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EVALUATION OF BIOLOGICAL TREATMENTS FOR AGRICULTURAL BY-PRODUCTS IN RUMINANTS FEEDING. I- LABOURATORIAL STUDY

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ABSTRACT

Laboratorial evaluation was carried out for the effect of biological treatments with *Pleurotus ostreatus, Trichodermal reesei* and *Chaetanium cellulyticum* of rice and peas straws and corn stalks, with or without different additives on chemical composition, cell wall constituents (CWC), gross energy and *in situ* dry and organic matter disappearance. The obtained results could be summarized in the following:

- Peas straw was the best roughage among the tested straws and stalks; it gave the highest contents of crude protein, gross energy and cellulose. It reflected also the highest *in situ* disappearance of either dry matter or organic matter.
- *Pleurotus ostreatus* was the best fungus among the tested fungi in increasing the treated roughage contents of organic matter, crude protein, NFE and gross energy. It was the best also in elevating dry matter and organic matter disappearance *in situ*. It led to the lowest CF and most of CWC.
- Fungus + soybean meal was the best treatment that led to the highest CP content and *in situ* disappearance of DM and OM as well as to the lowest CWC.

Conclusively, the biological treatment with the white rot fungi, particularly with the fungus *Pleurotus ostreatus* of the field wastes (roughages) can improve their chemical composition and nutritive value.

Keywords: Fungal treatments, Field wastes, Chemical composition, Cell wall constituents, Nutritive value.

INTRODUCTION

In Egypt, the agricultural by-products are considered as stable source of ruminant feeds and now a days interest in their effective utilization is increasing all over the world due to economical factors and pollution. Shortage in animal feeds has been found to have a negative impact on the development of animal production in Egypt. Non traditional feed resources such as crop residues and Agro-industrial by-products must searched in order to decrease the relay on traditional resources, to fill the gap and to decrease feeding costs (Zaza, 2005). Sugar beet plulp is a by-product remains after extraction of sugar from sugar beet tubers (Talha *et al*., 2002). Utilization of by-product can not only be used in favor of solving feed shortage problem but also as a method to control environmental pollution (Zaza, 2004). Feeding is the most important cost item for livestock production which represents about 70% of the total production costs (Borhami and Yacout, 2001). The major

limitations of using these agricultural residues as feed are poor in nutrients such as protein content and vitamins and they are rich in fibers with low digestibility, or law palatability and high lignin contents. The degree of lignification is relatively more important in controlling hydrolysis rate in animal digestive tract (Fan *et al*., 1981). Therefore, biological treatment is used for increasing the nutritional value of many by-products, because they have significant concentrations of simple carbohydrates, such as mono-and disaccharides. For these reasons the microbial conversion of these wastes can improve their nutritional value and transforming them into animal feed with high quality (Villas-Boas *et al*., 2002). Many efforts have been employed to remove the lignin and/or to break up the linkages between lignin and carbohydrates and to increase their feed values by biological treatments (Abo-Eid *et al*., 2007; El-Shafie *et al*., 2007 and Abo-Eid, 2008). The main objectives of this study were to evaluate the effect of biological treatments of peas or rice straws and corn stalks with three fungal (F) strains (*Pleurotus ostreatus, Trichoderma reesie* and *Chaetonium cellulolyticum*) and F + additives (Control, Fungus, F + 2.5% soy bean meal, F + 3% molasses (M) and F + 2.5 soybean meal + 3% M) on chemical composition, gross energy and cell wall constituents as well as on the *in situ* dry and organic matter disappearance.

MATERIALS AND METHODS

The present laboratory study aimed to evaluate the effect of biological treatment with three fungal strains for three crop-residues (rice and peas straws and corn stalks) on their chemical composition, gross energy, cell wall constituents and *in-situ* digestibility.

Crop residues preparation:

The crop residues (rice and peas straws and corn stalks) were chopped (approximate 1-3 cm) and each one was divided into 5 treatments [Untreated roughage (control). Fungal treated roughage with *Pleurotus ostreatus (P.o).* Fungal treated roughages with *P.o* + 2.5% soybean meal. Fungal treated roughages with *P.o* + 3% molasses. Fungal treated roughages with *P.o* + 2.5% soybean meal + 3% molasses. The same treatments were repeated with the other two fungal species].

