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ScienceDirect
Animal Feed Science and Technology
140 (2008) 67–77
ANIMAL FEED SCIENCE AND TECHNOLOGY
www.elsevier.com/locate/anifeedsci

Effects of *Aspergillus oryzae* extract and a *Saccharomyces cerevisiae* fermentation product on intake, body weight gain and digestibility in buffalo calves

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Received 31 July 2006; received in revised form 14 February 2007; accepted 21 February 2007

Abstract

An *Aspergillus oryzae* fermentation extract and a *Saccharomyces cerevisiae* culture product were added to a pelleted calf starter, respectively, at 6.0 and 26 g/kg of dry matter (DM) to determine effects on faecal score, DM intake, body weight (BW) gain and *in vivo* digestibility. Treatments were control groups with the control starter alone (CSt) or with free access to ryegrass hay (CStH) and combinations with the fungal supplemented starter (ExpSt and ExpStH). Forty buffalo calves (12 male, 28 female; 10 calves per treatment) were started on the experiment at 10 days of age and for 12 weeks. Faecal scoring was conducted twice weekly, and DM intake was measured weekly. Every 2 weeks, BW gain was recorded and grab faecal samples were collected to examine for the presence of intestinal parasites and *Salmonella*. At the end of the experimental period, on a subset of 20 calves (*i.e.*, five per treatment), nutrient *in vivo* digestibility was measured by using acid-insoluble ash as internal indigestibility marker. Starter DM intake was unaffected by fungal supplementation, but inclusion of fungal additives in the starter resulted in increased apparent total tract digestibility,

Abbreviations: ADFom, acid detergent fibre exclusive of residual ash; ADG, average daily gain; AIA, acid-insoluble ash; BW, body weight; CP, crude protein; CSt, control starter; CStH, control starter with free access to ryegrass hay; DM, dry matter; DMI, DM intake; ExpSt, experimental starter; ExpStH, experimental starter with free access to ryegrass hay; FE, feed efficiency; Lignin(sa), lignin determined by direct solubilization of cellulose with sulphuric acid; NDFom, neutral detergent fibre exclusive of residual ash; OM, organic matter

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0377-8401/$ – see front matter
doi:10.1016/j.anifeedsci.2007.02.010

regardless of the presence of hay in the diet. Calves fed ExpStH diet *versus* the CStH and ExpSt diets had improved faecal consistency, higher average daily gain and higher total tract digestibility of fibre. Supplementation of starter with *A. oryzae* fermentation extract and *S. cerevisiae* culture may improve calf digestive efficiency if forage is in the diet.

Keywords: *S. cerevisiae* culture; *A. oryzae* fermentation extract; *in vivo* digestibility; Calf

1. Introduction

Weaning is a critical transition in the life of calves. The transition from milk to solid food may cause a lag in growth, especially when the milk allowance is high (Hepola, 2003). Numerous attempts have been made to stimulate rumen development in pre-ruminants in order to wean them at an earlier age and to avoid digestive disorders due to feed transition. Supplementation of diets with feed additives that promote rumen metabolic development could be a useful tool to achieve these goals.

Microbial products claiming to improve ruminant performance by modulating rumen function, and the activities of its microflora, are based either on live yeasts or yeast cultures (*Saccharomyces cerevisiae*) or on spent culture medium from growth of *Aspergillus* species. Effects of both materials on production traits have been studied in most domestic ruminants. Research with fungal additives has mainly focused on milk production in dairy cattle, with contradictory results (Williams et al., 1991; Wallace and Newbold, 1995; Lesmeister et al., 2004).

A number of studies have investigated effects of fungal cultures in pre-ruminant dairy calves (Wagner et al., 1990; Beharka et al., 1991; Quigley et al., 1992; Seymour et al., 1995; Kumar et al., 1997; Agarwal et al., 2002; Lesmeister et al., 2004). As in dairy cattle, results of these studies have been variable, perhaps due to the effects of ration composition, level of fungal culture inclusion, and source of the fungal culture examined. Beharka et al. (1991) reported earlier weaning and increased ruminal microbial activity when *Aspergillus oryzae* was fed. Wagner et al. (1990), Quigley et al. (1992), Seymour et al. (1995) and Agarwal et al. (2002) reported no effect of brewery yeast or yeast culture on calf growth or rumen microbial population and fermentation pattern of young calves. In contrast, Kumar et al. (1997) found increased ruminal bacteria numbers and a modified volatile fatty acid production when a yeast culture was fed to buffalo calves. When *S. cerevisiae* culture was included at 20 g/kg of dry matter (DM) in a dairy calf starter, DM intake (DMI) and growth were enhanced (Lesmeister et al., 2004).

