulate in the form of ATP. Microorganism growth is limited by quantity of the available ATP [14]. The ruminant creates living conditions necessary for them, but microorganisms allow for the decay of large quantities of the fibrous fodder taken by the animal and, after their death, are a source of bacteria protein of greater biological value than the protein contained in vegetable fodder for the ruminant. The rumen is a capacious fermentation chamber inside the ruminant organism where, apart from the fermentation of easily soluble hydrocarbons, the decay of cellulose and other, difficult to digest, polysaccharides, takes place aided by the specific microorganisms' enzymes [48]. Multi-chambered stomach is indispensable for the ruminant [75]. Total energetic demand of the ruminant is met with the energy released inside tissue during oxidation-phosphorylation of the rumen fermentation products in 66-88%. Thanks to fermentation processes inside rumen the ruminants stay alive on the diets that would not be suitable for other animals because of energy and protein contents.

Fodder contentss are transformed during fermentation into products useful for the host (VFA, bacteria protein, group B vitamins), useless (CH_4, CO_2) or even harmful (ammonia, nitrates). Fodder saccharides, proteins and fats, complex before fermentation, are broken into simple molecules (monomers) as it happens, for example, before absorption in the small intestine. VFA, CH_4, CO_2 and ammonium acid among others, belong to the final, continuously removed products of rumen fermentation. CO_2 and H_2O and not volatile fatty acids, constituting the main source of energy available to the ruminant, would be rumen digestion products in the presence of O_2[47]. Composition and activity of the rumen flora vary daily, especially after grazing. Bacterial population of the rumen reacts with the greatest alterations in case of changing of one fodder to another (for example, from volumetric to protein one) and in case of changes in the fodder contents and its physical form [18, 37, 82]. Fodder taken by an animal, its quantity, kind, alimentary components' contents (especially hydrocarbons), the degree of fragmentation and other factors have a decisive influence on the generation of

volatile fatty acids. The inhibitory influence of the excess of fatty acids on motor activity of the rumen and its contents pH is also known. The strongest and the longest lasting inhibitory activity is caused by the following acids in this order: butyric acid, acetic acid, lactic acid and propionic acid [11, 77].

Protozoa constitute the rumen microfauna. Their number varies from 10^3 to 10^6 in 1 ml of the rumen liquid [2] (according to Yokoyama and Johnson [87] from 10^5 to 10^6 in 1 g of the rumen contents). It is assumed that there are 1000 times less protozoa than bacteria in the rumen liquid but their body mass results in the volume almost equal to that of the bacteria mass. Protozoa of the ciliates (*Ciliata*) subtype are found as a rule in the domestic animals rumen. The rumen protozoa, like bacteria, are unable to live outside the ruminant organism. They are transplanted ("transmitted") through the direct contact of the animals. Their total number is variable and depends on the fodder, season of the year and the animal's age [23]. About 5%-15% of the rumen contents is composed of Infusoria and their mass renews approximately 1 a day. A few day long one-sided feeding of the ruminant leads to reduction of the great number of particular kinds of protozoa and after 4-5 days fasting they disappear completely from the rumen contents. It is thought that protozoa and bacteria alike belong to the factors stabilizing rumen fermentation [10].

Other microorganisms important for digestion processes of the forestomachs are:
- **Fungi -** anaerobic, cellulolytic of *Neocallimastix sp*. and *Piromonas sp*. kinds [49, 69], after feeding the ruminants with a diet rich in straw and hay and such, their number might reach 8% of the microbiological mass present in the rumen (10^4 in 1 ml of the rumen contentss). Though they are very important for the rumen ecosystem, their role still isn't definitely explained; they probably digest cellulose and xylenes which is essential for the digestion of fibers.
- **Mycoplasms -** absolutely anaerobic mycoplasms were found in the rumen of cattle and sheep [2, 38, 48]. Two species were isolated, identified and named *Anaeroplasma bactoclasticum* and *Anaeroplasma abactoclasticum*.
- **Bacteriophages -** those found in the rumen of cattle and sheep are present there in minimal numbers of $5x10^7$ up to 10^{12} phages/ml of the rumen liquid. About 125 different morphological types were identified. Up till now no specific or significant role of the rumen bacteriophages has been proved [2, 69].

Rumen fermentation influences creation of the acid-base equilibrium (abe) of blood [11, 34, 35, 79, 89]. The intensity of the fermentation processes causes appropriate modification of the abe parameters. Speed and intensity of the blood abe disorders depends on the speed of acids absorption from the rumen and on the continuous dissociation of particular acids. Acid absorption in the acidic environment depends on their solubility in fats and therefore on the length of their carbon chain. The fastest diffusing is butyric acid, next propionic and then acetic acid. [9, 11, 65]

It was not find any works concerning the influence of the low fodder temperature on the rumen fermentation processes and acid-base equilibrium parameters in sheep in the available literature, many authors, however, describe the influence of the environment temperature on digestion or on the energetic metabolism of the ruminants [8, 22, 31, 44, 67, 85]. Graham et al. [31] examined energy level changes in sheep at three levels of feeding and at seven temperatures. They proved that metabolic energy of the animals kept at lower temperatures ensured heat corresponding to the minimal metabolism. Baxter et al. [8] examined termoregulation in sheep using three feeding levels and different environment temperatures.

