PJN
ISSN 1680-5194

PAKISTAN JOURNAL OF NUTRITION

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
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Pakistan Journal of Nutrition 8 (3): 273-278, 2009
ISSN 1680-5194

Effect of Yeast (*Saccharomyces cerevisiae*) on Apparent Digestibility and Nitrogen Retention of Tomato Pomace in Sheep

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Abstract: Twenty mature rams were used to determine the effects of yeast (*Saccharomyces cerevisiae*) on digestibility and nitrogen retention of tomato pomace. The animals were assigned randomly to one of the four different treatments (0, 2, 4 and 6 g/head/day yeast) with five rams per treatment in a completely randomized design. Each diet was fed for 14-day adaptation following a 7-day collection period whereas tomato pomace digestibility was measured by difference method using alfalfa as the basal diet. Yeast supplementation significantly ($P < 0.05$) increased digestibility of dry matter (DM), organic matter (OM), crude protein (CP), NDF and ADF of tomato pomace where the gross digestibility derived from the supplementation was superior in 4 gram yeast compared to the control group. In addition, sheep fed yeast had a marked increase in energy digestibility of tomato pomace at 4 gram level, however, yeast did not affect energy digestibility at 2 and 6 gram. Also, the nitrogen retention of tomato pomace was improved by *Saccharomyces cerevisiae* adding at 2 and 4 gram levels. The observed results were attributed to better digestibility and nitrogen retention of tomato pomace of 4 gram yeast group, possibly due to its better supply of rumen fermentation and microorganisms' activity of digestive tract.

Key words: Digestibility, *saccharomyces cerevisiae*, sheep, tomato pomace

Introduction

The production of juice and other products from tomatoes is a major industry in some areas of the world. After the juice is extracted, a residue, tomato pomace, which primarily consists of water, tomato seeds and peels is left. The high water content (ca. 75%) of this by-product limits its length of storage. Fresh tomato pomace would spoil in two days if exposed to the air. Because of storage problems, tomato pomace is often dried. Dried tomato pomace has been fed to dairy cows and sheep (Belibasakis, 1990; Fondevila *et al*., 1994). Although the tomato pomace has a moderate concentration of CP, it has high concentrations of water, NDF and lignin. The concentration of ADIN also is high, suggesting that some of the lignin might have been an artifact (Weiss *et al*., 1997). Increasing the digestibility of ruminant diets and thereby improving nutrient efficiency is an important aspect of ruminant nutrition. This improvement in nutrient utilization must initially come from an improved rumen digestion and utilization. Yeast products especially *Saccharomyces cerevisiae* have been added to the diets of ruminant animals for many years to improve their efficiency with a variety of results. Some of the benefits associated with *Saccharomyces cerevisiae* include: increased DM and NDF digestion (Carro *et al*., 1992), increased some nutrient digestibility (Dawson, 1993; Weiss *et al*., 1997; Kim *et al*., 2006), increased initial rates of fiber digestion (Williams *et al*., 1991), increased milk production in dairy cattle (Harris and Webb, 1990; Kung *et al*., 1997), improved ruminal fermentation plus different ruminal bacteria (Lynch and Martin, 2002; Miller *et al*., 2002;), increased dry matter intake and average daily gain (Fadel, 2007; Haddad and Goussous, 2005; Jahnson and Robs, 2003).

It seems that we can increase digestibility of by-products like tomato pomace by adding additives such as yeast products. This work therefore, has the objective of evaluating the supplemental effect of yeast (*Saccharomyces cerevisiae*) as an additive on digestibility and nitrogen retention of tomato pomace in sheep.

Materials and Methods

Animals, housing and diet: Twenty mature rams were used in this experiment. The animals were housed in individual metabolic pens allowing collection of feed refusals, feces and urine. They were assigned randomly to one of four different treatments with five rams per treatment following the completely randomized design. The animal on the different treatments received the same basal diet of alfalfa hay (50%) plus tomato pomace (50%) and supplemented with 0, 2, 4 and 6 g/day yeast (*Saccharomyces cerevisiae*) respectively. Tomato pomace digestibility was measured by difference method using alfalfa as the basal diet. Yeast was added to 6g of wheat bran as an inert material in addition 5ml of molasses as appetizer and fed shortly after offering the diet. The animals were housed in individual metabolic pens allowing collection of feed refusals, feces and urine. The ingredient compositions of the tomato pomace and alfalfa hay are presented in Table 1. Feed was offered twice a day at 7:30h and 19:30h at 1.56% (as-fed basis) of live body weight and sheep had *ad libitum* access to clean water during the experiment. Each diet was fed for 14-day adaptation following a 7-day collection period. The body weight of the animals was recorded at the beginning and the end of the experimental period.

