EFFECT OF TWO BIOLOGICAL TREATMENTS FOR SOME AGRICULTURAL RESIDUES, (RICE STRAW AND COTTON STALKS) ON THEIR NUTRITIVE VALUES

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ABSTRACT

The present study was carried out in the Central Laboratory for Food and Feed (CLFF) to study the effect of treated rice straw and cotton stalks with white-rot fungi (Pleurotus ostreatus) alone, added yeast (Saccharomyces Cervisiae), incubated yeast for 15hr and combination of fungi and yeast on chemical composition, fiber fractions, digestibility of dry matter (IVDMD), digestibility of organic matter (IVOMD), true digestibility (IVTD), pH and microflora of ruminal fluid (Enterococci count, lactic acid bacteria count and total anaerobic bacteria count). The results of chemical composition showed in case of RS incubated with SC for 15hr increase in protein content (from 4.6 to 6.2%), and in case CS incubated with SC for 15hr (from 3.5 to 5.9%) however, additional increase in case of spent RC (from 4.6 to 7.6%) and spent CS (from 3.5 to 10.9%). The results of fiber content were decrease in general, in case of RS incubated with SC for 15hr decrease in fiber content (from 34.8 to 33.4%), and in case of CS incubated with SC for 15hr (from 48.2 to 45.9%). However, additional decrease in case of spent RC (from 34.8 to 16.4%) and spent CS incubated with SC (from 48.2 to 30.9%). Each of fiber fraction, lignin content in both experimental materials showed considerable decrease refers to the treatment with fungi or yeast or both. The result of IV-DMD showed highest value with the treated rice straw with fungi + yeast (52.24%) and the same trend with IV-OMD (60.54%). However, the highest IV-true digestibility value was with treated rice straw with fungi and incubated yeast for 15hr (66.28%). No effect of yeast treatment on the pH value of the rumenal fluid over all the experiment. The administration of SC to small ruminants stimulates the growth of the beneficial rumenal bacteria which consequently positively affects the rumenal fiber degradation i.e 30x10³ vs. 56x10³, 30x10³ vs. 55x10³ and 20x10³ vs. 52x10³ for enterococci count, lactic acid bacteria count and total anaerobic bacteria count, respectively. The results revealed that the cultivation of fungi and inculation or addition with yeast could improve the nutritive value of rice straw and cotton stalks. Hence, the biological treatment, in addition, of sharing it for improvement of nutritive value of these residues to be suitable for animal feeding, the treated residues share in reducing the environmental pollution by avoiding burning it.

Keywords: Rice straw, Cotton stalks, *Pleurotus Ostreatus, Saccharomyces cerevisiae, In Vitro*, Chemical composition, Nutretive value, Rumen

microorganism.

INTRODUCTION

For long time ago, several researches and reports entitled with the problem of animal feed shortage as the main constraint which limits the development of animal production all over the world. The present animal feed status seems to meet 50-60% of the requirements of existing animals, ruminants and monostomach.

It should be pointed out that about 14 million tons of plant residues are available annually in Egypt (Hathout and El-Nouby, 1990 and El-Shinnawy1998). However, only 4.0 to 4.3 million tons may be used in feeding

ruminants and the remainders are burned or wasted, and hence lead to environmental pollution and consequently health hazards.

The most fibrous plant residues are available as cheap roughage resources, including different straws and other materials i.e. rice straw, wheat straw, bean straw (stalks), com stalks, stovers, corn cobs, rice hulls, banana leaves and plantain, cotton stalks and others.

Nowadays, several trails have been established to improve nutritive value of lignocelluloses crop residues by the biological treatments with various yeast, moulds, algae and bacteria, Abdul-Aziz et al (1997); El-Ashry et al., (1997); Henderson and McDonald (1977); Khorshed (2000); El-Tahan (2003), El-Tahan and Mohammadi (2005) and Akila S. Hamza et al. (2006). Saccharomyces cerevisiae culture has been used as supplement for ruminant's dietary for many years. However, interest in Sacc. cerevisiae culture as a potential alternative to antimicrobial feed additives has increased within the past 10 years. Some of the benefits associated with the use of Sacc. cerevisiae included the increase of dry matter and NDF digestion (Carro et al, 1992), initial rates of fiber digestion (Williams et al 1991), and milk production in dairy cattle (Harris and Webb 1990; Kung et al 1997; Piva et al 1993 and Williams et al 1991).