They described in detail heat losses and critical temperatures. Rogerson [67] conducted research of beef earlings and did not find any influence of the environment temperature within 20-40°C range on heat production in the animals on the low feeding level. Delfino et al. [22] examined beef earlings' weight gain and found lower utilization of the metabolic energy in the animals kept in the cold. Similarly, Keyserlingh and Mathison [44] observed decrease of digestibility and utilization of fodder in sheep kept at 4°C. Westra and Christopherson [85] examined digestibility, utilization and retention time of the various kinds of fodder in sheep kept at the environment temperatures of 0.8°C, 10.1°C and 17.7°C. They found a depressive influence of the low environment temperature on fodder digestibility and its passage through digestive tract. The influence of the fodders subject earlier to low temperature was also examined. Pasha et al. [62] fed sheep with hay which was earlier frozen or vacuum stored. It was brought to the environment temperature before feeding. The authors observed shortening of retention time and decrease of digestibility parameters of hay stored by freezing. Beever et al. [5, 6] conducted similar experiments determining nitrogen solubility and energy digestibility in the forestomachs and small intestine after feeding with previously dried or frozen fodder (grass soilage). Sheep were fed the fodder brought to the environment temperature. The authors found that significant differences of particular parameters exist only between the fodder dried at high temperature and the fresh or frozen one. Digestibility factors and nitrogen degradation were similar in case of fresh and frozen fodder. Thomson et al. present similar conclusions in their study edited by Ruckebusch [70] showing fodder preserved by freezing as the equivalent of the fresh and in comparison to frozen one. Loesche et al. [51] examined utilization of soy oil from previously frozen beans by sheep. The oil was administered in quantity of 0%, 7%, 14% and 21%. Decrease of VFA, NH_3 and metabolism energy were observed in case of fodder sets with 14 and 21% of frozen soy bean oil contents. Eriksson et al. [25] examined a possibility of stress occurrence after intraruminal administration of cold water (at 10°C and 7.5-10% of body weight) in goats. They observed increase of blood adrenaline and noradrenaline levels afterwards with clinical symptoms of stress, which is why they advice supplying liquids at body temperature.

The aim of this work was to determine the influence of feeding sheep with frozen fodder on the chosen parameters of rumen fermentation: pH, temperature, VFA production, fermentation efficiency, NGGR factor, protozoa number and venous blood acid-base equilibrium parameters.

MATERIALS AND METHODS

The research was conducted on 7 healthy sheep of Polish Merino race, aged 3 to 5 years and weighing between 40 to 45 kilograms. The sheep had cannulas installed in their rumen before the experiment [21]. They were fed with hay and protein fodder mixture C-J according to feeding standards [7, 40, 71]. This fodder set was supplemented with fresh (in the first part of the experiment) and frozen and fragmented (in the second part of the experiment) sugar beet pulp in the quantity of 0.5 kg/sheep. Daily fodder dose was between 1.8 do 2.3 kg and was fed to the sheep in even doses twice a day, at 8.00 a.m. and 2.00 p.m. The sheep ate frozen and fragmented fodder in 2-5 minutes. Continuous, round-the-clock measurements of rumen pH and temperature were taken. Microprocessor pH-meter PM 600 with combined electrode ERH-123-6 type (especially longer) and temperature sensor PT 100 were used for this purpose [66]. The pH-meter was calibrated to pH 4 and pH 7 at 39°C before each measurement. The records were not corrected. The electrode was washed in 0.1 n HCl and distilled water after each measurement and then used for the next one. Samples of the rumen contentss were taken through cannula before feeding and then in 2 and 4 hours after feeding

(according to many authors [28, 60, 86, 91] the peak fermentation moment occurs 4 hours after feeding); at the same time blood for acid-base equilibrium assay was drawn. The rumen contentss taken for the analysis of volatile fatty acids was stabilized with formic acid (0.25 ml of formic acid was added to 5 ml of rumen contents), and for protozoa number assessment - with 4% foramen (2.5 ml of formaldehyde were added to 2.5 ml of rumen contents). Blood acid-base equilibrium was assayed directly after drawing the blood sample from external jugular vein. The blood was anaerobically drawn into heparinized syringes. The samples were placed in a thermos flask at +4° - +8°C for transfer to the laboratory. Administration of the frozen fodder was repeated 6 hours after the morning feeding and sample taking 2 and 4 hours afterwards. In this way 2 "feeding cycles" in twenty four hours were obtained. The measurements were during 70 "feeding cycles" with 158 samples of the rumen contents and blood alike taken. The abe assays were conducted with type BME 33/BGA 3 Astrup apparatus made by Radiometer [46, 52, 72]. Volatile fatty acids of the rumen contentss preserved with formic acid were assayed with gas chromatography method [15, 26] using Hewlett-Packard HP5890A apparatus. Gas chromatography conditions were as follows: split-splitless feeder, split set for 1:50, feeder temperature of 200°C, FID detector, temperature 250°C, HP20M column (Carbowax 20000) 25m x 0.32mm, film 0.3μm, isotherm 120°C, hydrogen carrier gas. Fermentation parameters such as: fermentation efficiency (FE), nonglucogenic: glucogenic volatile fatty acid ratio (NGGR) were then calculated according to formulas given by Chalupa [13] and Czerkawski [17] and Church [14] and presented below:

VFA (FE) fermentation efficiency [%]

$$\frac{0.62 \times \text{acetic acid} + 1.09 \times \text{propionic acid} + 0.78 \times \text{butyric acid} \times 100}{\text{acetic acid} + \text{propionic acid}}$$

NGGR ratio

$$\frac{\text{acetic acid} + 2 \times \text{butyric acid} + \text{valeric acid}}{\text{propionic acid} + \text{valeric acid}}$$

Protozoa number was calculated with chamber method (samples were preserved with formaldehyde). The rumen contentss was filtered through 4 layers of surgical gauze and then 2.5 ml of 4% formalin were added to 2.5 ml of the sample and tightly closed with cork. Formalin causes death of protozoa and allows preservation of the sample which then might be stored for a long time. The sample was poured into a vessel with tight-fitting, ground glass stopper and supplemented with 20 ml of distilled water which washed the remaining contentss, before counting. In this way a tenfold dilution of the sample was obtained. After closing the vessel with stopper, its contentss was thoroughly mixed and then a protozoa suspension was drawn and put under Fuchs-Rosenthal chamber cover glass placed under the microscope. The protozoa were counted in 20 small rectangular prisms at 100 x magnification after falling to the bottom of the chamber. Protozoa from the same sample of the rumen contentss were counted four times. Total count was conducted in 80 small rectangular prisms in four consecutive assays (each of 20 rectangular prisms). A total of the protozoa in 80 rectangular prisms of Fuchs-Rosenthal chamber corresponds to their number in 0.001 ml of the rumen contentss in tenfold dilution. The number of protozoa in 1 ml of the undiluted rumen contentss was obtained by multiplication of the above number by 1000:

$$X = n \times 1000 = \text{number of protozoa in 1 ml of the undiluted rumen contentss}$$

Mean values and standard deviations were calculated through mathematical analysis [73]. Apart from that, the obtained results were also verified with Student t-test. Levels of significance were determined with Student t-test. The differences of $p \leq 0.001$ were adopted as extremely highly significant, $p \leq 0.01$ as highly significant and $p \leq 0.05$ as significant. Values obtained after 2 and 4 hours were compared with the initial values (that is before feeding), then values obtained after 2 and 4 hours were compared between themselves. Next, values obtained during the first stage were compared to the corresponding values from the second stage. In this way we obtained a picture of relations which might occur depending on time passed after feeding in case of one fodder kind and the influence of various fodder kinds (fresh and frozen).