Sample collection: At the time of weighing feeds, the feed samples were collected into plastic bags, labeled and stored until chemical analysis. Daily feces and orts from each animal during data collection period were weighed, mixed, a 20% sample were taken and stored in freezer (-20^o Centigrade). A 20% urine sample (volume/volume) was taken and placed in freezer (-20^o Centigrade) until analysis for N balance measurement. After urine collection, containing 20ml sulfuric acid (normal) was added into collection container to prevent any ammonia loose. The added sulfuric acid volume was detected from sheep urine in the next collection day. Data collection was done for 7 days starting from 14th adaptation day.

Chemical analysis: Partial dry matter (DM) of samples was determined after drying at 105^o Centigrade for 24 hours. Dried samples were ground to pass a 1-mm screen, were analyzed for DM, organic matter (OM) and nitrogen according to AOAC (1978). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined according to Robertson and Van Soest. Gross energy was determined on a parr bomb calorimetric.

Statistical analysis: Data collected from the various parameters were subjected to analysis of variance (ANOVA) using completely randomized design (CRD). The differences between means were evaluated by LCD. The analysis was performed using WINKS SDA software.

Results and Discussion

Digestibility: The results of the yeast supplementation indicated in Table 2 shows that *Saccharomyces cerevisiae* yeast supplementation significantly (P < 0.05) tended to increase digestibility of dry matter (DM), organic matter (OM), crude protein (CP) and NDF of tomato pomace at 2 and 4 gram yeast and rams fed 4 gram yeast produced the best digestibilities. The Table 2 shows that supplementation at 6 gram brings no profit while supplementation above 2 gram increased the digestibilities compared to the control. Therefore, digestibility of these parameters was influenced by adding *Saccharomyces cerevisiae* at 2 and 4 gram yeast per head per day but, they were not influenced by adding *Saccharomyces cerevisiae* at 6 gram. ADF digestibility was significantly (P < 0.05) affected by dietary yeast but, there was no significant difference between levels 2 and 4 whreae, yeast supplementation did not significantly affect ADF digestibility at 6 gram level. Sheep fed yeast had a marked increase (P < 0.05) in energy digestibility of tomato pomace at 4 gram level but, yeast did not affect energy digestibility at 2 and 6 gram.

Table 1: The chemical composition of tomato pomace and alfalfa

Item	tomato pomace	alfalfa hay
DM (%)	93.62	92.2
OM (%)	95.36	93.06
CP (%)	23	13.81
CF (%)	25.92	30.12
GE (cal/g)	4274.71	4295.3
NDF (%)	54.33	51.02
ADF (%)	45.67	48.98
Ash (%)	6.8	8.82
EE (%)	10.02	2.32
DE (mcal/kg)	2.62	2.53

These results agreed with other studies (Fadel, 2007; Kim *et al*., 2006; Haddad and Goussous, 2005; Lesmeister *et al.,* 2004; Ando *et al*., 2004; Miller *et al*., 2002; Wiedmeier *et al*., 1987; Birick and Yavuz, 2001; Newbold *et al*., 1990; Newbold and Wallace, 1992) whereas, others (Mruthunjaya *et al*., 2003; Enjalbert *et al*., 1999; Erasmus *et al*., 1992; Wohlt *et al*., 1991; Chademana and Offer, 1990) have recorded no effect.

The tomato pomace had high concentrations of water, NDF, lignin and fatty acids and a moderate concentration of CP (Table 1). Overall, values were similar to reference values (NRC, 1985). Some authors (Fondevila *et al*., 1994; Gasa *et al.,* 1989) reported that the lignin concentration was much higher than published values of 7-11%. The concentration of ADIN also was high, suggesting that some of the lignin might have been an artifact (Weiss *et al*., 1997). The effect of yeast on fiber digestion were highly variable, with some authors recording increases on the fiber digestion of low quality forages (Lynch and Martin, 2002), while others (Hadjipanayiotou *et al*., 1997; Avendano *et al*., 1995; Enjalbert *et al*., 1999) have not recorded any increasing effect.

Studies on ruminal degradation of corn silage as a feed containing high fiber by yeast culture supplementation (Yeast Culture Laboratory Research, 1998), demonstrated that yeast culture increased ruminal degradation of dry matter, NDF and hemicellulose. Other researchers (Fadel, 2007; Birick and Yavuz, 2001; Enjalbert *et al*., 1999; Erasmus *et al*., 1992; Miller *et al*., 2002) have also confirmed these results using *in vivo* and *in vitro* experiments. Williams *et al*. (1991) also indicated that the initial rate of degradation, rather than the potential degradability of the forage, was affected. Fadel (2007) have also reported that NDF digestibility and rumen fermentation of forage sorghum hay in Nubian goat's kids affected by yeast (*Saccharomyces cerevisiae*) addition.

