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EFFECT OF FUNGUS TREATMENTS OF COTTON STALKS ON SHEEP PERFORMANCE

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SUMMARY

This study was carried out at (El-Serew Research Station, Domuat Governorate) to study the effect of fungus treatments (*Cheatomium cellublyticum, Trichoderma viride* and *Cheatomium cellublyticum* with *Trichoderma viride*) of cotton stalks on feed intake, digestibility coefficients, nutritive value, nitrogen balance and some rumen liquor and blood parameters. Four treatments were tested, the first treatment (NC) was the negative control, second treatment (CC) was cotton stalks treated with fungus (*Cheatomium cellublyticum*) offered *ad. Libitum*. Third treatment (TV) was cotton stalks treated with fungus (*Trichoderma viride*) and fourth treatment (CO) was cotton stalks treated combination (*Cheatomium cellublyticum* with *Trichoderma viride*).

Twenty four rams mean weighed 20.0 kg were used in feeding trial lasted 120 days to evaluate the effect of biological treatments on the nutritive value of cotton stalks increased daily gain (165.8, 193.3, 211.6 and 136.6) for CC, TV, CO and NC, respectively. The results indicated that CC, TV and CO increased (P<0.05) crude protein of cotton stalks and decreased (P<0.05) dry matter, crude fiber, NFE, NDF, ADF, ADL and cellulose contents than the control group. Dry matter intake (DMI) increased (P<0.05) in the groups fed cotton stalks treated with *CC, TV* and *CO* than the control group. Fungus treatments increased (P<0.05) digestibility coefficients of CF, ADF, NDF, ADL, cellulose and hemicellulose than the untreated cotton stalks. Total digestible nutrients (TDN) and digestibility of crude protein (DCP) for CO, TV and CC were higher (P<0.05) than untreated cotton stalks.

***Keywords**: cotton stalks, fungus, biological treatments, feeding value, sheep.*

INTRODUCTION

The shortage of feeds in general and protein in particular attracted the attention of many research workers towards the unconventional sources of feed crop residues. They are mainly fibrous materials of agricultural by-products which represent a high percentage of the total volume of the feeds produced annually in Egypt. However, only 4.0 to 4.3 million tons of crop residues out of 13.7-15.2 million tons produced are used for feeding ruminants (Hathout and El-Nouby, 1990).

Residues are burned or wasted, and hence lead to environmental pollution and health hazards. The main factors limiting the utilization of crop residues are of low digestibility, low protein content and some time low palatability.

This study aimed to investigate, the ability of biological treatments to improve nutritive value as total digestible nutrient (TDN) and digestibile crude protein (DCP) of cotton stalks as crop residues and the effect of biological treatments of cotton stalks on chemical composition, nutrient digestibility, the rumen parameters and blood parameters of sheep fed on it.

MATERIALS AND METHODS

Microorganisms:

Cheatomium cellublyticum NRRL 6102 and *Trichoderma viride* F-516 were obtained from Microbial Chemistry Department, National Research Center, Dokki, Egypt. The microorganisms were maintained on agar medium composed of (g/L) malt extract, 30.0; mycological peptone, 5.0 and agar 20.0.

Preparation of fungal inoculum:

Three days old slant (20 x 200mm) of *Cheatomium cellublyticum* NRRL 6102 and *Trichoderma viride* F-516 into a flask containing 25 ml of sterilized water. The inoculum was used to inoculate 500ml capacity conical flasks containing 20g of cooled sterilized residue by (autoclaving on 121°C for 30 minutes while moistened by basal medium containing 4% molasses, 0.4% urea, 0.2% KH_2PO_4 and 0.03 $MgSO_4.7H_2O$ in solid liquid ratio 1:2 by 10% (v/w). The inoculated flasks were incubated in adjusted temperature incubator at 30 °C±2 for 5 days.

Crop residues preparation:

Cotton stalks were chopped into 3-5 cm until moisture level was (65-70%) and put specific fungal spawn and left for 21 days.