RESULTS AND DISCUSSION

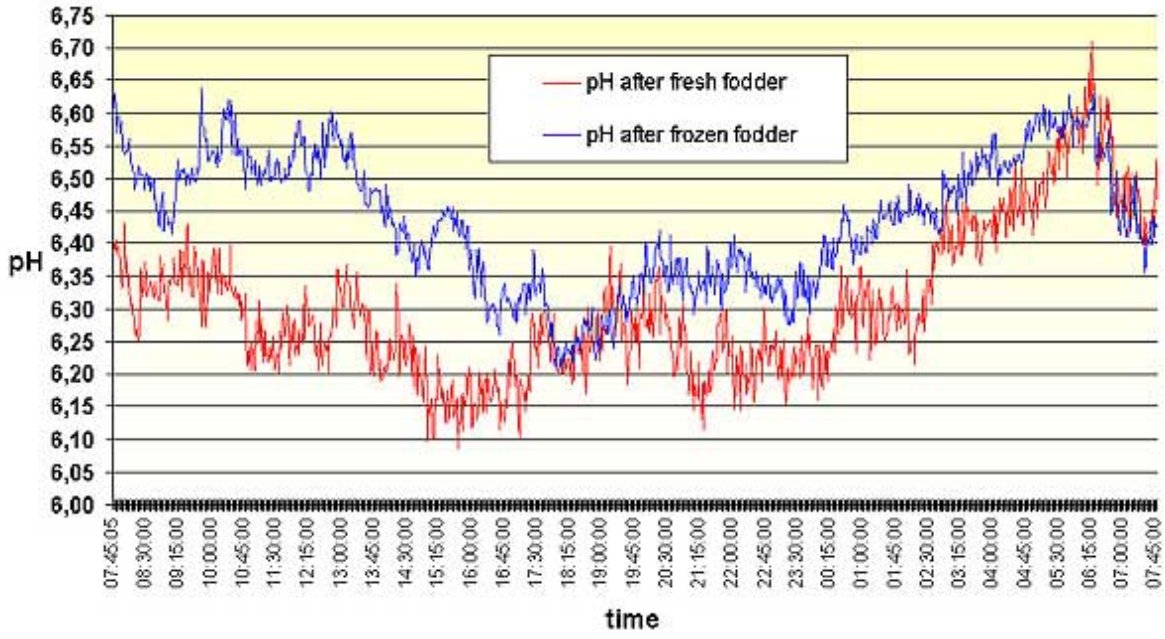
The problem of rumen fermentation in sheep and acid-base equilibrium of those animals blood under the influence of low temperature fodder was not described by the available Polish and foreign literature, as was mentioned before.

Rumen contents pH

Rumen contents reaction, or pH, is undoubtedly one of the most important parameters describing rumen fermentation. This parameter values obtained during first stage of the experiment were 6.10 to 6.70 and 6.20 to 6.65 during the second stage. Fluctuations of the rumen contents pH value of cattle [42] and sheep [60] during round-the-clock measurements have already been described by other authors. The relationship with feeding time, rumen movements and ruminating are considered among the factors conditioning this phenomenon [42, 60, 86]. It was determined that rumen contents pH after feeding changes in a characteristic way, that is begins decreasing reaching its lowest value about 4 hours after feeding (fermentation peak and the greatest VFA production); then increases gradually until reaching again initial values [86, 28]. Okamoto [60] observed pH fluctuation cyclicity synchronized with rumen movements. Ruminating caused pH increase in a few minutes after the beginning of this process and decrease after its conclusion [60]. The authors also describe significant pH changes of the rumen contents during periods without rumination and rumen movements which suggests the existence of still different factors influencing transitory pH changes. Most of the observed changes depended on the sensitivity and kind of the electrodes used for measurements. Johnson and Sutton [42] made "continuous" pH measurement records, yet did not notice most of the changes because, it seems, of too slow reaction of the used electrode (steel housing, silver capsule). Okamoto [60] increased frequency of the measurements to 15 minutes which allowed him to observe the above mentioned phenomena. In own research was used measuring-recording microprocessor set which registered the measurements every 90 seconds (960 measurements of pH value were made during 24 hours). Such high measurement sensitivity allows to call it continuous and shows how often slight pH value fluctuations occur. Round-the-clock measurements results were averaged and shown in the graph. In our own research we proved that feeding with frozen fodder causes significant increase of the rumen contents pH. In order to better present the experiment and for statistical purposes 10 minute time intervals were chosen just after administering the fodder, after 2 hours and after 4 hours from feeding. Average values of pH ratio in those intervals were for the first part of the experiment respectively: 6.29; 6.26; 6.24; and for the second part of the experiment: 6.49; 6.45; 6.40. These values remain within the generally accepted standards considered as physiological [27, 41, 60, 63, 66, 91]. It is impossible to exclude the influence of the electric potential of the rumen muscular layers and other electrophysiological factors. It

is supposed that rumen contents pH fluctuations testify mainly to the changes of VFA quantity which are produced inside rumen and which was observed in experiment. Dependence on the quantity of the produced saliva is also described. The produced saliva quantity is clearly bound to physical characteristics of the fodder (such as its fragmentation or compression) and rumination frequency [60].

