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THE EFFECT OF BIOLOGICAL TREATMENT ON CHEMICAL COMPOSITION, DIGESTIBILITY AND FEEDING VALUES OF COTTON STALKS AND RICE STRAW.

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ABSTRACT

The present work was carried out to study the effect of incubated cotton stalks and rice straw with white-rot fungi growth (*Pleurotus ostreatus*) (P.O) on the digestibility (*In vitro* and *in vivo*) and feeding value of cotton stalks and rice straw.

Results showed that CP was increased by 39.80% and 56.40% due to the growth of P.O on cotton stalks and rice straw, respectively, whereas CF was decreased by 42.55% and 53.54% for cotton stalks and rice straw, respectively.

The *in vitro* dry matter (DM) digestibility of treated rice straw and cotton stalks was significantly increased ($P < 0.05$) by 30 and 23.2% respectively, while *in vitro* OM digestibility was increased ($P < 0.05$) by 37.7 and 36.4%, respectively.

Compared with untreated materials, most of nutrient digestibility coefficients of rations contained treated materials were significantly higher ($P < 0.05$) than those contained untreated ones. Likewise, the feeding value as TDN and DCP% was improved as a result of biological treatment. It could be concluded that biological treatment could be used successfully to enrich poor quality roughages and improved digestibility coefficients and feeding values of treated materials and helpful to eliminate environmental pollution.

INTRODUCTION

One of the most important limiting factors for animal production in Egypt is the availability of feedstuffs. There is however, considerable amounts of crop residues (about 26 million tons) can be used after treatments as feed for livestock to get rid of their environmental pollution and to fill part of the gap in animal feeds.

Several agricultural residues have been used to produce the edible mushroom and animal feed. Among these residues, the use of cotton stalks as animal feed was impossible because of it's high content of lignin which makes it very hard to be utilized by the animal.

The annual cultivated area from cotton in Egypt is about 700.000 feddans. Based on this figure the total annual neglected yield of cotton stalks is about 1155000 tons. Whereas the annual cultivated area from rice in Egypt in about 1507000 feddans. Based on this figure the total annual neglected yield of rice straw is about 2262000 tons.

Cohen *et al.* (2002) reported that one of the most important species of *Pleurotus spp.* is related to the use of their ligninolylic system for a variety of applications, such as the bioconversion of agricultural wastes into valuable products for animal feed. Traditionally, the cultivation of *Pleurotus sp.* is performed on different composted and pasteurized agricultural wastes such as banana leaf, corn cobs, rice hulls and sugarcane bagasse (Evelise *et al.* 2005). Most of these wastes contain large amount of cellulose, hemicellulose and lignin, which support the growth of mycelium of the Oyster mushroom (*Pleurotus ostreatus*) and fruit bodies' production (Senyah and Robinson 1988).

Patrick *et al.* (2003) reported that the inoculation of forages with lingocelluloytic fungi for improving quality without adding chemical products is recommended. Substrate quality influences fungal activity and end product quality. A linear increase over time was observed for crude protein whereas a linear decrease was observed for NDF, cellulose and hemicellulose contents. The biological treatment caused degradation of fibrous fraction and increased CP content. *Pleurotus ostreatus* have the ability to secrete extra cellulotic enzymes during growth to degrade and digest lignin and cellulose. The protein content of the substrate increased by 110% and the cellulose content was decreased by 30% (Patrick *et al.*2003).

Jalc *et* al. (1996) reported the increase of crude protein and ash contents in fungus treated straw. Also IVDMD values were increased in straws treated by *Pleurotus ostreatus*. Beside that the detergent fiber content, natural detergent fiber (NDF) and acid detergent fiber (ADF) was reduced in fungus treated straw and out of three fractions, hemicellelose and lignin showed the largest proportionate loss (Jalc *et al.* 1996).

Racz (1998) reported that, when mushrooms are grown on rice straw, cellulose and hemicellulose in the substraté are readily broken down but metabolism of lignin is slower.

