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Effect of processed lemon pulp with *Saccharomyces cerevisiae* yeast on protein and energy metabolism in Raini goats

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ABSTRACT

The aim of present study is investigating effect of treated lemon pulp by *Saccharomyces cerevisiae* yeast on protein and energy metabolism in goats was fed with this product. In this experiment 8 goats from raini breed were used for 21 days period; 16 days for adaptation and 5 days for sampling, to investigate the effect of processing lemon pulp by *Saccharomyces cerevisiae* yeast on protein receivement, retention and excretion and also energy metabolism. Goats were put in metabolic cages equipped to separate urine and feces collection system and they were fed with a diet containing lemon pulp and alfalfa with 40:60 proportion in maintenance limit. Collected data were analyzed statistically as a completely randomized design. After processing lemon pulp crude protein was increased whereas crude fat and nitrogen free extract were decreased significantly ($P< 0.05$). Digestibility of treated lemon pulp´s crude protein was higher but digestibility of treated lemon pulp´s crude fat was lower ($P< 0.05$). Consumptive nitrogen, feces nitrogen and urinary nitrogen were increased significantly whereas nitrogen retention did not change significantly also creatinin and total urinary protein were increased significantly. But uremic nitrogen and total blood protein did not change. Entirely processing lemon pulp with *Saccharomyces cerevisiae* yeast moreover increasing crude protein percent cause to increase digestive coefficient of lemon pulp´s protein.

Key words: lemon pulp, *Saccharomyces cerevisiae* yeast, Raini goat, Protein metabolism, Energy metabolism.

INTRODUCTION

Citrus fruit product is one of the most abundant fruit crops among the world with more than 88 million tones world's annual production, which about 15 million tons of citrus pulp is estimated as waste (Marin et al, 2007). Dried Citrus pulp contains various soluble and non-soluble carbohydrate polymers that make it an ideal animal feed and good substrate for biomass conversion to biofuels such as ethanol and biogas (Wilkins et al, 2007, Naparaju, and Rintala, 2006). Citrus pulp is a valuable edible material that includes a wide range of energy nutrients for rumen microorganisms (Miron et al, 2002, Scerra et al, 2001 and Tripodo et al, 2004). Every year large amounts of fresh citrus pulp is added to the diet of ruminants. These citrus are those which are not marketable or due to the large amount of their production for human or citrus juice industries were not useable (Volanis et al, 2004). Another important factor for using processing byproducts as an animal feed is the low cost of them. (Ammerman and Henry, 1991). Wet

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citrus pulp processing for increasing the protein content will cause increase their efficiency in animal nutrition. Research results indicate that processed citrus pulp by using the fungus increased crude protein in them. Because the fungi is converted rapid fermentable and lignocellulose materials in the pulp into energy, protein and carbon dioxide by extracellular enzymes (Scerra et al,1999). Citrus pulp is suitable source of soluble carbohydrate and NDF (Miron et al, 2002), these carbohydrates are energy sources that are available for rumen microorganisms. The purpose of this experiment was to investigate the effect of processing of lemon pulp with *Saccharomyces cerevisiae* yeast on protein and energy metabolism in goats fed with this product.

MATERIAL AND METHODS

Samples processing with *Saccharomyces Cerevisiae*

Lemon pulp was collected, dried, and then a sample was examined to define the chemical compositions. The temperature, humidity and acidity of lemon pulp were adjusted to prepare optimal conditions for *Saccharomyces cerevisiae* growth (dadvar et al., 2011). After being dried, the pulp was mixed with water at 1:2 ratio (pulp: water) to supply relative humidity of 85 percent. The measured pH was 3.6. The optimum pH for yeast activity is between 5 and 6. Therefore, 6.4% bicarbonate was added to lemon pulp to increase the pH. The lemon pulp samples then were placed in 35 º C oven in which yeast could grow. In order to achieve high level of crude protein, a pre-test was carried out to determine the optimum processing time and required level of yeast. Also, different times of processing and levels of yeast were examined. The results are presented in Table 1. According to these results, it was decided to use 4% yeast and 24 hours processing time in this experiment. After processing, the samples were placed in oven, and their dry matter was determined.