Biological treatments:

The tested fungi were obtained from the National Center of Agricultural Utilization Research Service, USA, Department of Agriculture, Peario, Illinois, USA. The strains were maintained on potato dextrose agar medium (PDA), grown at 24 – 28^{o}C for 48 – 72 hrs, then stored at 4^{o}C. Medium, which used throughout the current work consists of potato dextrose agar medium according to Difco manual (1979). The medium used for the maintenance of the fungi consists of (g/l) potato extract (4.0 g), glucose (20.0 g) and agar (20.0 g). The pH value was adjusted to 5.6 before autoclaving at 121^{o}C for 20 minutes. Precultures of the fungal strains were prepared by inoculating 250 ml

conical flasks containing 50 ml nutrient glucose broth medium (Fouda *et al*., 1960) with mycelial discs (5 mm diameter) of 7 days old culture. The inoculated flasks were incubated on a rotary shaker (200 r.p.m) at 28°C for 7 days. The treated sorghum grains were used for inoculation of the pretreated substrates at 3% fresh weight basis (Garcha, 1981). The tested crop-residual (rice or peas straws and corn stalks) samples were treated with *Trichoderma reesei* NRRL 3653. The fungal strain was cultured in a medium (per one liter)of glucose 10 g + Yeast extract 3 g + Malt extract powder 3 g + peptone 5 g. Fifty ml of the previous media were introduced into 250 ml conical flask, the flasks were autoclaved at 121° C for 20 minutes. Sterilized flasks were incubated with a fungus loop of 7 days old cultured slants. The flasks were incubated in a rotary shaker at 200 r.p.m for 20 minutes at 25°C ± 2 for 7 days. The mycelia of growing fungi were used to inoculate the experimental flasks at 10% (V/W). Twelve grams of sugar beet pulp were introduced into 250 ml conical flask containing 15 ml water. The flasks were autoclaved at 121°C for 30 minutes. The flasks were cooled then inoculated by the above prepared inoculums. The inoculated flasks were incubated statically in an incubator adjusted to 28 – 32° C for 7 days.

Solid state cultivation technique:

About 6.5 Kg of dry corn stalks, rice straw and peas straw were soaked in water for 4 hrs. at room temperature, then spread on a plastic sheet to adjust the moisture content at (60 – 65%). Therteen samples of prepared straws (500 g of each) were packed in plastic bags for each crop residue then autoclaved at 121°C for 30 minutes. Next day, the bags were inoculated by solid state fermentation system (SSFS) with spawn of *P. ostreatus*, *Tr. Reesei* and *Ch. cellulyticum* (10% wet weight basis) and incubated at 28°C for 21 days. The samples were dried at 60°C for 24 hrs then at 105°C for 3 hrs for studying the chemical composition, fiber fractions, growth energy and *in situ* disappearance.

Criteria measured:

Proximate chemical analysis of raw and treated crop residues samples in triplicates per each determination was carried out for dry matter (DM), crude protein (CP), ether extract (EE), crude fiber (CF) and ash content according to the A.O.A.C. (1990). The nitrogen free extract (NFE) was calculated by subtracting the summation percentages of CP, EE, CF and ash from one hundred. Untreated and treated crop-residues samples, except crop-residues samples after soaking were analyzed according to Goering and Van Soest (1970) to determine neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL). Hemicellulose and cellulose were determined by difference. The gross energy was calculated according to Blaxter (1961). Degradability of DM and OM for all crop by-products (untreated or treated with different fungal strains) in the rumen of buffalo was estimated. The *in situ* technique was carried out as described by Cherney *et al.* (1990); Hussein *et a*l. (1991) and Bowman and Firkins (1993) to study the effect of rumen fluid on digestibility of cellulosic and hemicellulosic substances. Dacron polyester bags (6 cm x 10 cm) with an average pore size