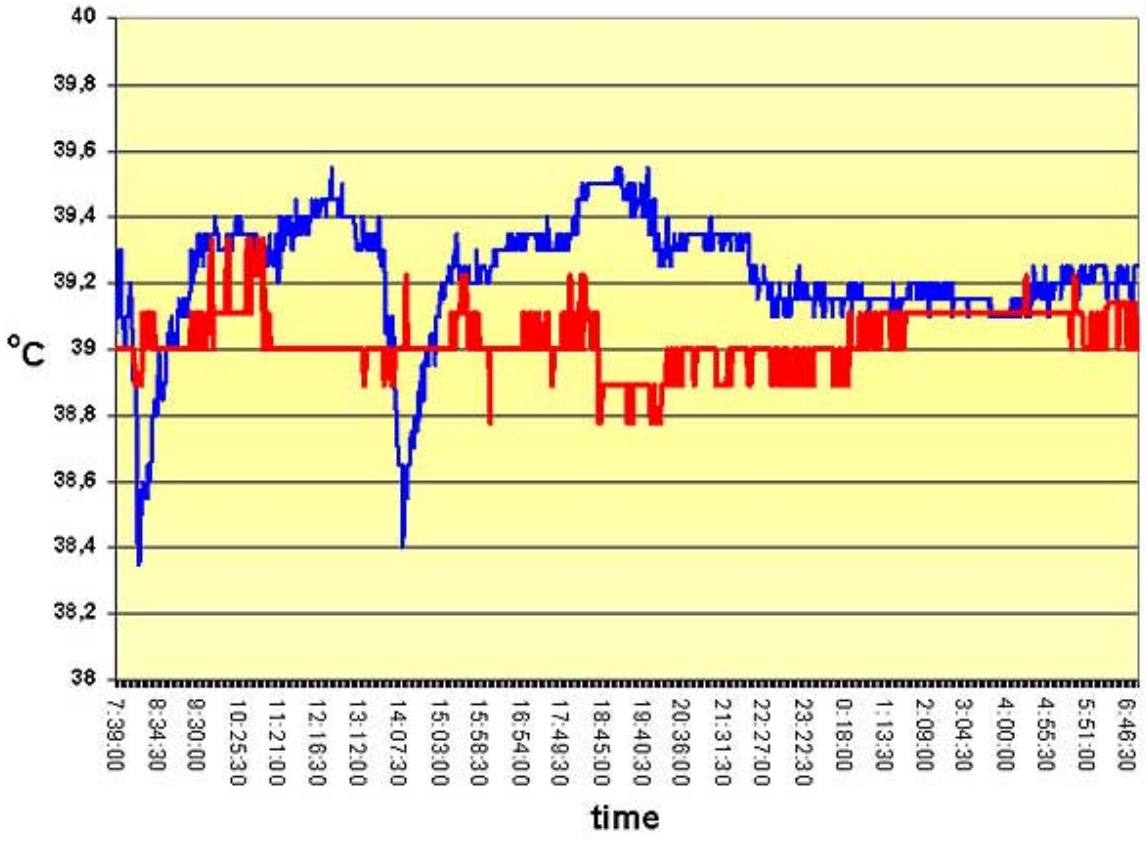
Graph 1. Average rumen contents pH values of the sheep of groups I and II in the continuous round-the-clock measurement.



Rumen contents temp erature

Rumen contents pH measurement was conducted simultaneously with control and recording of the temperature inside rumen (graph 2). It was very important since in order to recognize the results of rumen contents acidity measurements as objective it is necessary to relate them to the actual temperature inside the rumen. While conducting measurements at the temperature different from calibration temperature, the used pH-meter PM 600, made compensation of the electrode characteristics slope basing on the conventional method of the slope increase with value of 0.1984 mV/deg. The second reason for temperature measurement was a possibility of observation of the eventual changes of rumen contents temperature after feeding with a portion of frozen fodder (at -4°C). Rumen contents temperature obtained in the first part of the experiment was on the average 39.0°C $\pm$ 0.2 which corresponded to the results obtained by the author earlier [66]. O'Kelly, examining rumen contents of the different races of cattle [59], obtained similar measurement results - from 37.5°C to 38.8°C. Measurements of this parameter during the second part of the experiment showed temperature decrease of about 1°C, maintained for about 15 - 25 minutes, directly after feeding with frozen fodder. It shows how fast the rumen ecosystem homeostasis is restored. It certainly depends on the quantity of the "transformed" fodder - in this case with lower temperature, however, the effort was made in this experiment to create conditions close to natural ones, where quantity of the fed fodder was limited by the alimentary dose.

Graph 2. Average temperature values inside the sheep rumen after feeding with fresh and frozen fodder.