Beside that, due to the mushroom cultivation, the concentration of minerals such as N, P, Ca was increased with a reduction in the C:N ration in the spent rice straw. Thus the spent straw can be suitable to be used as cattle feed (Jadhav *et al.* 1998).

Yoshida *et al.* (1993) mentioned that when rice straw inoculated with *Pleurotus ostreatus* and incubated for 8 weeks the hemicellulose content decreased by more than 40 % while the cellulose content of straw relatively stable, though cellulose content of straw substrates decreased at fruit body formation. The rate of decrease of acids detergent lignin was reduced. They added that the *In vitro* DM digestibility by cellulose of straw decreased early in the incubation. Digestible DM of straw substrate increased by 11%.

Baldrian *et al.* (2005) said that, *Pleurotus ostreatus* produces the cellulolytic and hemicellulolytic enzymes endo-1,4-beta–glucanase, exo-1,4-bet-glucanase, endo-1,4-beta- glucosidase, endo-1,4-beta-mannosidase, endo-1,4-beta-mannanase and 1,4-beta-mannosidase and ligninolytic enzymes Mn-peroxidase and laccase during growth on wheat straw. In the same time (Line *et al.* 2001) reported that, the increase of protein content and the reduction of lignocellulose content contributed to the increase in the dry matter digestibility of the spent substrate, making it possibly acceptable as a potential ruminant feed. This could provide an alternative to environmentally sound use of *P. ostreatus* spent substrate. In this study the genus *Pleurotus* comprises a group of edible ligninolylic mushrooms with medicinal properties and important biotechnological and environmental applications.

The main target of this experiment was to investigate the ability of white – rot fungi *(Pleurotus Ostreatus)* to degrade liqnocellulotic material in cotton stalks and rice straw. The effect of this fungus on nutrients digestibility coefficients and feeding values were also studied.

MATERIALS AND METHODS

The present work was carried out in the Central Laboratory for Food and Feed (CLFF), Agriculture Research Center during 2004, 2005.

Production of inoculums:

Pleurotus ostreatus culture obtained from faculty of Agriculture – Ain Shams University, Cairo, Egypt was used in the present study. The production of inoculums in Petri dishes and its conservation in test tubes was preformed according to Bononi *et al.* (1995).

Preparation of mother spawn substrate:

The mother spawn was prepared according to Kumar and Mujal (1975) and Quimio (1986). To prepare the mother spawn, sorghum was washed thoroughly, then soaked over night. Dead seeds or those that float on water were carefully removed. Then, the grains were drained. Precipitated chalk (CaCO3) and $CaSo_4$ 4% "1% each w/wet basis" were added to the grains. Fill the jars two third from the prepared grains then plugged with aluminum paper. The grains were sterilized under pressure for one hour at 121°C. The jars were then cooled at room temperature for inoculation.

Inoculation of mother spawn with agar plugs:

Inoculation was performed inside laminar flow cabinet. One grain jar was planted with at least two mycelial plugs.

Incubation:

Inoculated jars were incubated at 25°C for 15 days. When the jars were fully covered with the mycelium, the jar was used to inoculate 10 jars. This planting spawn was used in mushroom cultivation after incubated 15 days at 25 °C until the grains were covered with mycelium biomass.

Preparation of substrate for mushroom cultivation:

Rice straw and cotton stalks were obtained from a field attached to the Agriculture Research Center. They were sun dried and chopped (ca 2-6 cm). Chopped substrates were soaked in tap water until moisture content 60-70%, followed by soaking in boiled water for 2 hours according to Balasubramanya and Kathe (1996); Sakar *et al.* (1988); and Meera *et al.* (1989) to decrease contamination. Calcium carbonate 1% (w/w) was used to adjust the pH to 5.5.

Incubation of substrate:

The substrate was cooled to room temperature and drained until moisture reached 65 - 70%. The pasteurized substrate was manually packaged into 100cm x 50cm transparent polyethylene bags with 5 kg wet substrate per bag, together with 250 g spawn (5% in relation to the wet mass of the substrate).

Mycelial growth:

The inoculated bags incubated at room temperature (20°C ± 5°C). At the end of incubation period (4 weeks) the mycelial growth of the tested cultures covered the substrate. The Plastic bags were opened and used for chemical analysis and digestibility trial by sheep.