Analytical methods:

Chemical composition of feed, feces and urine were determined according to A.O.A.C (1990). Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) were determined by the methods of Van Soest (1982).

Digestibility trial:

Twelve adult rams were divided into four similar groups and used to carry out four metabolic trials using three animals for each group as follows: NC: Negative control. CC: Cotton stalks with *Cheatomium cellublyticum*. TV: Cotton stalks with *Trichoderma viride*. CO: Cotton stalks combination with *C. cellublyticum* + *T. viride*.

Preliminary period lasted 21 days followed by 7 days for collection. The nutrient intake and excretion were determined to calculate their digestibility and utilization by conventional balance trial method. The experimental animals were fed concentrate feed mixture (CFM) to fit the maintenance requirement (NRC, 1985). All groups were fed roughage *ad. Libtum*. Feces were collected quantitatively every day during the collection period. At the end of the period, feces samples for each ram were ground and mixed well and kept in the refrigerator for chemical analysis. Samples of feed, feces and urine were analyzed according to A.O.A.C. (1990). Rumen liquor samples were taken from each animals at the end of collection period at 0, 3 and 6 hours after feeding by stomach tube. Rumen liquor pH and ammonia nitrogen (NH_3-N) were immediately determined according to Conway (1963). Frozen rumen liquor samples were analyzed for total volatile fatty acids (TVFA's) by steam according to Abou-Akkada and Osman (1967).

Total fungal counts and total bacterial counts were measured according to (Difco, 1984) and microbial protein was measured by sodium taugistate methods according to Shultz and Shultz (1970).

Feeding trial:

Twenty-four lambs were taken from the station herd and were divided into four similar groups, according to weight. Average initial live body weight was 20.0 kg/head and animals groups were fed the four respective rations in 2 meals/day for 120 days. All lambs in the trial were given requirements according to NRC (1985) recommendation and cotton stalks either treated or untreated were fed *ad. Libitum.*

The data were statistically analyzed according to Sendecor and Cochran (1980) using SAS (1985). The difference between means was tested by Dancan's multiple range test (1955).

RESULTS AND DISSCUSSION

1- Chemical composition:

Chemical composition of experimental cotton stalks is presented in Table (1). The cotton stalks untreated (NC), cotton stalks treated with CC, cotton stalks treated with TV and cotton stalks treated *CO* resulted in decreasing (P<0.05) dry matter (DM) being 91.1, 90.5, 88.1 and 90.0%, respectively. The substantial increase (P<0.05) in crude protein (CP) of the fungal treated cotton stalks against other treatments being 3.7, 10.0, 8.8 and 11.2%, respectively. Ash content for biological treatments of cotton stalks were higher than control, being 12.5, 11.3, 9.4 and 7.6, respectively. Treatments of cotton stalks with *CC* and *TV* resulted in reducing (P<0.05) NDF, ADF, ADL, Cellulose and hemicellulose. These observations were in agreement with Soliman *et al.* (1979) and Gado (1999).

The degradation of various fiber fractions increased with increasing level of hemicellulose. It was noticed that, all treatments; *CO*, *CC* and *TV* were greater in NDF, ADF, ADL, cellulose and hemicellulose levels indicating their influence on hemicellulose breakdown as effect of the treatment. These results are in agreement with Gado (1999). The biological treatment with *CO*, *CC* and *TV* which led to crude protein augmentation and reduce the fiber fractions.

ADF content of cotton stalks treated with *CO* recorded the lower value than those of untreated and treated with *CC* or with *TV*, being 43.1, 45.2, 44.5 and 43.2%, respectively. *CO* treatment of cotton stalks contained the least percentages of ADL than NC being 9.01and 9.89%, respectively.

2- Digestibility coefficients and nutritive value:

Nutrient digestibility coefficients and nutritive value, have been affected by biological treatments, are presented in Table (2). Coefficients of digestibility for DM increased significantly (P<0.05) for treated; cotton stalks CO (68.4%) and TV (63.7%) than the control (54.6%).

