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***Full Length Article***

# Biologically Treated Sugar Beet Pulp as a Supplement in Goat Rations

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## ABSTRACT

This study was carried out to determine the use of biologically treated sugar beet pulp as a supplement in goat rations. Fungus treatments (*Trichoderma viride*) fermented with sugar beet pulp was supplemented at 0.3, 0.6 and 0.9% (w/w) to complete feed mixture and mixed well just before feeding for treatments 2, 3 and 4, respectively. While, un-supplemented complete feed mixture as a control group ($T_1$), the effect of different treatments on feed intake, growth performance, digestibility coefficients, nutritive value, nitrogen balance and some rumen liquor also studied. The average daily gain (104.83 g), feed conversion (8.89 kg DMI $kg^{-1}$. gain) were significantly better for goats fed $T_4$ followed by $T_3$ compared to $T_2$ and control group. The digestion coefficients percentage of DM (70.99); OM (73.67); CP (71.38); CF (66.43); EE (72.43) and NFE (73.29) were significantly higher ($P < 0.05$) in $T_4$ compared to $T_2$ and control group. Its worthy to note that the improving digestibility of nutrients lead to improvement in nutritive value of diets. The best TDN and DCP were observed with $T_4$ followed by $T_3$ compated to $T_2$ and control group. All biological treatments had significantly ($P < 0.05$) improved nitrogen balance compared to the control gorups. The $T_4$ group was of more pronounced effect on improving N-balance. Ruminal total volatile fatty acids values were significantly higher for goats fed on biologically treated rations than the control group. Ammonia-nitrogen of treatments $T_3$ and $T_4$ were higher than values of the control and $T_2$ groups. In conclusion, biological treatments of sugar beet pulp could be used to enrich roughages and improved nutritive value and animal performance of goats.

**Key Words:** *Trichoderma viride*; Goats; Digestibility; Nitrogen balance; Daily gain; Rumen liquor parameters

## INTRODUCTION

In Egypt, there is a gap between animal requirements and the available feeds, so there is an urgent need to search for more available and cheaper roughage particularly agricultural by-products as groundnut vins hay for animal feeding. Biological treatments are preferable than other treatments such chemical and physical treatments for better and clear environment. Burning of residues lead to environment pollution and consequently health hazards. The major limitations of using these agricultural residues as feed are its low palatability and low digestibility, low protein and high fiber contents. Recently, the production of microbial protein from agricultural waste products has received the attention of several workers (Bellamy, 1975; Dunlop, 1975; Han & Anderson, 1975; Moo-Young *et al.*, 1978; Garg & Neclantan, 1981). Much interst has been evinced in new biotechniques for improving the nutritive value of lignocelluloses using biological treatment in solid substrate fermentation system under non-sterile conditions (Gupta *et al.*, 1985). Shoukry *et al.* (1985), Khorshed (2000), El-Ashry *et al.* (2003) and El-Kady *et al*. (2006) used biological treatments such as *Trichoderma viride* to improve the nutritive value and digestibility of poor quality roughages. Exogenous enzymes obtained from fungal treatment will lead to the beneficial effects on animal performance. Thus, such treatments are likely to be of great value for ruminants in positive nitrogen blance. *Trichoderma sp*. and *Aspergillus sp. were* used in different studies to enhance the protein content of SBP an to hydrolyse pectin and lingo- cellulolytic bonds, which in turn increase the soluble carbohydrate fraction and decrease the structural carbohydrate particularly, hemicellulose (Blumenthal, 2004; Mohamed, 2005).

This study was to investigate the ability of biological treatments as a supplement to improve nutrient digestibilities, nitrogen metabolism and ruminal parameter of goats fed on complete feed mixture.

## MATERIALS AND METHODS

This study was carried out in the Nubaria Experimental Station belonging to Animal Production Department.

**Biological Treated Sugar Beet Pulp**

**Microorganisms.** *Trichoderma viride F.516* was obtained from the Microbial Chemistry Department, National Research Center, Dokki, Cairo, Egypt. The organisms were maintained on medium. Sugar beet pulp (SBP) the secondary by- product of sugar industry from sugar beet was obtained from El-Fayoum sugar Factory-El-Fayoum, Egypt.

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**Preparation of fungal inoculum.** The fungal inoculum was prepared in 250 mL capacity conical flasks containing 50 mL of a medium of (g $L^{-1}$) petome, 5.0 yeast extract, 3.0, malt extract, 3.0 and sucrose, 10.0. The flasks were sterilized by autoclaving at 121°C for 15 min. The cooled sterilized flasks were inoculated by a loop of 3 days old fungal cultures. The inoculated flasks were incubated in a rotary shaker (GFL) 150 rpm at 30°C for 48 h. The fungal mycelia were used to inoculate the experimental flasks at 10 (v/w).