In vitro experiments have also reported that, in some cases, S. cerevisiae culture favorably altered the mixed ruminal microorganism fermentation and stimulated lactate uptake and cellulose digestion by pure cultures of predominant ruminal bacteria (Callaway and Martin 1997; Martin and Nisbet 1992; Nisbet and Martin 1991, and Nisbet and Martin 1993). Unfortunately, in-vivo and in-vitro effects of Sacc. cerevisiae culture are not always consistent (Martin and Nisbet 1992).

Recent research (Callaway and Martin 1997) demonstrated that, Sacc. cerevisiae culture stimulated growth of the predominant ruminal anaerobic bacteria such as Selenomonas ruminantium, Megasphaera elsden, Fibrobacter succinogenes and Ruminococcus albus. Sacc. Cerevisiae culture increased the initial rate but not the extent of cellulose digestion by F. succinogenes and Ruminococcus flavefaciens (Callaway and Martin 1997). Sullivan and Martin 1999 reported that pure culture results, found that Sacc. cerevisiae culture on the in vitro increase mixed ruminal microorganism fermentation of rice straw and cotton stalks.

The objective of present work is to study the effect of incubating rice straw or cotton stalks either with oyster mushroom alone (*Pleurotus ostreatus*) or bacter's yeast alone (*Sacharomyces cerevisiae*) or fungal yeast treatment on the improvement of nutritive values, and also on *in vitro* mixed ruminal microorganism

MATERIALS AND METHODS

The present work was carried out in the Central Laboratory for Food & Feed (CLFF), Agriculture Research Center during 2006.

Two microorganisms have been used:-

1-The strain of *Pleurotus ostreatus* used in this experiment was obtained from the Department of microbiology laboratory, Faculty of Agriculture, Ain Shams University. 2-The strain of Saccharomyces Crevisiae ATCC 0000 was obtained from Food Safety Laboratory, Central Laboratory for Food and Feed, Agriculture Research Center.

Pleurotus ostreatus cultivation:-

1- Preparation of mother culture

The preparation of mother culture was grown on Potato Dextrose Agar (PDA) media. After preparing the media (41 gm from PDA / 1 litter distilled water), stir on heating until boiling. Fill glass test tubes to one third with the media, cover with cotton wool then aluminum paper. Autoclaved at 121 °C under pressure for 20 min. Lay tubes at 45 ° angles until cooled at room temperature, then inculated using the mother culture which incubated at 25°C for 15 days.

2-Preparation of mother spawns substrate

The mother spawns was prepared according to Kumar and Mujal (1975) and Quimio, (1986). To prepare the mother spawn, sorghum was washed thoroughly, and then soaked over night. Dead seeds or those that float on water were carefully removed. Then, the grains were drained. Precipitated chalk (CaCO3) and gypsum "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.

3- Inoculation and incubation

The jars were inoculated inside laminar flow cabinet and 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 plantedspawn was used in mushroom cultivation after being incubated 15 days at 25 °C until the grains were covered with white cotton shaped mycelium biomass.

Preparation of substrate for mushroom cultivation

Raw rice straw and cotton stalks were obtained from field attached to the Agriculture Research Center. Raw cotton stalks was sun dried. Raw rice straw and cotton stalks were chopped (ca 2-6 cm). The chopped materials were soaked in tap water until moisture content 60–70 % excess water out of substrate, followed by soaking in boiled water (95-100°C), this process is carried out to reduce the count and of other microorganisms that may compete with growth of fungi thin all containers of substrate must be closed tightly and kept in sterilized room, leave the substrate till reach room temperature (6-8 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 spread in one layer (15 cm thick) into plastic containers (40x25x30 cm). The spawn was distributed over the substrate at a rate of 5% (w/w). A five cm thick layer of substrate was added to cover the spawn. Total amount of substrate used was 2.0 k g for each container. Twelve containers from each strain were prepared. Mycelial growth

The inoculated containers were covered tightly with plastic sheets and incubated at room temperature (20 °C ± 5°C). At the end of incubation

period "3weeks", the mycelial growth of the tested cultures covered the substrate. The Plastic sheet was removed; relative humidity of the room was adjusted " $70\% \pm 5$ %" by watering the containers once daily and spraying the floor with water.