of 52 + 16 µm were used. Samples of experimental feedstuffs and treatments materials were ground using a 1-mm screen and 2 gm were weighed into each bag. Bags were tied to nylon lines at three ends. Bags were removed from rumen either after 12, 24, 48 or 72 hr post-immersion, washed with tap water and squeezed until the runoff was clear. Bags were then dried at 105^{o}c for 24 hours and weighed for calculating DM residual and burned in a muffle at 600^{o}C to 4 h for determination the ash content. The *in situ* technique was conducted on two consecutive days to obtain triplicate measurements for determination of *in situ* DM and OM disappearance.

Statistical analysis:

The obtained data were analyzed according to Statistical Analysis System user's Guide (SAS, 1998) for one way analysis of variance. Separation among means were carried out by using Duncan's (1955) multiple range test. Data of chemical composition, gross energy, fiber fractions and in situ dry and organic disappearance were analyzed according to factorial design.

RESULTS AND DISCUSSION

Table 1 illustrates the effects of crop residues, fungal strains, and additives on the chemical composition and gross energy. Except the ether extract, all other nutrients were significantly ($P \leq 0.01$) affected by various studied variables. Peas straw was the highest in crude protein and gross energy. Corn stalks were the best in organic matter and nitrogen free extract. But, rice straw was the highest in dry matter and ash contents. Meanwhile, *Pleurotus ostreatus* realized the highest OM, CP, NFE and GE and the lowest CF contents than the other two fungi (*Trichoderma reesei* and *Chaetonium cellulyticum*). But *Trichoderma reesei* resulted in the highest DM, CF and ash contents. Concerning the additives, all of them led to lower OM, CF and energy contents. Yet, fungi + soybean meal treatment increased CP level; whereas, fungi alone increased NFE content and fungi + soybean meal + molasses elevated the ash percentages. The interactions among the variables studied were significant ($P \leq 0.01$) for all components of the chemical composition and gross energy content, except for EE. Also, corn stalks and rice straw showed some significances for the interaction effects on chemical composition and gross energy contents as affected by residual type, fungal strain, and additive used in the present study. All cell wall constituents were significantly affected also by different variables studied (Table 2). RS was high in hemicellulose, CS was high in NDF and ADF, and PS was high in ADL and cellulose. NDF, ADF, ADL and cellulose increased by the treatment with *Trichoderma reesei,* while hemicollulose improved by using *Chaetonium cellulyticum* for the biological treatment. All the tested additives were responsible for lowering all cell wall constituents comparing with the control. Data of the interaction effects among different variables studied on cell wall constituents showed significant differences.

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Data of *in situ* dry matter and organic matter disappearance % are given in Table 3. The results of both of them were statistically ($P \leq 0.001$) affected, whether by crop residual type, fungal strain, additives used, or time of incubation in rumen. PS was more digestible than CS and RS. *Pleurotus ostreatus* was more effective than *Trichoderma reesei* and *Chaetonium cellulyticum*. The multi – additives (fungi + soybean + molasses) were better affecting than all other individual additives (fungi alone, fungi + molasses alone, or fungi + soybean alone). The *in situ* disappearance of DM and OM increased by the incubation time. The interaction effects of fungal strain, additives, and incubation time on DM and OM disappearance of PS, CS and RS were significant.

Forages (Abdelhamid and Gabr, 1993), grasses (Abdelhamid and Gabr, 1991a,b & 1993; Abdelhamid and Topps, 1991 and Shehata *et al.,* 2001), field by-products and agro-industrial wastes (Abdelhamid and El-Ayoty, 1988 and Abdelhamid, 1990 & 1992) are used as conventional feeding stuffs for ruminants. Yet, other unconventional substances may be used too (Abdelhamid, 2004 and Abdelhamid *et al*., 2006 & 2007).