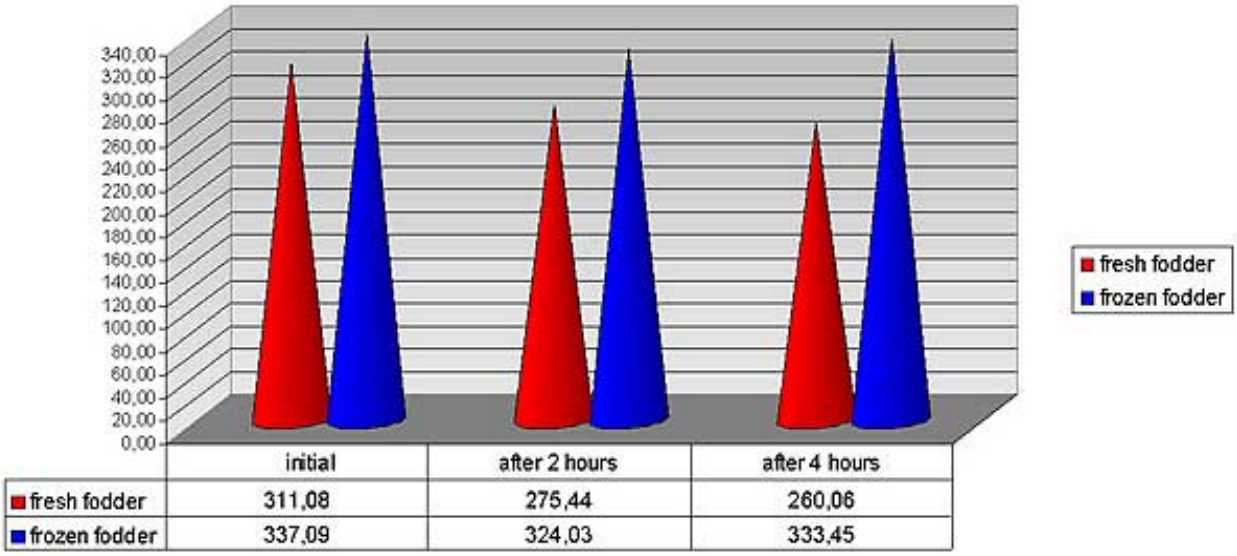


Number of protozoa

Numbers of protozoa observed during the first part of the experiment (fresh fodder) were: 311.0 thous/ml, 275.5 thous/ml, 260.0 thous/ml (respectively: before feeding, after 2 hours and after 4 hours from feeding). After feeding with frozen fodder (second stage of the experiment) these numbers for the similar time intervals as before were respectively: 337.09 thous/ml, 324.03 thous/ml, 333.45 thous/ml. The values oscillate within the lower ones considered as physiological. The authors point to the different fauna concentrations in the rumen contents. Anderson [2] adopts $3.00x10^5$/ml - $6.00x10^5$/ml as a standard, Franzolini et al. [30] reports about values of $3.61x10^5$/ml to $6.42x10^5$/ml or even to $8.05x10^5$/ml in his results depending on the contents of concentrates in the fodder. Close relationship between the number of protozoa in the rumen contents and dietary supplements was also described by Grubb and Dehority [33] and by De Semet et al. [19]. Abe et al. [1] obtained $3.99x10^5$/ml and Michalowski [56] from $4.8x10^5$/ml to $12.5x10^5$/ml depending on the time of day and feeding frequency. Bieganska and Szynkiewicz [7] report, like Hungate [39], the value of 10^6/ml as the average. Low numbers of protozoa might be explained by the adaptation of microorganisms to the kind of fodder. It is a long lasting process which was proved by Mackie et al. [54]. A marked tendency to the increased number of protozoa after feeding with frozen fodder, especially in relation to the corresponding time intervals in the first part of the experiment, might be related to the observation made by O'Kelly [59] while comparing rumen fermentation parameters of different cattle races. The author showed that permanently lower rumen contents temperature (of about 0.5°C) in some races was positively correlated with greater protozoa number (of about 30%). The consequences of decreased protozoa number might also be observed in the rumen pH values. Veira et al. [81] and Rowe et al. [68] describe

a decrease of pH at the peak of fermentation in defaunated sheep (after 2 - 4 hours from feeding). A similar relationship was observed in our own experiment.

Graph 3. Protozoa numbers in the sheep rumen contents after feeding with fresh and frozen fodder

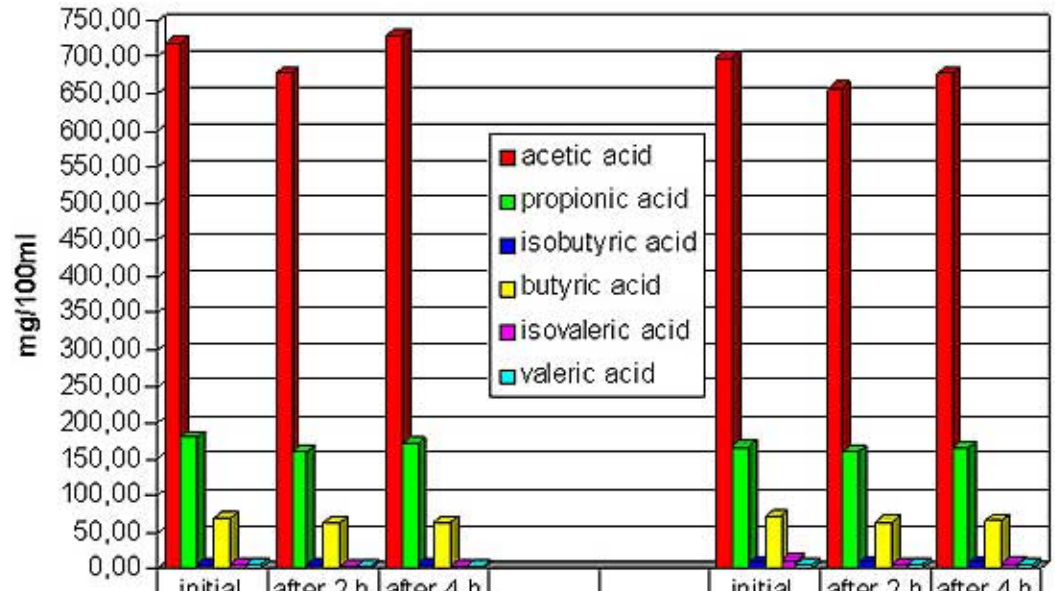


	initial	after 2 hours	after 4 hours
fresh fodder	311,08	275,44	260,06
frozen fodder	337,09	324,03	333,45

Volatile fatty acids (VFA)

Volatile fatty acids' (VFA) levels depend on the rumen contents pH, diet kind and time interval between feeding and sample taking [76]. The relation between VFA level and protozoa number is also described [17, 55]. Mean values of the assayed volatile fatty acids (acetic, propionic, isobutyric, butyric, isovaleric and valeric) in mg/100 ml of the rumen contents were shown in graph 4. The obtained VFA levels do not differ from those obtained by other authors [27, 29, 76, 86, 91]. Some scientists adopt total VFA level as a comparative coefficient in their experiments, however, assays of particular acids' fractions seem necessary for the observation accuracy. Perier et al. [64] observed during their research of volatile fatty acids' absorption that particular VFA concentration in the rumen contents differed depending on the kind of fodder, while their total pool remained unchanged. A visible tendency of acetic acid level decrease after 2 hours from feeding and then its increase after 4 hours in our own research is similar to that described by Fenner et al. [28] and Gray and Pilgrim [32] with regard to sheep. Stewart et al. [78] described a similar situation in cattle after 1-2 hours from feeding. The observation concerning iso-acids' forms (isobutyric acid and isovaleric acid) seems very interesting. In spite of their small quantities their level determines effective utilization by protozoa. Acetic and butyric acids are used by protozoa and bacteria in very small quantities. The observed decrease of isobutyric and isovaleric acids' level after 2 and 4 hours from feeding during the first and the second part of the experiment is also described by other authors [32, 78].However, after feeding with frozen fodder the observed level decrease was smaller. (Relatively greater quantities of iso-acids could influence their utilization by microorganisms which seems to be reflected in protozoa numbers). In his research of defaunated sheep Mendoza [55] proved interdependency of certain VFA levels and protozoa numbers. He observed significantly lower levels of butyric, isovaleric and valeric acids in defaunated sheep. Czerkawski also made similar observations [18].

