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Population of ciliates, rumen fermentation indicators and biochemical parameters of blood serum in heifers fed diets supplemented with yeast (S*accharomyces cerevisiae*) preparation

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Evaluated was the influence of live cells and metabolites of yeast (*Saccharomyces cerevisiae)* in the diet on number of ciliates, concentration of volatile fatty acids (VFA) in the rumen and biochemical parameters of blood of heifers. The experiment was carried out on three rumen-fistulated heifers. The animals were fed diet consisting of 88% meadow hay and 12% concentrate. A dose of 10 g of live yeast or their metabolites – 60 g, were introduced into the rumen. The preliminary feeding period of animals lasted three weeks and was followed by sampling of rumen fluid (RF), rumen contents (RC) and blood. The number of protozoa in RC and concentration of VFA in RF as well as total protein, triacylglycerol, total cholesterol, HDL (high density lipoprotein), LDL (low density lipoprotein) and VLDL (very low density lipoprotein) in blood serum were determined. Supplementation with metabolites of yeast significantly increased the number of genus *Entodinium* compared to animals fed control diet or live cell of yeast. The number of representatives of the genus *Diplodinium* was similar in heifers fed control diet or metabolites of yeasts and was significantly higher than when the live yeast was applied to the diet. The number of ciliates from genus *Ophryoscolex* and *Dasitricha* significantly increased, when heifers were fed diet supplemented with live cells of yeast. Addition of fungal preparations to the diets increased RF pH compared to animals fed control diet. The administration of yeast metabolites to heifers increased molar concentration of acetate and acetate to propionate ratio, but decreased molar proportions of propionate and butyrate. The total protein, triacylglycerol and total cholesterol concentrations decreased significantly when live yeast cells were added comparing to animals fed control diet or metabolites of yeast.

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Products containing yeast, particularly live yeast cells and yeast metabolites of *Saccharomyces cerevisiae* have been used to increase growth rate and milk production in domestic ruminants [Pinos-Rodríguez *et al.* 2008; Mašek *et al.* 2008]. Beneficial effects of these supplements have been associated with their abilities to alter rumen functions. Addition of *Saccharomyces cerevisiae* preparation to the ruminant diets improved digestibility of structural carbohydrates and stimulated activity of cellulolytic bacteria, in particular of *Fibrobacter succinogenes* and *Ruminococcus albus* [Callaway and Martin 1997, Newbold *et al.* 1995]. Furthermore, yeast additives can reduce the pH decrease and lactate accumulation in the rumen by increasing number of lactate-utilizing bacteria, particularly *Selenomonas ruminantium* and *Meganosphera elsdenii* [Callaway and Martin 1997] and also by inhibiting the activity lactate-producing bacteria, particularly *Streptococcus bovis* [Chaucheyras *et al.* 1996]. Influences of *Saccharomyces cerevisiae* preparation on the number and genus of protozoa are inconsistent. Brossard *et al*. [2006] and Plata *et al.* [1994] observed increase in the total number of rumen ciliates of cows fed yeast preparations. According to Doreau and Jouany [1998] addition of yeast preparation did not change the concentration of *Ophryoscolecidae* family represented by the genus *Entodinium, Epididinium* and *Diploplastron.* On the other hand, the family *Isotrichidae,* represented by the *Isotricha* spp., was present in higher numbers when the diet was supplemented with the diet. Several studies [Marden *et al*. 2008, Doležal *et al.* 2005, Khadem *et al.* 2007] have shown that adding of yeast preparation to the diet resulted in changing of the volatile fatty acids (VFA) concentration in the rumen. The elevated levels of propionate, butyrate and valerate can slow down the triacylglycerol and cholesterol synthesis in liver cells and may change the lipid profile of blood. According to Pysera and Opałka [2001] lipid profile and lipoproteins (HDL, LDL, VLDL) in blood serum of calves are modified by the age of animals, amount and type of fat or feed additives.

The aim of this study was to evaluate the influence of live cell or metabolites of yeast (*Saccharomyces cerevisiae*) in the diet on number of *ciliates* and concentration of VFA in the rumen, as well as some biochemical parameters in blood serum of heifers.