There were significant variations in chemical composition, energy content, cell wall constituents and in situ disappearance of DM and OM due to variations in crop residual type, fungal strain used in the biological treatment as well as to the additives used. In this respect, numerous fungal species were used for biological treatments of roughages, particularly Pleurotus ostreatus (Abdelhamid *et al*., 2006 & 2007), *Trichoderma reesie* (Gado, 1999), and *Chaetonium cellulyticum* (Kim *et al.,* 1985). Since, biological treatments improve the roughage palatability (Abdelhamid *et al.,* 2006), crude protein and energy contents (Bassuny *et al.,* 2003 a &b), digestibility and voluntary intake and thus nutritive values (Bader, 2001) and growth (El-Ashry *et al*., 2001).

However, Dhanda *et al*. (1994) reported some increases in crude protein contents of biologically treated roughages. Salem (2003) found that crude fiber decreased in biologically treated field by-products. Generally, contraventions among results are clear, concerning biological treatments effect on chemical composition of the treated wastes (Abd El-Aziz, 2002). Moreover, the changes severity in chemical composition depends on the roughage type (Abo-Eid *et al.*, 2007), incubation period (Zaza *et al*., 2008) and the microorganism used it self (Mahrous *et al*., 2005).

Cell wall constituents are influenced too, so biological treatments were found to decrease, NDF, ADF, ADL, cellulose and hemicellulose (Belewu, 2006). The opposite trend was recorded by Hamza *et al*. (2003). These degradation was dependent on the substrate (Hassan *et al*., 2005), the microorganism (Younis and El-Faramawy, 2003) and incubation period (Zaza *et al*., 2008).

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تقييم المعاملات البيولوجية للمخلفات الزراعية فى تغذية المجترات:
١- دراسة معملية

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استهدفت تقييم أثر المعاملات البيولوجية باستخدام ثلاثة سلالات من الفطريات (فطر بلروتس أستراتس – التريكوديرما ريساى – شيتنيم سللتكم) على بعض المخلفات الزراعية تحت الدراسة (قش أرز – حطب ذرة – تبن فاصوليا) بعد التحضين لمدة واحد وعشرين يومياً مع إضافة أو بدون إضافة مصدر طاقة سهل وبروتين (فطر، فطر + كسب فول صويا، فطر + مولاس، فطر + كسب فول صويا + مولاس) وأثر ذلك على التركيب الكيماوى – والطاقة الكلية – ومكونات جدر الخلايا ومعدل اختفاء المادة الجافة والعضوية داخل كرش الحيوان خلال فترات تحضين مختلفة (١٢ – ٢٤ – ٤٨ – ٧٢ ساعة). وكانت أهم النتائج أن أدت المعاملة البيولوجية بإضافة أو بدون إضافة إلى زيادة محتوى المخلفات من البروتين الخام – والرماد – المستخلص الخالى من الآزوت – والمادة الجافة الكلية ، كما أدت الى انخفاض نسبة الألياف الخام – المادة العضوية – الطاقة الكلية – الألياف الذائبة فى القلويات – الألياف الذائبة فى الأحماض – اللجنين الذائب فى الأحماض – السليلوز – والهيميسليلوز مقارنة بالمخلفات غير المعاملة، زيادة معدل اختفاء ا لمادة الجافة والعضوية تدريجياً بزيادة فترة التحضين داخل كرش الحيوان، فكان أعلى معدل اختفاء حتى ٧٢ ساعة، وكانت أحسنها مع تبن الفاصوليا مع فطر بلروتس أسترتس بإضافة أو بدون إضافة، ثم يلى بعد ذلك قش الأرز ثم حطب الذرة مع نفس الفطر. ويستخلص من هذه الدراسة أن استخدام المعاملات البيولوجية بالفطريات *Trichoderma reesei, Pleurotus ostreatus, Chaetanium cellulyticum* مع قش الأرز وحطب الذرة وتبن الفاصوليا يعمل على رفع القيمه الغذائية من زيادة نسبة البروتين الخام وخفض الألياف الخام ومكوناتها، ورفع معاملات الهضم للعناصر الغذائية، وأن تطبيق ذلك على نطاق واسع يسهم فى تقليل الفجوة العلفية الموجودة فى مصر بتحسين الاستفادة من المخلفات الزراعية، وأن استخدام المعاملة البيولوجية للمخلفات الزراعية وسيلة آمنة لمعظمة الاستفادة منها، والقضاء على التلوث البيئى الحادث من تراكمها أو حرقها.

