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Effect of Dietary Supplementation with Biologically Treated Sugar Beet Pulp on Performance and Organs Function in Goat Kids

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Abstract: Due to the importance of roughages in ruminant diets, the possibility of improving its nutritive value by biological treatments should be explored. Sixteen goat kids were used to evaluate the effects of diets containing different levels of fungal (*Trichoderma viride*)-treated SBP on growth performance, blood metabolic profile indicating for liver, kidney and endocrine functions and rumen fermentation. The animals were distributed randomly on four equal feeding trials, lasting for 120 days: replacement of concentrate feed mixture (CFM) by 0% (R_1) as control, 0.3% (R_2), 0.6% (R_3) and 0.9% W/W (R_4) treated-SBP. Kids weights were recorded at the beginning of experiment and thereafter biweekly till the end of experiment. Blood sera were analyzed at zero-time and at the end of the experimental period for some serum biochemical parameters and metabolic hormones. Ruminal fluid samples were collected at the end of experiment from all kids. The highest values of daily body gain (g), total body weight gain(kg), dry matter intake(g/h/d) and feed conversion (Kg DMI/Kg gain) were recorded for goats in the trial R_4 compared to other groups. There were no significant ($P>0.05$) differences in serum levels of glucose, total cholesterol, triglycerides, HDL, LDL, AST, ALT, urea and creatinine among all trials. Metabolic hormone assay revealed that thyroid hormones (TSH and T_3) and IGF-I significantly ($P<0.05$) elevated for goats in trials R_3 and R_4 compared with R_2 and R_1, while T_4 and insulin did not affected. Ruminal fluid pH significantly ($P<0.05$) decreased, while TVF acids and NH_3-N concentrations were significantly ($P<0.05$) higher for goats fed the treated rations; R3 and R4 than the R2 and control ones. It was concluded that fungal-treated SBP as supplement in CFM can improve nutritive value of rations and had beneficial effects on the performance and metabolic hormones of goat kids. However, less than 0.6% in CFM seems to be ineffective.

Key words: Goats · *Trichoderma viride* · Exogenous enzymes · Growth performance · Serum biochemistry · Metabolic hormones · Rumen fermentation

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, non conventional and cheaper sources particularly agricultural by-products as sugar beet pulp (SBP). The major limitation of using these agricultural residues as feed, are its low palatability and low digestibility, low protein and high fibre-contents [1]. Many attempts have been done to improve the nutritive value and digestibility of poor quality roughages by using biological treatments [1-3].The microbial treatment is principally based on inoculating certain strains of fungi to grow on a moist substrate and under aerobic condition in a process called solid state fermentation [4]. *Tricoderma sp.* and *Aspergillus sp.* were used in different studies to enhance the protein content of SBP and to hydrolyse pectin and lingo-cellulolytic bonds, which in turn increase the soluble carbohydrate fraction and decrease the structural carbohydrate particularly, hemi cellulose [2, 5]. Exogenous enzymes obtained from the fungal treatment will led to beneficial effects on animal performance [2].

The mechanism by which the enzymes improve the nutritive value of feeds was discussed by Rode *et al.* [6]

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and Yang *et al.* [7]. Their assumption was that the beneficial effects of such enzymes are mainly due to increased fiber and total tract digestibility in ruminants, thereby improving the digestion and absorption of nutrients. Moreover, earlier studies indicated that such effects were mostly due to increased fibrolytic microbial population and microbial colonization of rumen content following enzymes feeding and thus can increase the rate of degradation in the rumen [8, 9].

Animal performance is closely related to the regeneration of metabolism and functioning of the endocrine system. A close relationship between thyrotropin- releasing hormone (TRH) - thyroid stimulating hormone (TSH) - triiodothyronin (T_3) - thyroxin (T_4) and growth hormone (GH) had been found and this axis play an important role in growth [10]. Few studies have been carried out to determine the effects of these enzymes on the organ functions including liver, kidney and endocrine glands. Therefore the present study had an interest in this issue.

MATERIALS AND METHODS

This study was carried out at Sheep and Goat Research Unit, belongs to the Animal Production Department, National Research Centre (Nubaria Experimental Station at Abd EI-Menem Riad Village).

Biological Treated Sugar Beet Pulp

Microorganisms: *Trichoderma viride F.* 516 was obtained from the Microbial Chemistry Department, National Research Centre, Dokki, Giza, Egypt. The organisms were maintained on PDA medium. Sugar beet pulp (SBP) the secondary by product of sugar industry from sugar beet was obtained from EI-Fayoum sugar Factory-EI-Fayoum, Egypt. Studied fermentation factors and preparation of the growth media were based on those reported by Nigam [11].

