Bioconversion of Some Agricultural Wastes into Animal Feed by *Trichoderma* spp.

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Abstract: To improve the protein content and nutritional value of some agricultural wastes; tomato leaves, sugar beet leaves, sugar beet pulp, rice straw and sugarcane bagasse, fungal strains *Trichoderma viridi*, *T. harzianum* and *T. reesei* were used. In this experiment, crude protein contents recorded 3.75, 5.62, 10.62, 14.31 and 15.12% of the raw cellulosic wastes sugarcane bagasse, rice straw, sugar beet pulp, tomato leaves and sugar beet leaves, respectively. Results showed that, pretreatment of wastes with acid (0.5 N H_2SO_4) and boiling for 60min. of tomato leaves increased crude protein content in fermented substrate using *Trichoderma viridi*, *T. harzianum* and *T. reesei* , 15.12 to 18.53, 18.52 and 18.25% after 5, 10 and 15 days, of fermentation time respectively. Where sugar beet leaves yielded the highest crude protein content (14.2%) after 5 days with *T. reesei*. Treated sugar beet pulp was the most efficient pretreatment for the production of maximum crude protein content (17.9%) with *T. reesei* after 5 days. Whereas, Rice straw supplemented with ammonium sulphate increased crude protein content to 7.92, 7.83 and 7.79% for *T. reesei*, *T. viridi* and *T. harzianum*, respectively after 10 days of fermentation period. From the biological assay in which albino mice were used, except for 40% which was not economically efficient ,it is recommended to use diet supplemented with 10, 20% of fermented cellulosic wastes to improve the nutritive value of the studied cellulosic wastes as animal feed .

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1. Introduction

A challenging problem today is to ensure a maximum production of proteins to meet the nutritional demands of the growing human population especially if it is from a renewal resource. One possible solution to this problem is single cell protein (SCP) which is used as a protein source in human food and animal feed (Adedayo *et al.,* 2011).

Approximately half of the agricultural biomass produced is not used as a food or feed or for the production of textiles, representing lignocellulosic residues (Smith *et al.,* 1988 and Foyle *et al.,* 2007). Among the main recovery products; activated carbon, degradable plastic composites, cosmetics, bio sorbent, resins, medicines, foods, feeds, methane, bio pesticides, bio promoters, fertilizer and other miscellaneous products are produced (Galbe & Zacchi, 2007 and Demirbas, 2008). Presently, these wastes are disposed off through burning, creating environmental problem and disturb the rich soil biodiversity through heating (Vibha and Sinha, 2005). In Egypt, agriculture wastes accumulate in huge quantities ranging from 22 to 26 million dry tons per year (Ministry of Agriculture report, 2009, Abou El- Azayem and Abd El-Ghani, 2010). Moreover, bioconversion of agricultural and industrial wastes to protein-rich food and fodder stocks has an additional benefit of making the final product cheaper (Anupama and Ravindra, 2000 and Mosier *et al.,* 2005).

Physical pretreatment of wastes includes mainly two categories: mechanical pretreatment and thermal pretreatment. The use of mechanical chopping (De Sousa *et al.,* 2004); milling (Guerra *et al.,* 2006 and Inoue *et al.,* 2008) have successfully proved as a low cost pretreatment strategy. Hot water treatment can help to solubilize all of the hemicellulose and one to two-thirds of the lignin in certain biomass sources (Liu and Wyman, 2003 and Mosier *et al.,* 2005). Combined chemical and physical treatment systems are of importance in dissolving hemicellulose and alteration of lignin structure, providing an improved accessibility of the cellulose for hydrolytic enzymes (Sun and Cheng, 2002 and Hendriks and Zeeman, 2009).

Many researchers were investigated alkali pretreatment that could lead to facilitate the degradation and better enzymatic hydrolyses of agricultural wastes e.g., wheat straw, spruce wood waste, sugarcane, cassava and peanuts wastes and corn cob. (Carrillo *et al.,* 2005, Zhao *et al.,* 2007, Martin and Thomsen, 2007 and Torre *et al.,* 2008)

Fungal pretreatment of agricultural residues is a new method for the improvement of digestibility (Sinegani *et al.,* 2005). White-rot fungi are the most effective basidiomycetes for biological pretreatment of

lignocellulosic materials (Sun and Cheng, 2002). Recent studies have shown that *Aspergillus terreus* (Emtiazi *et al.,* 2001); *Trichoderma spp.* (Pérez *et al.,* 2002); *Cyathus stercoreus* (Keller *et al.,* 2003); *Lentinus squarrosulus* (Shide *et al.,* 2004); *L. edodes* (Songulashvili *et al.,* 2005 and Brienzo *et al.,* 2007); *Trametes pubescens* (Melamane *et al.,* 2007); *Pleurotus spp.* (Belewu, 2006 and Locci *et al.,* 2008) and *Penicillium camemberti* (Taşeli, 2008); were efficiently used in the biological treatments of agricultural wastes.