**Experimental flasks.** Five hundred capacity conical flasks containing 25 g moisting at solid: liquid ratio 1:2 with salt medium of (g $L^{-1}$) urea 5; ammonium sulphate, 75, $KH_2PO_4$, 5 magnesium sulphate 0.125 in 0.05 m citrate buffer pH 5.2, The flasks were autoclaved at 121°C for 30 min. The cooled sterilized flasks were inoculated with above inoculum and then incubated under static condition 30°C ± 2 for 72h. The fermented substrate was used inoculum for the following containers.

**Scaling up methodology of fungal biomass.** The treatment was scaled up in 20 L capacity flasks each containing 400 g SBP moistened with above basal liquid medium at solid liquid ratio 1:2 (The moistened SBP was sterilized in heating bags at autoclaving for 121°C for 30 min). The containers were sterilized by ethanol. The above growing fungal spores at 10% (w/w) inoculated the flasks. The inoculated flasks were incubated at room temperature (28-34°C) for 5 days to obtain sufficient amount of solid state fermented SBP.

**Harvesting.** At the end of incubation period the fermented SBP was dried in conditional airflow at 20°C tell constant weight. The fermented sugar beet pulp (SBP) was supplemented at 0.0, 0.3, 0.6 and 0.9% (w/w) to complete concentrate feed mixture (CFM) and mixed well just before feeding for groups, respectively.

**Growth trial.** Sixteen growing male kids aged (7-9 months) and had almost the same weight (17 kg) were divided into four similar groups (4 kids in each group) and housed in semi-opened pens where they were individually fed. The experimental kids were fed on the complete tested feed mixtures offered *ad-lib*. The growth trials lasted for 120 days. Tested rations were offared twice daily in two equal portions at 8.00 a.m. and 2.00 p.m. while feed residues were removed and weighed once daily before morning feeding to estimate daily feed intake. The experimental kids were individually weighed bi-weekly and offered feeds were weekly adjusted according to changes of body weight. Mineral blocks and fresh water were freely available at all time.

**Digestibility trials.** At the end of the growth, trial 3 kids of each group were chosen randomly and used to carry out four metabolism and nitrogen balance trials. Animals were housed in metabolic cages for ten days as a preliminary period followed by a seven days collection period. Drinking water was freely available.

On the last day of the digestibility trial, rumunial content samples were taken at 3 h post feeding via stomach tube and strained through four layer of cheesecloth. Samples were separated into 2 portions, the first was used for immediate determination of ruminal pH using digital pH-meter and ammonia-nitrogen ($NH_3$-N) according to AOAC (1996), while the 2nd portion was stored at -20°C after adding few drops of toluene and a thin layer of paraffin oil till analysis for TVFA's according to Warner (1964). Feed, fecas and urine were analyzed according to AOAC (1996) methods. Data were subjected to statistical analysis using SAS (1998), while differences among means were tested using Duncan (1955).

## RESULTS AND DISCUSSION

**Chemical composition.** The chemical composition of unsupplemented and biological treated sugar beet pulp as a supplement to complete concentrate feed mixture is presented in Table I. The fungal treatment as a supplement increased CP and ash contents while, OM, CF and NFE were decreased comparing with un-treated materials. The increase of CP content in the treated ration was due to the capture of access nitrogen by aerobic fermentation, also from the residual to urea from the media of fungi, El-Shafie *et al.* (2007). On the other hand, the decreasing of CF values in the experimental rations could be as results of the cellulase enzymes secreted by cellulolytic bacteria. It clear that ash values increased in treated rations there effects were due to added media of growing fungi. Similar results obtained by Shoukry *et al.* (1985); Khorshed (2000) El-Marakby (2003) and Abo-Eid *et al.* (2007). They found that, fungal treated wheat straw with biological treatment, increased total ash content, which reflect to lowering OM content. On the other hand, the biological treatment increased crude protein content of treated materials with some strains of fungus. These results agreed with those obtained by Shoukry *et al*. (1985); Khorshed (2000); El-Marakby (2003) and El-Shafie *et al.* (2007).