Since fungal additives may stimulate rumen development and calf growth, we reasoned that the combination of the two product types could enhance the effect of their supplementation. Thus, this study was conduced to evaluate effects of inclusion of an *A. oryzae* fermentation extract and a *S. cerevisiae* culture product in a calf starter on faecal consistency, body weight (BW) gain, DMI and *in vivo* digestibility.

2. Materials and methods

2.1. Diets and animals

A pelleted calf starter (Svezzocotton, Petrini, Bastia Umbra, Perugia, Italy) was supplemented (experimental starter—ExpSt) with 6 g/kg of DM of a microbial product containing *A. oryzae* fermentation extract (Seb Diet Dry; AKRON, Arese, Milan, Italy) and 26 g/kg of DM of a microbial product containing *S. cerevisiae* culture (Diamond V XP^{TM} LS, Diamond V Mills Inc., Cedar Rapids, IA, USA). Neither additive maker claims to supply live microbes. ExpSt was not a commercially available product. The unsupplemented starter was used as the control (CSt). The study was completed from July to September 2005 on a commercial dairy buffalo (*Bubalus bubalis*) farm near Caserta in southern Italy. Forty buffalo calves (12 male and 28 female) were separated from their dams by 24 h postpartum, housed in a well-ventilated barn and kept in individual calf cages (*i.e.*, 1.2 m wide by 2.4 m long) that were elevated 40 cm above the ground. Plastic covers suspended 5 cm below the cage floor served to collect the faeces. Cages were made of galvanized metal, had fully slatted floors and were equipped with external feed and water troughs. All pens were cleaned daily. Calves were fed colostrum by nipple pail until 5 days of age and then received a commercial acidified milk replacer diet (Sloten Italia, Crema, Italy), containing per kg of DM; 237 g of crude protein (CP), 237 g of fat, 79 g of ash and 1 g of crude fibre, reconstituted to 180 g/l and fed at approximately 18 °C. Milk replacer was prepared fresh daily and offered at a rate of 100 ml/kg of initial BW for the first 2 weeks (on average 4 l/day) and thereafter according as: 5 l/day from week 4 until week 10, 4 l/day at week 11 and 3 l/day at week 12. Milk replacer was offered twice a day (at 08:00 and 15:00 h) by nipple pail and it was entirely consumed by all calves. Starting at 10 days of age, calves were assigned within sex and body weight into one of the following four treatment groups: control starter (CSt); control starter with free access to ryegrass hay (CStH); experimental starter (ExpSt); experimental starter with free access to ryegrass hay (ExpStH). From day 21 of life, milk replacer was supplemented with the starter on an *ad libitum* basis with or without chopped ryegrass hay access according to the experimental treatment. The daily ration was present in two equal feedings (at 08:00 and 15:00 h). The experimental period was 12 weeks.

2.2. Experimental measurements

Each calf was weighed at group formation (average BWs 46.2, 46.6, 46.1, 46.7 kg for groups CSt, CStH, ExpSt, ExpStH, respectively) and thereafter every 2 weeks in the morning, just before feeding, when grab faecal samples were also collected to examine for the presence of intestinal parasites and *Salmonella* spp. Individual DMI was measured weekly, beginning at grouping, by difference between feed offered and feed refused. Representative samples of feed were collected once per week.

The consistency of faeces was assessed twice weekly, using a numerical score of 0–4 (*i.e.*, 0 = severe scours, 1 = scours, 2 = soft, 3 = normal, 4 = firm). No treatment for scour was initiated if diarrhoea was assessed to be occurring.

2.3. Digestion study

At the end of the 12-week feeding period, five buffalo calves from each treatment were randomly selected to measure nutrient *in vivo* digestibility by using acid-insoluble ash (AIA) as internal indigestibility marker. The experimental protocol included a 6 days collection preceded by a 7 days preliminary period. During the collection period calves were offered experimental diets about 900 g/kg of *ad libitum* DMI, an allowance established during preliminary period. Feed consumption was measured daily along with collection of representative samples of faeces and feed. Faeces and feed samples were immediately analysed for DM. Samples of feeds offered and faeces from each calf were composited over the 6 days faeces collection period and stored until further analyses.