Single Cell Protein (SCP) production from low cost wastes offers a potential substrate for conversion of low-quality biomass into an improved animal feed and human food (Anupama and Ravindra, 2000). The crude protein (CP) content was increased from 3.10% in wheat straw to 10.91% in spent wheat straw (Bakshi and Jangar, 1991, Bajwa *et al.,* 1991). Ghanem (1992) used Beet pulp (BP) as a medium cultivated with mixed culture of *Trichoderma reesei* and *Kluyveromyces marxianus.* Mixed culture for high SCP yields, Bhalla and Joshi (1994) improved the protein content of apple pomace by cultivating cellulolytic filamentous fungi (*Thrichoderma* and *Aspergillus niger*) and yeasts (*Saccharomyces cereviseae*, *Candida utilis* and *C. tropicalis*) in different combinations. The co-culture of *C. utilis* and *A. niger* was the best combination, resulting in a 200% increase in protein content after only 7 days of solid-state fermentation. Dhanda *et al.* (1994) fermented wheat straw with white rot fungi and observed that the CP content was increased from 3.42 to 6.18%. Vibha and Sinha (2005) evaluated SCP production of six cellulolytic fungi viz., *T. harzianum*, *Penicillium citrinum*, *Curvularia lunata, Aspergillus flavus*, *A. niger* and *Alternaria alternate* from pre-treated rice stubble. Only *T. harzianum* significantly showed high SCP production with crude protein and biomass, especially when potassium nitrate was used as a nitrogen source, whereas the lowest protein and biomass were recorded in case of *A. niger*.

The aim of the present study is to investigate the efficiency of different *Trichderma* species in converting some agricultural wastes into a useful product for animal feed.

2. Material and Methods

2.1 Cellulosic raw materials

Sugarcane bagasse was collected from sugar-cane juice shops of different districts in Fayoum city, and sugar beet pulp was collected from Fayoum Sugar Factory, Kasr El-basel, Etsa, Fayoum, Egypt, while, sugar beet leaves, rice straw and tomato leaves were collected from different Fayoum farms. The raw materials were cleaned, air-dried, and finely powdered by a laboratory mill.

2.2 Microorganisms and Media used

Trichoderma viride AUMC 28020, *Trichoderma harzianum* AUMC 20476 and *Trichoderma reesei* AUMC 5829 were collected from Mycological Center, Faculty of Science, Assiut University, Assiut, Egypt. Potato dextrose agar medium (PDA) (Bridson, 2006) was used as a maintenance medium for fungal cultures. The inoculation medium was used as described by Abd El-Bakey (1974), Basal mineral medium (Mandels and Weber, 1969) was used for the fermentation course.

For inoculum preparation, cultures from each of the selected strains, were suspended in 10ml sterilized distilled water (0.01% Tween 80). One ml of the spore suspension was used to inoculate 100ml of the medium and then incubated on a shaker incubator, at 100 rpm, for 24 hours at 30±2°C. Fungal spore counts were determined by haemo-cytometer and the inoculums density was 2×10^8/ml.

Physiochemical treatment:

Sugarcane bagasse and rice straw were soaked in 0.5N H_2SO_4at 1: 10 (substrate: solution) ratio. The treated substrate was boiled at 100˚C for 30 (T_1) or 60 (T_2) min, then washed with double distilled water for removal of chemicals. The treated substrate was filtered and neutralized by washing with dilute aqueous sodium hydroxide followed by washing with double distilled water until the filtrate becomes neutral. The substrate was dried at 50˚C for 12 hours on hot air fan oven before used for fermentation process. The same procedure was conducted for sugar beet leaves, sugar beet pulp and tomato leaves. The C/N ratios of different wastes used in the experiments were adjusted to give a final C/N ratio of 30:1.

