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# INFLUENCE OF FODDER SILAGE TYPE ON FERMENTATION PROCESSES AND RUMEN MICROORGANISMS

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## ABSTRACT

The study was conducted on four beef cannulae put into forestomach of 500 kg mass in the Latin square system. The control group, apart from corn silage, was fed with hay, while experimental groups were fed trefoil silage, darnel or meadow trefoil-darnel mixture. The parameters characterizing changes taking place in the rumen as well as the number of bacteria and protozoa were determined in the rumen fluid collected before feeding and 1.5 h and 3 hours after feeding. The substitution of hay with silage caused the increase of N-$NH_3$ concentration in the rumen and changed the fermentation profiles expressed by the increase of the amounts of the following acids: propanoic, pentanoic and iso-pentanoic, and the decrease of the following acids: acetic and butyric. The silage of meadow trefoil caused the increase of bacteria and protozoa count, while the darnel silage and meadow trefoil-darnel mixture only caused the increase of bacteria count, and reduced the protozoa count.

**Key words:** rumen, fermentation, bacteria, protozoa, silage.

## INTRODUCTION

The high genetic potential of cows can be fully used under the condition of applying high quality fodders. It is difficult due to great diversity of volumetric fodders as well as their variable chemical composition and alimentary value [4, 25]. Difficulties in the balancing of alimentary doses for cows also result from a limited ability of fodder uptake and the fact that their type is closely related to the fermentation processes taking place in the rumen. There is a complete agreement with regard to the opinion that particular fodders have a considerable influence on rumen microorganisms controlling these processes. The populations of bacteria and protozoa react very quickly to all changes of the alimentary dose, and especially to the content of protein and energy in it as well as their source of origin. The problem of rumen microorganisms is undoubtedly very complex and significantly less frequently approached in studies, particularly in Poland. This complicated biological symbiotic system of animal – microorganisms is more sensitive to the amount and source of particular dietary components [6, 8, 13, 14, 20, 29]. There is a general opinion that it is possible to maintain optimal balance between the rumen microorganisms, which influences the metabolism of alimentary components and, in this way, conditions the high yield of the animals through proper selection of fodders and their balancing in the dose [4; 15; 19; 25; 28;]. As results from the studies, the role of protozoa in the metabolic processes taking place in the rumen, and their importance for cattle have been so far less known that these of bacteria, despite the fact that the activity of these microorganisms is similar [17; 21; 23; 27].

Silage is used most frequently in dairy cows feeding, irrespectively of the production level [3; 11; 18;]. Even green forage during the summer period is often eliminated in such feeding models. Apart from corn silage, also grass silage, originating from papilionaceous plants or mixtures of grasses and papilionaceous plants is used in such cases. This type of feeding system enables more equal composition of the alimentary dose throughout the year, since the production of grass silage and of papilionaceous plants is more reliable than hay production. This is not neutral for bacteria and protozoa, whose amount in the rumen is closely related to the type and quality of fodders.

This study presents research results in which meadow hay in alimentary doses was substituted with silage, and the influence of such substitution on the rumen fermentation products and on the populations of bacteria and protozoa was determined.

## MATERIALS AND METHOD

Meadow hay prepared by means of a traditional method (on swaths) during good weather as well as silage of corn, darnel, meadow trefoil and a mixture of meadow trefoil and darnel (1 : 1) was used in the study. Corn of Polish Elza (FAO 260) variety was ensilaged during wax maturity of grain, while darnel – during coming into ear, and meadow trefoil and its mixture with darnel (1 : 1) – during the initial phase of trefoil blooming. Grass, trefoil and a mixture of these plants were ensilaged with the addition of Mikrosil (1l/t) after previous withering on swaths to about 30-35% of dry matter. Meadow hay used in the experiment was prepared with a traditional method on swaths during good weather. The basic alimentary components were determined in all fodders used during the experiment [2], which were applied while calculating the alimentary value of fodders in INRA'88 system. Also the NDF neutral-detergent fraction and ADF acid-detergent fraction were determined according to Soest and Wine [26]. In silages, the pH reaction was determined potentiometrically [2], and the organic acid content was determined as well [12].

The dietary experiments were conducted in the Latin square system on four beef cannulae of black-white breed with over 50% contribution of HG gene, and body weight of 500 kg, which were equipped with permanent rumen forestomach [9]. The animals were drawn in the Latin square system and located in separate stalls preventing the contact between individuals. The animals were fed with balanced doses at the level of the living demand, according to the system provided (table 1), and had permanent access to water and salt-licks.