Coefficient digestive by in vivo techniques

Processed and unprocessed lemon pulps were mixed with alfalfa and were fed to eight adult male goats. The goats were about 3-4 years old with a mean weight of 39±1.5 kg. The experimental design was a completely randomized design with 2 treatments and 4 replicates for a 28-day period. The first twenty one days were for adaptation and the last seven days were for collecting samples. Initial weights of the goats were recorded and they were housed in individual metabolic cages equipped with separate collection of urine and feces. In order to determine the effect of lemon pulp on digestibility of the diet, digestibility of alfalfa was measured separately at the beginning of the experiment. The experimental diets contained 40% alfalfa hay and 60% lemon pulp (processed and unprocessed). Goats were fed twice a day (morning and afternoon) at a level of 40 g per kg metabolic weight. Fresh, clean water was available all the times. The animals were maintained according to the guidelines set by the Iranian Council on Animal Care (1995). All the feces was collected and weighed at 8 a.m. and 10% of the feces were sampled, packed in nylon bags and protected at -20˚C. Feed intake and residue were recorded in every day of the experiment. At the end of the period, feces and feed samples and feed residue of each goat were separately mixed, and one sample per goat was considered for chemical analyses. Total diet digestibility was determined as:

Total diet digestibility = [(40% lemon pulp) × X] + [(60% alfalfa) × alfalfa digestibility]

X: lemon pulp digestibility At the end of each period, goats were bled and plasma was collected in tubes containing EDTA. Then, the tubes were placed on ice and centrifuged at 3000 × g for 15 min. Feed digestibility for each animal were determined as follow (McDonald et al, 2011):Apparent digestibility = [(feed intake – feces weight)/feed intake] × 100.

At the end of each period, goats were bled and plasma was collected in tubes containing EDTA. Then, the tubes were placed on ice and centrifuged at 3000 × g for 15 min.

N retention was calculated as fallow:

$$\text{N Retention} = \text{N intake} - (\text{Fecal N} + \text{Urinary N})$$

Statistical Analyses

Data of chemical composition (5 replicates) were analyzed according to completely randomized design. Data of in vivo test were analyzed using the PROC MIXED procedure of SAS (2005), with the statistical model: $Y_{ij} = \mu + T_i + e_{ij}$, where Y_{ij} is the measured trait for ith treatment and jth animal, μ is the overall mean, T_i is the effect of treatment on the observed trait, and e_{ij} is the residual. The means across treatments were compared by Tukey test at 0.05% level

RESULTS AND DISCUSSION

Chemical composition

Processing of lemon pulp significantly ($p<0.05$) decreased DM content (Table 1). It was because of using pulp compositions by yeast as substrate. After internal metabolism and respiration of yeast, some amounts of carbon in lemon pulp go out as carbon dioxide, so DM percentage of treated lemon pulp decreases. Shojaosadati et al. (1999) also reported a decrease in dry matter of treated pulp. Crude protein of lemon pulp increased significantly after processing. It could be due to growth and division of the yeast that has increased the pulp protein content. Fungi and yeast use easily-digestible materials in lemon pulp by extra cellular enzymes, and produce protein (Shojaosadati et al., 1999). In the present study, ether extract and nitrogen-free extract decreased significantly in treated pulp.

The metabolisable energy of pulp decreased significantly ($p<0.05$) probably due to the use of easily digestible carbohydrates by yeasts. Kayouli and Stephen (2000) reported that citrus wastes contain high-energy content for ruminants. They reported the energy content of 10.3 and 2.4 Mcal/kg for dry and wet pulp respectively.