Preparation of Fungal Inoculum: The fungal inoculum was prepared in 250 mL capacity conical flasks containing 50 ml of a medium of (g/L) petome, 5.0 yeast extract, 3.0 malt extract 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 48h. The fungal mycelia were used to inoculate the experimental flasks at 10% (V/W).

Experimental Flasks: Five hundred capacity conical flasks containing 25g SBP moisten at solid: liquid ratio 1:2 with salt medium of (g/l) 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, then incubated under static condition 30°C±2 for 72h. The fermented substrate was used as 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 flasks were inoculated by the above growing fungal spores at 10% (w/w) and 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 air flow at 20°C tell constant weight.

Experimental Animals: Sixteen growing male kids aged 7-9 months and weighing in average 17.0 kg, were divided into to four equal groups (n = 4) for four feeding trials; (R_1, R_2, R_3 and R_4), extended for 120 days. The animals were protected against parasitic infections by drenching of albendazole (Pharma-CID) at dose level of 1ml/10kg BW and injection of ivermection (Ivomic Super, Merial) S/C at dose level of 1ml/50 kg, before the beginning of experiment. All animals were housed in semi-opened pens where they were individually fed and kept under close clinical observation [12] and were not exposed to either stresses or pathogens during the period of experiment.

Feeding and Experimental Rations: The fermented 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 R_1, R_2, R_3 and R_4 trials, respectively. The experimental kids fed on the complete tested feed mixtures at 2% level of their body weight *ad-lib.* Chemical composition of feeds was determined [13]. Mineral blocks and fresh water were freely available all time.

The Growth Trial: Tested rations were offered twice daily in two equal portions at 8.00 a.m. and 2.00 p.m. The offered and refused feeds were daily recoded before morning feeding to estimate daily feed intake. Kid weights were recorded at the beginning of the experiment and thereafter at biweekly intervals till the end of the experiment after water and feed were at withdraw for 12 h.

Sampling and Analytical Technique

Blood Samples: Blood samples were collected by jugular vein puncture on day-zero before initiation of experiment and at the end of experimental period (120 days), into plain vacutainer tubes for serum separation which was stored at -20°C for biochemical and hormonal assay. Serum glucose was determined as soon as possible after sampling.

Biochemical and Hormonal Assay

- Sera were analyzed for glucose, total cholesterol, triglycerides, high-density lipoprotein (HDL), low-density lipoprotein (LDL), alanine aminotransferase (ALT), aspartate amino transferase (AST), urea and creatinine using automated analyzer (Olympus chemistry analyzer AU 400, Olympus optical, 0.LTD) [14]. The activity of γ-glutamyl transferase (GGT) in serum was assayed spectrophotometrically using commercial kit [15].
- Sera were also analyzed for thyroid stimulating hormone (TSH), triiodothyronin (T_3), Thyroxin (T_4) using Enzyme Immunoassay test kit (Monobind, INC, Costa Mesa, CA 92627, USA) and Eliza Reader Stat Fax-2100 [16]. Insulin and insulin like growth factor-I (IGF-I) were analyzed by radio immune-assay (RIA) kits obtained from Diagnostic System Laboratories (DSL), Corporate USA. A Gamma Counter (Multicrystal Gamma Counter Perthold LP 2103, Germany) was used. Insulin and Insulin growth factor-1 (IGF-I) were determined according to Gerich [17] and Daughaday and Rofwein [18], respectively.

Rumen Fluid Samples: At the end of experimental period, ruminal content samples were taken at 3 hrs 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) [13], while the 2nd portion was stored at -20°C after adding few drops of toluene and a thin layer of paraffin oil till analysis of total volatile fatty acids (VFA'S) [19].

Statistical Analysis: Data were subjected to statistical analysis using SAS [20], while differences among means were tested using Duncan [21].

RESULTS AND DISCUSSION

Although dramatic progress has been made in the past decade on the use of enzymes in the diets of livestock, extensive studies will be needed to exploit the full potential of this very powerful and beneficial technology.

Chemical Composition of Experimental Rations: The chemical composition of the control un-treated ration and the fungal - treated SBP as a supplement in three levels to CFM is shown in Table 1. The fungal - treated as a supplement, increased CP and ash contents while, OM, CF and NFE were decreased comparing with un-treated ration. The increase of CP content in the treated rations was due to the capture of access nitrogen by aerobic fermentation, also from the residual to urea from the media of fungi [22]. On the other hand, the decreasing of CF values in the experimental rations could be as a result of the cellulase enzymes secreted by cellulolytic bacteria. Similar results were obtained by Abo-Eid *et al.* [3], Khorshed [23] and El-Marakby [24].