Table 2: The effect of yeast (Saccharomyces cerevisiae) on tomato pomace digestibility

Item	Yeast supplement (g/head/day) 0	2	4	6	SEM	Significance level
DM (%)	56.62[a]	65.42[b]	68.24[c]	58.12[a]	1.25	*
OM (%)	59.33[a]	63.52[b]	66.73[c]	59.54[a]	1.22	*
CP (%)	56.54[a]	62.89[b]	66.39[c]	57.53[a]	1.27	*
NDF (%)	53.37[a]	58.27[b]	60.5[c]	53.91[a]	0.94	*
ADF (%)	49.65[a]	53.29[b]	54.15[b]	50.53[a]	0.72	*
GE (%)	42.7[a]	43.52[a]	46.78[b]	43.83[a]	0.64	*

[abc]Means with different superscript in the same row differ significantly *: $P < 0.05$. SEM: Standard error of a mean.

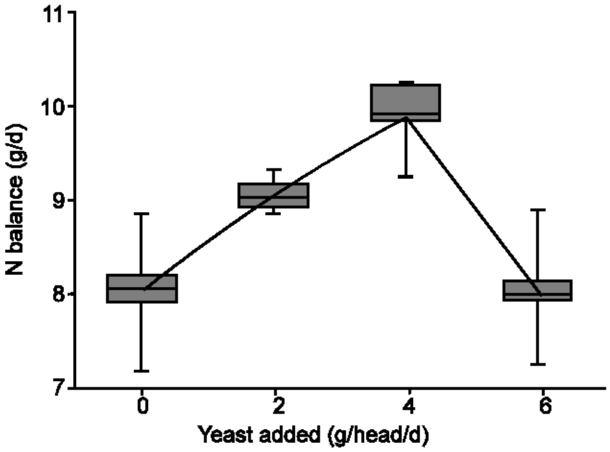

Fig. 1: Effect of yeast (*Saccharomyces cerevisiae*) on nitrogen retention of tomato pomace in sheep.

Newbold and Wallace (1992) suggested that, differences between control and yeast culture groups were not related to the number of viable yeast cells in the preparations and their ability to stimulate rumen fermentation may be related to difference in metabolic activity. It is also likely that the method of growing, harvesting and storing the culture affects the final activity (Miller *et al*., 2002).

Fadel (2007) demonstrated that *Saccharomyces cerevisiae* improved OM plus NDF digestibility compared with control diet. Some researches have shown that treatment with some yeast cultures increased the number of total and cellulolytic bacteria in the rumen and in some cases increased cellulose degradation (Miller *et al*., 2002; Dawson, 1990; Newbold, 1990; Newbold *et al*., 1990). Newbold *et al*. (1990) suggested that Aspergillus oryzae fermentation extract and *Saccharomyces cerevisiae* culture stimulated fiber digestion by ruminal microorganisms. Therefore, it is suggested that, in a diet containing tomato pomace, *Saccharomyces cerevisiae* probably alter the rumen fermentation which it can accelerate nutrient digestion in rumen due to increase microorganisms' ability.

As mentioned above in the present study, feeding 2 and 4 gram yeast daily to rams improved measured digestibility parameters but rams fed greater level of yeast had similar digestibility to control. These results suggest that yeast increased digestibility parameters at an optimum level and its effect will reduce exceed of this optimum level that probably refer to ruiminal fermentation and rumen microorganisms' activity. It seems that the rumen fermentation will alter by adding more yeast and digestibility will alter too. The initial studies suggested that the effects of yeast cultures on fiber digestion were modulated via an effect on PH in rumen. The yeast appeared to increase rumen PH so, it was suggested that the effects of yeast on fiber digestion in the rumen might be mediated via an effect on rumen PH. However, Chademana and Offer (1990) found that yeast stimulated dry matter digestion over a range of forage to concentrate ratios, with a little effect on rumen PH. Indeed, Koul *et al*. (1998) suggested that the increasing in rumen PH, in animal fed yeast was itself a secondary effect. Yeast culture stimulates the growth of lactic acid utilizing bacteria (Newbold *et al*., 1990) while, preventing acid production from hexose fermenting bacteria (Chaucheyras *et al*., 1995). Thus, it appears that the stimulation of fiber degradation in the rumen caused by yeast can not be explained by a simple increase in rumen PH, rather the effect is modulated via an increase in the number and activity of cellulolytic bacteria (Williams *et al*., 1991). This effect on fiber digestion appears to differ according to the fiber source. Roa *et al*. (1997) showed that quality of the forage affects NDF digestion respect to yeast culture, with more benefits of good quality forages.