Metabolism trials:

Two digestibility trials were carried out for the compost cotton stalks and rice straw cultivated by *Pleurotus ostreatus* to determine nutrient digestibility coefficients, feeding value of untreated and treated substrates.

Six mature Rahmani rams with average live body weight of 45 Kg were used in four digestibility trials. The rams were weighed at the start of the trial and at the end of the collection period. They were divided into two similar groups, on the basis of average live body weight. The animals were individually housed in metabolic cages. Two trials was conducted with rice straw (untreated or treated) followed by two trials with cotton stalks in two sequance periods. During trials all roughages were fed along with commercial concentrate feed mixture (CFM) at the rate 42 : 58% (R : C ratio). The CFM contained: 89.2, 86.83, 18.39, 12.69, 1.54, 13.17 and 54.22 for DM, OM, CP, CF, EE, Ash and NFE, respectively.

Each trial lasted for 21 days of which 14 days were preliminary period during which the animals were adapted to consume the experimental rations, followed by an experimental period of 7 days, during which feces were collected, mixed and then dried at 60 °C for 48 hours using an electric oven with fan. Dry matter was determined at 105 °C for three hours. Dried feces were ground and stored for running the chemical analysis. For urine, one twentieth of the daily urine excretion was stored in a well stoppered polyethylene bottle containing 50ml. H_2 So_4 (1:1) and used for nitrogen determination.

Chemical analysis:

Chemical analyses were performed on raw cotton stalks and rice straw and the compost substrates stalks incubated for 4 weeks. The substrates were dried in oven at 60 C and ground. Crude protein (NX 6.25) and ash were determined according to A.O.A.C. (1990).

Samples of different used rations, feces and urine were analyzed for moisture, crude protein (CP), crude fiber (CF), ether extract (EE), and ash according to (A.O.A.C., 1990). Nitrogen free extract was obtained by difference. Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) of raw and compost substrates were determined according to Van Soest and Bretson, (1979) Hemicellulose was calculated as the difference between NDF and ADF and cellulose, was calculated as the difference between ADF and ADL. In vitro dry matter and organic matter digestibility were performed on the raw and spent rice straw and cotton stalks according to Menke *et al.*, (1979) and the description of Karl and Herbert (1989).

Statistical analysis:

Statistical analysis of the collected data was carried out according to procedures of General Linear Models (GLM) by using SAS (1996) computer program system. The differences among means were tested according Duncan (1955) Maltiple Range Test.

RESULTS AND DISCUSSION

Chemical analysis.

Data in Table (1) showed that protein content of both raw rice straw and cotton stalks are nearly similar. However biological treatment with *Pleurotus ostreatus* increased protein content of rice straw and cotton stalks by 56% and 124.7%, respectively. Crude fiber of both rice straw and cotton stalks decreased by 47.4% and 34.86%, respectively as a result of biological treatment. Ash content of biological treatment of both roughages was higher than untreated materials.These results are in agreement with Gado (1999) and Mahrous (2005).

The natural detergent fiber (NDF) and acid detergent fiber (ADF) were reduced in fungus treated rice straw and cotton stalks by about 21%, 8% and 22%, 21%, respectively. The hemicellulose content of rice straw decreased by 45%, while it is reduced by 21% with cotton stalks . Concerning the cellulose content in sbent rice straw there is a limited decrease (6%) while it was remarkable with cotton stalks, the raduction reached 20%. These results are in agreement with those recorded by Jalc *et al.* (1996) and Patrick *et al.* (2003).

Lignin content of raw rice straw is very limited when compared with that of cotton stalks as shown in Table (1). Improvement of rice straw quality by lignin degradation (10.2%) was noticed after biological treatment, while it reached 22.4% with cotton stalks. Such treatment could show the great effect of Oyster mushroom on nutrient availability of low agricultural wastes.

Table (1): The effect of biological treatment by *Pleurotus Ostreatus* on chemical composition (%)of rice straw and cotton stalks.