The first fruiting bodies were harvested 10-12 days latter. Four harvests ($1^{st} = 50\%$, $2^{nd} = 25\%$, $3^{rd} = 15\%$ and $4^{th} = 10\%$ approximately) at intervals of about 5-7 days were carried out during the next 60 days. The raw materials (rice straw and cotton stalks) and spent materials (rice straw and cotton stalks) have been divided into three parts:

1- Chemical analyses were performed from raw and spent materials (rice straw and cotton stalks). The substrates were dried in oven at 60 °C and ground. Dry matter, crude fiber, ether extract, crude protein (N X 6.25) and ash were determined according to A.O.A.C. (1990). Nitrogen free extract was calculated by difference.

2- Fiber fractions:

Neutral detergent fiber, acid detergent fiber, acid detergent lignin, acid insoluble ash, hemicellulose, cellulose and lignin were determined according to Van Soest and Breston (1979)

3- In Vitro Fermentation studies:

The *in vitro* dry matter disappearance was determined on the raw rice straw, cotton stalks and treated rice straw, cotton stalks with *P. ostreatus* as will as with and without yeast (Saccharomyces cerevisiae) according to Menky *et al.*, (1979).

Three adult rams' Rahmany sheep (average 65 kg BW), fed 2% of BW (consists of 40% barssem hay and 60% of concentrate mixture. Before the morning feeding (0900), approximately 500 mL of ruminal fluid was drawn from each of the rams with a rubber stomach tube, deposited into a vacuum flask that had been previously flushed with O2-free CO2, mixed, and immediately transported to the laboratory. The mixed sample was strained through four layers of surgical gauze into an Erlenmeyer flask under continuous flushing with CO2, and efforts were made to maintain the temperature at 39 C. The fluid was then mixed with buffer (pH 6.9; containing [per liter] 292 mg of K2HPO4, 240 mg of KH2PO4, 480 mg of (NH4)2SO4, 480 mg of NaCl, 100 mg of MgSO4 7H2O, 64 mg of CaCl2 2H2O, 4,000 mg of Na2CO3, and 600 mg of cysteine hydrochlonde) in a 1:4 ratio. After mixing, 50 mL of diluted ruminal fluid was anaerobically transferred to 60-mL fermentation bottles containing 0.5 g of each substrate on a DM basis, incubated at 390C in a water bath for 48h and mixed periodically. Indigested residue was collected on a pre-weighed oven dried Whatman number 1 filter paper by vacuum filtration. The filter paper and undigested residues were then oven-dried at 105 □C for 24 h to remove excess moisture and then weighed. In vitro DM disappearance (IV-DMD) was calculated as original dry sample weight minus dry residue weight divided by the original sample weight. This value was then multiplied by 100 to derive the IV-DMD percentage.

 $\%IVOMD = 100 \times (initial substrate DM) - (substrate residue OM - blank residue OM - ash residue OM) / (initial substrate DM).$

For estimation of true digestibility, remove flasks from water bath after digestion or from refrigerator if stored. Wash with 100ml neutral detergent solution into 600ml Berzelius beaker to make a total volume of 150ml.Add 2 ml decahydronaphthalene. Reflux for 1 hour, and filter into a 50ml, 40mm plate, and coarse porosity fritted – glass crucibles. Wash twice with hot water, twice with acetone and suck dry. Dry in oven at 105 over night and weight. Blank are not necessary.

The calculation of true dry matter digestibility had been done by step one (after 24h incubation) Goering and Van Soest (1970), and the calculation as follows:

100-% ND residue= % true DM digestibility

Using Saccharomyces cerevisiae as bropiotic:

Subculture from the selected Saccharomyces cerevisiae was inoculated in Brain Heart Infusion broth Lenette et al. (1980), and incubated at 25°C for 24 hours then the total viable count was determined according to NMKL (2005). The freshly prepared suspension was added as follows to determine the in- vitro fermentation:-

- 1- Seventeen µl of freshly prepared suspension contained 5x10⁵ cfu was inoculated on 50 ml of (40 ml buffer solution +10 rumenal fluid) added to ½ gm crushed sample (raw, spent cotton stalks and raw, spent rice straw) and incubated for 48 hrs at 39oC in a water bath. Sub-samples were collected at 0, 3, 6, 9, 24 and 48 hrs intervals from which pH, total anaerobic bacteria (according to method no. 56 1994), Lactobacillus species (according to method no. 140 1991) and *Enterococci* species (according to NMKL method no. 68 2005) were counted.
- 2- Fifty grams of raw and spent samples under investigation were inoculated each with 1 ml of Saccaromyces suspension contained 5x10⁵ cfu (colony forming unit) and incubated at 25°C for 15 hrs then 0.5 gm of each sample was mixed with the 50 ml of suspension consists of (40 ml buffer solution +10 rumenal fluid) and incubated for 48 hrs at 39oC in a water bath. Sub-samples were collected at 0, 3, 6, 9, 24 and 48 hrs intervals from which pH, total anaerobic bacteria, Lactobacillus species and Enterococci species were counted according to the above mentioned methods.