2.4. Analytical methods

Weekly feed samples were composited over the entire feeding period. The AOAC (1990) official methods were used to determine DM, ash and CP contents in feedstuffs as described by procedures 930.15, 927.02 and 976.05, respectively. The organic matter (OM) content was calculated as the difference between DM and ash contents, with ash determined by combustion at 550 °C overnight. Neutral detergent fibre (NDFom) and acid detergent fibre (ADFom) exclusive of residual ash were determined by methods of Van Soest et al. (1991), without the use of an amylase and sodium sulphite for NDFom. Lignin(sa) was determined by solubilization of cellulose with sulphuric acid according to Robertson and Van Soest (1981). The gross energy content was determined by calorimetry (IKA C400, Staufen, Germany) with a benzoic acid standard. All analyses were completed at least in duplicate. Samples of feed, orts and faeces collected throughout the digestion study were subjected to the same assays as feed samples. Feed and faecal samples were analysed for AIA by the 2N hydrochloric acid procedure of Van Keulen and Young (1977).

2.5. Statistical analyses

Data were analysed using the Statistical Analysis System (SAS) package (1990), with calf used as the experimental unit. Data on faecal score, average daily gain (ADG), total DMI and starter DMI underwent analysis of variance for repeated measures with treatment (CSt, CStH, ExpSt and ExpStH) as a non-repeated factor and week of observation and week of observation × treatment as repeated factors.

Feed conversion efficiency (FE), expressed as the amount of feed consumed divided by BW gain, total BW gain and the apparent digestibility coefficients were analysed by one-way analysis of variance with treatment as the factor. When significant effects (*i.e.*, $P<0.05$) of the treatment occurred, orthogonal contrasts (*i.e.*, CSt *versus* ExpSt, CStH *versus* ExpStH and ExpSt *versus* ExpStH) were used to determine differences between treatments. Hay DMI was analysed using ANOVA for repeated measures with treatment (CStH and ExpStH) as a between-animal factor and week of observation and week of observation × treatment as within-animal factors. Where appropriate, a *t* test was used to identify differences between least-square means. Significance was declared at $P<0.05$, and a tendency toward significance was declared at $0.05<P<0.10$.

Table 1
Chemical composition of the control and experimental starters, of the ryegrass hay and of the diets used in the digestibility study

	Starter[a]		Ryegrass hay	Diet[b]	
	Experimental[c]	Control		Hay supplementation	No hay supplementation
Dry matter (g/kg)	862	872	898	908	907
105 °C DM (g/kg)					
Organic matter	915.9	921.8	901.9	915.1	919.5
Crude protein	180.4	177.2	100.0	176.9	200.7
Fat	44.8	45.2	22.3	99.0	117.2
NDFom	267.9	279.2	577.0	265.6	170.7
ADFom	111.4	118.9	328.7	133.6	71.8
Lignin(sa)	29.2	25.7	38.1	21.6	17.1
Calcium	11.1	11.0	4.7	8.9	10.4
Phosphorus	6.5	6.5	2.1	5.9	7.1
Sodium	2.6	2.5	1.2	3.1	3.7
Chlorine	5.2	5.1	5.7	5.9	5.9
Potassium	10.8	10.8	15.2	12.3	11.3
Magnesium	5.3	5.4	2.2	5.3	6.3
105 °C DM (mg/kg)					
Iron	12.1	12.0	612.0	417.0	312.0
Zinc	40.4	40.2	21.2	231.5	276.9
Manganese	25.4	25.2	83.0	176.5	190.1
Gross energy (MJ/kg DM)	19.04	19.32	18.18	20.08	20.57

[a] Based on: maize grain, soybean meal, barley, wheat middlings, molasses, flaked maize, whole cotton seed, crushed soybeans, sodium bicarbonate, calcium carbonate, sodium chloride, vitamin and mineral supplement.
[b] Hay supplementation: diet based on starter, milk replacer and hay supplementation; no hay supplementation: diet based on starter and milk replacer without hay supplementation.
[c] Fungal supplemented starter.

3. Results

Chemical compositions of the feedstuffs and diets fed in the digestibility study are in Table 1. The experimental and control starters had very similar chemical composition. Hay supplementation increased NDFom, ADFom and lignin(sa) of diet, and decreased CP.