Growth Performance: Average DM intake, average daily body gain and feed conversion of the experimental rations are presented in Table 2. Results revealed that daily gain, DMI and feed conversion are significantly ($P<0.05$) higher for goats feed R_4 following by those fed R_3 than those of R_2 and R_1. The differences between R_1 and R_2 were not significant suggesting that level lower than 0.6% of fungal-treated SBP supplement did not induce beneficial effects. These results were in agreement with Ganong *et al.* [3], Mahrous *et al.* [25] and

Table 1: Chemical composition of the experimental rations on DM basis

	Experimental rations			
Items	R_1	R_2	R_3	R_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

DM = Dry matter-OM = Organic matter-CP = Crude protein-EE = Ether extract-NEF = Nitrogen free extract

The concentrate feed mixture (CFM) consists of yellow corn 20%, soybean meal 15%, wheat bran 21.5%, ground nut vins hay 40%, limestone 2% and Vit + Min. Mix 1.5%

R_1 = un-supplemented complete CFM (control)

R_2 = 0.3% fermented SBP supplemented to CFM

R_3 = 0.6% fermented SBP supplemented to CFM

R_4 = 0.9% fermented SBP supplemented to CFM

Table 2: Growth performance of goats fed the experimental rations

	Experimental rations				
Items	R_1	R_2	R_3	R_4	+ SEM*
No of animals	4.00	4.00	4.00	4.00	-
Experimental period day	120.00	120.00	120.00	120.00	-
Initial live body weight (IBW), kg	17.00	17.00	17.00	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/d)	88.58^c	89.17^c	95.25^b	104.83^a	2.56
Dry matter intake (DMI) (g/h/d)	885.00^c	898.00^c	908.00^b	932.00^a	3.65
Total DMI/BW%	3.970	4.000	4.000	4.050	-
Feed conversion (kg DMI/kg gain)	9.99^a	10.07^a	9.530^b	8.890^c	0.14

Means in the same row with different superscripts differ significantly (P<0.05) *Standard error of means

Table 3: Some serum biochemical parameters in growing goat kids fed rations supplemented with biologically treated sugar beet pulp

	Zero-time					Post-120 days				
Item	R_1	R_2	R_3	R_4	±SEM	R_1	R_2	R_3	R_4	±SEM*
Glucose (mg/dl)	56.53	61.38	71.03	61.70	5.510	67.83	64.13	65.50	73.50	8.630
Total cholesterol (mg/dl)	97.88	100.25	86.88	102.13	8.570	93.63	25.50	85.00	86.25	5.760
Triglycerides (mg/dl)	33.38	31.75	29.63	30.12	2.630	29.37	28.68	29.00	32.06	2.390
HDL-cholesterol (mg/dl)	31.75	27.84	31.58	34.80	3.040	29.38	32.50	31.88	35.25	4.080
LDL-cholesterol (mg/dl)	36.37	40.63	38.80	42.50	4.150	34.87	36.12	42.75	32.87	4.080
ALT (IU/L)	24.12	23.28	20.75	19.83	3.130	19.50	22.70	18.13	20.75	3.660
AST (IU/L)	102.13	98.03	111.33	120.00	15.210	96.88	89.25	101.63	113.25	16.220
GGT (IU/L)	39.03	36.88	29.65	35.13	6.673	41.38	41.75	37.88	35.20	5.985
Urea (mg/dl)	36.75	31.25	35.50	33.00	4.215	35.93	34.25	37.50	31.75	4.245
Creatinine (mg/dl)	0.61	0.67	0.59	0.70	0.108	0.68	0.73	0.78	0.57	0.111

Means in the same row with different superscripts differ significantly (P<0.05) *Standard error of means

Bassuny *et al.* [26]. They indicated that daily gain, feed intake and feed conversion of biologically treated roughage improved as compared with untreated roughages.

Serum Biochemistry: Table 3 demonstrates the values of some serum biochemical parameters related to liver and kidney functions. There were no significant (P>0.05) differences among different experimental groups concerning serum glucose levels and lipid profile. Also the treatments did not alter the activities of AST, ALT and GGT enzymes, or the concentrations of serum urea and creatinine. Generally all these parameters were within the normal range of goats [12, 27]. The obtained results indicated that the fungal-treated rations had no adverse effect on liver or kidney functions. Similar results were obtained in lambs [28], cattle [29] and buffalo-calves [2] supplemented with exogenous enzymes.

Metabolic Hormone Levels: Table 4 shows the metabolic hormonal levels including thyroid hormones (TSH, T_3 and T_4), insulin and IGF-I among the different experimental groups. Thyroid hormones, which are mainly functional in the regulation of tissue growth and metabolism, are influenced by many factors including nutrition [30]. This is supported by the finding of Ganong [31], who concluded that the increase in the basal metabolic rate is accompanied by increased appetite and subsequent with increased body weight.