Material and methods

Animals and feeds

The experiment was carried out on three permanently rumen-fistulated Jersey heifers with an average live weight of 350 kg and similar body condition. The animals were fed either a control diet (7.0 kg DM·d $^{-1}$) consisting of 88% meadow hay and 12% concentrate or two experimental diets that were composed of the control diet supplemented with live yeast *Saccharomyces cerevisiae* (Levucell® SC, *S. cerevisiae* CNCM I-1077) or their metabolites (Diamond V Mills XP®, Cedar Rapids, IA), which were supplied in doses of 10 and 60 g·d $^{-1}$ respectively. The composition of the diet is

given in Table 1. The daily ration was divided into two equal parts and fed at 7.00 and 15.00. Both additives were administrated directly through the rumen fistula. Drinking water was available *ad libitum*.

Table 1. Composition of the ration

Item	% of r matter
Meadow hay	87.5
Crushed barley	10.0
Rapeseed oilmeal	1.0
Soyabean oilmeal	1.0
Vitamin-mineral premix[1]	0.5
Nutrient analysis	
organic matter	93.0
crude protein	15.0
N – free extracives	52.0
crude fibre	24.0
NDF	55.0
ADF	31.0
ADL	5.0
cellulose	26.0

[1]Premix contents per kilogram: Ca – 246g, Na – 80g, P– 20g, Mg – 30g, S -1,2g, Zn – 1g, Cu – 30mg, Mn – 60 mg, Se – 30mg, vitamin A – 700 000j.m., vitamin D_3 – 140 000j.m., vitamin E – 1 500j.m., niacin – 500mg; NDF – neutral detergent fiber, ADF – acid detergent fiber, ADL – acid detergent lignin.

Diamond V Mills XP® is produced by fermenting liquid and cereal grain raw ingredients with yeast *S. cerevisiae* and drying the entire culture medium without destroying components associated with yeast such as B vitamins and other fermentation products [Lynch and Martin 2002].

The preparation of Levucell® SC, *S. cerevisiae* CNCM I-1077, contain only select viable *S. cerevisiae* lyophilize cell.

Experimental design and sampling

The experiment was performed in 3 x 3 Latin square design. Preliminary feeding lasted three weeks and was followed by sampling period. The samples of rumen fluid (RF) and of rumen contents (RC) were withdrawn just before the morning feeding and 2, 4 and 8 h thereafter on three consecutive days. The samples of RC for protozoa counting (about 5 g) were fixed with aqueous formaldehyde solution (4% vw) while those of RF for determination of volatile fatty acids (VFA, about 5 ml) were preserved with 0.5 ml formic acid. To get the representative samples, the RF and RC were taken from different places in the rumen, thoroughly mixed and the appropriate samples were collected. The remaining material was immediately transferred back to the rumen. The blood samples were withdrawn from the jugular vein, just before and two h after the morning feeding into heparinized centrifuge tubes and centrifuged at 2 500 g for

15 min. Serum was stored at -80 ^{0}C until analysed for total protein, triacylglycerol (TG), total cholesterol (Chol) and HDL. LDL was calculated according to pattern: Chol – HDL – TG/2.2 and VLDL according to: TG/2.2.

The concentration of VFA in RF was determined by gas chromatography according to Ziołecki and Kwiatkowska [1973] using Hewlett Packard 5890 apparatus. The pH of RF was measured using Beckman pH-meter. The protozoa were identified and classified according to Dogiel [1927] and counted under light microscope according to Michałowski *et al.* [2001]. The total protein, triacylglycerol, total cholesterol and HDL fraction were estimated using a VITROS DT 60 II analyzer. Feed analysis were conducted using AOAC methods [2005].

Statistical

The mean values and the significance of differences between them were computed using the Statistica 9.0 software package. The normality of distribution of variables was tested by the Shapiro-Wilk test. Mean values were calculated by ANOVA and the significance of differences was determined by Schaffé test for normal distribution. Variables without normal distribution were evaluated by the nonparametric Mann-Whitney U test. Treatment effects were considered to be significant at $P<0.05$.