Concerning digestibility coefficient of crude protein (CO) had the highest value 69.4% as shown in Table (2). Digestibility coefficient of CF was significantly (P<0.05) higher for cotton stalks treated with *CO*, being 68.7% and the lowest CF digestibility coefficient was recorded for the control group (45.0%). The improvement in DM, CP and CF digestibility coefficients over a wide range of low quality roughages due to fungus treatments were observed by (Gado. 1999). *CO* treatment increased the digestibility coefficient of fiber component (Table 3). The NDF digestibility of mixed *CO* was

Table (1): Chemical composition (%on DM bases) of untreated and treated cotton stalks and concentrate feed mixture.

Item	NC	CC	TV	CO	CFM
DM	91.1	90.5	88.1	90.0	88.8
OM	92.4	90.6	88.7	89.5	87.3
CP	3.7	10.0	8.8	11.2	16.2
CF	45.0	40.1	39.8	38.5	13.0
EE	1.2	1.4	1.5	1.5	3.2
NFE	42.5	39.1	38.6	36.3	54.8
Ash	7.6	9.4	11.3	11.5	12.6
NDF	77.0	75.0	74.2	73.1	32.7
ADF	45.2	44.5	43.2	43.1	10.4
ADL	9.8	9.6	9.3	9.0	3.4
Cellulose	35.3	34.9	33.8	34.1	6.9
Hemicellulose	31.8	30.4	31.0	30.0	22.2

Table (2): Effect of biological treatments on digestibility and nutritive value.

Item	NC	CC	TV	CO	± SE
Digestibility:					
DM	44.6^{b}	55.3^{b}	63.7^{a}	68.4^{a}	±1.38
OM	50.7^{d}	65.4^{c}	68.6^{b}	70.1^{a}	±1.23
CP	44.7^{c}	50.2^{b}	50.2^{b}	56.4^{a}	±0.47
CF	45.0^{c}	52.6^{c}	60.7^{b}	68.7^{a}	±0.96
EE	55.3^{c}	65.5^{b}	67.3^{b}	72.7^{a}	±0.43
NFE	60.3^{c}	67.2^{b}	72.3^{a}	74.4^{a}	±1.51
NDF	48.6^{d}	52.4^{c}	58.1^{b}	62.9^{a}	±1.13
ADF	39.0^{d}	46.4^{c}	54.1^{b}	65.4^{a}	±0.27
ADL	27.4^{d}	31.0^{c}	33.7^{b}	47.9^{a}	±0.78
Cellulose	47.7^{d}	50.3^{c}	57.3^{b}	64.9^{a}	±0.26
Hemicellulose	60.6^{d}	63.8^{c}	71.5^{b}	78.5^{a}	±1.20
Nutritive value %:					
TDN	50.5^{d}	55.8^{c}	59.4^{b}	61.8^{a}	±0.62
DCP	4.3^{d}	6.2^{c}	7.4^{b}	9.3^{a}	±1.23

a, b ,c and d Means with differet superscripts in the same row differ significantly (P<0.05).

Table (3): Effect of biological treatments on nitrogen balance.

Item	NC	CC	TV	CO	±SE
Nitrogen Intake (NI) (g/h/d)	14.1^{d}	17.7^{c}	20.0^{b}	22.9^{a}	±1.20
Urinary Nitrogen (UN) (g/h/d)	8.5^{d}	8.7^{c}	10.0^{b}	10.3^{a}	±0.88
Feces Nitrogen (FN) (g/h/d)	4.5^{d}	5.6^{c}	5.8^{b}	5.9^{a}	±1.21
Nitrogen Digested (ND) (g/h/d)	9.5^{d}	14.0^{c}	16.6^{b}	17.5^{a}	±0.91
Nitrogen Balance (NB) (g/h/d)	$+1.0^{d}$	-3.3^{c}	$+4.2^{b}$	$+6.8^{a}$	±1.30

a, b ,c and d Means with differet superscripts in the same row differ significantly (P<0.05).

significantly higher (P<0.05) than each alone treatments and control.