Statistical analysis

Data obtained were subjected to analysis of variance according to procedures outlined by Snedecor and Cochran (1982).

RESULTS AND DISCUSSION

Data obtained from biological treatments presented in Table 1 and 3 showed that protein content of raw rice straw and raw cotton stalks are 4.6 and 3.5% respectively. However biological treatment with *Pleurotus ostreatus* increased protein content of all substrates after harvesting the fruits (three months) Results in the tables showed that, the highest increase was recorded 194.3% with *Pleurotus ostreatus* cultivated on cotton stalk followed

by rice straw when cultivated with Pleurotus ostreatus 58.7 %. Where as when pleurotus ostreatus followed by Saccaromycies servisea and incubated for 15 h, the protein content increase from 194.3% to 211.4% and from 58.7% to 65.2% on cotton stalks and rice straw respectively. These results recorded by Shoukry et al. (1985), and Akila S. Hamza et al. (2006) who mentioned that, crude protein and In-vitro dry matter digestibility were increased gradually with increasing incubation period. This increase may be attributed to the presence of the fungus mycelium during incubation with Pleurotus ostreatus which is rich in crude protein (35%) and Saccarmyeies serevicia (48.1%), beside that, Deraz and Ismail (2001) since they conclude that, the elaborated white-rot fungus succeeded in increasing protein content due to the enzymes which secret by this kind of fungus. Also they reported that, CF content decreased while CP content increased in roughage when treated with different species. Concerning crude fiber of rice straw and cotton stalks, it decreased by 52.9% and 36.3% when cultivated with Pleurotus ostreatus followed by incubation the spent rice straw and spent cotton stalks with Saccaromyces serevisea for 15 hr, respectively, this could be attributed to the utilization of carbohydrate by the fungus as an energy source. Insoluble ash content of all used substrates was higher in treated substrates than untreated materials. These results are in agreement with Mahrous (2005).

Concerning the fiber fractions content which presented in Tables 2 and 4 data showed that, NDF, cellulose, hemi cellulose and lignin were reduced in fungus treated materials. Concerning the NDF of rice straw and cotton stalks it is reduced 21.7% and 13.8% respectively when cultivated with Pleurotus ostreatus and followed by incubation period (15h) with Saccaromycies sereviciae.

Table (1): The effect of biological treatments by *Pleurotus ostreatus* and Saccaromyces cerevicea (SC) on chemical composition of rice straw

Substrate	DM%	OM%				NFE%	Ash%
Raw rice straw (RRS)	92.8	74.3	4.6	34.8	0.5	34.4	18.5
RRS incubated with SC for 15 hr	92.2	73.4	6.2	33.4	0.6	33.2	18.8
Spent rice straw (SRS)	94.5	63.3	7.3	16.4	1.2	38.4	31.2
SRS incubated with SC for 15 hr	90.2	57.8	7.6	16.2	0.8	33.2	32.4

DM = Dry matter, OM = Organic matter, CP = Crude protein, CF = Crude Fiber, EE = Ether extract, NEF = Nitrogen free extractive

Table (2): The effect of biological treatments by *Pleurotus ostreatus* and *Saccaromyces cerevicea* (SC) on fiber fractions of rice straw

Substrate	NDF %	ADF %	ADL %	Hemi. %	Cellulose %	Lignin %	AIA %
Raw rice straw (RRS)	76.4	45.5	8.2	30.9	37.3	4.3	3.9
RRS incubated with SC for 15 hr	73.5	45.1	10.1	28.4	35.0	4.5	5.6
Spent rice straw (SRS)	55.6	39.4	17.6	16.2	21.8	2.5	15.1
SRS incubated with SC for 15 hr	59.8	44.6	19.0	15.2	25.6	2.9	16.1