Items	Raw rice straw	Spent rice straw	Raw cotton stalks	Spent cotton stalks
DM	93.47	93.50	89.4	89.20
Chemical analysis on DM basis:				
OM	80.31	74.57	89.18	86.20
CP	4.98	7.79	4.94	11.10
CF	35.36	23.03	44.13	23.06
EE	2.19	1.01	1.36	1.02
NFE	37.78	42.43	39.75	51.02
ASH	19.69	25.74	10.82	13.80
NDF	83.42	65.81	92.40	72.90
ADF	54.16	49.87	75.60	59.70
ADL	25.11	22.54	33.90	26.30
Cellulose	29.05	27.33	41.7	33.4
Hemicellulose	29.26	15.94	16.80	13.20

***In vitro* dry matter (IV–DMD) and organic matter (IV–OMD) digestibility:**

Results in Table (2) showed that high increased (P < 0.05) of IV-DMD and IV-OMD were observed in treated rice straw and cotton stalks than that of untreated substrates. The two roughages responded differently to the treatment.The treatment increased the IV-DMD by 29.87% and 23.20% in both rice straw and cotton stalks, respectively .Whereas the treatment increased the IV-OMD by 37.69% and 36.36 % in both rice straw and cotton stalks, respectively.

Table (2): The effect of biological treatment by *Pleurotus ostreatus* on *In vitro* dry matter and organic matter digestibility of rice straw and cotton stalks.

Items	Raw rice straw	Raw cotton stalks	Spent rice straw	Spent cotton stalks	± SE
IV-DMD	26.08c	29.27b	33.87a	36.06a	0.86
IV-OMD	31.39b	32.54b	43.22a	44.37a	1.11

a,b,c Means with the same letter within each raw are not significant at P < 0.05

The chemical composition of experimental rations is presented in Table (3). Each pair of rations (ration 1 & 2 and 3 & 4) was practically similar in their chemical composition, especially CP content. However, ash content of ration 2 and 4 contained treated materials was higher than those of 1 and 3. These observations are in agreement with Mahrous (2005).

Table (3): The chemical composition (%) of the rations fed during the metabolism trials using rice straw and cotton stalks.

Items	Ration(1)	Ration(2)	Ration(3)	Ration(4)
DM	92.05	92.07	89.29	89.20
Chemical composition on DM basis (%)				
OM	84.51	82.71	87.82	86.56
CP	10.83	12.00	15.22	15.41
CF	23.41	18.28	24.21	17.05
EE	1.51	1.30	1.46	1.32
NFE	48.76	51.13	46.92	52.79
ASH	15.49	17.29	12.18	13.44

Digestibility coefficients and feeding values:

Nutrient digestibility coefficients and nutritive values are presented in Table (4). Generally, most of nutrient digestibility coefficients of rations contained treated materials (R2 and R4) of both roughages were significantly higher (P < 0.05) than those of contained untreated ones. However, the biological treatment on rice straw and cotton stalks had a positive effect on DM Digestibility. The value was increased from 58.42 % to 63.68 and 55.73 to 65.05 respectively. Also, organic matter digestibility of treated rice straw and cotton stalks by P.O was increased by about 11% and crude protein content of rice straw increased by 15.5 %, while little increase (4.5%) was shown with cotton stalks.

Digestibility coefficient of CF was not significantly affected by biological treatment of rice straw (55.5 vs 55.92%), while, it was higher (P < 0.05) by about 17.74% for cotton stalks than untreated one. Finally NFE digestibility was increased by about 11.86% and 15.65% with ration containing treated rice straw and cotton stalks, respectively. These results agreed with those of Mahrous (2005) and Tahan and Mohamadi (2005).

It is of interest to note that the TDN value of ration contained cotton stalks (R4) was significantly higher than that contained untreated one R3 but no significant difference between ration 1 and 2 was observed in this respect (Table 4). However, the reverse picture was true in case of DCP content, since the difference was not significant between rations contained cotton stalks (R3 and R4), but was significant (P < 0.05) between R1 and R2 contained treated and untreated rice straw, respectively. The results showed that rations containing treated rice straw and cotton stalks showed high feeding values than that of control rations. The TDN values of rations containing spent rice straw and cotton stalks were increased by about 6.92% and 13.24% respectively. The DCP of treated rations was higher than that of control by about 28.0% and 5.90% for rations contained rice straw and cottons stalks, respectively.