The ADF digestibilities of treated stalk were significantly higher (P<0.05) than the control treatment. Cellulose digestibility coefficient of cotton stalks treated with *CO* was 64.9% and that treated with *TV* was 57.3% but cotton stalks treated with *CC* was 50.3% being increased slightly than the control group (47.7%). Hemicellulose digestibility was also the highest (78.5%) for CO followed by TV (71.5%) and CC (63.8%) and the lowest value was recorded for the control treatment (60.60%). The results agree with Deraz and Ismail (2001) who reported that fungus treatments had the effect of loosening lignucelluletic bonds and solublize some of the hemicellulose content. The nutritive value of treatments as TDN was the highest for CO, TV (61.8%), (59.4%) and CC (55.8%) where they significantly higher (P<0.05) than the control (50.5%). Also, DCP were significantly higher (P<0.05) with fungus treatment than the control. These results agree with Hammouda (1996) and Al-Ashry *et al.* (1997) and Fouad *et al.* (1998). They rep rted that fungi treatment had the effect of lossening lignocellulatic bonds and solublize some of the hemicellulose content.

Thus, it could be concluded that biological treatment of cotton stalks could increase the digestibility coefficients of most nutrients and thus their feeding values as TDN and DCP compared with untreated cotton stalks. The amount of increase, however, differs amang the treatments used. These results are in agreement with those reported by several researcher worked on stalks as Singh and Gupta (1994) and Azzam (1992).

3- Nitrogen balance:

Data of nitrogen balance measured in the metabolism trial for sheep fed the experimental rations are presented in Table (3). All treatments showed a positive nitrogen balance. Nitrogen balance ranged from (+1.0) to (+6.8) g/h/d. There were significant (P<0.05) differences between biological treatment tested. Results showed that the nitrogen balance (g/h/d) for CO (+6.8) was higher than CC (+3.3) and TV (+4.2). The nitrogen balance for the control (+1.0) was lowers (P<0.05) than all biological treatments. The same trend was detected for nitrogen digested (ND), nitrogen intake (NI) and nitrogen balance (NB) as % of NI and NB as % of ND.

Therefore, ration containing biologically treated crop-residues showed positive nitrogen balance which in agreement with that of Langer *et al.* (1982) and Ahuja *et al.* (1986).

Marwaha *et al.* (1990) conducted a feeding trail with growing Jersey calves fed fungal treated wheat straw. They found that retained nitrogen was 27.7% of NI.

Singh *et al.* (1990) also, found a positive nitrogen balance when they fed calves on fungal treated wheat straw. This observation was in agreement with Kakkar *et al.* (1990) and El-Ashry *et al.* (1997).

4- Rumen liquor parameters:

The rumen liquor parameters are shown in Table (4). Ammonia-N (NH_3-N) concentration reached the maximum after 3 hours of feeding in all groups. However, NH_3-N concentrations were significantly higher (P<0.05) in the treated groups than the control group. After 6 hours of feeding NH_3-N concentration tended to decrease in all groups. This agree with Williams and Newbold (1990) who reported that the reduction of ammonia in the rumen liquor appear to be the result of increased incorporation of ammonia into microbial protein and it was considered as a direct result to stimulated microbial activity.

Cotton stalks treated with *CO* maintained highest value of rumen TVFA's after 3 hr. of feeding followed by cotton stalks treated with *TV*, cotton stalks treated with *CC* and the lowest values recorded for the control group. These results agree with those obtained by Henics (1987) who found that the level of ruminal TVFA's reached its maximum at 3 hr. after feeding for lambs fed *ad. libitum*. The results of biological treatments might be related to more utilization of dietary energy and positive fermentation in the rumen. These results agree with that obtained by Arambel and Kent (1988) whom reported that *A. orzae* increased rumen ammonia and branched-chain VFA's concentration. Since *A. orzae* promoted protein degradation and that branched-chain VFA's are produced from protein degradation.