No deaths occurred in the experimental groups, and no parasite eggs or *Salmonella* spp. were found in faeces throughout the 12 weeks experimental period.

For all variables reported in Table 2, the interaction week of observation × treatment was not significant. Inclusion of fungal additives in the starter led to a better faecal score only for ExpStH calves compared to CStH ($P<0.001$). ExpStH group had the highest faecal score, and the effect of week ($P<0.001$) may be because the incidence of diarrhoea declined as the study progressed.

As milk replacer intake for the first 2 weeks was determined by the initial BW of the calves, and thereafter was the same for all calves, it could not be affected by microbial supplementation. However, starter, hay and total DMI increased ($P<0.001$) throughout the experimental period (Figs. 1 and 2).

Table 2
Faecal score, average daily gain, final body weight and feed efficiency of calves fed with different treatments

	Treatment[a]				SE	Probability[b]			
	CSt	CStH	ExpSt	ExpStH		Week	CSt *vs.* ExpSt	CStH *vs.* ExpStH	ExpSt *vs.* ExpStH
Faecal score	1.37	1.32	1.56	1.83	0.112	0.0003	0.125	0.0018	0.048
Dry matter intake (g/day)									
Starter	517	403	527	434	17.2	0.0001	0.67	0.204	0.0002
Hay	–	252	–	290	8.3	0.0001	–	0.047	–
Total	1307	1407	1320	1478	18.0	0.0001	0.60	0.0057	0.0001
ADG[c] (g/day)									
Weeks 1 to 12	451	464	502	529	13.6	0.0001	0.009	0.001	0.16
Final BW (kg)	88.7	90.1	93.2	95.9	2.36	–			
Feed efficiency[d]	0.25	0.26	0.22	0.24	0.009	–			

[a] CSt: control starter; CStH: control starter with hay access; ExpSt: fungal supplemented starter; ExpStH: fungal supplemented starter with hay access.
[b] Probability of effect of week. When treatment effect was $P<0.05$, orthogonal contrasts were used to separate mean differences.
[c] Average daily gain.
[d] kg of DM/kg of gain.

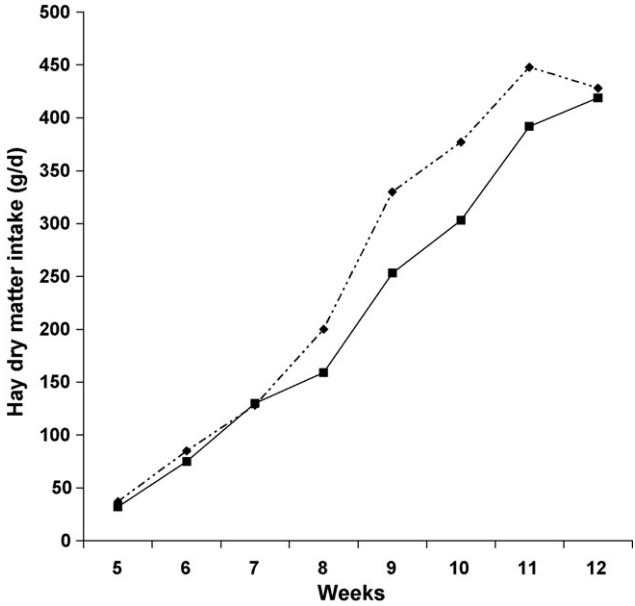

Fig. 1. Least-square means of hay dry matter intake throughout the experimental period. CStH (■): control starter with hay access; ExpStH (♦): experimental starter with hay access.

Starter intake was unaffected by use of fungal additives, but calves that were provided with hay consumed about 20% (P<0.0001) less starter compared to those fed exclusively with starter. From weeks 9 to 11, hay intake was higher (P<0.015) in ExpStH *versus* CStH groups, resulting in a higher total DMI (Figs. 1 and 2).

