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Fecal nitrogen excretion in dairy cows supplemented with feed additive containing active dry yeast

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Abstract

The aim of the study was to determine the fecal excretion of N (TN and NH_4^+-N) in dairy cows receiving the feed supplement Biolife® (Eurovix S.p.A., Italy) containing active dry yeast *Saccharomyces cerevisiae*. Two groups of 14 Holstein cows (B – experimental; C – control) at tie-stalled breeding participated in the experiment. Group B received the feed supplement through the total mixed ration (TMR). Fresh feces samples from both groups were collected on d 7, 14, 21, 28, 35 from the start of supplementation. Mean values of fecal pH and excreted DM did not differ between groups. The concentration of NH_4^+-N in the fecal samples of group B was reduced by 28.05%, average for the entire studied period (P<0.05). In the period d 21-35, this reduction was 35% (P=0.095). Excreted TN, averaged over the entire period, was reduced by 16.18% in group B, but the difference was not statistically significant. The reduction of TN content in feces from group B was more pronounced in the period d 21-35, when the reduction was 31.99% compared to group C (P=0.084). A period of at least 14 days is required for adaptation to the supplement and for an occur effect on N excretion.

Keywords*:* fecal nitrogen excretion; dairy cows; active dry yeast; feed additive

Introduction

Plant nitrogen (N), mostly in the form of protein, is an essential feed component for animal growth and development. Most of the consumed N is excreted, providing nutrients in the manure needed for crop growth. The problem with this cycling of N is that large losses usually occur, contributing to environmental pollution (Rotz, 2004). N losses and ammonia emissions from animal husbandry represent a significant water and air pollutant (Hristov, 2013). Besides increasing the agricultural land involved in manure use, the application of feeding and management techniques capable of increasing N retention efficiency and reducing N release per unit of animal product should be promoted (Xiccato et al., 2005). Improving N utilization by dairy cows and especially reducing N excretion in fecal masses is desirable because of concerns about the contribution of agriculture to nitrogen pollution, especially ammonia (Kebreab et al., 2002). Reference values for N excretion from different livestock systems are required for the implementation of the Nitrate Directive (91/676/EC) (Xiccato et al., 2005).

Active dry yeast is a widely used feed additive in the dairy industry (Li et al., 2021), with varying success for favorably modifying the rumen environment and promoting microbial growth (Hristov et al., 2010). The yeast *Saccharomyces cerevisiae* stimulates microbial activity in the rumen, and thus more forage N is incorporated into the microbial fraction and the flow of microbial N reaching the small intestine is increased (Williams and Newbold, 1990; Williams et al., 1990; Karr et al., 1991; Erasmus et al., 1992; Newbold et al., 1995, 1998; Hristov et al., 2010; Tristant and Moran, 2015). An increase in the total number of bacteria in the rumen as well as specific groups of microbes has been observed in a number of studies (Wiedmeier et al., 1987; Harrison et al., 1988; Dawson et al., 1990; Williams and Newbold, 1990; Erasmus et al., 1992; Newbold et al., 1998; Li et al., 2021). Greater bacterial biomass in the rumen will improve forage digestibility (Newbold et al., 1998), which may reduce urinary N losses (Hristov et al., 2010).

Supplementation with the yeast *Saccharomyces cerevisiae* reduced rumen ammonia concentration in some studies (Harrison et al., 1988; Erasmus et al., 1992; Piva et al., 1993; Enjalbert et al., 1999; Kamra et al., 2002; Alshaikh et al., 2002; Doležal et al., 2005; Moallem et al., 2009; Pinloche et al., 2013; Aoki et al., 2021). Lower ammonia concentrations are consistent with an observed increase of bacteria concentrations in rumen, which is associated with increased absorption of NH_3-N into microbial proteins and increased influx of bacterial N to the small intestine (Williams and Newbold, 1990; Erasmus et al., 1992; Hristov et al., 2010; McAllister et al., 2011). Microbial protein synthesis in rumen is an effective indicator of N utilization (Chen and Gomes, 1992; Dias et al., 2018). The concentration of NH_3-N in the rumen can be used to predict the efficiency of incorporation (absorption and retention) of dietary N into microbial protein (Bach et al., 2005), and therefore for studying feed efficiency.