5-Total fungal, total bacterial and microbial protein counts:

The data presented in Table (5) shows that the total fungi counts ranged from 1.20 to 1.90 (x 10^3 cfu/ml). All treated stalks were significantly higher in fungus count than control. Meanwhile, they differed significantly in between. CO was the highest, followed by TV then CC. This result is in harmony with those reported by Singh and Gupta (1994) and Ibrahim (2001).

Total bacterial counts ranged from 0.60 to 0.92 (x10^3 cfu/ml). Results indicated that total bacteria counts were significantly higher ($P<0.05$) for sheep fed biologically treated rations (TV, CO and CC) than control (NC). The result obtained in this study is in harmony with those reported by Singh and Gupta (1994) and Ibrahim (2001) who showed that total counts of bacteria were about 20-fold greater than initial viable counts when the buffaloes were given a diet of roughages only and they were about 10-fold greater when a diet of concentrate and roughage was given.

The data of microbial protein are shown in Table (5) indicate significant ($P<0.05$) differences among the values of microbial protein ranged from 0.24 in NC to 0.50 in CO (g/100ml). The highest values for microbial protein was found in the treated cotton stalks with the two fungus CO (0.40), than TV (0.33), CC (0.24) and the least the control (0.10). Striskandara *et al.* (1982) and Ibrahim (2001) concluded that the efficiency of microbial protein synthesis in the rumen was not limited by the supply of peptides and amino acids.

Vicent and Kelly (1994) found that cows fed bromegrass hay plus fungi decreased ruminal fermentation of hemicellulose and microbial N-flow at the duodenum and tended to decrease microbial efficiency, but when alfalfa hay was fed, there were no effects of fungi on site or extent of digestion or on microbial N synthesis.

6- Feeding trial:

The average DM intake expressed as (g/h/d), average daily body gain and feed conversion for the experimental groups are presented in Table (6). The result revealed that the average DMI as (g/h/d) of lambs during 120 days of experimentation was higher for lambs supplemented with *CO* (1291g/h/d) followed by that supplemented with *TV* (1244 g/h/d) than with *CC* (1150 g/h/d), then the least one NC (1040 g/h/d). The results of feed conversion (g DM/g gain) showed that bio treatment recorded the best value (*CO* followed by single treatments with *TV* and *CC* while control was the least. Mohamed *et al.* (1998) indicated that feed conversion of treated cotton stalks was better compared with untreated cotton stalks.

Table (4): Effect of biological treatments on rumen liquor and blood parameters.

Item		NC	CC	TV	CO	± SE
Rumen parameters:						
	0	7.2^{c}	7.2^{c}	7.4^{b}	7.6^{a}	±0.64
pH	3	6.7^{c}	6.8^{c}	7.1^{b}	7.5^{a}	±0.60
	6	7.4^{c}	7.4^{c}	7.5^{b}	7.7^{a}	±0.80
	0	11.8^{a}	10.8^{b}	10.8^{b}	10.9^{b}	±0.02
NH_3-N (mg/100ml)	3	17.8^{d}	22.0^{b}	20.6^{c}	25.8^{a}	±0.03
	6	13.2^{d}	19.7^{b}	17.6^{c}	20.4^{a}	±0.02
	0	9.5^{d}	10.0^{c}	10.3^{a}	10.1^{b}	±0.52
TVFA's /mg/100ml)	3	12.5^{d}	13.0^{c}	13.5^{b}	14.4^{a}	±0.82
	6	9.2^{d}	9.4^{c}	9.7^{a}	9.6^{b}	±0.13

a, b ,c and d Means with differet superscripts in the same row differ significantly (P<0.05).

Table (5): Effect of biological treatments on total fungal, total bacterial and microbial protein counts.