NDF= neutral detergent fiber, ADF= Acid detergent fiber, ADL=Acid detergent fiber. NDF-ADF=Hem. ADF-ADL=Cell ADL-AIA=Lignin

Table (3): The effect of biological treatments by *Pleurotus ostreatus* and Saccaromyce scerevicea (SC) on chemical composition of cotton stalks

Substrate	DM%	OM%	CP %	CF%	EE%	NFE%	Ash%	
Raw cotton stalks (RCS)	94.0	89.5	3.5	48.2	1.2	36.6	4.5	
RCS incubated with SC for 15 hr	91.3	85.3	5.9	45.9	2.0	31.5	6.0	
Spent cotton stalks (SCS)	95.2	80.5	10.3	30.9	1.2	38.1	14.7	
SCS incubated with SC for 15 hr	91.4	85.6	10.9	30.7	1.3	42.7	5.8	

DM = Dry matter, OM = Organic matter, CP = Crude protein, CF = Crude Fiber, EE = Ether extract, NEF = Nitrogen free extractive

Table (4): The effect of biological treatments by *Pleurotus* ostreatus and Saccaromyces cerevicea (SC) on fiber fractions of cotton stalks

Substrate	NDF %	ADF %	ADL %	Hemi. %	Celiuiose %	Lignin %	AIA %
Raw cotton stalks (RCS)	82.6	64.9	33.6	17.7	31.3	31.1	2.5
RCS incubated with SC for 15hr	68.8	53.0	13.9	15.8	39.1	13.2	0.7
Spent cotton stalks (SCS)	71.9	54.4	15.0	17.5	39.4	9.8	5.2
SCS incubated with SC for 15hr	71.2	54.3	15.4	16.9	38.9	10.2	5.2

NDF-ADF=Hem. ADF-ADL=Cell ADL-AIA=Lignin

Concerning lignin contents of raw cotton stalks and raw rice straw are high when compared with other used substrates as shown in Tables 2 and 4. Improvement of cultivated substrates by lignin degradation was noticed after cultivation and collecting the fruits. The highest degradation were noticed in lignin content (68.5%) on cotton stalks and (41.1%) on rice straw after cultivated with white-rot fungi, such treatment could show the great effect of oyster mushroom on nutrient availability of farm wastes. Also this improvement in fiber fraction (cellulose, hemicellulos and lignin) could be attributed to the enzymes secreted (Laccase, Cellulolase, Manganeze peroxidase and others) during the fungi growth and could be also due to break of the lingo-cellulose bonds and cellulose can be hydrolyzed by fungi as stated by Deraz and Ismail (2001), Abdul-Aziz et al. (1997), Bader (1993) and El-Ashry et al. (1997).

In vitro dry matter disappearance:

Table (5) showed that the highest significant increase of IV-DMD was that for raw rice straw and added yeast (sacaromycese), followed by raw rice straw with inject yeast, being 17.37% and 3.11%, respectively than that in raw. There were increases in IV-OMD content by inject yeast of rice straw, being 6.3% than that in raw. Highest rate of IV-OMD was that of rice straw with added yeast being, 10.53 %. Al-Dabeeb and Ahmed (2002) fed sheep base! ration containd Rhodes grain hay, wheat bran and barley grins at the ratio of 2:1:1, respectively. The IV-DMD and IV-OMD of the base! ration as affected by the yeast culture supplementation level 10g was significantly (p<0.05) improved from 65.76% and 67.56% to 75.78% and 76.58%, respectively. Raw rice straw with inject yeast resulted a highly increased in IV- true digestibility followed by raw rice straw with added yeast ,the values

were 61.84 %, 49.90 %, respectively than that raw without yeast was 48.09%.

The effect of incubation period and harvested fruiting bodies by biological treatments *p. ostreatus*, and combination with *p. ostreatus* and yeast are shown in Table (5). Which showed the best effect of incubation of rice straw by biological treatments with *p. ostreatus*, than that the raw being 24.63%. These results are due to the effect of biological treatments on fiber breakdown and the release of the soluble component. This result is in agreement with that of Zadrazil (1977). Many other outhors reported an increase IV-DMD for biological treated group residues.