**Growth performance.** Average DM intake, average daily body gain and feed conversion of the experimental rations are presented in Table II. The results revealed that daily gain and DMI were significantly higher for goats feed $T_4$ following by those fed $T_3$, but the differences between $T_2$ and $T_3$ were not significant suggesting that level lower than 0.6% of sugar beet pulp treated with fungi supplemented with concentrate feed mixture diets did not increase the beneficial effects of the fermented preparation used. Data of feed conversion recorded the best value with $T_4$ followed by $T_3$ than $T_2$ and $T_1$ the control treatment. These results were in agreement with Mahrous and Abou Ammou (2005), Bassuny *et al*. (2005) and El-Kady *et al*. (2006). They indicated that daily gain, feed intake and feed conversion of biological treated roughage were improved compared with un-treated roughage.

**Digestibility coefficients and nutritive value.** Nutrient digestibility coefficients and nutritive value of the experimental rations are shown in Table III. The digestion coefficient values of DM, OM, CP, CF, EE and NFE were signficiantly higher (P<0.05) in $T_4$ and in almost nutrient for $T_3$ ration than that of the other groups ($T_2$ & $T_1$). Data of experimental rations with level lower than 0.6% fermented SBP with fungi as a supplement to complete concentrate feed mixture diet did not showed any beneficial effects of the biological preparation used. On the same trend several authors showed an increase in DM, OM, CP, CF and NFE, digestibility when fermented SBP with *T. viride* were supplemented in animals diets (Titi & Lubbadeh, 2004; Wang *et al.,* 2004; Dean *et al*., 2005; Eun & Beauchemin, 2005; Yu *et al*., 2005; Mohamed *et al*., 2005; El-Kady *et al*., 2006). Deraz and Ismail (2001) reported that *Trichoderma* treatments had the effect of loosening lignucelluletic bonds and solublize some of the hemicellulose content. However, Mora-Joimes *et al.* (2002) suggest that alpha-amylase from fungi. *A. niger* could be used as additives in improve ruminal digestibility of songhum grain starch. Gutierrez *et al*. (2005) reported that both amylolytic thermostable enzymes optained from fungus treatment have the potential to become feed additives to improve ruminal digestibility of corn and sorghum, and are stable at low humidity conditions, which may facilitate incorporation with grain during feed processing. El-Kady *et al*. (2006) found that the supplementation with *T. viride* treatment increased (P<0.05) digestibility of almost nutrients with buffalo calves compared to the control group.

Its worthy to note that the improving digestibility of nutrients lead to improve the nutritive value of diet. Therefore, the supplementation with *Trichoderma* treatment increased the nutritive value of the different biological treatment supplementation. The best TDN and DCP were observed with $T_4$ followed by $T_3$ but no significant difference was observed between $T_2$ and $T_1$ groups.

Similar results were observed with Dean *et al*. (2005) and El-Kady *et al*. (2006) they found that the nutritive value and fermentation of Bermuda grass silage or pennisentum glaucum (pearl millet) could be improved by *Trichoderma* treatment supplementation compared with control group.

Biological treatment of sugar beet pulp improved significantly (P<0.05) the digestibility coefficients, nutritive values and growth performance. These results agreed with those found by Pulatov *et al.* (1983) that the enzymatic treatment of roughage improved the digestibility of ration and slowing feed passage time through out the digestive tract that reflected better absorption.

The highest digestibility coefficient of CP in the ration treated could be an indicator of increasing the microorganism's biomes, while the highest digestibility of CF may be due to the increase of the activity of enzymes produced by microorganisms, Gado *et al*. (2007a). Also, Colombatto *et al*. (2003) reported that enzymes were more efficient in degrading fibers without increasing methane production in the rumen of the animal.

**Nitrogen balance.** Data of nitrogen balance are presented in Table IV. All treatments showed positive nitrogen balance. The estimated daily nitrogen balance ranged from (+3.08) to (+4.66) g. All biological treatments had significantly (P < 0.05) improved nitrogen balance compared to the control group. $T_4$ group showed more pronounced effect on improving N-balance. Such improvement was highly significant, compare with other treatments, when related to percent of N-intake. The improved positive nitrogen is in

**Table I. Chemical composition of the experimental rations (on DM basis)**

| Items | Experimental rations | | | |
|---|---|---|---|---|
| | $T_1$ | $T_2$ | $T_3$ | $T_4$ |
| DM | 87.20 | 88.10 | 88.43 | 88.51 |
| OM | 88.39 | 87.78 | 87.59 | 88.01 |
| Ash | 11.61 | 12.22 | 12.41 | 11.99 |
| CP | 16.00 | 16.58 | 16.72 | 16.79 |
| EE | 2.72 | 2.76 | 2.56 | 2.61 |
| CF | 16.47 | 15.87 | 16.07 | 16.11 |
| NFE | 53.20 | 52.57 | 52.24 | 52.50 |

$T_1$ = Unsupplemented complete feed mixture(control) consist of yellow corn 20%, soybean meal 15%, wheat bran 21.5%, ground nut vines hay 40% limestone 2% and vit + Min. Mix 1.5%

$T_2$= 0.3% fermented sugar beet pulp (SBP) supplemented to complete feed mixture.

