Rumen fermentation characteristics in pre-weaning calves receiving yeast culture supplements

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ABSTRACT: In an experiment with 45 neonatal male Holstein calves, effects of yeast cultures Yea-Sacc® 1026 (SC) containing *Saccharomyces cerevisiae* (strain 1026) and Vitex (KF) containing *Kluyveromyces fragilis* (strain Jürgensen) on rumen fermentation characteristics were determined. From day 4 to day 56 of age, the calves were allocated to one of the three dietary treatments (Control, SC, and KF) of 15 animals each, placed in individual pens, and fed 4 l of whole milk twice daily and a basal concentrate mixture ad libitum as calf starter feeds. The control treatment was not supplemented with yeast culture. The yeast culture supplements Yea-Sacc® 1026 and Vitex were top-dressed at 10 g/calf daily on the basal concentrate mixture of treatments SC and KF, respectively. At the end of the experiment (day 56), all calves were slaughtered and the rumen fluid was analysed. The administration of yeast cultures Yea-Sacc® 1026 and Vitex to calves did not affect final body weight (BW), BW gain, dry matter intake, feed conversion ratio, ruminal pH, lactic acid concentration and the molar proportion of propionic acid, but it decreased ($P < 0.05$) the total volatile fatty acid concentration and the molar proportion of butyric acid, and increased ($P < 0.05$) the molar proportion of acetic acid and the acetate to propionate ratio. In addition, the microbial cellulolytic activity was higher in calves that received both yeast cultures compared to the control treatment. The results of this study suggest that the ruminal fermentation was more stable in calves receiving yeast culture supplements.

Keywords: Holstein calves; *Saccharomyces cerevisiae*; *Kluyveromyces fragilis*; performance; ruminal pH; volatile fatty acids; lactic acid; microbial cellulolytic activity

In the neonatal ruminant, the forestomach, which comprises the rumen, the reticulum and the omasum, is relatively underdeveloped because the suckling animal primarily depends on the abomasum and intestine for digestive functions (Leek, 1993; Pond et al., 1995). Although the forestomach develops innately with aging, the critical period for establishing the potential degree of development is between 3 and 8 weeks of the calf age, when intermediary metabolism moves away from being glucose-based towards being volatile fatty acid (VFA)-based (Leek, 1993). There is a relationship

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between the forestomach development and microbial activity because the consumption of solid food by the young ruminant promotes microbial fermentation and VFA production, and physical stimulation of the ruminoreticular papillae and of the omasal leaves (Leek, 1993; Van Soest, 1994). Inadequate forestomach development can lead to increased health problems (i.e. diarrhoea, bloat) in the calf and can postpone the weaning age (Beharka et al., 1998; Magalhães et al., 2008). Therefore, the understanding of factors responsible for the initiation and control of forestomach development, and especially of rumen development, in neonatal calves is of primary importance.

Products that improve animal production through ruminal and intestinal flora manipulation have been used for decades. In recent years, probiotics, being considered 'natural' products, have received increasing attention. Probiotics are live microbial feed supplements that beneficially affect the health and well-being of the host animal by improving its gastrointestinal microbial balance (Fuller, 1989). The majority of probiotic products are based on *Lactobacillus* spp., *Streptococcus* spp., and *Bacillus* spp., although microscopic fungi, including *Saccharomyces cerevisiae* and *Kluyveromyces fragilis* yeasts, are also used (Cheeke, 1999; Spais et al., 2001). The inclusion of yeast probiotics in feeds benefits the host cattle with already developed rumen by increasing palatability, stimulating cellulolytic bacteria, and thus the rumen fermentation, and improving nutrient digestibility (Wiedmeier et al., 1987; Harrison et al., 1988; Koul et al., 1998; Cheeke, 1999; Marciňáková et al., 2008). In contrast, other researchers showed that the yeast culture had no effect on counts of cellulolytic bacteria (Erasmus et al., 1992) and ruminal digestion (Mir and Mir, 1994; Olson et al., 1994; Putnam et al., 1997). Moreover, Homolka et al. (2002) and Homolka et al. (2008), using enzymatic *in vitro* methods, evaluated crude protein (CP) degradability and intestinal digestibility of protein undegraded in the rumen for Vitex fodder yeast that contains *K. fragilis*. However, to our knowledge there is no published information on effects of *S. cerevisiae* and *K. fragilis* on rumen fermentation characteristics of calves at week 8 of age. Thus, the objective of this study was to evaluate the use of *S. cerevisiae* and *K. fragilis* in starter feeds of calves, receiving no forage, relative to their performance, ruminal pH, volatile fatty acid and lactic acid concentration, as well as the cellulolytic activity of rumen bacteria as a percentage of digested pure cellulose.