Table 1- Chemical composition of untreated and treated lemon pulp with *Saccharomyces cerevisiae* (DM basis)

	lemon pulp			
	Untreated	Treated	SEM (n=5)	P Value
Dry matter (%)	29.6 ^{a}	19.5 ^{b}	1.2	0.005
Crude protein (%)	8.36 ^{b}	14.31 ^{a}	0.951	0.006
Ether extract (%)	7.06 ^{a}	3.68 ^{b}	0.426	0.005
Nitrogen-free extract (%)	67.69 ^{a}	43.22 ^{b}	0.649	0.0001
Metabolisable energy[1] (Mcal/ kg DM)	3.04 ^{a}	2.61 ^{b}	0.022	0.0002

a'b' Values within rows with different superscript letters indicate a significant difference at $p<0.05$

[1] Metabolisable energy (Mcal/kg DM)=10× [(3.5×crude protein)+(8.5×crude fat)+(3.5× Nitrogen-free extract)]

Determination of digestibility coefficient by in vivo technique

The results of digestibility determined on animals are shown in table 2. Digestibility coefficients of DM were not affected by processing. Digestibility of crude protein increased significantly, however, digestibility of crude fat decreased ($P<0.05$). Processing of lemon pulp with *Saccharomyces cerevisiae* increased digestibility of crude protein, which consequently increased availability of crude protein in the rumen for microorganism utilization. Fegeros et al. (1995) reported that digestibility of crude protein in citrus pulp is around 85% of that in corn, that digestibility As a whole, fermented feedstuffs have better digestibility, and it is due to different microorganisms and their enzymes (McDonald et al., 2011). Miron et al. (2002) added dry citrus pulp to total mixed rations of dairy cows. They observed that replacing of 11% dry citrus pulp with corn seeds resulted in better conditions for rumen microorganism and increased digestibility of cell wall and crude protein. In another study, Madrid et al. (1996) fed goats with lemon pulp and alfalfa with ration 50:50 and digestibility of dry matter, organic matter, crude protein; NDF and ADF were 72.9, 78.1, 45.3, 66.5 and 68.5 %, respectively. O'Mara et al. (1999) reported that citrus pulp digestibility of organic matter, crude protein and NDF were 84.4, 50.5 and 71 % for sheep and 82.6, 42.2 and 69 % for cow, respectively. Dadvar et al (2011) were measured the digestibility of metabolizable energy of processed lemon pulp with *Saccharomyces cerevisiae* yeast by In vitro technique and observed that during processing lemon pulp with yeast, metabolizable energy was significantly reduced due to the reduction of digestible organic matter in dry matter.

Table 2- In-vivo digestibility and total digestible nutrients coefficients of untreated and treated lemon pulp with *Saccharomyces Cerevisiae*

	Lemon pulp			
	Untreated	Treated	SEM (n=4)	P Value
Dry matter (%)	58.03	62.4	1.86	0.08
Crude protein (%)	60.53 [b]	71.23 [a]	2.12	0.015
Crude fat (%)	94.8 [a]	86.06 [b]	0.71	0.007

a'b' Values within rows with different superscript letters indicate a significant difference at p<0.05

Nitrogen retention

Mean nitrogen intake, urinary nitrogen and fecal nitrogen in goats fed with processed pulp were significantly higher (P<0.05) (Table 3). With processing Lemon pulp, the crude protein was increased, so that the goats were fed diets containing processed lemon pulp were received more protein or nitrogen than the other group. With increasing nitrogen consumption in this group, the metabolism of protein in goat's body and urinary nitrogen were increased. Ben-Ghedalia et al (1989) were reported an increment in nitrogen flow to the duodenum with feed containing dried citrus pulp compared with barley feed. Kay (1969) was reported that the amount of fermentable substrate which reaches to the end of intestine can be increased fecal nitrogen and reduced apparently digestibility of nitrogen. The fecal nitrogen that contains metabolic nitrogen will be changed by dry matter intake (AFRC, 1993). It has been estimated that 21 to 40 grams of nitrogen per kg of dry matter intake is excreted metabolically (NRC, 1988). Coombe et al (1980) were reported that a high fermentable substrate will minimize the PH increment associated with rapid hydrolysis of urea in the rumen. It seems that high concentrations of rumen ammonia were led to a net absorption of nitrogen and converting it into urea and its loss in the urine (Waldo et al, 1968). With respect to these points, diets containing adequate nitrogen, leading to a decrease in blood urea nitrogen should cause a relatively large amount of nitrogen retention.