A meta-analysis by Desnoyers et al. (2009) showed an increased digestibility of organic matter (OM) in whole tract and dry matter intake (DMI) in ruminants supplemented with *Saccharomyces cerevisiae*. In a number of experiments with cows supplemented with *S. cerevisiae*, crude protein (CP) digestibility was improved (Wiedmeier et al., 1987; Erasmus et al., 1992; Panda et al., 1995; Wohlt et al., 1991, 1998; Yoon and Stern, 1996; Miller-Webster et al., 2002; Lascano et al., 2012; Leicester et al., 2016; Perdomo et al., 2020; Li et al., 2021; Phesatcha et al., 2022). Based on the mechanisms of action, described in the literature of *S. cerevisiae* supplements, we set ourselves the goal of determining nitrogen excretion in the feces of dairy cows supplemented with the Biolife® feed additive containing active dry yeast *Saccharomyces cerevisiae*.

Material and methods

For the purposes of the present study, fresh fecal samples obtained from an experimental dairy cows fed with the feed additive Biolife® (manufacturer: Eurovix S.p.A., Italy) were examined. The supplement contains dehydrated yeast of *Saccharomyces cerevisiae*, malted barley, soya lecithin, and flour of seaweed. According to the manufacturer's description (Eurovix: Biolife – Technical data sheet, *Mod. 416*), a continuative use of the product in the feed ration improves the functional balance of intestinal microflora, preventing formation of toxins that may alter the organic balance of the animal; more over the better using of the feed contribute to reduce the faecal emissions of undesired compounds (above all the ammonia) reducing the environmental impact and also the olfactive pollution in animal farms.

Two groups of Holstein cows (B – experimental; C – control) of 14 animals per group were kept tie-stalled until the end of the experiment. The Biolife® feed additive was added to the concentrate mixture at a level of 500 g/ton, and supplied to the experimental group of cows with the total mixed ration (TMR). Fresh feces samples from both groups were taken in the morning before feeding on days 7, 14, 21, 28 and 35 from the start of Biolife® supplementation. After rapid unification and homogenization of the individual samples, mid-samples were separated, sealed and de-aerated in freezer bags and delivered to a laboratory for pH, dry matter (DM), total N (TN) and ammonium N (NH_4^+-N) analysis.

The pH values were determined after dilution, homogenization and stabilization of the samples with deionized water in a ratio of 1:2.5 (feces:water) and measured using a pH meter. Dry matter content (DM, %) was determined after drying in an dryer at 105 °C to constant weight. The ammonia complex (NH_4^+-N, mg/kg) was extracted with 2.0 mol/L KCl and subjected to colorimetric analysis (Nesslerization method).

Total nitrogen content (TN, %) in feces was determined by the Kjeldahl method (AOAC International, 2002). The obtained results were summarized using the statistical package MYSTAT 12 (SYSTAT Software, Inc., 2007).

Results and discussion

Table 1 presents the results of the study, statistical analysis was performed for both periods whole experiment (d 7-35) and for the period from the 21st to the 35th day (d 21-35).

The mean pH values of the freshly excreted feces did not differ between the control (C) and the experimental (B) group of cows, and they were 6.68 and 6.66, respectively, for the entire measurement period. For the period d 21-35, a slight decrease of 0.12 pH-units was reported in group B (Figure 1). The determined pH values of the feces were in the reference range for lactating cows (Palladino et al., 2022) with a daily variation of 5.80 to 6.76.

There were also no differences in DM content in fecal samples from groups C and B (on average 20.58% and 21.44%, respectively, for the whole period). However, in the period d 21-35 there was a more pronounced but insignificant increase in excreted dry matter in group B. Overall, the variation in fecal DM content was

Table 1. Effect of the feed additive Biolife® on fecal pH, DM, NH_4^+-N, and TN in dairy cows (mean ± SD)

Variable	Period and treatment					
	d 7 to 35 (n=5)			d 21 to 35 (n=3)		
	C	B	*P*-value	C	B	*P*-value
pH	6.68±0.17	6.66±0.34	0.827	6.63±0.16	6.51±0.25	0.394
DM (%)	20.58±1.18	21.44±2.14	0.490	20.47±0.81	22.00±2.51	0.295
NH_4^+-N (mg/kg)	265.69±79.24	191.16±64.34	0.019*	239.67±89.54	155.77±58.87	0.095
TN (%)	0.686±0.040	0.575±0.165	0.232	0.695±0.050	0.472±0.103	0.084