MATERIAL AND METHODS

Yeast cultures

Two yeast cultures (Table 1) were used in an experiment with newborn calves at the Czech University of Life Sciences, in Prague (Czech Republic). The yeast culture Yea-Sacc® 1026 (SC) containing a minimum of 5×10^9 colony forming units (CFU)/g *S. cerevisiae* (strain 1026) was obtained from Alltech Inc. (Nicholasville, KY, USA), whilst the yeast culture Vitex (KF) containing *K. fragilis* (strain Jürgensen) was obtained from Biocel Company, a.s. (Paskov, Czech Republic). Vitex is a pure, genetically modified organisms (GMO)-free, primary nutritional yeast with yeast cells grown in a medium obtained directly from spruce wood under conditions which permit the highest quality standards (Biocel, 2009).

Table 1. Chemical composition[1] (g/kg) of yeast cultures[2] and cow milk (as fed basis)

	Yea-Sacc® 1026	Vitex	Cow milk
Dry matter	921.8	924.0	126.0
Crude protein	185.4	513.2	31.7
Fat	49.4	29.6	36.6
Ash	6.9	6.3	8.7

[1]values represent duplicate assays of two samples for each material

[2]the yeast culture Yea-Sacc® 1026 contained *Saccharomyces cerevisiae* (strain 1026) and the yeast culture Vitex contained *Kluyveromyces fragilis* (strain Jürgensen)

Experiment on calves

Effects of dietary supplementation of yeast cultures on ruminal pH, volatile fatty acid and lactic acid concentration, and microbial cellulolytic activity were investigated in 45 neonatal male Holstein calves in an 8-week study. All calves used in the experiment were cared for according to applicable recommendations of the U.S. National Research Council (1996). All calves used in this study were removed from their dams before suckling and, after weighing, were randomly allocated to one of the three dietary treatments (Control, SC, and KF) of 15 animals each and placed in individual pens. At the beginning of the experiment (day 4 of age), the mean body weight (BW) of male calves for the three treatments was 42.4 ± 0.35 kg. All 45 pens were identical in relation to the direction, orientation and covered area (i.e. 2.6 m^2/calf) and were equipped with similar troughs for feeding grain concentrates and water. All calves were bottle fed 4 l of colostrum in four feedings on the first day after birth, and for three consecutive days. From day 4 to day 56 of age, calves were fed 4 l of whole cow milk twice daily at 07:00 and 18:00 h and a basal concentrate mixture (Table 2) *ad libitum* as calf starter feeds according to National Research Council (2001) nutrient requirements. The control treatment was not supplemented with yeast culture. The yeast culture supplements Yea-Sacc® 1026 and Vitex were top-dressed at 10 g/calf daily on the basal concentrate mixture of treatments SC and KF, respectively. Concentrate intake was measured daily on a pen basis, and cow milk and concentrate dry matter (DM) intake and feed conversion ratio (FCR) were calculated. At the end of the experiment (day 56), all calves from each treatment were fasted for 15 h (water was allowed), weighed and slaughtered at a commercial slaughterhouse. Sample of whole ruminal content was obtained from each calf and strained through cheesecloth, and transferred in the laboratory into a closed test tube, placed in a thermos with controlled temperature of 39°C and CO_2 atmosphere, for analyses.

Table 2. Concentrate composition of calf starter feed

	Basal concentrate mixture
Ingredient composition (kg/t, as mixed)	
Barley grain (ground)	83
Maize grain (ground)	200
Oats grain (ground)	100
Wheat grain (ground)	160
Sugar beet pulp (dried)	35
Wheat bran	100
Soybean meal (440 g/kg CP)	250
Sugar beet molasses	40
Limestone	12
Monocalcium phosphate	10
Salt	4
Vitamin-trace mineral premix[1]	6
Chemical composition[2] (g/kg, as fed)	
Dry matter	878
Crude protein (CP)	187
Crude fat	26
Neutral detergent fibre	134
Acid detergent fibre	60
Ash	37
Calcium	9.1
Phosphorus	6.7
Sodium	2.4
Magnesium	2.4
Net energy for gain (MJ/kg)	5.02