2.3 Fermentation process

Fermentation was conducted in 500 ml flasks containing 15 gm oven dry milled cellulosic wastes and 235 ml of basal medium mentioned above. The pH of the mixture was adjusted to 4.5 and the flasks were autoclaved at 121°C for 15 minutes. Each flask was then inoculated with 20 ml of the fungal culture using, *T. viride*, *T. harzianum* and *T. reesei* each separately. All flasks including control were set in triplicates and incubated at 30° C for 15 days, excluding the control (raw material) which was not treated and inoculated with different fungal strains. Crude protein samples were taken at 5, 10 and 15 days of the incubation period. The biomass produced in each flask for every treatment was collected through filtration then dried at 50°C for 24 hours. The increase percentage in biomass was calculated according to the following equation:

$$= \frac{\text{Biomass produced weight (gm)} - \text{Waste weight (gm)}}{\text{Waste weight (gm)}} \times 100$$

Chemical analysis of cellulosic wastes:

Moisture, ash contents and Crude fiber content were determined as described by, A.O.A.C. (1970). Total nitrogen was colorimetrically determined in dry cellulosic materials powder by using the Orange G dye colorimetric method (Hafez and Mikkelsen, 1981). Organic carbon determination was carried out as described by Black *et al.* (1965).

Biological experiment design

Eighty male albino mice with body weight of 21-24g were obtained from the Vacsera, Ministry of Agriculture, Egypt, and were acclimated to animal house conditions for one week prior to the experiment. They were housed in groups of five each in universal polypropylene cages at room temperature and at a 12h/day photoperiod. Animals were fed on standard laboratory (El-Nasar Lab. Chem. Co., Egypt) and water *add libitum* till the end of the experiment (five weeks). The mice were divided into sixteen experimental groups of five mice each, as follows:

Group I	(Control) fed with normal basal diet daily.
Group II	fed on a diet containing 10% treated sugarcane baggase daily.
Group III	fed on a diet containing 20% treated sugarcane baggase daily.
Group IV	fed on a diet containing 40% treated sugarcane baggase daily.
Group V	fed on a diet containing 10% treated rice straw daily.
Group VI	fed on a diet containing 20% treated rice straw daily.
Group VII	fed on a diet containing 40% treated rice straw daily.
Group VIII	fed on a diet containing 10% treated tomato leaves daily.
Group IX	fed on a diet containing 20% treated tomato leaves daily.
Group X	fed on a diet containing 40% treated tomato leaves daily.
Group XI	fed on a diet containing 10% treated sugar beet leaves daily.
Group XII	fed on a diet containing 20% treated sugar beet leaves daily.
Group XIII	fed on a diet containing 40% treated sugar beet leaves daily.
Group XIV	fed on a diet containing 10% treated sugar beet pulp daily.
Group XV	fed on a diet containing 20% treated sugar beet pulp daily.
Group XVI	fed on a diet containing 40% treated sugar beet pulp daily.

2.4 Biological assay

The effect of feeding on the best material that gave the highest crude protein from previously treated agricultural wastes on body weight, liver and kidney function efficiency of albino mice was studied to assure the safety limits and nutritive value of these agricultural wastes as suitable animal feed.

Body weight (BW): Body weight (BW) was recorded at weekly intervals during the experimental period (5 weeks).

Body weight gain (BWG): Body weight gain (BWG) was calculated by subtracting the initial body weight (BW_0) at the beginning of each period from the final body weight (BW_5) at the end was calculated as in this formula ($BWG = BW_5 - BW_0$).

Growth rate (GR): GR was calculated per mice during the experimental period according to the following equation (Brody, 1945):

$$GR = \frac{(W_2 - W1)}{0.5\,(W_1 + W_2)} \times 100$$

Where, W_1= initial weight (g) and W_2= final weight (g).

Protein efficiency ratio (PER): Protein efficiency ratio (PE) was calculated according to the following equation:

$$PER = \frac{\text{Weight gain g/mice during the whole period}}{\text{Total protein g/mice during the same period}}$$

At the end of the experimental period (5 weeks) animals were fasted overnight, and blood samples were collected in tubes containing gel called vacutainer and left at room temperature. Plasma was obtained by centrifugation at 3000 rpm for 15min and then stored at (-20°C) until analysis. Liver and kidney organs were excised, rinsed in chilled saline solution, and then plotted on filter paper, each weighed to calculate the absolute organs weight.