**Table 1. Feeding rations used in the first experiment (kg/head/d)**

| Item | Feeding groups | | | |
|---|---|---|---|---|
| | (KS) control group | (KKZ) | (KKO) | (KM) |
| Maize silage | 20 | 18 | 18 | 18 |
| Meadow hay | 3.5 | | | |
| Perennial ryegrass silage | | 10.5 | | |
| Red clover silage | | | 11 | |
| Perennial ryegrass and red clover silage | | | | 11 |

The daily dose was divided into two feedings: morning one (7.00 a.m.) and afternoon one (4.00 p.m.). In each experiment, a 21-day initial period was separated, necessary for the rumen microorganisms adaptation, and a 5-day proper period, during which the rumen fluid samples were taken through the forestomach: directly before the morning feeding and 1.5 and 3 hours after feeding. The fluid samples were collected from the upper, middle and lower part of the rumen in the amount of 150–200 ml, making sure that the depth of the fluid collection and its amount were identical each time. Samples were taken from the biological material collected in this manner, and the following parameters of the samples were determined: protozoa and bacteria count, and the basic parameters of the rumen metabolism. The following parameters were determined in the rumen fluid: reaction and ammonia nitrogen [7] (determined potentiometrically), and the total of volatile fatty acids on gas chromatograph of Varian 3000 type.

Dogiel's key was used while determining protozoa [10]. These microorganisms were systematized within the scope of five types: *Isotricha, Diplodinium, Entodinium*, *Epidinium* and *Ophryoscolex*. They were determined under a light microscope with the use of Fuchs – Rosenthal camera. The determination of bacteria count was conducted on the basis of the method adopted by Zawadzki [30].

The results concerning the rumen metabolism factors as well as bacteria and protozoa count were analyzed statistically by means of STATGRAPH software. The dependencies between particular rumen fermentation indexes versus protozoa types and bacteria count were also determined (STATGRAPH).

## RESULTS AND DISCUSSION

The results of the studies conducted indicate that the use of silages in the alimentary dose as a substitute of meadow hay has no influence on the pH reaction of the rumen fluid, while it influences the profiles of individual metabolites. These observations are confirmed by other authors' research [27; 31; 32;].

The level of pH in the rumen was characteristic of animals fed exclusively with volumetric fodders and corresponded to the results obtained by Varel and Kreikemeier [27]. This is very important since low pH usually reduces the activity of cellulolytic bacteria and deteriorates the use of cellular wall polysaccharides, and may also reduce the synthesis of Allen microbiological protein [1]. The amount of ammonia nitrogen in the animals' rumen was similar and only to a slight degree depending on the alimentary dose. However, the substitution of meadow hay with silages of trefoil, and a mixture of trefoil with darnel caused the increase of ammonia nitrogen level, which can be explained by higher content of easily soluble total protein than in hay or darnel silage [23; 24; 31; 32]. The increase of this basic component of nitrogen metabolism in the rumen may also prove high proteolytic activity of the rumen microorganisms and worse utilization of hydrocarbons for microbiological synthesis. It is worth noting that protozoa were negatively correlated with N-$NH_3$, while bacteria – positively (table 3).

**Table 2. Effect of silages used on the rumen fermentation parameters**

| Determined parameters | Feeding rations | | | |
|---|---|---|---|---|
| | KS | KKZ | KKO | KM |
| pH | 7.00$^{a}$ | 6.87$^{a}$ | 6.85$^{a}$ | 6.78$^{a}$ |
| N-$NH_3$ (mM/dl) | 10.62$^{a}$ | 10.50$^{a}$ | 11.03$^{b}$ | 11.30$^{b}$ |
| Total VFA | 7.50$^{a}$ | 10.45$^{b}$ | 11.40$^{c}$ | 10.25$^{b}$ |
| Molar ratio of VFA (%) | | | | |
| acetic acid | 70.18$^{c}$ | 67.37$^{ab}$ | 65.77$^{a}$ | 68.38$^{b}$ |
| propionic acid | 15.73$^{a}$ | 20.29$^{b}$ | 20.83$^{b}$ | 18.54$^{b}$ |
| butyrc acid | 11.03$^{b}$ | 6.94$^{a}$ | 8.07$^{a}$ | 7.79$^{a}$ |
| izo-butyric acid | 1.00$^{a}$ | 0.92$^{a}$ | 1.05$^{a}$ | 1.09$^{a}$ |
| valeric acid | 0.78$^{a}$ | 3.25$^{b}$ | 2.87$^{b}$ | 2.75$^{b}$ |
| iso-valeric acid | 1.25$^{a}$ | 1.23$^{a}$ | 1.41$^{a}$ | 1.45$^{a}$ |
| acetic/propionic ratio | 4.46$^{c}$ | 3.32$^{ab}$ | 3.16$^{a}$ | 3.69$^{b}$ |