[1]the premix supplied per kg of concentrate: 7 500 IU vitamin A; 1 020 IU vitamin D_3; 27 mg vitamin E; 0.5 mg Co; 15 mg Cu; 1 mg I; 30 mg Fe; 60 mg Mn; 0.24 mg Se; 70 mg Zn
[2]the concentrate was analysed for dry matter, crude protein, crude fat and ash according to AOAC (1990), and for neutral detergent fibre and acid detergent fibre according to Van Soest et al. (1991). All other values were calculated from NRC values (2001)

Chemical analyses

The yeast cultures Yea-Sacc® 1026 and Vitex, as well as the concentrate mixture were analyzed for DM by drying at 102°C for 16 h in a forced air oven, and for crude protein (CP), fat, and ash according to methods 976.06, 920.39, and 942.05, respectively, of AOAC (1990). Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were also determined in the concentrate mixture according to Van Soest et al. (1991). NDF was analysed without sodium

sulphite or *a*-amylase, and NDF and ADF were expressed without residual ash.

The cow milk sample was analysed for DM by drying at 102°C for 16 h in a forced air oven, and for crude protein, fat, lactose, and solid-not-fat (SNF) by IR spectroscopy (Milkoscan 4000; TESCO, Denmark) according to method 972.16 of AOAC (1990). Ash was calculated as SNF minus protein and lactose.

The chemical composition of the yeast cultures Yea-Sacc® 1026 and Vitex, and of cow milk, is in Table 1, while that of the concentrate mixture is shown in Table 2. Crude protein and fat content of cow milk used in this study was similar to common milk obtained from Holstein cows (Brouček et al., 2004; Pešek et al., 2005; Liu et al., 2008).

The strained rumen fluid was analyzed within 1 hour for pH with an InoLab 720 pH-meter (Wissenschaftlich Technische Werkstätten GmbH and Co, Weilheim, Germany). The rumen fluid was also analyzed for acetic, propionic, butyric, and lactic acid composition by the high pressure liquid chromatography (HPLC) method of Mudřík and Vrátný (1990).

Microbial cellulolytic activity

The cellulolytic activity of rumen bacteria was determined according to Akin (1980) methodology as a percentage of digested pure cellulose. The determination of cellulolytic activity was done in

Table 3. Effects of yeast cultures on body weight (BW), body weight gain, dry matter (DM) intake, feed conversion ratio (FCR) and rumen fermentation characteristics of calves

	Treatment[1,2]			SEM
	control	SC	KF	
Initial BW (kg)	42.4	42.7	42.2	0.35
Final BW (kg)	62.7	62.8	62.6	0.37
BW gain (g/day)	384	381	384	4.59
DM intake (kg/day)				
Cow milk (Table 1)	0.50	0.50	0.50	–[3]
Concentrate (Table 2)	0.61	0.62	0.62	0.02
Total	1.11	1.12	1.12	0.02
FCR (kg DM intake/kg BW gain)	2.93	2.96	2.92	0.09
Ruminal pH	5.06	5.13	5.07	0.029
Total volatile fatty acids (VFA, mmol/l)	1.11[a]	0.84[b]	0.87[b]	0.037
Individual VFA (mol/100 mol)				
Acetic acid	46.38[a]	53.11[b]	50.63[b]	0.899
Propionic acid	37.90	35.49	37.84	0.773
Butyric acid	15.72[a]	11.40[b]	11.53[b]	0.552
Acetate:propionate ratio	1.23[a]	1.50[b]	1.34[ab]	0.055
Lactic acid (mmol/l)	0.019[ab]	0.013[a]	0.024[b]	0.003
Microbial cellulolytic activity (digested pure cellulose)	0.095[a]	0.133[b]	0.125[b]	0.004

[1]control = control treatment, SC = treatment with 10 g/calf/day Yea-Sacc® 1026, KF = treatment with 10 g/calf/day Vitex

[2]the yeast culture Yea-Sacc® 1026 contained *Saccharomyces cerevisiae* (strain 1026) and the yeast culture Vitex contained *Kluyveromyces fragilis* (strain Jürgensen)

[3]due to equal limit feeding among treatments, no statistical analysis was completed

[a–b]means within each row with different superscripts are significantly different ($P < 0.05$)

three separate repetitions for each test animal. The cellulolytic activity was calculated as the difference between the original weighing of pure cellulose and the remains of undigested cellulose.