Graph 4.



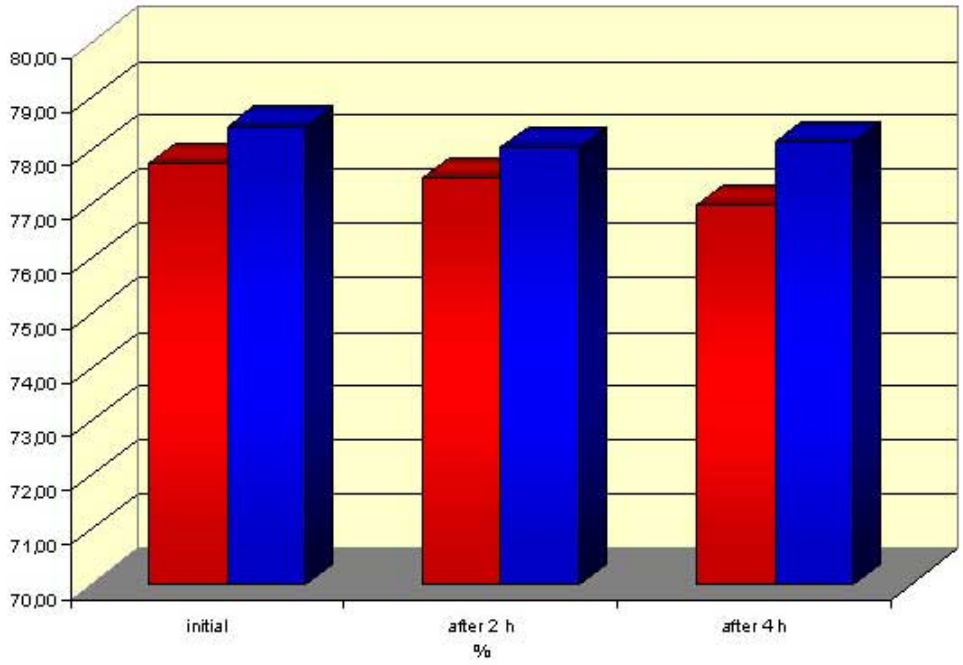
	initial	after 2 h	after 4 h			initial	after 2 h	after 4 h
acetic acid	718,82	677,21	728,71			699,02	656,41	677,15
propionic acid	180,05	160,87	171,48			167,16	160,02	166,97
isobutyric acid	6,58	5,70	5,79			9,45	8,73	8,81
butyric acid	68,69	63,16	63,12			71,70	64,87	65,45
isovaleric acid	6,77	4,47	4,56			11,02	7,43	7,89
valeric acid	6,34	4,77	3,96			7,58	7,18	7,18

Some authors obtained in their experiments great spread of the results and their unquestionable interpretation is rather difficult [45]. Therefore, it seems that the use of formulas and ratios suggested by Chalupa [13], Czerkawski [17] and Orskov [61], such as FE - fermentation efficiency and NGGR - nonglucogenic : glucogenic ratio, seems justified. Taking into account contribution of the particular VFA they allow for better reflection of the whole of rumen processes.

Fermentation efficiency /FE/

Values obtained in our own experiment after feeding with fresh fodder are respectively: 77.77%; 77.51% after 2 hours and 77.00% after 4 hours. After feeding with frozen fodder FE values were: 78.45%, 78.08% and 78.16%. These values are similar to those obtained by Zawadzki [91]. Czerkawski [17] adopt 75% as a minimal FE value. A tendency to FE value decrease was observed after feeding with fresh fodder, while no such tendency appeared after feeding with frozen fodder. However, it must be stressed that a difference had already existed before feeding the animal (higher FE in the group later fed with frozen fodder).

Graph 5. Rumen fermentation efficiency (FE) in sheep after feeding with fresh and frozen fodder

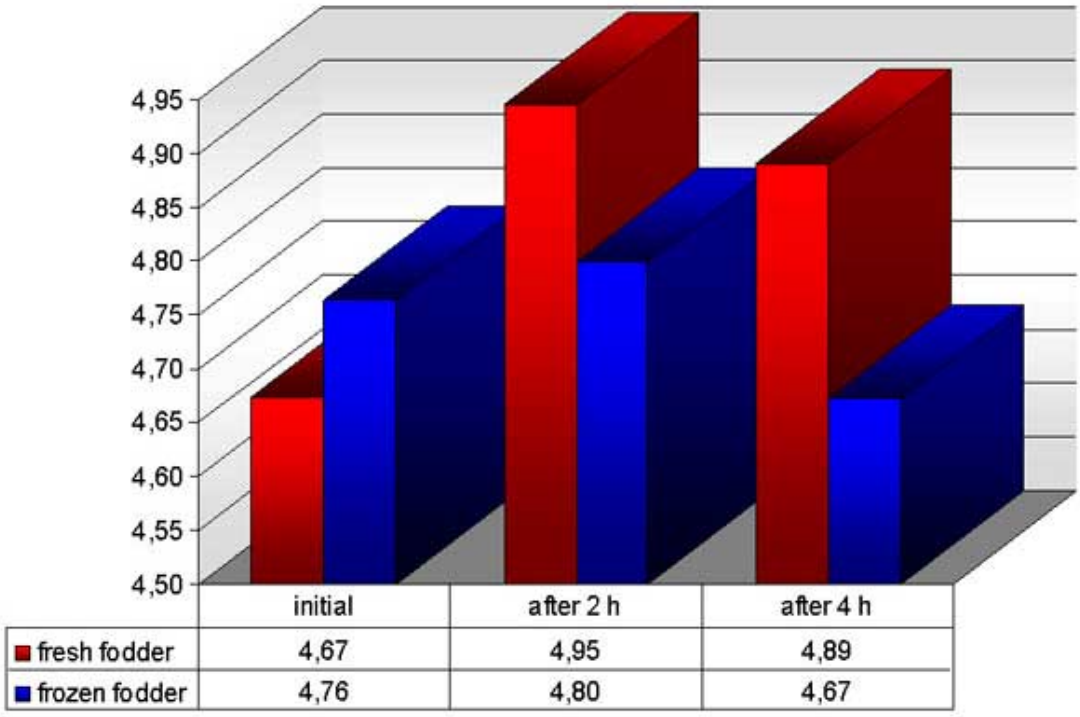


	initial	after 2 h	after 4 h
fresh fodder	77.77	77.51	77.00
frozen fodder	78.45	78.08	78.16