*C – Control; B - Biolife®; * - P<0.05*

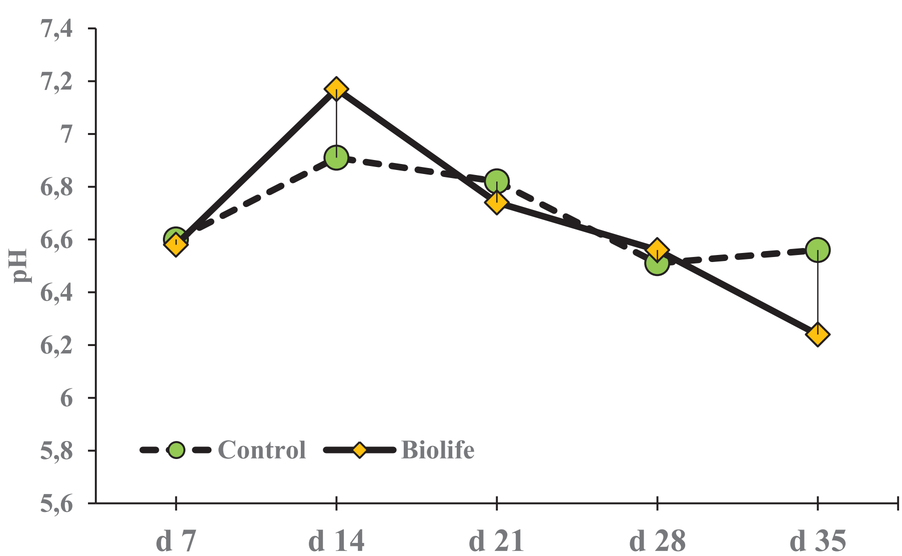

Fig. 1. Dynamics of pH values in fresh excreted feces of dairy cows, supplemented with Biolife®

low, and within the range published by Weiss (2004) from 11.9%. up to 20.9% in lactating Holstein cows.

The concentration of NH_4^+-N in fecal samples of group B (191.16 mg/kg) was reduced by 28.05% compared to that of group C (265.69 mg/kg), average over the entire study period (P<0.05). In the period d 21-35, this reduction was even higher (35.00%), however, it was not statistically significant (P=0.095). In general, for the entire experimental period, NH_4^+-N values had a moderately high degree of variation in both groups (CV=29.8% in group C and 33.7% in group B). Figure 2 reflects the dynamics in fecal concentrations of NH_4^+-N. In a study by Hristov et al. (2010), manure from dairy cows fed with a supplement of *Saccharomyces cerevisiae* fermentation product has a reduced ammonia emission potential.

Excreted total nitrogen (TN) were average 0.686% for group C and 0.575% for group B, over the entire experimental period, presenting a 16.18% reduction in TN in animals receiving Biolife®, but the difference was without statistical significance (P=0.232). TN excretion in group C maintained a constant level (Figure 3) with minimal variation (CV=5.8%), which may reflect constant daily dry matter intake (DMI) and ration crude protein (CP) during the experiment, due to the established a positive correlation between daily fecal N excretion and individual variables such as DMI, N intake and ration CP content (Bougouin et al., 2022). The low variation in excreted DM (CV=5.8%) in group C may also be a result of constant dry matter intake.

The reduction of TN in fecal samples from group B was more pronounced in the period d 21-35 (Figure 3), when the reduction was 31.99% compared to group C, but the difference again did not reach statistical significance (P=0.084). It can be concluded that a minimum period of 14 days is necessary for animals to adapt to the supplement and to report an effect on N excretion. The values for excreted TN in our study overlap with those published by Brown (2013) for dairy cows, who from an analysis of 278 fecal samples (DM 18-30%) obtained a mean value of 0.69% for TN.