Statistical analysis

Rumen fermentation characteristics of the calves were statistically analyzed by one-way analysis of variance with each calf being the experimental unit, while significant differences among treatment means were tested using Duncan's test at the 5% probability level (Steel and Torrie, 1980). The statistical analysis was done with the help of the SPSS Statistical Software Package (2008).

RESULTS

The results for the production parameters and the rumen fermentation characteristics of calves are shown in Table 3. These results suggest that the supplementation of yeast cultures to the diet for pre-weaning calves had no significant effect ($P > 0.05$) on final BW, BW gain, DM intake and FCR. The ruminal pH and the lactic acid concentration of male Holstein calves was unaffected by dietary supplementation of yeast cultures Yea-Sacc® 1026 and Vitex, while the lactic acid concentration was higher ($P < 0.05$) in treatment KF compared to treatment SC. In contrast, total VFA concentration decreased ($P < 0.05$) in treatments SC and KF supplemented with yeast cultures compared to the control treatment. Moreover, the molar proportion of acetic acid in treatments SC and KF and the acetate to propionate ratio in treatment SC increased ($P < 0.05$) compared to the control treatment. The molar proportion of propionic acid remained unaffected and that of butyric acid decreased ($P < 0.05$) in treatments with yeast supplementation. In addition, the microbial cellulolytic activity (digested pure cellulose) was higher ($P < 0.05$) in treatments SC (0.133) and KF (0.125) compared to the control treatment (0.095).

DISCUSSION

In our previous study (Hučko et al., 2004), regarding the effect of the ingredient composition of calf diets on the cellulolytic activity of rumen microorganisms, we found that the diet that included lucerne hay negatively impacted the cellulolytic activity by 23.89% compared to a diet without hay. Therefore, all calves in this study were fed whole milk and a basal concentrate mixture from day 4 to day 56 of age, without any forage supplementation, so that the effects of yeast culture supplements on rumen fermentation characteristics would be maximised.

In the present study, final BW, BW gain, DM intake and FCR were not affected by dietary yeast supplementation. Our results are consistent with the findings of Magalhães et al. (2008), who reported that the incorporation of *S. cerevisiae* at 20 g/kg of the diet DM fed to dairy calves from 2 to 70 days of age did not alter final BW, BW gain, DM intake and FCR. In a similar study with Holstein calves, Pinos-Rodríguez et al. (2008) found that dietary supplementation of *S. cerevisiae* (1 g/day/calf) did not affect BW, BW gain, and FCR, but increased starter DM intake by 8.2%. Moreover, Lesmeister et al. (2004) reported that dietary supplementation of *S. cerevisiae* (20 g/kg of diet DM) increased final BW, BW gain, and DM intake of dairy calves, but did not affect FCR, while no differences occurred with dietary supplementation of the yeast culture at 10 g/kg of diet DM.

In our study, the ruminal pH values in calves from all three treatments were lower than the pH values obtained by Göpfert et al. (2006) for calves at 8 weeks of age, but similarly low like the pH values obtained by Beharka et al. (1998) for male calves fed a ground diet at 8 weeks of age. In agreement with our results, other researchers reported that ruminal pH was not affected (Wiedmeier et al., 1987; Erasmus et al., 1992; Olson et al., 1994; Yoon and Stern, 1996; Putnam et al., 1997; Pinos-Rodríguez et al., 2008) while others found that ruminal pH increased (Williams et al., 1991; Koul et al., 1998) or decreased (Harrison et al., 1988; Corona et al., 1999; Doležal et al., 2005) after the yeast culture supplementation to ruminant diets. It is possible that yeast cultures influence rumen pH, which supports the bacteria using lactic acid as a source of energy.