Volatile fatty acids' utilization ratio /NGGR/

Volatile fatty acids' utilization ratio takes also into account valeric acid levels which allows for a more thorough analysis of the rumen fermentation processes. Czerkawski [17] adopts the ratio value of about 3.5 as optimal. Orskov [61] regards similar values as optimal. Higher values testify to uncomplete utilization of fatty acids (which would be especially important in case of animals with productivity related to fat). The results concerning volatile fatty acids utilization - NGGR obtained in own research are shown in graph. During the first part of the experiment they were: 4.67 after feeding, 4.95 after 2 hours and 4.89 after 4 hours, respectively. After feeding with frozen fodder and within the similar time intervals they were: 4.76; 4.80; 4.67. These values are slightly higher than those obtained by Czerkawski [17] and Orskov [61] but close to those obtained by Zawadzki [91]. A significant increase of NGGR ratio (that is worse utilization) was observed after 2 and 4 hours from feeding with fresh fodder. Frozen fodder did not cause such differences but a significant decrease of NGGR value after 4 hours from feeding was observed in this case. In spite of the differences in the initial stage where the utilization was worse during the second stage, a distinct improvement occurs after feeding with frozen fodder. However, this interesting observation must be analyzed with regard to particular fodder and in this case it was frozen sugar beet pulp. It is not known whether a different fodder kind would give a similar NGGR reflection. The improvement of VFA utilization can be related to protozoa pool, which was higher within the analogical time, and to higher levels of iso-acids.

Graph 6. Nonglucogenic : glucogenic volatile fatty acids' utilization ratio /NGGR/ after feeding sheep with fresh and frozen fodder.



Venous blood acid-base equilibrium

Acid-base equilibrium (abe) of venous blood was based on the comparison of four parameters, that is pH, pCO_2, BE and HCO_3 [34, 43, 53, 57, 58, 74]. Reference values indicated by the authors or obtained by them during their own experiments with healthy sheep as the initial ones are shown in table 1.

Table 1. Reference or initial values of abe parameters

Authors	pH	pCO_2 mmHg	BE mEq/l	HCO_3 mmol/l
Schalm et al. [74] Kaneko [43]	7.32 – 7.50	38		21 – 28
Hejlasz and Nicpon [34]	7.48	36.1	+4.8	27.6
Nicpon [58]	7.43	37.6	+4.1	25.13
Lukomski [53]	7.35 – 7.44	30 – 45	-3.4 - +4.3	19.9 – 29.3

1 mmHg = 0.133 KPa

It appears from the presented results that sheep are characterized by high pH values as species. Lukomski [52, 53] showed it especially clearly conducting comparative research of sheep, horses and cattle and obtaining the highest pH values in sheep. Venous blood pH values obtained during the first part of the experiment were as follows: 7.45 just before feeding, 7.47 after 2 hours from feeding and 7.47 after 4 hours from feeding. After feeding with frozen fodder and at the analogical time intervals they were: 7.44; 7.47 and 7.47, respectively. Thus, no significant differences caused by fodder kind were observed.

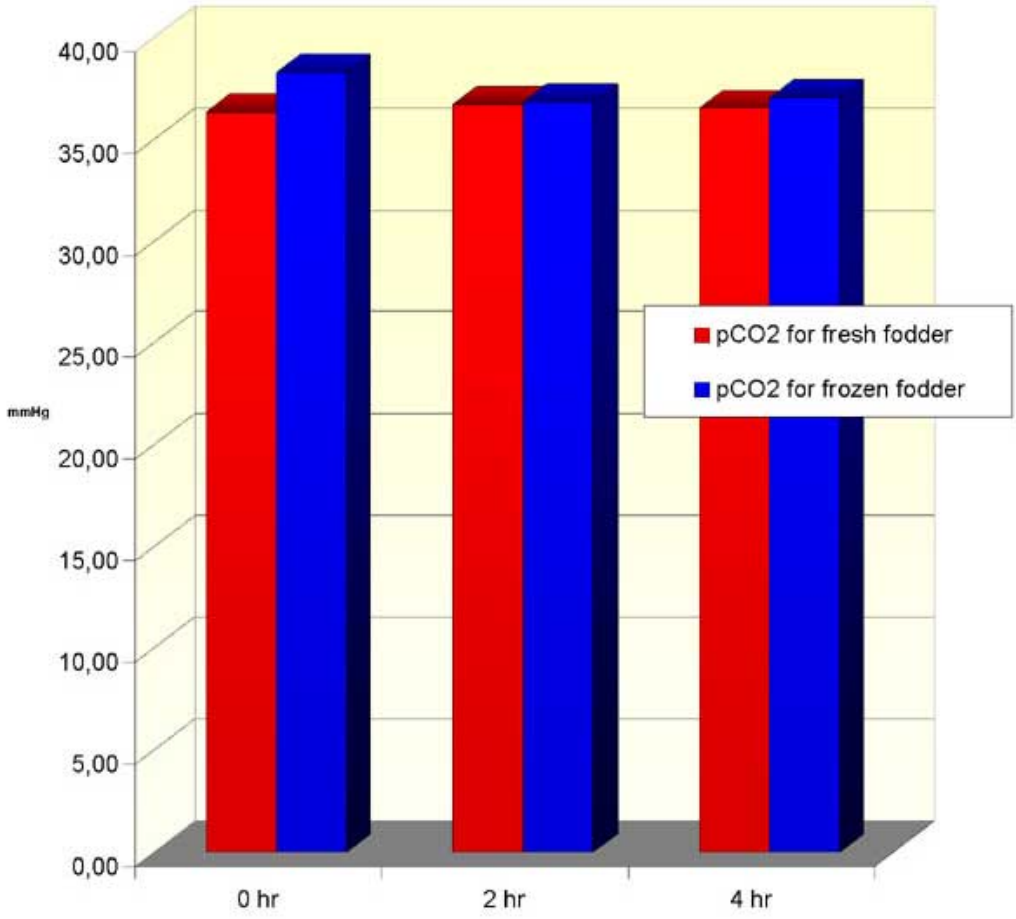
Graph 7. Venous blood pH in sheep after feeding with fresh and frozen fodder (mean values, n=42)

	0 h	2 h	4 h
pH after fresh fodder	7.45	7.47	7.47
pH after frozen fodder	7.44	7.47	7.47

All abe measurements results obtained during the experiment (in the first and in the second stage) do not exceed reference values suggested by the above mentioned authors.