The spent rice straw with added yeast was slightly increased than that spent rice straw, however, the values of IV-DMD and IV-OMD was increased from 41.69% to 44.17% and 46.19% to 48.36%, respectively. Compare with incubated. El-Ashry et al (2002) reported that significant (p<0.01) improvement for IV-DMD and IN-OMD. The improvement rate was higher when rice straw and cotton stalks were treated with coculture of fungus and yeast as it were (42.52 vs. 56.42%) and (62.45 vs. 72.43%) for rice straw and (40.43 vs. 69.16%) and (73.46 vs. 80.87%) for cotton stalks, respectively. The best result of True – digestibility was recorded with spent rice straw with incubated yeast (66.28%) than those spent rice straw and spent with added yeast (54.40%). However in case of cotton stalk, the spent cotton stalk gave the highest true digestibility (73.98%).

Table (5): The *in vitro* dry matter (IV-DMD), organic matter (IV-OMD) disappearance and IV-True digestibility for raw rice straw, spent rice straw with added yeast or incubated yeast.

Ingredient	IV-DMD%	IV-OMD%	IV-True Digestibility%
Raw rice straw	33.45°	41.28°	48.09 ^t
Raw rice straw with added yeast	40.48°	46.14 ⁵	49.90°
Raw rice straw with incubated yeast	34.81	44.05°	61.84°
Spent rice straw	41.69°	46.19°	54.40⁵
Spent rice straw with added yeast	52.14ª	60.54ª	56.88 ^c
Spent_rice straw with incubated yeast	44.17°	48.36 ⁵	66.28ª

The *In vitro* dry matter disappearance of the effect of biological treatments *p. ostreatus*, and combined *p. ostreatus* with yeast *S. Cerevisiae* are shown in table (6) The highest significant increase was that for raw cotton stalks with incubated *S. Cerevisiae*, followed by raw cotton stalks with added yeast *S. Cerevisiae*, being 57.2 % and 22.23%, respectively than that the raw cotton stalks. There were significant increases in IV-DMD by *P. ostreatus* than that in raw, being 32.06%. This result is in agreed with that of Zadrazii (1977). Highest rate of IV-DMD for spent cotton stalks with injected *S. Cerevisiae* are 52.66%, followed by spent cotton stalks with added *S. Cerevisiae* are 42.47% than that the spent cotton stalks without yeast. The IV-OMD values were decreaded of raw cotton stalks by added yeast by 10.6% than that the raw, followed by raw cotton stalks with injected yeast by

21.88%. The result was increased in IV-OMD of spent cotton stalks than that the raw cotton stalks, being 32.08% but the spent cotton stalks with added and injected yeast were decreased than that the spent cotton stalks.

The yeast was improved the IV_ true digestibility of raw cotton stalks with incubated yeast 61.19%, followed by raw cotton stalks with added yeast than that the raw without yeast. The spent cotton stalks was increased of IV_ true digestibility than that the raw, being 21.73%, but slightly decreased of spent cotton with added and incubated yeast

Table (6): The *in vitro* dry matter (IV-DMD), organic matter (IV-OMD) disappearance and IV-True digestibility for raw cotton stalks, spent cotton stalks with added yeast or incubated yeast.

Ingredient	IV-DMD	IV-OMD	IV-True Digestibility
Raw cotton stalks	28.02°	36.84 ^b	52.25°
Raw cotton stalks with added yeast	34.25°	32.93°	53.39°
Raw cotton stalks with incubated yeast	44.06 ^b	28.78°	61.19 ^b
spent cotton stalks	41.21°	54.24ª	73.98ª
spent cotton stalks with added yeast	42.47°	46.13°	70.25a
spent cotton stalks with incubated yeast	52.66ª	38.61°	72.49 ^a

The incorporation of S. cerevisiae culture into mixed ruminal microorganism fermentations of ground com, maltose, or lactate had little effect on final pH and fermentation products. However, in the presence of alfalfa hay or Coastal bermudagrass hay S. cerevisiae culture increased concentrations of several fermentation products and numerically increased in vitro dry matter disappearance of forage fiber. IV-DMD of Coastal bermudagrass hay was numerically increased in the presence of S. cerevisiae culture. Concentrations of other fermentation products were not altered by S. cerevisiae culture n vitro dry matter disappearance of both alfalfa hay and Coastal bermudagrass hay did increase over time. Recent research showed that a filter-sterilized filtrate of Diamond V XP S. cerevisiae culture stimulated growth of pure cultures of ruminal bacteria on either lactate or cellobiose and increased the initial rate of cellulose digestion by cellulolytic ruminal bacteria However, in the presence of alfalfa hay or Coastal bermudagrass hay S. cerevisiae culture increased concentrations of several fermentation products and numerically increased IV-DMD of forage fiber.