$T_3$= 0.6% fermented sugar beet pulp (SBP) supplemented to complete feed mixture.

$T_4$= 0.9% premented sugar beet pulp (SBP) supplemented to complete feed mixture.

**Table II. Growth performance of goats fed the experimental rations**

| Items | Experiment rations | | | | |
|---|---|---|---|---|---|
| | $T_1$ | $T_2$ | $T_3$ | $T_4$ | +SEM* |
| NO of Animals | 4 | 4 | 4 | 4 | - |
| Experimental period day | 120 | 120 | 120 | 120 | - |
| Initial live body weight (IBW),kg | 17.0 | 17.0 | 17.0 | 16.75 | - |
| Final live body weight,(FBW),kg | 27.63$^c$ | 27.90$^b$ | 28.43$^b$ | 29.33$^a$ | 0.35 |
| Total gain, Kg | 10.63$^c$ | 10.70$^c$ | 11.43$^b$ | 12.58$^a$ | 0.26 |
| Av. Daily gain (g $h^{-1}$ $d^{-1}$) | 88.58$^c$ | 89.17$^c$ | 95.25$^b$ | 104.83$^a$ | 2.56 |
| Dry matter intake (DMI) (g $h^{-1}$ $d^{-1}$) | 885$^c$ | 898$^c$ | 908$^b$ | 932$^a$ | 3.65 |
| Total DMI/BW% | 3.97 | 4.00 | 4.00 | 4.05 | - |
| Feed conversion (kg DMI $kg^{-1}$ gain) | 9.99$^a$ | 10.07$^a$ | 9.53$^b$ | 8.89$^c$ | 0.14 |

a,b and c Means with different superscripts in the same row differ signficiantly (P < 0.05)

* Stander error of means

**Table III. Nutrient digestibilities and nuritive values of different tested rations consumed by goats**

| Items | Experiment rations | | | | |
|---|---|---|---|---|---|
| | $T_1$ | $T_2$ | $T_3$ | $T_4$ | +SEM* |
| **Digestion coefficients:** | | | | | |
| DM | 67.43$^b$ | 67.88$^b$ | 70.30$^a$ | 70.99$^a$ | 0.56 |
| OM | 68.31$^c$ | 69.75$^c$ | 71.38$^b$ | 73.67$^a$ | 0.53 |
| CP | 66.38$^b$ | 66.84$^b$ | 69.45$^a$ | 71.38$^a$ | 0.48 |
| CF | 63.21$^b$ | 63.29$^b$ | 65.33$^a$ | 66.43$^a$ | 0.41 |
| EE | 68.41$^c$ | 68.57$^c$ | 70.09$^b$ | 72.43$^a$ | 0.52 |
| NFE | 68.04$^c$ | 68.35$^c$ | 71.38$^b$ | 73.29$^a$ | 0.54 |
| **Nutritive values, %** | | | | | |
| TDN | 61.39$^c$ | 61.30$^c$ | 63.42$^b$ | 65.40$^a$ | 0.39 |
| DCP | 10.62$^b$ | 11.08$^b$ | 11.61$^a$ | 11.98$^a$ | 0.03 |

a,b and c Means within a row with different superscripts are significantly different at (P<0.05).

* SEM: standard error of means

**Table IV. Nitrogen balance and rumen fluid parameter of goats fed experimental rations**

| Items | Experiment rations | | | | |
|---|---|---|---|---|---|
| | $T_1$ | $T_2$ | $T_3$ | $T_4$ | ±SEM* |
| N-intake (g $d^{-1}$) | 21.75 | 22.07 | 22.31 | 22.90 | - |
| Fecal N (g $d^{-1}$) | 9.86 | 9.67 | 10.01 | 10.03 | - |
| Urinary N (g $d^{-1}$) | $9.09^a$ | $9.32^a$ | $8.37^b$ | $8.21^b$ | 0.43 |
| N balance (g $d^{-1}$) | $+2.80^d$ | $+3.08^c$ | $+3.94^b$ | $+4.66^a$ | 0.27 |
| N-balance % of N-intake | $12.87^d$ | $13.96^c$ | $17.62^b$ | $20.35^a$ | 0.23 |
| **Rumen fluid parameters at 3 hrs post feeding** | | | | | |
| pH | $6.81^a$ | $6.71^a$ | $6.45^b$ | $6.47^b$ | 0.08 |
| TVFA's meq $d^{-1}$ $mL^{-1}$ | $10.49^b$ | $10.48^b$ | $12.73^a$ | $12.81^a$ | 0.49 |
| $NH_3$-N mg $d^{-1}$ $mL^{-1}$ | $19.84^b$ | $19.93^b$ | $21.01^a$ | $23.57^a$ | 0.53 |

a,b,c and d Means with different superscripts with same row differ significantly ($P < 0.05$)