a,b,c,d $P< 0.05$

**Table 3. Correlation indexes for metabolites and microorganisms of the rumen content of animals fed with various fodder silage**

| Details | Entodinium | Diplodinium | Holotrocha | Epidinium | Total | Bacteria |
|---|---|---|---|---|---|---|
| Acetic acid | 0.00 | 0.42 | 0.21 | 0.88* | 0.09 | 0.80* |
| Propanoic acid | -0.18 | -0.27 | -0.02 | -0.90* | -0.26 | 0.83* |
| Butyric acid | 0.64* | 0.14 | -0.30 | 0.93* | -0.72* | -0.94* |
| pH | 0.27 | 0.71* | 0.29 | 0.91* | -0.43* | -0.93* |
| N-$NH_3$ | 0.41 | -0.84* | -0.72* | -0.71* | -0.27 | 0.63* |
| VFA total | -0.03 | -0.51* | -0.26 | -0.93* | -0.14 | 0.86* |
| Entodinium | 1.00 | -0.43 | -0.82* | 0.35 | 0.99* | -0.45* |
| Diplodinium | | 1.00 | 0.87* | 0.47* | -0.28 | -0.46* |
| Holotrocha | | | 1.00 | 0.07 | -0.71* | -0.01 |
| Epidinium | | | | 1.00 | 0.47* | -0.99 |
| Total | | | | | 1.00 | -0.58* |
| Bacteria | | | | | | 1.00 |

Volatile fatty acids constitute a basic source of energy for ruminants. Their total production in the rumen of the animals fed with silages was higher that in case of those fed with a dose with hay contribution. Such dependencies were also observed by other authors [22; 27]. Feeding with hay caused the increase of acetic acid concentration in the rumen, but reduced the amount of propanoic acid, which was certainly related to a higher content of structural hydrocarbons in this fodder. This caused the spread of a molar ratio to 4.46, while in the groups fed with silages the proportions of these acids ranged from 3.16 to 3.69. Similar regularities were observed by other authors [5; 22; 27]. It is also worth stressing that these acids were significantly correlated with *Epidinium*, acetic – positively (0.88) and propanoic – negatively (-0.90) (table 3).

A thesis has been confirmed that the substitution of hay with silages in dietary doses has a significant influence on the population of bacteria settled in the rumen. It is interesting that in the rumen fluid of the animals fed only with

silages the total of bacteria was significantly higher than in those animals that were also fed with meadow hay (table 4). The bacteria population determined was dominated by cocci, whose count in the rumen fluid was subject to the same changes, depending on the alimentary dose, as the total bacteria count. In case of bacilli count, which was little in the rumen in comparison to cocci, bigger differences occurred. From complete absence, in the animals fed with darnel silage (KKż), up to 0.30 x 10 in the group kept on a dose with meadow trefoil silage contribution (table 4). This may suggest that bacilli are more demanding with regard to the type of silage than cocci.

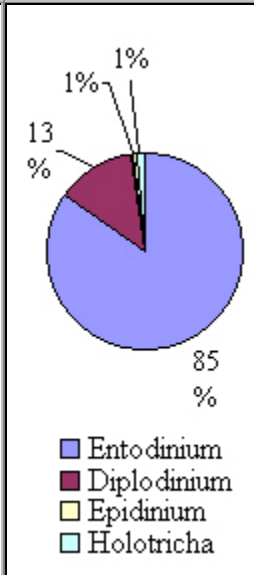
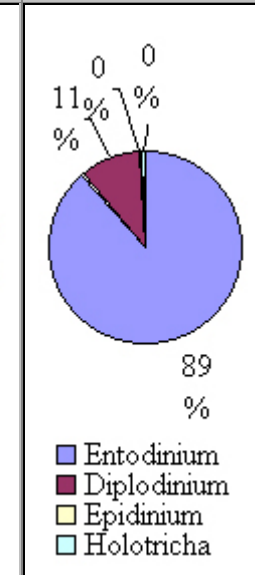
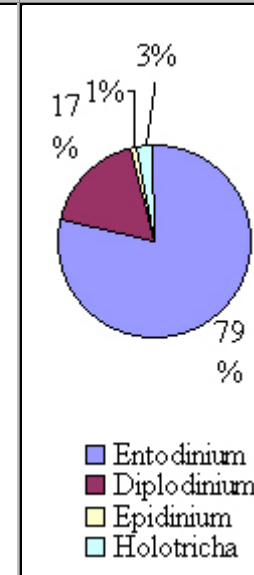
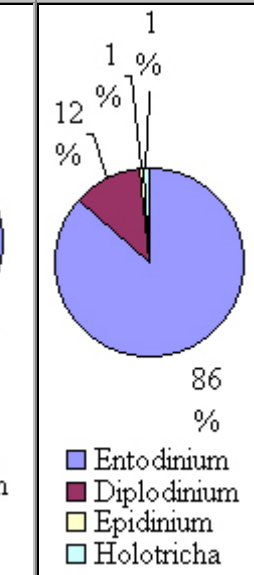
**Table 4. Number of bacteria in 1 ml of rumen fluid (x$10^9$)**