Biochemical analysis

Plasma enzyme activities of aspartate aminotransferase (AST, E.C.2.6.1.1.) and alanine aminotransferase (ALT, E.C.2.6.1.2.) (As indicators for liver damage or injury from different types of diseases) were measured according to the method

described by Reitman and Frankel (1957). Total protein, albumin, cholesterol and urea were determined according to the methods described by Gornall *et al.* (1948), Doumas *et al.* (1971), Carr *et al.* (1993) and Fawcett and Soctt (1960), respectively using enzymatic colorimetric procedures Kits from Bio-Diagnostic Co., Egypt. Globulin was determined by difference between total protein and albumin. Albumin: globulin ratio was also calculated.

Statistical analysis:

Data were analyzed using General Linear Models (GLM) procedures of Statistical Package for Social Science (SPSS) software (SPSS, 2010). Duncan's multiple range tests were used for comparing means (Duncan, 1955).

3. RESULTS AND DISCUSSION

3.1 Chemical composition of cellulosic wastes

The results shown in Table (1) indicated that sugarcane bagasse contained the highest amount of crude fiber (36.8%) followed by rice straw, sugar beet pulp, tomato leaves and sugar beet leaves (34.7, 27.7, 14.5 and 7.9%, respectively). It is also clear from results that sugar beet pulp contained the highest organic carbon content (47.0%) followed by sugarcane bagasse, tomato leaves, rice straw and sugar beet leaves (43.3, 42.0, 34.9 and 29.7%, respectively). Regarding total nitrogen content, data revealed that tomato leaves, sugar beet pulp and sugar beet leaves contained the highest amount of total nitrogen (2.42, 2.29 and 1.75%, respectively) compared to those of rice straw and sugarcane bagasse which were 0.9% and 0.6% respectively. With respect to ash content, data showed that rice straw, tomato leaves and sugar beet leaves contained high amount of ash 14.60, 13.56 and 12.50%, respectively. While, sugar beet pulp and sugarcane bagasse have moderate content of ash being 5.5 and 2.4%, respectively. The moisture content was almost the same in all cellulosic wastes except sugar beet leaves; it was slightly higher, whereas sugar cane bagasse contained the lowest content being 6.5%.

Table (1): Chemical composition of various cellulosic wastes (%).

Component (%) / Cellulosic materials	Moisture	Ash	Total nitrogen	Crude fiber	Organic carbon	C/N
Tomato leaves	10.60	13.56	2.42	14.5	42.0	17.36
Sugar beet leaves	12.5	12.50	1.75	7.9	29.7	16.97
Sugar beet pulp	9.8	5.5	2.29	27.7	47.0	20.50
Rice straw	8.7	14.60	0.9	34.7	34.9	38.77
Sugar cane bagasse	6.5	2.4	0.6	36.8	43.3	72.17

The results shown in Tables (1) indicated that, the tested cellulosic wastes are rich in energy materials; In addition, these wastes are poor in crude protein except tomato leaves, sugar beet leaves and sugar beet pulp respectively. The complexity of different cellulosic wastes makes them unsuitable for animal feeding; therefore, some chemical and biological pretreatments should be take place (Farghaly, 1993 and El-Sayed, 2001).

3.2 Effect of different pre-treatments on production of protein enriched product

Cellulosic wastes such as tomato leaves, sugar beet leaves, sugar beet pulp, rice straw and sugarcane bagasse represent inexpensive and ready available carbon source. Because, a unique characteristics of high amount of lignocellulose (mainly cellulose and small amount of lignin), suitable pretreatment is needed before bioconversion of such cellulosic wastes into protein enriched product. Consequently, the effects of some applicable pretreatments such as mechanical, physical and chemical treatments before fermentation were investigated. Bioconversion of cellulosic wastes was increased with pretreatment severity, but in various degrees. Cellulosic wastes grinding process, as a mechanical pre-treatment, leads to disruption of plant cell walls which enables microbial enzymes to penetrate plant tissues and consequently may affect the bioconversion rate of wastes into a highly protein enriched product through fermentation process.

The significant increase in protein content due to physiochemical treatments may be attributed to the chemical pretreatment that led to the breakdown of the ligno-cellulosic bonds due to hydrolysis process which later on resulted in the release of available fermentable carbohydrates (Abd El-Baki *et al.,* 1984). The efficiency of such heat and physiochemical pretreatments may be due to the disruption of the cellulose- hemicellulose- lignin complex to provide components rich in either cellulose or hemicellulose which are subsequently enzymatically hydrolyzed by the fungal enzymes providing xylose and glucose for fermentation.