Table (1): Effect of crop residual type, treatment and some additives on percent chemical composition (on dry matter basis, means ± SE).

Items	Crop residual type			Treatment			Additives				
	RS	CS	PS	T1	T2	T3	C	F	F + SY	F + M	F+SY+M
DM	92.04A ± 0.60	91.79B ± 0.54	91.14C ± 0.46	91.46C ± 0.55	91.80A ± 0.68	91.70B ± 0.69	91.36D ± 0.13	91.59C ± 0.79	91.95A ± 0.62	92.77B ± 0.74	91.61C ± 0.66
OM	78.99C ± 2.13	88.67A ± 0.99	88.30B ± 2.19	85.81A ± 4.44	85.13B ± 5.23	85.02C ±4.93	87.81A ± 4.77	85.39B ± 4.65	84.88C ± 5.22	84.31D ± 4.84	84.21E ± 4.19
CP	8.96B ± 3.28	8.15C ± 2.57	11.54A ± 3.63	10.24A ± 3.38	9.17C ± 3.12	9.23B ± 3.87	4.58E ± 1.01	8.86D ± 1.74	12.49A ± 2.80	10.19C± 2.29	11.63B ± 2.30
CF	29.99B ± 6.14	30.48A ± 3.12	30.48A ± 3.12	29.79C ± 4.66	30.41B ± 4.53	30.80A ± 4.03	38.13A ± 2.40	29.74B ± 0.73	27.49B ± 2.32	28.96C ± 1.78	27.35E ± 1.39
EE	0.91^{A} ± 0.03	0.91^{A} ± 0.03	0.91^{A} ± 0.03	0.91^{A} ± 0.03	0.91^{A} ± 0.03	0.92^{A} ± 0.04	0.91^{A} ± 0.03	0.91^{A} ± 0.03	0.90^{A} ± 0.02	0.92^{A} ± 0.04	0.91^{A} ± 0.03
NFE	39.14^{C} ± 2.63	49.13^{A} ± 1.08	45.32^{B} ± 2.87	44.88^{A} ± 4.44	44.65^{B} ± 4.50	44.08^{C} ± 5.31	44.20^{B} ± 6.37	45.89^{A} ± 4.03	43.99^{C} ± 4.02	44.27^{B} ± 4.17	44.33^{B} ± 4.80
Ash	21.01^{A} ± 2.13	11.33^{C} ± 1.00	11.70^{B} ± 2.14	14.19^{C} ± 4.44	14.87^{B} ± 5.23	14.98^{A} ± 4.93	12.19^{E} ± 4.77	14.61^{D} ± 4.65	15.12^{C} ± 5.22	15.69^{B} ± 4.84	15.79^{A} ± 4.18
GE, Kcal/Kg	3463.07^{C} ± 75.95	3853.23^{B} ± 49.93	3887.21^{A} ± 62.52	3765.19^{A} ± 183.03	3720.32^{A} ± 219.54	3718.01^{B} ± 206.45	3764.96^{A} ± 211.44	3726.31^{C} ± 206.40	3760.17^{B} ± 226.49	3725.22^{C} ± 168.81	3695.88^{D} ± 205.94

R.S = Rice straw C.S = Corn stalks P.S = Peas straw T_1 = *Pleurotu ostreatus* T_2 = *Trichoderma reesci*
T_3 = *Chaetonium cellulyticum* C = Control F = Fungi SY = Soybean meal M = Molasses
A,B,C,D and E: Means in the same row with different superscripts differ significantly (P < 0.01).

Table (2): Effect of crop residual type; treatment and some additives on cell wall constituent % on dry mater basis (means ± SE).