Table (4): The effect of biological treatment by *Pleurotus ostreatus* on digestibility coefficients of and feeding values rice straw and cotton stalks.

Items	Ration (1)	Ration (2)	Ration (3)	Ration (4)	± SE
DM	58.42b	63.68a	55.73b	65.05a	0.94
OM	63.38b	70.15a	61.36b	70.26a	1.22
CP	60.00c	69.30a	64.60b	67.54ab	1.19
CF	55.52a	55.92a	29.20c	24.32b	9.80
EE	83.41a	74.30b	76.6b	67.75c	1.17
NFE	67.12c	75.08b	68.77c	79.53a	1.17
Feeding values % on DM basis:					
TDN	54.91ab	58.71a	51.94b	58.82a	1.05
DCP	6.50c	8.32b	9.83a	10.41a	0.45

a,b,c Means with the same letter within each raw are not significant at P < 0.05

Nitrogen balance:

Data of nitrogen balance (NB) measured in the metabolism trials for sheep fed the experimental rations are presented in Table (5).

The nitrogen balance was carried out to make sure that the experimental animals maintained their weight and had a balanced maintenance rations during the experimental period. The results showed that the N retention was positive with the all rations. The highest nitrogen balance values were recorded (P < 0.05) with rations containing treated rice straw and cotton stalks. The same trend was detected for NB as % of nitrogen intake and digested. This observation was in agreement with those reported by EL-Ashry *et al.* (1997) and Mahrous (2005).

Table (5): Nitrogen utilization of different experimental rations

Items	Expermental rations				
	Rice straw		Cotton stalks		
	Untreated	Treated	Untreated	Treated	± SE
Nitrogen intake (g/h/d)	11.90	13.20	16.74	16.95	
Fecal nitrogen (g/h/d	4.76b	4.05c	5.93a	5.50a	0.18
Digested nitrogen (g/h/d)	7.14d	9.15c	10.81b	11.45a	0.18
N. excreated in urine (g/h/d)	4.24c	4.55bc	5.89a	5.47ab	0.22
N. balance (g/h/d)	2.90c	4.60b	4.92b	5.98a	0.22
N.B. % of intake	24.37c	34.85ab	29.40b	35.28a	1.44
N.B. % of digested	40.62c	50.27ab	45.51bc	52.23a	1.75

a,b,c,d Means with the same letter within each raw are not significant at $P < 0.05$

CONCOLUSION

In general, it is clear from the obtained results that the biological treatment with *Pleurotus ostreatus* improved the quality of both cotton stalks and rice straw. Therefore by this technique an important amount of treated roughages especially cotton stalks would be available to be utilized as animal feed which is also helpful to eliminate environmental pollution.

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تأثير المعاملة الحيوية علىالتركيب الكيميائى و القيمة الهضمية والغذائية لكل من حطب القطن وقش الأرز

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***المعمل المركزى للأغذية والأعلاف – مركز البحوث الزراعية – جيزة – مصر**

**** قسم الانتاج الحيوانى – جامعة المنصورة – مصر**

أجريت هذه التجارب لدراسة تاثير نمو فطر عيش الغراب المحارى (بلوروتس اوستريتس) على كل من حطب القطن وقش الأرز بعد التلقيح ومرور فتره التحضين (٤ أسابيع) لدراسة مدى التحسن فى التركيب الكميائى لهذين المخلفين وإجراء تجارب لتقدير معامل الهضم المقدر معمليا (In-Vitro) وكذلك اجراء تجارب تحليل غذائى على كباش الرحمانى لدراسة معاملات الهضم وميزان الأزوت والقيم الغذائية للعلائق التى تحتوى على قش الارز وحطب القطن غير المعامل أو المعامل.