3.3 Effect of biological treatment on boiled wastes treated with acid on protein production efficiency

Data in Tables (2 & 3) indicate that the maximum crude protein (18.53%) was produced after 5 days incubation with T_2 for *T. viride*, while it was 18.52% and 18.25% for *T. harzianum* and *T. reesei* after 10 days of incubation on tomato leaves,

respectively with the same treatment. Similar results by Dhillon *et al.* (1980) were found who ascribed similar reasons for the slight decrease in crude protein after the fifth day of incubation.

Table (2): Crude protein produced by different fungal strains after acid and heat treatment for 30 minutes.

Trichoderma spp. used	Periods (days	Crude Protein %									
		Tomato leaves		Sugar beet leaves		Sugar beet pulp		Rice straw*		Sugarcane bagasse *	
		C	T	C	T	C	T	C	T	C	T
T. viride	0	15.12	15.12	10.62	10.62	14.31	14.31	5.62	5.62	3.75	3.75
	5	16.84	18.33	11.65	13.23	15.32	17.34	6.18	7.25	4.36	5.02
	10	16.56	18.10	11.79	13.66	15.20	16.52	6.30	7.59	4.52	5.45
	15	16.23	17.30	11.04	12.32	14.85	15.39	6.05	6.66	4.16	4.41
T. harzianum	0	15.12	15.12	10.62	10.62	14.31	14.31	5.62	5.62	3.75	3.75
	5	16.33	18.01	12.15	12.53	15.23	16.20	6.12	7.24	4.20	5.15
	10	16.77	18.12	12.50	13.23	15.39	16.85	6.25	7.48	4.45	5.45
	15	16.11	17.07	11.46	12.40	14.43	15.59	6.08	6.70	4.02	4.95
T. reesei	0	15.12	15.12	10.62	10.62	14.31	14.31	5.62	5.62	3.75	3.75
	5	16.58	17.16	12.36	13.53	15.75	17.40	6.35	6.70	5.44	5.92
	10	16.68	18.10	12.25	12.94	15.29	16.85	6.44	7.28	5.60	6.71
	15	16.25	17.00	11.20	12.35	14.42	15.73	6.12	6.42	5.29	5.76

C= control & T= treated; *Supplemented with ammonium sulfate

Amongst the various tested fermentation periods, the 5th day was suitable for maximum crude protein production from sugar beet leaves with T_2 (14.20%) in case of *T. reesei*. While it was 14.10 and 13.95%, for *T. harzianum and T. viride,* respectively after 10 days of incubation. Furthermore, the increase in incubation period did not enhance crude protein production where slight decrease in crude protein was observed.

The persistent growth of all tested strains was due to the availability of nutrients in the substrate and the stability of growth to a certain time was due to decline in the available carbon and energy sources needed for the metabolic processes. Continuous growth after stationary phase was due to the fact that the organism was able to utilize its metabolic end-products which if accumulated may be inhibitory or toxic to the organism (Yabaya and Ado, 2008).

Results for sugar beet pulp indicated that .*T. reesei* and *T. viride* showed highest protein concentration on the fifth day. Similarly, Nigam (1994) reported that optimum incubation period for conversion of beet pulp to high biomass through solid state fermentation by *Trichoderma* sp. was 5 days. In case of *T. reesei* the maximum crude protein production (17.9%) was also recorded on the 5th incubation day while in *T. harzianum* (17.73%) was detected later; on the 10th day of incubation.

Table (3): Crude protein produced by different fungal strains after acid and heat treatment for 60 minutes.