Results and discussion

Rumen ciliates

The density of rumen ciliates is presented in Table 2. The total protozoa count did not differ between dietary supplements; however, diets supplemented with metabolites of yeast led to numerically higher ciliate numbers than those identified in animals fed the control diet without yeast preparations – *i.e.* the one supplemented with live *Saccharomyces cerevisiae***.** The effect of yeast preparation on the total protozoa found in our experiment was in accordance with the relationships described by Arakaki *et al*. [2000], who reported that total protozoa count numerically increased when metabolites

Table 2. The concentration of ciliates ($x10^3 \cdot g^{-1}$ digesta) in the rumen of heifers

Item	Control	Live yeast	Yeast metabolites	Standard error of mean
Total ciliate	233	225	286	7.2
Entodinium	181^b	184^b	235^a	6.8
Diplodinium	40^b	18^a	39^b	2.7
Ophryscolex	4^b	10^a	3^b	0.5
Isotricha	4	6	5	0.3
Dasitricha	4^b	7^a	4^b	0.4

^{ab}Means bearing different superscripts differ significantly at $P<0.05$.

were introduced into steers diet. On the other hand, Dobicki *et al.* [2006] observed the elevated number of ciliates in the rumen when dried yeast was added to the cows'diet. In the present study, numerical increase in the population density of rumen protozoa was accompanied by increased number of genus *Entodinium* in animals fed the diet supplemented with yeast metabolites.

Supplementation the diet with yeast metabolites significantly increased concentration of genus *Entodinium* compared to animals fed control diet or diet supplemented with live yeast. The concentration of protozoa from the genus *Diplodinium* were significantly lower in heifers fed live yeast than control (without supplements) or yeast metabolites supplemented diets. Arakaki *et al*. [2000] reported that number of *Diplodinium* spp. in steers fed metabolites of yeast increased by 4%. Dobicki *et al*. [2006] have also found that metabolites of *Saccharomyces cerevisiae* in diets increased the population of *Diplodinium* spp. compared to control cows. These results suggest that metabolites of *Saccharomyces cerevisiae* contained soluble factors (*i.e*. vitamins B, amino acids, organic acids – fumarate, malate, aspartate) as well as cell membrane components (mannanes and β-glucanes) and could stimulate growth of *Entodinium spp*. In contrast, Arakaki *et al.* [2000] reported that metabolites of *Saccharomyces cerevisiae* had reduced the *Entodinium* spp. count in the rumen.

In the current study, the number of ciliates of *Ophryoscolex* genus significantly increased when heifers were fed the diet supplemented with live cell of yeast. However, Galip [2006] did not observe respective changes in the number of the organism in question. Brossard *et. al*. [2006] suggest that number of protozoa from the family of *Ophryoscolecidae*, which represent approximately 90 % of total rumen protozoa, probably increased with the addition of *Saccharomyces cerevisiae.*

Supplementation of the diet with live *Saccharomyces cerevisiae* significantly increased population of *Dasitricha* compared to heifers fed diet without yeast preparations and supplemented with metabolites of yeast. The number of *Isotricha* did not differ among the diets. According to Doreau and Jouany [1998] and Brossard *et al.* 2006] the addition of live *Saccharomyces cerevisiae* to the diet led to the tendency of the higher number of *Isotrichidae* in the rumen after feeding. Abe *et al.*[1981] reported that increasing number of *Isotricha* spp. and *Dasitricha* spp. in the rumen, may be caused by their sequester from the reticulum wall and migration into the rumen for a few hours after feeding.

Fermentation products

The rumen pH and molar VFA values in fistulated heifers are given in Table 3. Preparations of yeast added to the diets increased the pH of RF compared to animals fed control diet. This is in accordance with the results of Khadam *et al.* [2007] and Křížová *et al*. [2011], who reported much higher rumen pH in animals fed yeast-supplemented ration than in the control group. The fungal preparations added to the ruminant diet could minimize fluctuations in rumen pH and reduce the risk of acidosis because of reduction availability of glucose for lactate synthesis by *Streptococcus*

Table 3. Rumen pH, concentration of total volatile fatty acids (mmol·L^{-1}) and molar proportion of particular acids in the rumen of heifers (% of total VFA)