* SEM: standard error of mean

agreement with Langer *et al*. (1982) Marwaha *et al*. (1990) and Bakshi and Janger (1991) conducting a feeding trial growing Jersy calves fed fungal treated wheat straw. They found that retained nitrogen was 27.7% of N-intake. However, Walli *et al*. (1991) noticed a positive N-balance when they fed calves on fungal treated wheat straw. The present observation is in agreement with Kakkar *et al*. (1990); El-Ashry *et al.* (1997) and Gado *et al*. (2007b).

Generally, the superiority of N-retention in a specific ration is affected by several factors. These include possible production of microbial protein synthesis and increased presence of fermentable energy (Hagemeister *et al*., 1981), differences in availability of fermentable energy (Tagari *et al*., 1976), variability in nitrogen, which might escape fermentation from the rumen an increased utilization of ammonia in the rumen (Holzer *et al*., 1986) and the effect of the free fats in protein synthesis (Sutton *et al*., 1983).

**Rumen liquor parameters.** Data illustrated in Table IV indicated that ruminal pH were significantly lower for biologically treated ($T_3$ & $T_4$) than $T_2$ and $T_1$ groups after 3 h post feeding. These results may be related to fermentation process of both non-structural and structural carbohydrates and production of volatile fatty acids, which affected the pH to some limit until they were proportionally and relatively absorbed from the rumen wall. This assumption is in agreement with the conclusion of Reddy and Reddy (1985) who stated that pH values were inversely related to TVFA's concentration in the rumen.

Ruminal TVFA's values obtained in this study were within the normal levels (10.5–12.8 meq $d^{-1}$ $mL^{-1}$ of rumen liquor) reported by Kandil *et al*. (1996). Total volatile fatty acids values for $T_4$ and $T_3$ were higher ($p < 0.05$), while there was non-significant differences between $T_2$ and $T_1$.

The anaerobic fermentation of biological treatment ($T_4$ & $T_3$ groups) was more efficient faster yielding more TVFA's than that in $T_2$ and control groups. Also, it may be due to the increase of digestibility of organic mater similar results obtained by EL–Ashry *et el*. (2003). So the TVFA's concentration in rumen is affected by many factors such as DM digestibility, rate of absorption, rumen pH, transportation of the digesta from the rumen to other parts of the digestive tract and the microbial population in the rumen and their activities similar results obtained by Allam *et al.* (1984). One factor or more of these cases could change its pattern with proceeding time and might affect the total concentration of TVFA's found in the rumen media.

Values of rumen liquor ammonia-nitrogen Table IV of treatments $T_4$ and $T_3$ were higher ($P<0.05$) than that of $T_2$ and $T_1$ groups. These results indicted that biological treated ration ($T_3$ & $T_4$) groups fed to goats caused as increase in rumen $NH_3$-N compared to the un-treated ration.

The higher values observed with the biologically treated based rations especially $T_4$ indicated that the release of ammonia-nitrogen from those rations were easier Pujszo (1964) than control rations, or that treated ration were it is well utilized by rumen microbes. Other investigators attributed the increase in ammonia – nitrogen concentration in the rumen media to reduction of ammonia – nitrogen absorption by rumen epithelium or to a decrease in the efficiency of microbial protein synthesis (Smith *et al.*, 1980; Ikwuegbu & Sutton, 1982). Moreover, EL-Ashry *et al.* (1997) and Khorshed (2000) noticed significant increases in rumen ammonia-nitrogen concentration with fungal treated residues. Yadav and Yadav (1988) noticed that increased ruminal ammonia-nitrogen concentration might be due to higher intake of nitrogen and higher crude protein digestibility this finding agree, well with the present results.

It could be concluded, that biological treatments could be used successfully to enrich roughages and improved the nutritive value and animal performance of goats given rations supplemented with sugar beet pulp treated with *T. viride*. In addition, biological treatments are preferable than other treatments such as chemical and physical treatments for better and clear environment besides less possible negative side effects.

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