Trichoderma spp. used	Periods (days	Crude Protein %									
		Tomato leaves		Sugar beet leaves		Sugar beet pulp		Rice straw*		Sugarcane bagasse *	
		C	T	C	T	C	T	C	T	C	T
T. viride	0	15.12	15.12	10.62	10.62	14.31	14.31	5.62	5.62	3.75	3.75
	5	16.84	18.53	11.65	13.51	15.32	17.73	6.18	7.46	4.36	5.59
	10	16.56	18.40	11.79	13.95	15.20	17.08	6.30	7.83	4.52	5.94
	15	16.23	17.92	11.04	12.76	14.85	16.36	6.05	7.31	4.16	4.60
T. harzianum	0	15.12	15.12	10.62	10.62	14.31	14.31	5.62	5.62	3.75	3.75
	5	16.33	18.40	12.15	13.81	15.23	16.76	6.12	7.52	4.20	5.90
	10	16.77	18.52	12.50	14.10	15.39	17.73	6.25	7.79	4.45	6.36
	15	16.11	17.70	11.46	12.95	14.43	15.98	6.08	7.14	4.02	5.70
T. reesei	0	15.12	15.12	10.62	10.62	14.31	14.31	5.62	5.62	3.75	3.75
	5	16.58	17.88	12.36	14.20	15.75	17.90	6.35	7.48	5.44	6.52
	10	16.68	18.25	12.25	13.90	15.29	17.21	6.44	7.92	5.60	6.99
	15	16.25	17.61	11.20	13.11	14.42	16.36	6.12	7.45	5.29	5.92

C= control & T= treated; *Supplemented with ammonium sulfate

These findings are in agreement with the results reported by Israilides *et al.* (1994) who found that growing *T. reesei* on sugar beet pulp at 28°C led to 9.9% increase in the protein content of the resulting biomass. T_2 treatment produced the highest crude protein crop, so it is recommended to be used in mass production for feeding.

Rice straw was supplemented with ammonium sulfate to enrich the medium to be suitable for fungal growth. The maximum crude protein, 7.90%, was recorded after 5 days for *T. reesei* followed by 7.52 and 7.50% for *T. viride* and *T. harzianum,* respectively. The results revealed that the best incubation period was the 5th day for the maximum crude protein production. The physiochemical treatment significantly enhanced fungal growth when compared to untreated rice straw. As the crude protein was increased from 6.20, 6.36 and 6.15% in untreated to 7.90, 7.52 and 7.50% for *T. reesei*, *T. viride* and *T. harzianum* treatments, respectively which means that this kind of pre-treatment improved the biological treatment. The obtained results are in agreement with Bisaria *et al.,* (1997) who used *Pleurotus saja-caju* as a fungal strain and urea as nitrogen source to upgrade crude protein content of wheat straw and rice straw to 10.25 and 9.50%, respectively.

Sugarcane bagasse was supplemented with ammonium sulfate as mentioned before to be suitable for fungal growth. Pretreatment of sugarcane bagasse with dilute acid (0.5 N H_2SO_4) and boiling at 100°C for 60 min. resulted in earlier swelling and softening of lignocellulosic bond making it easily accessible to microbial enzyme resulting in subsequent breakdown and further utilization for building microbial protein.

The crude protein production increased from 5.59, 5.9 and 6.52 % after 5 days incubation to 5.94, 6.36 and 6.99 % after 10 days for *T. viride*, *T. harzianum* and *T. reesei*, respectively. These results agreed with Nigam, (1990) who studied the bioconversion of sugarcane through mixed culture solid state fermentation, using *Trichoderma* sp and *Pleurotus ostreatus* and found that the maximum protein yield was obtained after 8 days from the start of the bagasse fermentation.

3.4 The effect of feeding mice on crude protein produced from the treated agricultural wastes on liver and kidney functions of albino mice

In order to determine the edibility and safety limits for feeding animals; mice were fed on normal diet supplemented with three levels of the five treated wastes.

The obtained results are shown in Tables (4 & 5). Among different experimental groups there were no significant differences between the mean value of initial body weight and organs weight (Table 4). While, significant effects were observed in final body weight, weight gain, growth rate and protein efficiency ratio in 40% of the treated group of each cellulosic waste relative to control group.

Results in (Table 5) showed that there were no significant changes in liver enzyme activities ALT or AST in treated groups compared with control mice. Also, the same trend in same table indicated that, there were no significant effects in levels of total protein, albumin, globulin, urea and cholesterol as compared with the control. Meanwhile, slight increase in urea, ALT and AST as shown in 40% concentration of each single treated group, which was not significant as compared with control. Further histological examination is needed to elucidate the effect of these treated cellulosic wastes on mice liver and kidney tissues.

Results also indicated that fed on different levels (10 and 20%) of the pretreated waste had no negative effects on body weight or liver and kidney weight and function parameters of male albino mice. In case of 40% concentration, significant differences were observed in final body weight, weight gain, growth rate and protein efficiency ratio among different experimental groups.