Item	NC	CC	TV	CO	±SE
Total fungi counts (x 10^3 cfu /ml)	1.20^{d}	1.35^{c}	1.68^{b}	1.90^{a}	±1.20
Total bacterial counts (x 10^3 cfu /ml)	0.60^{c}	0.82^{b}	0.91^{a}	0.92^{a}	±0.98
Microbial protein (g/100ml)	0.10^{d}	0.24^{c}	0.33^{b}	0.40^{a}	±1.30

a, b ,c and d Means with differet superscripts in the same row differ significantly (P<0.05).

Table (6): Effect of biological treatments on feed intake and feed conversion of experimental animals.

Item	NC	CC	TV	CO
No. of animals	6	6	6	6
Experimental period (days)	120	120	120	120
Initial weight (kg)	20.1	20.2	20.3	20.2
Final weight (kg)	36.5	40.1	43.5	45.6
Total gain (kg)	16.4	19.9	23.2	25.4
Average daily gain (ADG) (g)	136.6	165.8	193.3	211.6
DMI (g/d)	1040	1150	1244	1291
Concentrate feed mixture	660	660	660	660
Roughages	380	490	584	631
Feed conversion (g DM/g gain)	7.6	6.9	6.4	6.1

The biological treatments could be used successfully to enrich poor quality roughages (cotton stalks) and improved the nutritive value (digestibility coefficients and feeding value). In addition, biological treatments are preferable than other treatments such as chemical and physical treatments for better and clear environment besides less possible negative sideeffects.

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تأثير المعاملات الفطرية لحطب القطن على أداء الأغنام

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أجريت هذه الدراسة لمعرفة تأثير المعاملات البيولوجية بفطر *Cheatomium cellublyticum* وفطر *Trichoderma viride* وخليط بينهما على حطب القطن ودراسة تاثير المعاملات على كمية الغذاء المأكول ومعاملات الهضم والقيمة الغذائية وميزان الأزوت وبعض مكونات الدم وسائل الكرش ومعدلات النمو والكفاءة الغذائية للأغنام.

أستخدم عدد ١٢ كبش رحمانى لتجربة الهضم وقسمت الى ٤ مجاميع حسب النظام التالى:
المجموعة الأولى: كنترول (حطب قطن غير معامل). المجموعة الثانية: حطب قطن معامل بفطر *Cheatomium cellublyticum*. المجموعة الثالثة: حطب قطن معامل بفطر *Trichoderma viride*. المجموعة الرابعة: حطب قطن معامل بخليط من الفطرين.

وابرز النتائج التى ظهرت هى:

- أدت المعاملة بكلا الفطرين إلى زيادة معاملات الهضم لحطب القطن من المادة الجافة والمادة العضوية والألياف الخام والبروتين و ADF, NDF والسليلوز والهيميسليلوز واللجنين معنويا فى المجموعات الرابعة والثالثة والثانية مقارنة بمجموعة المقارنة.
- كان المجموع الكلى للمواد الغذائية المهضومة للمعاملة الرابعة أعلى معنويا عن مجموعة المقارنة والمجموعات الاخرى. وكذلك محتواها فى البروتين المهضوم كان أعلا فى المجموعة المعاملة بالخليط ثم المعاملات الاخرى ثم مجموعة المقارنة.
- بالنسبة لقياسات الكرش كان تركيز الامونيا – نيتروجين فى سائل الكرش للكباش المغذاة على حطب القطن المعامل أعلى معنويا مقارنة بالمغذاة على حطب القطن الغير معامل وكذلك مستوى الأحماض الدهنية الطيارة.
- كانت الزيادة فى معدلات النمو وكمية المأكول اليومى فى المجاميع المعاملة بيولوجيا أعلى من المجموعة الغير معاملة (المقارنة).

مما سبق يتضح ان المعاملة بالفطر تعمل على رفع القيمة الغذائية لحطب القطن واستفادة الأغنام منه. والمعاملة الفطرية لها القدرة على تكسير المواد اللجنوسليولوزية مثل السليلوز والهيميسليلوز واللجنين.