Items	Crop residual type			Treatment			Additives				
	RS	CS	PS	T_1	T_2	T_3	C	F	F + SY	F + M	F+SY+M
NDF	52.77^{B} ± 10.07	60.34^{A} ± 8.28	52.78^{B} ± 6.67	50.78^{C} ± 10.85	57.78^{A} ± 7.37	57.34^{B} ± 7.06	68.10^{A} ± 4.88	57.31^{B} ± 6.31	50.36^{D} ± 6.77	51.80^{C} ± 4.79	48.91^{E} ± 6.46
ADF	37.57^{C} ± 7.19	48.28^{A} ± 4.98	39.05^{B} ± 4.61	37.98^{C} ± 9.24	44.23^{A} ± 5.61	42.69^{B} ± 5.28	48.73^{A} ± 3.80	42.08^{B} ± 8.63	39.45^{D} ± 6.97	40.32^{C} ± 6.43	37.58^{E} ± 5.15
ADL	10.34^{C} ± 0.80	11.73^{B} ± 1.28	12.47^{A} ± 0.94	11.49^{A} ± 1.64	11.52^{A} ± 1.36	11.43^{B} ± 1.11	12.44^{A} ± 1.52	10.66^{E} ± 1.48	11.39^{C} ± 1.17	11.90^{B} ± 0.64	11.00^{D} ± 1.20
Cellulose	27.33^{B} ± 7.04	26.59^{C} ± 4.98	36.55^{A} ± 4.73	26.49^{C} ± 8.62	32.71^{A} ± 5.72	31.26^{B} ± 4.96	36.29^{A} ± 4.02	31.43^{B} ± 8.83	28.06^{D} ± 6.22	28.42^{C} ± 6.26	26.57^{E} ± 5.06
Hemicel-lulose	15.21^{A} ± 4.67	12.06^{C} ± 4.88	13.72^{B} ± 3.15	12.79^{C} ± 5.36	13.54^{B} ± 4.54	14.64^{A} ± 3.08	19.37^{A} ± 1.24	15.26^{B} ± 4.71	10.90^{E} ± 2.66	11.45^{C} ± 2.48	11.33^{D} ± 3.25

R.S = Rice straw C.S = Corn stalks P.S = Peas straw T_1 = *Pleurotu ostreatus* T_2 = *Trichoderma reesci*
T_3 = *Chaetonium cellulyticum* C = Control F = Fungi SY = Soybean meal M = Molasses
A,B,C,D and E: Means in the same row with different superscripts differ significantly (P < 0.001)

Table (3): Effect of crop residual type, treatment, additives and times (regardless to the other variables) *in situ* on dry and organic matter disappearance % (means ± SE).

Items	Crop residual type			Treatment			Additives					Times			
	Pea straw	CS	RS	P	T	Ch	C	F	F + M	F +SY	F+SY+M	12 hr	24 hr	48 hr	72 hr
DM	47.45^{A}± 0.740	43.23^{C}± 0.915	44.47^{B}± 0.931	46.02^{A}± 0.721	44.58^{B}± 0.900	44.54^{B}± 0.847	40.19^{E}± 1.068	44.67^{D}± 1.085	45.86^{C}± 1.106	46.48^{B}± 1.116	48.03^{A}± 1.147	27.73^{D}± 0.328	43.53^{C}± 0.464	52.58^{B}± 0.311	56.34^{A}± 0.238
OM	45.80^{A}± 0.721	43.42^{C}± 0.900	44.01^{B}± 0.847	45.80^{A}± 0.847	44.04^{B}± 0.815	43.83^{C}± 0.822	38.23^{E}± 1.019	44.24^{D}± 1.026	45.31^{C}± 1.023	46.29^{B}± 1.011	48.00^{A}± 1.041	28.52^{D}± 0.377	42.16^{C}± 0.316	51.53^{B}± 0.379	55.45^{A}± 0.290

PS = Peas straw C.S = Corn stalks R.S = Rice straw P = *Pleurotu ostreatus* T = *Trichoderm reesei* Ch=*Chaetonium cellulyticum*
C = control F = Fungal SY = Soybean meal M = Molasses hrs = hours
A,B,C,D and E: Means in the same row with different superscripts differ significantly (P < 0.001).