Table (4): Influence of feeding male albino mice on crude protein produced from different agricultural wastes on body, liver and kidney weights.

Group No.	Treatment	Initial body weight (g)	Final body weight (g)	Liver weight (g)	Kidney weight (g)	Weight gain (g)	Growth rate (%)	PER
Significant		NS	***	NS	NS	***	***	***
Group I	Control	24.39±3.89	31.40±4.82^{a}	1.32±0.44	0.39±0.06	7.00±1.19^{a}	28.82±3.32^{a}	1.31±0.08^{a}
Group II	SC 10%	22.56±1.49	28.97±0.98^{abc}	1.28±0.90	0.36±0.05	6.41±1.25^{ab}	28.72±7.45^{a}	1.10±0.20^{ab}
Group III	SC 20%	22.53±2.36	26.16±2.71^{bcd}	1.21±0.18	0.32±0.09	3.63±0.67^{cde}	16.15±2.61^{bcd}	0.65±0.12^{cdef}
Group IV	SC 40%	22.56±1.49	24.27±1.71^{cd}	0.99±0.16	0.27±0.03	1.70±0.44^{cefg}	7.550±1.77^{de}	0.30±0.10^{fg}
Group V	RS 10%	22.22±1.84	28.54±1.35^{ab}	1.29±0.22	0.35±0.01	6.32±1.13^{ab}	28.83±7.11^{a}	0.97±0.02^{abc}
Group VI	RS 20%	21.55±1.10	26.10±1.32^{bcd}	1.05±0.05	0.30±0.02	4.52±1.32^{bcd}	21.10±6.34^{ab}	0.85±0.09^{bcd}
Group VII	RS 40%	22.09±0.84	23.46±1.08^{d}	0.96±0.06	0.29±0.02	1.37±0.98^{fg}	6.29±4.62^{de}	0.29±0.18^{fg}
Group VIII	TM 10%	24.49±5.18	30.78±4.46^{ab}	1.31±0.04	0.39±0.04	6.29±0.89^{ab}	26.95±8.08^{a}	1.09±0.14^{ab}
Group IX	TM 20%	24.08±1.48	28.94±0.86^{abc}	1.43±0.05	0.36±0.03	4.86±1.93^{bcd}	20.59±9.52^{ab}	0.75±0.16^{cdef}

Group X	TM 40%	24.16±1.13	27.51±1.51^{abcd}	1.09±0.20	0.28±0.08	3.34±1.45^{cdef}	13.93±6.47^{bcde}	0.66±0.14^{bcde}
Group XI	SBL 10%	24.30±4.23	28.66±2.96^{abc}	1.50±0.06	0.42±0.02	4.36±1.30^{bcd}	19.01±8.11^{abc}	0.65±0.33^{cdef}
Group XII	SBL 20%	23.76±2.64	26.76±2.91^{abcd}	1.46±0.08	0.33±0.01	2.99±0.90^{def}	12.71±3.99^{bcde}	0.40±0.16^{efg}
Group XIII	SBL 40%	23.59±2.48	24.35±2.18^{cd}	1.11±0.19	0.29±0.01	0.75±0.80^{g}	3.372±3.54^{e}	0.15±0.19^{g}
Group XIV	SBP 10%	23.62±2.16	28.91±0.91^{abc}	1.39±0.16	0.37±0.02	5.29±2.38^{abc}	23.23±12.2^{ab}	0.94±0.50^{abc}
Group XV	SBP 20%	23.23±5.05	26.30±5.24^{bcd}	1.32±0.30	0.39±0.07	3.07±1.67^{def}	13.82±8.22^{bcde}	0.51±0.07^{defg}
Group XVI	SBP 40%	23.00±4.83	24.97±4.11^{cd}	1.32±0.05	0.34±0.04	1.97±1.00^{efg}	9.392±5.74^{cde}	0.46±0.05^{defg}

Values are expressed as mean ± S.D. (n=5).
Means having different superscripts within each item in the same column are significantly different.
NS: insignificant and ***: refers to significant decrease at $P \leq 0.001$.
SC: Sugarcane baggase; RS: Rice straw; TM: Tomato Leaves; SBL: Sugar Beet Leaves and SBP: Sugar beet pulp& PER: Protein Efficiency Ratio

Except 40% concentration, the body weight was increased and the edibility of these pretreated wastes was observed as the food consumption in 10 and 20% concentration caused significant weight decreases. The results of experimented animal indicated that the different levels (10 and 20%) treated wastes were safe, edible, economic and have the potential to be available protein supplement for livestock. Optimum levels may probably depend on other dietary components, the amount and the animal species to be fed. On the other hand 40% concentration of each treated cellulosic wastes did not have a pronounced effect on increasing the body weight. Hence, from an economic point; they are not worthy to be used.