Item	Control	Live yeast	Yeast metabolites	Standard error of mean
pH	6.8[a]	6.9[b]	7.0[b]	0.02
Total volatile fatty acids (VFA)	36.3	37.3	33.6	0.73
Acetate (A)	68.1[a]	68.2[a]	70.8[b]	0.41
Propionate (P)	16.3[a]	16.7[a]	15.0[b]	0.14
Butyrate	10.5[a]	10.7[a]	9.4[b]	0.13
Valerate	1.6	1.5	1.5	0.07
Isovalerate	1.9	1.5	1.9	0.06
Isobutyrate	1.6	1.4	1.4	0.04
A : P	4.1[a]	4.1[a]	4.6[b]	0.05

Explanations are at the bottom of Table 1.
[ab]Means bearing different superscripts differ significantly at $P<0.05$.

bovis. Furthermore, increase in rumen pH could be due to the lowered lactic acid concentration through ability of yeast cells to stimulate the activities of *Selenomonas ruminantium* and *Megasphaera elsdenii* which utilize lactic acid in the rumen [Nisbert and Martin 1991, Rossi *et al.* 2004]. The elevated rumen pH appears to be the result of the increase in number of total and cellulolytic bacteria in RF because decrease in rumen pH lowers the growth of population of rumen bacteria [Williams *et al*. 1991]. On the other hand, Doležal *et al.* [2005] reported that increasing doses of live cell yeast to the diets lowers the pH value of RF compared to control animals. In contrast to our experiment, it was reported that rumen pH dropped when diets contained preparation of yeast [Arcos-García *et al*. 2000].

In this study, molar concentrations of acetate increased when animals were administered metabolites of yeast, but those of propionate and butyrate decreased compared to control diet and diet with live yeast. Lynch and Martin [2002] reported that acetate concentration increased when rumen microorganisms were incubated *in vitro* in the presence of ground corn supplemented with metabolites of yeast compared to live *Saccharomyces cerevisiae*. Furthermore, metabolites and live cell of yeast had no effect on propionate and butyrate concentration. In contrast to our results, acetate concentration in cows fed live yeast was significantly higher compared to control animals in samples taken 2, 4, 7 h after feeding [Křížová *et al.* 2011]. On the other hand, propionate and butyrate concentration was higher in cows only 2 h after feeding. Doreau and Jouany [1998] concluded that the effect of yeast on VFAs concentration was transient. In this study, the molar concentration of acetate increased in heifers fed metabolites of yeast that may result from altered rumen microbial population. The elevation of acetate concentration might be due to the growth enhancement in number of cellulolytic bacteria. Moreover, Miller-Webster *et. al.* [2002] reported that the concentration of acetate and propionate depend on type of fungal culture in ration used for the cow. They found, that addition of yeast preparation - Diamond –V XP – to

the diet significantly reduced the molar proportion of acetic acid (47.1 % of total VFA) and increased that of propionic acid (32.0 % of total VFA) compared to A-Max yeast culture, (53.2 and 23.6 % of total VFA, respectively).

In this study, acetate to propionate ratio was lower in heifers fed control and live of yeast in the diet compared to animals receiving metabolites of *Saccharomyces cerevisiae* (Tab. 3). According to Mwenya *et al.* [2005] this could be the explanation of the tendency of dropping methane emission in animals fed control diet as related to rations supplemented with preparation of metabolites. Propionate production competes with methanogenesis for available hydrogen. The mentioned authors conclude that increased propionate concentration in the rumen resulted in lower methane emission.

The diet with *Saccharomyces cerevisiae* preparation did not affect the total VFA or molar proportion of valerate, isovalerate and isobutyrate.

Biochemical parameters of blood

The total protein content and lipid profile of blood serum in experimental heifers are presented in Table 4. Total protein, triacylglycerol and total cholesterol concentrations decreased significantly when live cell yeast additive was introduced to the diet compared to animals fed control diet and metabolites of yeast. The content of total cholesterol in blood serum was within the reference ranges, but concentration of total protein and triacylglycerol were both unsignificantly higher than the reference values [Winnnicka 2008]. The total protein of blood represents the sum of albumin and globulin. According to El-Sherif and Assad [2001], the decrease in serum protein was reflected by the globulin rather than by albumin level and resulted in the increase in albumin to globulin ratio. Contrary to this, Mašek *et al*. [2008] and Galip [2006], did not observe increase in concentration of albumin and globulin in blood of sheep fed live cells of yeast. On the other hand, higher total protein level of serum in heifers fed control diet and metabolites of yeast may be explained partially by the improved