وقد اظهرت النتائج مايلى:

أولا: التركيب الكميائى:

- زياده محتوى البروتين الخام بنسبة ٥٦ % ، ١٢٤ % على التوالى.
- إنخفاض الألياف الخام بمقدار ٤٧,٤% ، ٣٤,٨ % على التوالى.
- زيادة محتوى الرماد فى المواد المعامله .
- تحسين نوعية قش الأرز المعامل نتيجة تكسِّر اللجنين (١٠,٢%) بعد المعاملة بينما كان بلغ تكسير اللجنين فى حطب القطن(٢٢,٤%)
- إنخفاض محدود فى محتوى قش الأرز المعامل من السليولوز (٦%) بينما كان ملحوظا فى حطب القطن المعامل (٢٠%).

ثانيا: معامل الهضم المقدر معمليا:

ظهرت زيادة معنويه فى هضم المادة الجافة (مستوى ٠,٠٥) قدرها ٢٣,٢ ، ٣٠% علىالتوالى بينما كانت هناك زيادة معنويه (على مستوى ٠,٠٥) فى هضم المادة العضوية وصلت إلى ٣٧,٧ ، ٣٦,٤% على التوالى.

وقد اظهرت التجارب ان نمو فطر عيش الغراب المحارى(بلوروتس أوستريتس) على كل من حطب القطن وقش الأرز أدى إلى زيادة فى النسبة الهضمية لكل من الماده الجافة والماده العضويه والبروتين الخام بجانب ذلك انخفاض فى النسبة الهضمية للألياف الخام فى كل حطب القطن وقش الأرز.

ثالثا: معاملات الهضم المقدره على الحيوان والقيمه الغذائية:

- بالمقارنه بالمواد غير المعاملة أظهرت معظم العلائق التى تحتوى على مواد معامله زيادة معنويه (على مستوى ٠,٠٥) فى معاملات الهضم عن العلائق التى تحتوى على مواد غير معاملة.
- كان للمعاملة تأثير إيجابى على زيادة المهضوم من المادة الجافة حيث زادت من ٥٨,٢ – ٦٣,٧ و ٥٥,٧ – ٦٥,١% للعلائق التى تحتوى على قش الأرز وحطب القطن على التوالى.
- زيادة المهضوم من المادة العضوية بحوالى ١١% للعلائق التى تحتوى على قش الأرز وحطب القطن المعامل .
- زيادة المهضوم من البروتين الخام فى العلائق التى تحتوى على قش بمقدار ١٥,٥% بينما كانت الزيادة فى العلائق التى تحتوى على حطب القطن محدودة (٤,٥%).
- لم يكن هناك تأثير معنوي علي هضم الألياف في العلائق التي تحتوي علي قش الأرز ولكن بلغت الزيادة في العلائق التي تحتوي علي حطب القطن ١٧,٦%
- زيادة المهضوم من مستخلص خالي الأزوت بمقدار ١١,٧% و ١٥,٧% في العلائق التى تحتوى علي قش أرز معامل وحطب قطن معامل علي التوالي .
- كانت هناك زيادة معنوية للعلائق التي تحتوي علي مواد معاملة حيث بلغت الزيادة في المواد الكلية المهضومة ٦,٩% و ١٣,٢% والبروتين الخام المهضوم ٢٨,٠% و ٥,٩% في العلائق التي تحتوي علي قش الأرز المعامل وحطب القطن المعامل علي التوالي :

رابعا : ميزان الأزوت :

- كان ميزان الأزوت موجبا لجميع العلائق المستخدمة في التجربة .
- سجلت العلائق المحتوية علي قش الأرز أو حطب القطن المعامل أعلى ميزان أزوت

وتوصي الدراسة بأنه يمكن استخدام نمو فطر عيش الغراب المحاري (بلوروتس أو ستريتس) بنجاح علي كل من قش الأرز وحطب القطن الذي لا يتم استخدامة في العلائق نظرا لإرتفاع نسبة اللجنين ولإغناء تلك المواد الخشنة الفقيرة وتحسين معاملات هضمها وقيمها الغذائية لإستخدامها في تغذية المجترات والإسهام في الحد من التلوث البيئي