| Item | Feeding rations | | | |
|---|---|---|---|---|
| | **KS** | **KKZ** | **KKO** | **KM** |
| Bacilli | $0.25^b$ | $0.00^a$ | $0.30^c$ | $0.23^b$ |
| Coccus | $7.27^a$ | $10.12^b$ | $9.48^b$ | $10.04^b$ |
| Total number | $7.52^a$ | $10.12^b$ | $9.78^b$ | $10.27^b$ |

a,b,c,d P< 0.05

*Entodinium* (table 5) was the most numerously represented type out of the protozoa analyzed in the rumen of all animals. This kind of protozoa also dominated in animals in other studies which applied volumetric fodders [16; 20; 31].

**Table 5. Number of rumen protozoa in the rumen fluid (x $10^5$)**

| Listing | Dietary doses | | | |
|---|---|---|---|---|
| | I (KS) | II (KKo ) | III (KKż ) | IV (KM) |
| Entodinium | $1.88^c$ | $2.00^d$ | $1.36^a$ | $1.63^b$ |
| Diplodinium | $0.29^b$ | $0.24^a$ | $0.30^b$ | $0.22^a$ |
| Epidinium | $0.02^b$ | $0.01^a$ | $0.02^b$ | $0.01^a$ |
| Holotricha | $0.03^b$ | $0.01^a$ | $0.05^c$ | $0.02^b$ |
| Total | $2.22^c$ | $2.26^d$ | $1.73^a$ | $1.88^b$ |
| Protozoa contribution ( % ) |  |  |  |  |

a,b,c,d P < 0.05

The dietary doses applied in the experiment influenced particular types of protozoa. *Entodinium* occurred most numerously in the rumen of animals fed with corn and meadow trefoil silages (89%). A smaller count of *Entodinium* was found in the animals of the control group (85%) and fed with the dose including the trefoil-darnel mixture silage contribution (86%). The smallest count of these protozoa was present in the rumen of animals whose dietary dose of hay was substituted with darnel silage.

The calculated correlation indexes between particular metabolites in the rumen and the number of microorganisms proved significant in most cases. The bacteria were correlated with all rumen metabolism indexes, and especially highly negatively with acetic and butyric acids as well as pH, while positively – with propanoic acid and VFA total. *Epidinium* type had the biggest influence on the rumen metabolism among the protozoa. The more of these protozoa

multiplied in the rumen, the higher was the production of acetic and butyric acids, and the lower was the production of propanoic acid in the animals. *Entodinia* were positively correlated only with butyric acid, while *Diplodinia* positively with the rumen fluid reaction and, similarly as *Holotricha*, negatively to $N\text{-}NH_3$.

## CONCLUSIONS

1. The type of volumetric fodders applied in the dietary dose influences the direction of the rumen fermentation. The substitution of meadow hay with silages of withered meadow trefoil or trefoil-darnel mixture increases the concentration of $N\text{-}NH_3$ in the rumen as well as changing the fermentation profiles expressed with the increase in the amount of the following acids: propanoic, pentanoic and iso-pentanoic, and the decrease of the level of acetic and butyric acids.
2. The substitution of meadow hay with silages influences the rumen microorganisms in a non-uniform way. The meadow trefoil silage causes the increase of bacteria and protozoa count, while the darnel and trefoil-darnel mixture silages increase the bacteria count and reduce the protozoa count.
3. The dependencies between the rumen metabolism indexes and the amount of microorganisms have been proved. Bacteria were negatively correlated with acetic and butyric acids, while positively with propanoic acid and VFA total.
4. *Epidinium* type had the biggest influence on the rumen metabolism out of the protozoa. The more of these protozoa multiplied in the rumen, the higher was the production of acetic and butyric acids and the lower was the production of propanoic acid in the animals.

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