In this study, the dietary supplementation of yeast cultures Yea-Sacc® 1026 and Vitex to calves resulted in decreased total ruminal VFA concentration and molar proportion of butyric acid, and increased molar proportion of acetic acid and acetate to propionate ratio. Our results are supported by the findings of Williams et al. (1991) and Corona

et al. (1999), who showed that steers and sheep supplemented with 7.5 g and 3 g, respectively, of a yeast culture containing *S. cerevisiae* per day had lower total VFA concentration and molar proportion of butyric acid, respectively. In contrast, other previous reports indicated that total ruminal VFA concentration and molar proportions of acetic, propionic, and butyric acid were generally unaffected by yeast cultures (Wiedmeier et al., 1987; Erasmus et al., 1992; Mir and Mir, 1994; Yoon and Stern, 1996; Putnam et al., 1997; Pinos-Rodríguez et al., 2008). However, Koul et al. (1998) reported that total ruminal VFA increased in buffalo calves fed 5 g yeast culture containing *S. cerevisiae* per day compared to controls (132.2 versus 122.4 mmol/l, respectively). In addition, Doležal et al. (2005) reported increased VFA production with increasing doses of yeast culture containing *S. cerevisiae* strain SC-47. Furthermore, Harrison et al. (1988) found decreased molar proportion of acetic acid and acetate to propionate ratio, and increased molar proportion of propionic acid in the rumen fluid of Holstein cows supplemented with 114 g per day of a yeast culture containing *S. cerevisiae*.

In our study, the yeast supplementation of calf diets changed the pattern of the end products of ruminal fermentation, suggesting a shift in metabolic activities of ruminal microflora. The increased acetic acid production indicates that the number of cellulolytic bacteria was consequently increased. An increase in the number of total viable bacteria (Koul et al., 1998), cellulolytic bacteria (Wiedmeier et al., 1987; Harrison et al., 1988; Koul et al., 1998) and proteolytic bacteria (Yoon and Stern, 1996) was observed when the yeast culture was used as a feed supplement to cattle with already developed rumen. In addition, Yoon and Stern (1996), and Koul et al. (1998) reported that amylolytic bacteria and ciliated protozoa were not affected by supplemental yeast culture, while Doležal et al. (2005) reported increased numbers of rumen protozoa with increasing doses of yeast culture.

Along with VFAs, lactic acid is produced by amylolytic bacteria during starch degradation (Leek, 1993). The dietary supplementation of yeast cultures Yea-Sacc® 1026 and Vitex numerically decreased and increased, respectively, the ruminal lactic acid concentration in calves. Previous reports indicated that the ruminal lactic acid concentration was unaffected (Erasmus et al., 1992; Mir and Mir, 1994; Putnam et al., 1997) or decreased (Williams et al., 1991; Koul et al., 1998) by yeast cultures. However, the decreased lactic acid concentration in the latest studies may be attributed to the fact that cattle with the already developed rumen were used, while our experiment was conducted on developing calves until their weaning.

The higher cellulolytic activity of rumen bacteria as determined by digestion of pure cellulose in calves that received yeast cultures supports the finding of the increased acetic acid production and indicates that cellulose digestibility was improved. Olson et al. (1994) concluded that the yeast culture supplementation in steers could increase organic matter (OM) digestibility early in the grazing season. Moreover, the yeast culture addition to cow diets has been reported to increase apparent digestibility of CP, ADF and hemicellulose (Wiedmeier et al., 1987; Erasmus et al., 1992). In contrast, the addition of a yeast culture containing *S. cerevisiae* to cow diets did not affect apparent digestibility of DM, NDF, ADF, hemicellulose and starch (Harrison et al., 1988; Corona et al., 1999) and decreased *in vitro* cellulose degradation (Harrison et al., 1988). Furthermore, the dietary yeast culture supplementation to cows increased ruminal CP degradation (Yoon and Stern, 1996) and did not affect ruminal DM, OM, ADF and non-structural carbohydrate degradation (Putnam et al., 1997; Enjalbert et al., 1999). In addition, the dietary yeast culture supplementation did not affect (Enjalbert et al., 1999) nor decreased ruminal NDF degradation (Putnam et al., 1997).

CONCLUSIONS

The administration of yeast cultures Yea-Sacc® 1026 (*Saccharomyces cerevisiae*, strain 1026) at a level of 10 g/calf/day and Vitex (*Kluyveromyces fragilis*, strain Jűrgensen) at a level of 10 g/calf/day in male Holstein calves from day 4 to day 56 of age changed the pattern of the end products of ruminal fermentation and increased microbial cellulolytic activity, suggesting a shift in metabolic activities of ruminal microflora. The supplementation of Yea-Sacc® 1026 and Vitex to calf starter feeds resulted in positive effects on ruminal digestion.

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