Dry matter digestion was not stimulated by yeast culture. Williams et al. (1991) reported that yeast culture stimulated DM digestion in the rumen of hay-fed steers when the diet contained barley but not when it was absent. They attributed this difference to a stabilization of ruminal pH by yeast culture in animals receiving barley.

However, both treatments of *S. cerevisiae* live cells increased final pH and decreased acetate and in vitro dry matter disappearance. Neither yeast treatment had much effect on the Coastal bermudagrass hay fermentations. Some of the benefits associated with *S. cerevisiae* include: increased DM and NDF digestion Carro *et al.*, (1992), increased initial rates of fiber digestion Williams *et al.*, (1991) There was a small increase (P>0.05)

in H2 in the presence of 0.35 g/L of *S. cerevisiae* culture. Both concentrations of *S. cerevisiae* culture decreased (P>0.05) the final pH and IV-DMD, and the 0.73 g/L treatment decreased (P>0.05) the amount of acetate. Both treatments of *S. cerevisiae* live cells increased (P>0.05) the final pH and decreased (P>0.05) the acetate and IV-DMD. No treatment effects were observed for the other fermentation end products or IV-DMD, however, previous research has reported that *S. cerevisiae* culture does not significantly affect IV-DMD of either alfalfa hay or Coastal bermudagrass hay Sullivan and Martin, (1999). Furthermore, *S. cerevisiae* culture had little effect on the rate or extent of digestion of both forages by mixed ruminal microorganisms Sullivan and Martin, (1999).

Results of ruminal microorganisms

Data in Table (7) represents the effect of non fermented raw and treated rice straw (after collecting the fungal fruits) on the pH and the microflora of the ruminal fluid-Saccharomyces cerevisiae mixture which was performed in the in-vitro trial. It is clear from the table that, non of the used treatments had an effect on the pH values compared to that of ruminal fluid-Saccharomyces cerevisiae mixture illustrated in Table (11). It is clear also that, there was no difference in the effect of the used treatments on Enterococci, Lactobacilli and anaerobic bacteria counts of the ruminal fluid during this part of the experiment showing the normal microbial behavior but the total counts of the bacteria under investigation began to decline after a longer period (6 hrs for Enterococci count and 9 hrs for Lactic acid bacteria and anaerobic bacteria) compared to the its behavior in case of ruminal fluid-Saccharomyces cerevisiae mixture (6 hrs for all species, Table (11).

Table (7): Non fermented wastes (without Saccharomyces incubation for 15 hrs prior to the experiment)

item	Intervals/ hours	рH	Enterococc i count	Lactic acid bacteria count	Total anaerobic bacteria count
	0	6.98	40x10°	30x10 ⁵	51x10 ⁵
	3	6.94	11x10 ⁵	20x10 ⁵	50x10⁵
Raw Rice	6	7.12	90x10⁴	16x10 ⁵	17x10⁵
Straw	9	7.02	50x10⁴	20x10⁴	20x10⁴
Ī	24	6.96	33x10⁴	17x10⁴	12x10⁴
	48	7.06	20x10 ³	30x10 ³	40x10⁴
	0	6.91	10x10 ⁵	40x10 ⁵	13x10 ⁵
	3	6.85	70x10⁴	52x10 ⁵	56x10 ⁵
Treated	6	7.01	28x10 ⁴	15x10 ⁵	42x10 ⁵
Rice Straw	9	7.22	40x10 ³	16x10⁴	40x10⁴
•	24	7.48	90x10 ³	32x10⁴	70x10⁴
	48	7.51	60x10 ³	85x10 ³	12x10⁴

Data in Table (8) illustrates the effect of non fermented raw and treated cotton stalks (after collecting the fungal fruits) on the pH and the microflora of the ruminal fluid-Saccharomyces cerevisiae mixture which was performed in the *in-vitro* trial. It is clear from the table that, like in Table (7),

no significant change was recorded in the pH values all over this part of the experiment which clarifies that non of the used treatments had an effect on it. The microbial behavior in case of using raw cotton stalks had no marked difference compared to using raw rice straw. Using treated cotton stalks showed a marked increase of the bacterial species under investigation which begins to decline in count after longer period (24 hrs for Enterococci and 48 hrs for Lactic acid bacteria and anaerobic bacteria) compared to that in case of using rice straws (Table7) and also to the normal bacterial behavior illustrated in ruminal fluid-Saccharomyces cerevisiae mixture Blank(Table 11).