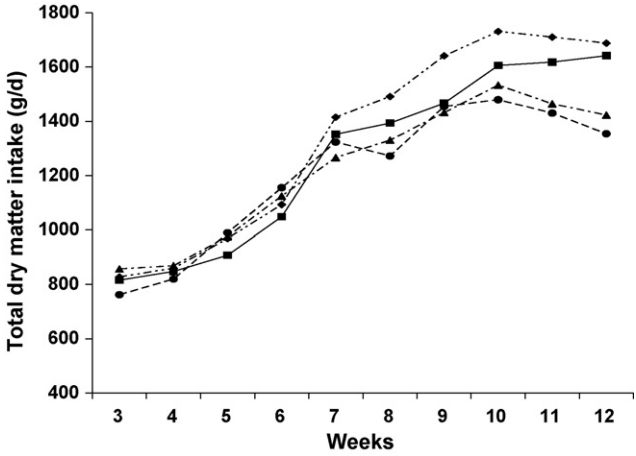

Fig. 2. Least-square means of total dry matter intake throughout the experimental period. CSt (●): control starter; CStH (■): control starter with hay access; ExpSt (▲): experimental starter; ExpStH (♦): experimental starter with hay access.

Table 3
Total tract apparent digestibility coefficients of calves fed with different treatments

	Treatment[a]				SE	Contrast probability[b]		
	CSt	CStH	ExpSt	ExpStH		CSt *vs.* ExpSt	CStH *vs.* ExpStH	ExpSt *vs.* ExpStH
Dry matter	0.79	0.69	0.81	0.71	0.008	0.04	0.046	0.0001
Organic matter	0.81	0.72	0.83	0.74	0.008	0.034	0.045	0.0001
Crude protein	0.82	0.60	0.85	0.64	0.010	0.031	0.01	0.0001
NDFom	0.32	0.47	0.41	0.56	0.027	0.029	0.028	0.0008
Energy	0.82	0.74	0.84	0.76	0.007	0.037	0.045	0.0001

[a] CSt: control starter; CStH: control starter with hay access; ExpSt: fungal supplemented starter; ExpStH: fungal supplemented starter with hay access.
[b] When treatment effect was significant (P<0.05) orthogonal contrast were used to separate mean differences.

Calf weight increased with week of observation (P<0.0001). The ExpStH group had higher ADG than CStH from week 7 until the end of the 12-week study (Table 2).

ExpSt and ExpStH calves had higher digestibilities compared to CSt and CStH groups (Table 3), with the biggest differences for NDFom. Digestibility coefficients of ExpStH calves *versus* ExpSt were lower for DM, OM, CP and energy (P<0.001), but higher for NDFom (P<0.001).

4. Discussion

During the experiment all calves were in relatively good health and free of parasitic infections. Nevertheless, inclusion of fungal additives improved faecal scores in ExpStH calves which, coupled with the tendency to a better score in the ExpSt *versus* CSt groups, seems to indicate a trend toward an improvement in faecal consistency in calves receiving fungal treated starter. Comparison of our results with previous findings must be made with caution since, to our knowledge, research into the effect of the combination of *S. cerevisiae* and *A. oryzae* has only been completed in dairy cows (Wiedmeier et al., 1987; Higginbotham et al., 1994; Yoon and Stern, 1996). However, since previous studies with calves used live strains of yeast and/or a single fungal supplement, some comparisons are possible. For example, Beharka et al. (1991) did not find an influence of dietary supplementation with *A. oryzae* on the faecal score of calves. However, contrasting reports on effects of *S. cerevisiae* on the occurrence of diarrhoea have been documented. Seymour et al. (1995) reported a numerical decrease in days with scours when brewer's yeast was included at 10 g/kg of dry feed, and attributed this effect to nutrients, mainly vitamins and amino acids, provided by *S. cerevisiae* which may have aided the growth of beneficial intestinal microorganisms and the establishment of normal fermentation in the intestine and subsequent reduction in stress and digestive upset. In addition, Agarwal et al. (2002) reported lower duration and incidence of diarrhoea in crossbred calves receiving live *S. cerevisiae*. In contrast, Lesmeister et al. (2004) found no effect of inclusion of yeast culture at 10 and 20 g/kg of DM in a calf starter on days scoured.

In our research, starter DMI was unaffected by inclusion of fungal additives. Contrasting results are reported by Beharka et al. (1991) who found no effect of *A. oryzae* supplement in an experiment with 112 calves. However, in a subset of 40 heifers, calves higher DMI was observed, perhaps because of less variation in this subgroup.