Callaway and Martin (1997) reported that *Saccharomyces cerevisiae* culture stimulated growth of the predominal ruminal cellulolytic bacteria *Fibrobacter succinogenes* and *Ruminococcus albus* on cellubiose in medium that did not contain yeast extract, *Saccharomyces cerevisiae* culture increased the initial rate but not the extent of cellulose digestion by *Fibrobacter succinogenes* and Ruminococcus falvefaciens.

It is assumed that, the yeast supplement may provides factors stimulatory toward proteolytic bacteria therefore, resulted in increase of CP digestion. The increase in proteolytic bacteria is more, when high concentrate diets are fed (Williams *et al*., 1991). Saccharomyces *cerevisiae* supplementation has been associated with an increased flow of microbial protein leaving the rumen and enhanced supply of amino acids entering the small intestine (Enjalbert *et al*., 1992).

Nitrogen retention: As shown in Fig. 1, supplementation of the tomato pomace with 2 and 4 gram yeast per head daily significantly ($P < 0.05$) improved nitrogen retention. In contrast, 6 gram yeast inclusion did not affect it significantly. This finding is in agreement with Cole *et al.* (1992) who reported that nitrogen retention was significantly affected by yeast in lambs fed it. The increased proteolytic and peptidolytic activity of the rumen microorganisms might be responsible for the high nitrogen retention observed in yeast-fed rams. Although, Newbold *et al.* (1990) suggested that, *Saccharomyces cerevisiae* had no effect on the proteolytic, peptidolytic or deaminative activity of the rumen microorganisms *in vitro*. However, several studies have recorded a decrease in the concentration of the rumen ammonia, when yeast culture was fed (Enjalbert *et al.*, 1999; Koul *et al.*, 1998; Newbold *et al.*, 1990). Although, some authors observed a significant increase in flow of undegraded dietary protein from the rumen of dairy cows fed yeast culture (Erasmus *et al.*, 1992; Putnam *et al.*, 1997), Doreau and Jouany (1998) found an increases in the rate of in situ nitrogen degradation in animals supplemented with yeast culture. In some studies this has been associated with an increased flow of microbial protein leaving the rumen, resulting an enhanced supply of amino acids entering the small intestine (Erasmus *et al.*, 1992). However, others (Jouny *et al.*, 1991) failed to find any increase in the flow of microbial protein leaving the rumen. An increase in microbial protein leaving the rumen any help explain the production benefits observed when yeast is added to the diet. Erasmus *et al.* (1992) suggested that an improved microbial activity was responsible for a greater incorporation of ammonia into microbial protein. Such an improvement in microbial protein synthesis was sustained by Mutsvangwa *et al.* (1992) who showed a tend towards increased allantoine secretion in sheep supplemented with yeast, but was not confirmed by Newbold *et al.* (1990) or El- Hassan *et al.* (1993). The more retention of N in sheep fed yeast can explain by reduced ammonia concentrations in the rumen that appeared to result from increased incorporation of ammonia into microbial protein that probably were the direct result of stimulated microbial activity. This increased flow of bacterial protein helps to explain some of the very positive responses observed with yeast supplementation in animals (Dawson and Hopkins, 1991). But in the present study nitrogen retention only increased at 2 and 4 gram levels not at 6 gram that probably refers to altering the rumen fermentation by adding more yeast. The effect of different doses of yeast culture *Saccharomyces cerevisiae,* strain SC-47 (0, 3, 6 and 12g of yeast/day, respectively) on the lactating performance of Holstein dairy cows was described by Nikkhah *et al.* (2004). They drew a conclusion that the yeast culture had a beneficial effect on the rumen health. Other available data indicated that in the rumen fluid of animals receiving supplements of yeast culture, the content of ammonia decreased (Enjalbert *et al.*, 1999; Kamra *et al.*, 2002; Nursoy and Baytok, 2003) in addition to, the total numbers of ruminal bacteria and infusoria significantly increased (Kamra *et al.*, 2002).

Conclusion: The present study concluded that yeast (*Saccharomyces cerevisiae)* supplementation to tomato pomace significantly affect digestion and nitrogen retention of this by-product at 2 and 4 gram per head per day although, yeast supplementation at a level above 4 gram did not bring additional profit. These effects may have been due to beneficial effects on rumen fermentation and microorganisms' activity.

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