Lascano et al. (2012) observed a quadratic decrease in fecal N excretion with increasing daily dose of live yeast *S. cerevisiae* (0, 10, 30, and 50 g/d) in Holstein heifers, but urinary N excretion was similar in different doses. This resulted in no effect of live yeast on total excreted N. However, total-tract N apparent digestibil-

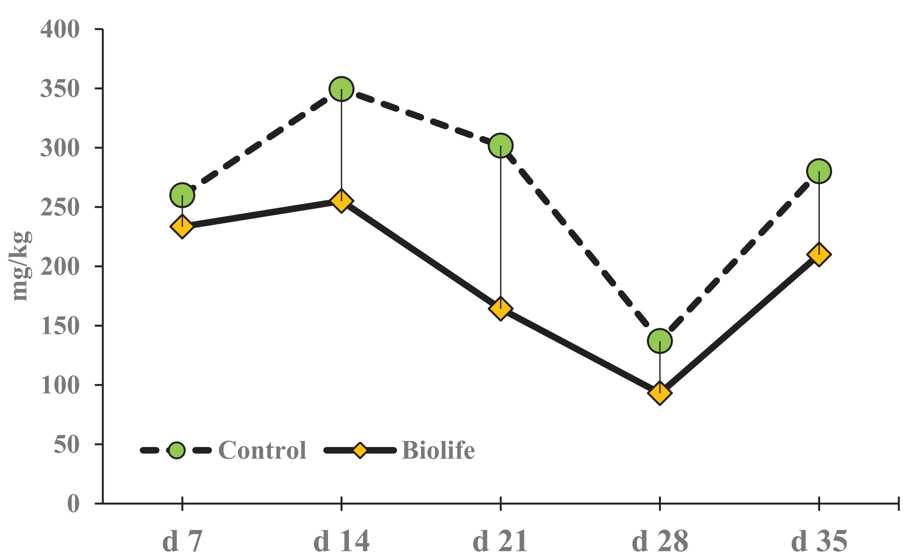

Fig. 2. Dynamics of NH_4^+-N in fresh excreted feces of dairy cows, supplemented with Biolife®

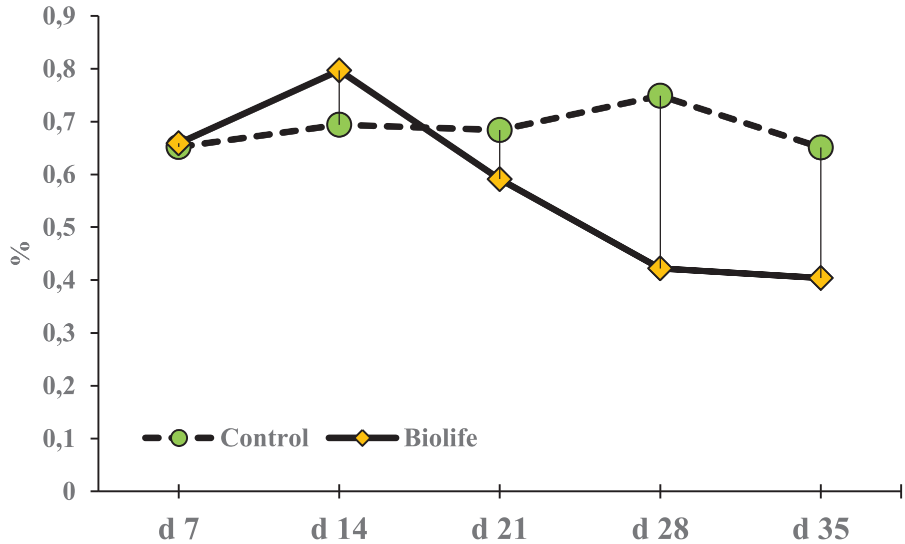

Fig. 3. Dynamics of TN in fresh excreted feces of dairy cows, supplemented with Biolife®

ity and retained N, expressed as a percentage of digested N, increased quadratically with increasing daily live yeast dose, reaching a maximum at 30 g/d. In a study by Chen et al. (2017), supplementation of Xiangzhong Black beef with *S. cerevisiae* significantly reduced fecal crude protein excretion, while Cunha et al. (2019) did not found effect of *S. cerevisiae* in Nellore heifers on N intake, fecal and urinary N excretion, N balance, urinary urea, N utilization efficiency (in relation to N intake and absorbed N), and microbial efficiency.

Conclusions

Supplementation of dairy cows with the feed additive Biolife® reduced fecal NH_4^+-N concentration by 28.05% for the period d 7-35 ($P<0.05$) and by 35.00% for the period d 21-35 ($P=0.095$). The supplement reduced TN excreted in feces by 16.18% average for the entire study period, but without statistical significance, while in the period d 21-35 the reduction of TN was by 31.99% ($P=0.084$). Mean values of fecal pH and excreted DM did not differ between groups. It can be concluded that a period of at least 14 days is needed to adapt to the feed additive and show an effect on nitrogen excretion.

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