Table 4. The total protein ($g \cdot L^{-1}$) and some of lipid parameters ($mmol \cdot L^{-1}$) in blood serum of heifers

Item	Control	Live yeast	Yeast metabolites	Reference value*	Standard error of mean
Total protein	84.42^{b}	77.83^{a}	84.41^{b}	51 - 71	1.092
Triacylglycerol	0.39^{b}	0.34^{a}	0.40^{b}	0.1 -0.3	0.007
Total cholesterol	2.60^{b}	2.39^{a}	2.56^{b}	1.8 -5.2	0.046
HDL	2.11	1.95	1.94		0.044
LDL	0.45	0.29	0.44		0.046
VLDL	0.17	0.15	0.18		0.005

Explanations are at the bottom of Table 1. HDL – high density lipoprotein; LDL – low density lipoprotein; VLDL – very low density lipoprotein. *According to Winnicka [2008].

utilization of dietary protein by animals. Moreover, the stimulation of rumen microflora may cause changes in bacterial protein synthesis and increase protein outflow of rumen. Contrary to the present study, several authors [Galip 2006, Mašek *et al*. 2008, Křížová *et al*. 2011] did not observe any decrease in serum protein concentration when live cell preparation of *Saccharomyces cerevisiae* was added to the ruminant diet.

In the present experiment, the decrease in triacylglycerol and total cholesterol level in blood serum of heifers fed diet with live cell *Saccharomyces cerevisiae*, could be caused by some positive changes in rumen fermentation and population of microorganisms (bacteria and protozoa). Additionally, several authors [Marden *et al*. 2008, Miller-Webster *et al*. 2002] observed changes in the concentration of rumen short chain fatty acids, particularly propionate, butyrate and valerate in animals fed diet supplemented with yeast. The increase in these acids is capable of reducing the synthesis of triglyceride and cholesterol in the liver cells and may change the lipid profile in blood. In current experiment, we found increase propionate and butyrate but not valerate concentration in rumen as well as decrease of triacylglycerol and total cholesterol concentration in serum of heifers fed live yeast preparation. Moreover, the cell wall of yeast is a rich source of β-glucans. According to Nicolosi *et al*. [1999], these polysaccharides reduce the total cholesterol of serum. Several other authors found no influence of live yeast on triglyceride and total cholesterol concentration of blood [Galip 2006, Mašek *et al.* 2008, Campanile *et al.*2008], but Payandeh and Kafilzadeh [2007] observed even increase in triacylglycerol level. The increase in triacylglycerol and total cholesterol in cows fed metabolites of *Saccharomyces cerevisiae*, compared to heifers fed live yeast, may explain enhanced activity of lipolytic enzymes and improved utilization of dietary lipid.

No effect of yeast preparations added to the diet was identified on the serum level of HDL, LDL and VLDL. This is in accordance with Campanile *et al.* [2008] who reported no effect of yeast additive to the diet on the HDL level in buffalo cows. According to Pysera and Opałka [2001], application of live yeast supplement modifies the lipid composition of the fractions VLDL and LDL as compared to control cows. However, in our study, LDL fraction level tended to be lower in animals fed live cell yeast compared to those kept on the control or yeast metabolites diet.

Results of this experiment suggest, that a choice of yeast preparation may differ depending on type of production (milk or meat) for cows; yeast metabolites can be more profitable than adding live yeast, because of increase of acetic and decrease of propionic and butyric acids, which levels in the rumen are very important for these animals.

We conclude that the administration of yeast Diamond V Mills XP® at 60 $g \cdot d^{-1}$ or *Saccharomyces cerevisiae* CNCM I-1077 at 10 $g \cdot d^{-1}$ to rumen-fistulated heifers, might have changed pattern of the fermentation as well as number and population of rumen ciliates. Moreover, supplementation of yeast CNCM I-1077 decreased concentration of triacylglycerol and total cholesterol of blood serum.

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