Differences of pCO_2 values were also observed, that is higher initial pCO_2 values after feeding with frozen fodder. These values given in mmol/l were as follows: 36.36; 36.74; 36.55 for the first stage and 38.31; 36.87 and 37.07 for the second stage. Mean BE values in mEq/l after feeding with fresh fodder were as follows: 1.43; 2.79 and 2.65 and after feeding with frozen fodder: 1.49; 3.30 and 3.44. HCO_3 levels in mmol/l after feeding with fresh sugar beet pulp were as follows: initial 25.12; 26.40 after 2 hours and 26.24 after 4 hours. After feeding with frozen fodder HCO_3 values in mmol/l were as follows: 25.55; 26.75 and 26.98, respectively. A statistically significant increase of base excess and actual hydroxide concentration after 4 hours from feeding with frozen fodder was observed in comparison of fodder kinds. These values, however, remain within the standard limits adopted by the above mentioned authors.

Graph 8. Venous blood pCO_2 mean values (in mm Hg) in sheep fed with fresh and frozen fodder

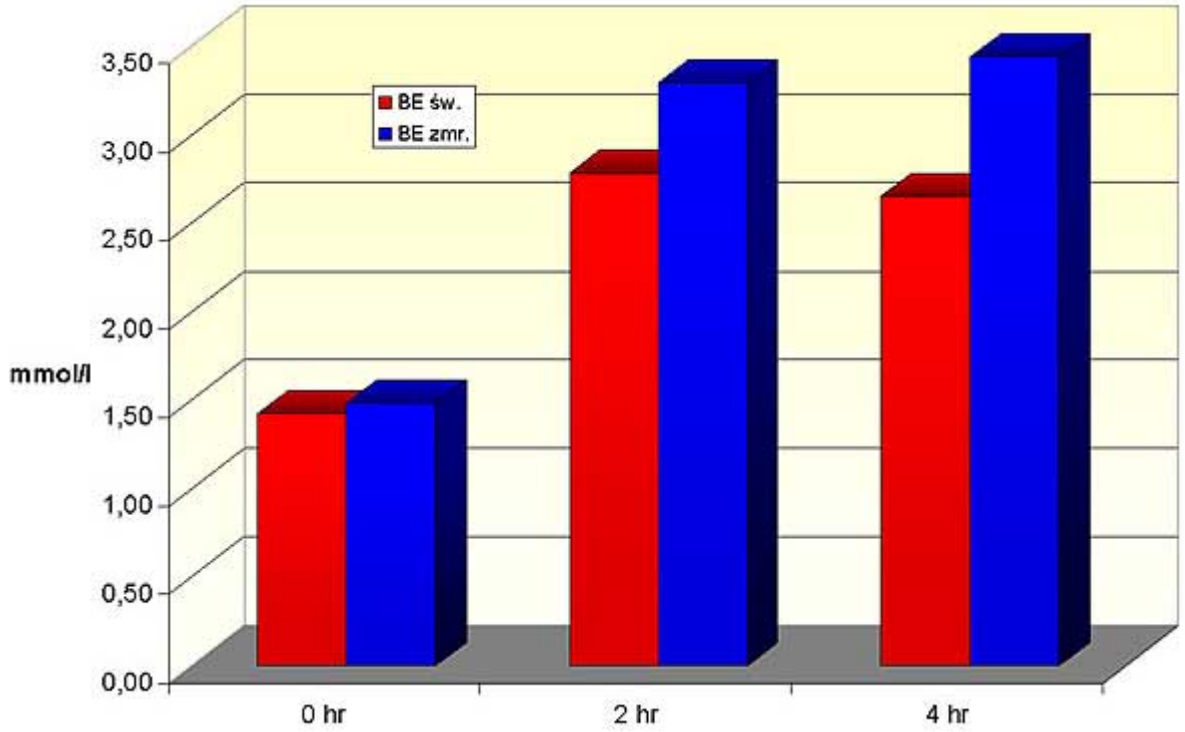


	0 h	2 h	4 h
PCO_2 for fresh fodder	36.36	36.74	36.55
PCO_2 for frozen fodder	38.31	36.87	37.07

The initial values of abe parameters and rumen fermentation obtained during this experiment cannot be considered as optimal (reference) for sheep because they were assayed for only 7 experimental sheep in order to conduct comparative analysis of their possible deviations during proper experiments. The fact that they differ from the results obtained by some scientists and are consistent with the results of the others means that conducting such measurements, and not only basing on reference values given by literature, was justified.

The results of the experiments shall be discussed only with regard to the used fodder, that is sugar beet pulp.

Graph 9. Venous blood BE mean values (in mmol/l) in sheep fed with fresh and frozen fodder



	0 h	2 h	4 h
BE for fresh fodder	1.43	2.79	2.65
BE for frozen fodder	1.49	3.30	3.44

The sheep examined in both stages of the experiment had no clinical changes or disorders which could be related to the used alimentary factor.

CONCLUSIONS

1. Feeding sheep with 0.5 kg of frozen sugar beet pulp caused production of isovaleric and isobutyric acids after 2 and 4 hours from feeding and the rumen contents pH increase,
2. In comparison to the first stage of the experiment, where the fodder was not frozen, greater number of protozoa was observed after 2 and 4 hours from feeding with frozen fodder.
3. Feeding with 0.5 kg/sheep of frozen fodder 2 a day caused base excess and actual bicarbonate concentration increase after 4 hours from feeding.
4. Feeding with frozen fodder causes changes which are quickly compensated by the rumen ecosystem and blood buffering systems.

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