In this experiment, nitrogen retention in goats fed diets containing treated and untreated lime pulp was not different because the goats have eaten more nitrogen, have a more nitrogen excretion.

Table3- Means of intake, fecal and urine nitrogen and nitrogen retention in goats fed with untreated and treated lemon pulp with *Saccharomyces Cerevisiae*

	Lemon pulp			
Parameters	Untreated	Treated	SEM (n=4)	P Value
Nitrogen intake (g/day)	9.26 [b]	12.09 [a]	0.254	0.0044
Fecal nitrogen (g/day)	3.09 [b]	3.68 [a]	0.076	0.0041
Urinary nitrogen (g/day)	4.43 [b]	6 [a]	0.157	0.0086
Nitrogen retention (g/day)	1.73	2.4	0.362	0.392

a'b' Values within rows with different superscript letters indicate a significant difference at p<0.05

Urinary nitrogen

Mean creatinine, total protein and urinary pH of goats fed experimental diets are given in Table 4. The creatinine and total protein of urine in goats fed with processing lemon pulp were significantly increased (p<0.05) While the urine acidity of goats was not different**.** As a result of increasing metabolism of nitrogenous compounds in the body the creatinine and total protein in the urine were increased. Increasing the Nitrogen consumption and increasing its excretion in the urine of goats occurred followed by goats fed diets containing pulp processed with yeast**.** This indicates that in these goats compared with goats fed diets containing untreated lemon pulp, more nitrogen metabolism has been occurred. Olomola et al (2008) reported that there is a significant correlation between urinary nitrogen and nitrogen consumption. Most of the nitrogen will excreted throughout excretion and urine. The minimize loss of urinary nitrogen from the body is endogenous nitrogen and it's the lowest loss of nitrogen from the body and can be used for estimating the requirements of nitrogen at maintenance level.

Tabte 4- Creatinine, total protein and urinary pH in goats fed with lemon pulp before and after processing

Parameters	Lemon pulp Untreated	 Treated	SEM (n=4)	P Value
Creatinine (mg)	77[b]	105[a]	4.32	0.01
Total protein (mg)	33.66[b]	49[a]	0.942	0.0003
pH	7.5	7.2	0.201	0.1014

a'b' Values within rows with different superscript letters indicate a significant difference at $p<0.05$

Blood nitrogen

None of the measured blood parameters in goats fed with rations containing lemon pulp was affected by processing with yeast and there was no significant difference between treated and untreated pulps (Table 5). Gholizadeh and Naserian (2010) reported that replacing dry citrus pulp with barley grain significantly increased blood urea nitrogen in goats. Waldo (1968) was reported that a high concentration of ammonia nitrogen in the rumen, leads to the net absorption of nitrogen, converting it to urea and increasing nitrogen of blood urea. However, Pinzon and Wing (1976) found a decrease in blood urea nitrogen after increasing dry citrus pulp.

Table 5- Blood parameters in goats fed with untreated and treated lemon pulp with *Saccharomyces cerevisiae*

Parameters	Lemon pulp Untreated	 Treated	SEM (n=4)	P Value
Blood urea nitrogen (mg/dl)	47.87	57.04	9.26	0.522
Total protein (mg/dl)	7.99	7.24	0.228	0.808

CONCLUSION

Processing of Lemon pulp with *Saccharomyces cerevisiae* yeast was increased the percentage of crude protein, digestibility of crude protein and decreasing metabolizable energy, as we suggest to study the effect of processing lemon pulp with yeast on the performance and growth of animals in subsequent experiments.

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