Table (5): Influence of feeding male albino mice on crude protein produced from different agricultural wastes on liver and kidney functions.

Group No.	Treatment	Total Protein (g/dl)	A albumin (g/dl)	Globulin (g/dl)	Alb/Glob Ratio	Cholesterol	Urea (mg/dl)	ALT (GPT) (U/L)	AST (GOT) (U/L)
Significant		NS	NS	NS	NS	NS	NS	NS	NS
Group I	Control	5.70±0.28	3.40±0.56	2.30±0.84	1.60±0.84	119.5±7.77	57.50±2.12	63.50±0.70	83.30±0.70
Group II	SC 10%	5.82±0.33	3.50±0.25	2.32±0.47	1.57±0.34	115.7±15.8	69.25±22.8	62.66±1.52	85.52±2.28
Group III	SC 20%	5.92±0.12	3.90±0.39	2.02±0.28	1.97±0.46	99.75±15.5	65.00±2.58	64.50±6.36	83.90±6.36
Group IV	SC 40%	5.80±0.36	3.43±0.11	2.36±0.25	1.46±0.15	99.33±6.65	66.66±7.76	67.00±0.00	87.70±0.00
Group V	RS 10%	5.85±0.63	3.70±0.28	2.15±0.91	1.92±0.95	129.0±7.07	52.5±10.60	66.50±9.19	85.05±10.3
Group VI	RS 20%	5.95±0.21	3.60±0.28	2.35±0.49	1.57±0.45	111.0±18.4	55.0±14.14	70.00±2.82	89.40±3.67
Group VII	RS 40%	6.15±0.07	3.70±0.42	2.45±0.35	1.53±0.39	126.0±11.6	64.0±5.656	77.00±0.00	92.30±0.00
Group VIII	TM 10%	5.77±0.59	3.57±0.56	2.20±1.04	2.01±1.13	123.5±43.9	66.50±13.2	60.33±5.50	79.13±5.50
Group IX	TM 20%	5.95±0.07	4.05±0.07	1.90±0.14	2.13±0.19	122.5±7.77	69.00±7.07	62.05±0.07	81.29±0.01
Group X	TM 40%	5.70±0.00	3.50±0.00	2.20±0.00	1.59±0.00	132.0±2.82	70.00±2.82	70.45±0.77	88.60±0.14
Group XI	SBL 10%	6.10±0.14	3.15±0.07	2.95±0.21	1.07±0.10	104.5±4.94	60.50±12.0	60.50±12.0	80.35±8.98
Group XII	SBL 20%	6.15±0.07	3.45±0.21	2.70±0.28	1.28±0.21	97.50±38.8	58.50±12.0	70.50±3.53	90.05±2.47
Group XIII	SBL 40%	5.97±0.03	3.40±0.14	2.57±0.17	1.35±0.14	92.00±2.82	67.50±2.12	72.50±0.70	93.25±1.48
Group XIV	SBP 10%	6.05±0.07	3.70±0.28	2.35±0.21	1.58±0.26	103.5±7.77	65.00±12.7	65.0±1.41	85.55±1.62
Group XV	SBP 20%	6.15±0.07	3.55±0.07	2.60±0.14	1.36±0.10	130.0±11.3	64.00±5.65	68.0±8.48	88.35±8.55
Group XVI	SBP 40%	5.55±0.49	4.00±0.28	1.55±0.21	2.59±0.17	128.0±11.3	77.50±30.4	74.0±2.82	93.30±2.12

Values are expressed as mean ± S.D. (n=5).
Means having different superscripts within each item in the same column are significantly different at $P \leq 0.05$

CONCLUSION

This study would be beneficial for producing large quantities of nutritional SCP from cheap carbon sources, which can also contribute to fulfill the protein demand of the world's ever increasing population. Abundant supply of good quality protein will certainly improve the quality of human and animal life on earth, presently and also in the future. This can also be used as a supplement and additive in the animal feed.

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