Reports on the effect of *S. cerevisiae* on DMI are conflicting. A reduction in DMI was observed in response to adding brewer's yeast (Seymour et al., 1995) or live yeast (Wagner et al., 1990) to a calf diet. The low inclusion level of yeast in calf starter (2 g/kg of DM) did not affect DMI (Quigley et al., 1992), but a 20 g/kg of DM dose led to higher starter DMI (Lesmeister et al., 2004). In the latter study, calves received no forage supplementation and the type of yeast culture and level of inclusion in the starter were similar to those utilized in the current study. However, in our study the calves were fed milk replacer throughout the experimental period, whereas in the study by Lesmeister et al. (2004) the calves were weaned at 5 weeks of age. Therefore, the post-weaning starter DMI was approximately four-fold higher than that observed at the same age in our study for calves fed ExpSt diet (on average 1538 g/day *versus* 402 g/day, respectively, corresponding to about 31 g/day *versus* 10 g/day of yeast culture). The effect of *S. cerevisiae* on DMI may be related to level of supplementation.

In our study, the use of fungal additives increased voluntary DMI of hay, resulting in higher total DMI in ExpStH *versus* CstH groups. It is possible that the yeast culture positively affected cellulolysis (Williams et al., 1991), thereby influencing hay intake *via* an increase in the rate of digestion in the reticulo-rumen.

Calves in ExpStH group had better ADG from week 7 to the end of study. Lesmeister et al. (2004) reported higher DMI and rates of BW gain in calves fed a yeast culture. Other studies have found no effect, a negative effect of brewer's yeast (Seymour et al., 1995), or a slight effect of live yeast on BW gain and DMI (Wagner et al., 1990; Quigley et al., 1992). In our study, fungal additives did not influence DMI of starter, but led to higher ingestion of hay and total DM. The higher intake of hay observed in the ExpStH group was not enough to explain the differences in ADG. Nevertheless, the higher fibre digestibility in ExpStH could have contributed to the higher growth rate, indicating higher metabolic activity by the cellulosolytic microflora in the calves rumen, resulting in higher availability of ruminal volatile fatty acids. Finally, factors such as hydration, water intake, waste excretion, ruminal content, and incidence of diarrhoea, may also influence BW gain.

To our knowledge, this study is the first to describe effects of fungal supplements on total tract digestibility in calves. In adult ruminants, effects of fungal additives on digestibility are variable, and often unpredictable, and much remains to be established about diet dependence of effects. Improvement in digestion, mainly using *S. cerevisiae*, has been previously reported (Dawson et al., 1990; Williams et al., 1991; Zinn and Borquez, 1993; Yoon and Stern, 1996). However, in some instances no effects of supplementation were observed on forestomach or total tract digestion (Adams et al., 1981; Dawson et al., 1990; Malcolm and Kiesling, 1990; Doreau and Jouany, 1998; Zinn et al., 1999).

The mode of action of fungal extracts on ruminal fibre digestibility proposed for adult ruminants are, for the *A. oryzae* extract, the action of extracellular enzymes remaining in the spent medium and, for the yeast, the presence of soluble growth factors (*e.g.*, organic acids, B vitamins, amino acids) or metabolic intermediates that stimulate growth of ruminal bacteria which utilize lactate and digest cellulose (Dawson et al., 1990; Varel and Kreikemeier,

1993; Callaway and Martin, 1996). This hypothesis is also supported by results obtained by Kumar et al. (1994, 1997), who observed (in both high concentrate and high forage diets) a large increase in concentrations of total bacteria and cellulolytic bacteria in the rumen fluid of buffalo calves fed a *S. cerevisiae* culture.

Calf apparent digestibility was affected by hay supplementation. The reduction in DM, OM, CP and energy digestibility in hay fed calves seems to have been predictable, but the higher NDFom digestibility could be due to the presence of hay in the rumen that stimulated growth of cellulolytic microorganisms to a greater extent than in calves not fed hay.

5. Conclusions

Inclusion of *S. cerevisiae* and *A. oryzae* in a calf starter improved total tract apparent digestibility of DM, OM, CP, NDFom and GE. When the experimental starter was supplemented with ryegrass hay, calves had a more favourable faecal quality, higher hay DMI, increased average daily gain and higher digestibility of NDFom. The positive effect of these additives may be related to stimulation of growth of cellulolytic bacteria, which led to increased hay intake and fibre digestibility. These fungal additives appear to have enhanced digestive efficiency of buffalo calves with hay in the diet. A better digestive efficiency can reduce the weaning stress occurring during the transition from milk to solid food.

Acknowledgements

The authors would like to thank Petrini Institute Inc. for providing starter and fungal products and Dr. G. Capurso for many useful suggestions.

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