In the present study the obtained results showed that the blood concentrations of thyroid hormones; TSH and T_3 were significantly higher (P<0.05) in goats fed the experimental rations R_3 and R_4 than R_1 and R_2, whereas that of T_4 did not seem to be affected. These results suggest that the enzymes directly or indirectly promoted an enhanced activity of deiodinase in liver and kidney tissues, promoting the transformation of T_4 into the

Table 4: Some serum metabolic hormone levels in growing goat kids fed rations supplemented with biologically treated sugar beet pulp

	Zero-time					Post-120 days				
Item	R_1	R_2	R_3	R_4	±SE	R_1	R_2	R_3	R_4	±SEM*
TSH (IU/ml)	1.38	1.06	1.44	1.59	0.23	1.25^{a}	1.55^{ab}	1.79^{ab}	2.13^{b}	0.25
T_3 (ng/ml)	0.89	0.93	0.79	0.88	0.10	0.91^{a}	1.16^{a}	1.36^{b}	1.50^{b}	0.09
T_4 (g/dl)	6.78	5.75	6.00	5.90	0.54	6.53	6.07	5.95	6.13^{ns}	0.47
Insulin (IU/ml)	5.81	6.69	5.98	7.05	0.71	5.96	6.32	6.16	7.19^{ns}	0.43
IGF-I (ng/ml)	96.86	117.5	97.38	86.25	10.36	97.63^{a}	97.50^{a}	137.13^{b}	142.20^{b}	9.02

Means in the same row with different superscripts differ significantly (P<0.05) ns = not significant

*Standard error of means

Table 5: Rumen fluid parameters of goats fed rations supplemented with biologically treated sugar beet pulp

	Experiment rations				
Items	R_1	R_2	R_3	R_4	±SEM*
Rumen fluid parameters					
at 3hrs post feeding pH	6.81^{a}	6.71^{a}	6.45^{b}	6.47^{b}	0.08
TVFA's meq/100ml	10.49^{b}	10.48^{b}	12.73^{a}	12.81^{a}	0.49
NH_3-N mg/100ml	19.84^{b}	19.93^{b}	21.01^{b}	23.57^{a}	0.53

Means with different superscripts with the same row differ significantly (P<0.05), *Standard error of mean

biologically active hormone (T_3) [31]. This in turn was responsible for enhanced rate of metabolism and accelerated growth in kids. Also, Hornick *et al.* [32] confirmed the present results, as they suggested that T_3 values are good indicators for growth changes. However, others reported that T3 values are not affected much in the cases of growth problem [33]. It seems that exogenous enzymes increased the blood concentrations of T_3 and correspondingly changed the concentrations of T_4 and TSH, suggesting that these enzymes have physiological effects similar to those of TRH.

The role of IGF-I is the regulation of tissue growth and differentiation and it was suggested that this hormone is regulated by nutrition [34, 35]. As shown in Table 4 serum levels of IGF-I elevated significantly (P<0.05) in the experimental groups R_3 and R_4 compared with R_2 and control groups.

In the current study the blood levels of T_3 and IGF-I were elevated simultaneously in the biologically treated diets. The crude-enzyme-microbial preparation may have had some growth-promoting substances that could have affected the cell receptors, thereby producing a physiological effect. The other possibility is that the enzyme preparation enhanced the digestion of feed [36], which in turn could have had an indirect effect on hormone concentrations. However, extensive studies will be needed to completely characterize these effects.

Rumen Liquor Parameters: Data presented in Table 5 indicate that ruminal pH were significantly (P<0.05) lower in goats fed R_3 and R_4 than R_2 and R_1 rations after 3h post feeding. These results may be related to fermentation process of both non-structural and structural carbohydrates and production of VFAs 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 [37], who stated that the pH values were inversely related to TVFAs concentration in the rumen.

In the present study, ruminal TVFA's values were within the normal levels (10.5-12.8 meq/dl) of rumen liquor [38].

Total TVFA's values for R_4 and R_3 were higher (P<0.05) than R_2 and R_1 rations, while there was non-significant difference between R_2 and R_1. The obtained results, indicated that anaerobic fermentation in rumen of kids in the trials R_4 and R_3 was more efficient faster, yielding more TVFA's than in R_2 and control ones. Also, it may be due to the increase of digestibility of organic matter [1, 36].

Rumen NH_3-N levels of goats fed R_3 and R_4 rations were significantly (P<0.05) higher than those of R_2 and control. The higher values observed with the biologically treated rations, especially R_4 indicated that the release of NH_3-N from these rations were easier [39]. Other investigators attributed the increase in NH_3-N concentration in the rumen media to higher intake of nitrogen and higher crude protein digestibility [40]. Similar results were obtained by Khorshed [23] and Al-Ashry *et al.* [41], who observed significant increases in rumen NH_3 -N levels with fungal treated residues. It could be concluded that the use of aerobic fermentation of SBP with *T. Viride* is a promising biological treatment

that can be successfully used to enrich roughages and improved the nutritive value and performance and metabolic status of goats.

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