Table (8): Non fermented wastes (without Saccharomyces incubation for 15 hrs prior to the experiment)

Item	Intervals/ hours	рН	Enterococci count	Lactic acid bacteria count	Total anaerobic bacteria count
	0	6.89	10x10⁵	30x10°	87x10 ⁵
l [3	6.94	11x10 ⁵	18x10°	26x10 ⁵
Raw Cotton	6	7.09	13x10 ⁵	17x10 ⁵	47x10°
Stalks	9	7.27	30x10⁴	30x10⁴	60x10⁴
] [24	7.27	20x10 ³	70x10 ³	40x10⁴
	48	7.26	30x10 ³	16x10 ³	60x10⁴
	0_	6.70	14x10 ⁵	70x10 ⁵	30x10 ⁵
Treated	3	6.99	12x10 ⁵	50x10 ⁵	54x10 ⁵
Cotton	6	7.09	22x10 ⁵	40x10 ⁵	31x10 ⁵
Stalks	9	7.06	60x10 ⁵	30x10°	16x10 ⁵
Starks	24	7.23	50x10⁴	21x10°	18x10 ⁵
	48	7.18	17x10⁴	52x10⁴	45x10⁴

Data in Table (9) illustrates the effect of fermented raw and treated rice straws (by incubation after Saccharomyces cerevisiae inoculation for 15 hrs) on the pH and the microflora of the ruminal fluid which was performed in the in-vitro trial. It is clear from that table that, no change was recorded in the measured pH values all over the period of the experiment compared to that of the ruminal fluid Blank (Table 11). The behavior of the Enterococci species in case of using fermented raw rice straws was similar to its behavior in the Blank sample (Table 11). The count of lactic acid bacteria showed -in case of fermented raw rice straw- a declined by one log after 3 hrs and stayed stable till the end of the experiment (48 hrs) while in case of Blank (Table 11), a gradual decline was noticed. Concerning the count of total anaerobic bacteria the count was stable till 9 hrs from the start then decreased one log to reach 104, and then stayed stable till the end of the experiment but in case of the Blank sample, a gradual decrease was noticed. Adding the fermented treated rice straw to the ruminal fluid showed the greatest effect on the count of the bacteria under investigation as all bacterial species remained at the same count as starting count till the end of the experiment which reflects the positive effect of this treatment on these types of microflora.

Data in Table (10) showed the effect of adding fermented raw and treated cotton stalks on the ruminal pH and the microflora present in it. It is clear that, no change in the pH values was recorded. Adding fermented raw

cotton stalks had a positive effect on the count of bacteria under investigation compared to that counted in the blank sample but adding the fermented treated cotton stalks showed higher bacterial counts compared to both raw cotton stalks and blank sample, Table (11) as the bacterial count still longer in the ruminal fluid.

Table (9): Fermented wastes (with Saccharomyces incubation for 15 hrs

prior to the experiment)

	buot to me	CAPCILLIC			
Item	intervals/ hours	рΗ	Enterococc i count	Lactic acid bacteria count	Total anaerobic bacteria count
	0	7.10	30x10°	90x10 ⁵	16x10°
	3	7.03	70x10⁴	59x10⁴	37x10 ⁵
Raw Rice	6	6.96	60x10⁴	20x10⁴	30x10 ⁵
Straw	9	7.65	60x10 ³	55x10⁴	13x10⁴
]	24	7.36	30x10 ³	13x10⁴	12x10⁴
	48	6.96	20x10 ³	15x10⁴	33x10⁴
	0	7.14	80x10 ⁵	40x10°	60x10 ⁵
1	3	7.03	40x10°	61x10°	34x10 ⁵
Treated	6	6.99	16x10°	13x10 ⁵	48x10 ⁵
Rice Straw	9	7.65	42x10°	21x10 ⁵	12x10°
}	24	6.84	12x10°	10x10 ⁵	24x10°
	48	6.66	20x10°	15x10 ⁵	42x10 ⁵

Table (10):Fermented wastes (with Saccharomyces incubation for 15

hrs prior to the experiment)

ltem	Intervals/ hours	рН	Enterococc i count	Lactic acid bacteria count	Total anaerobic bacteria count
	0	7.10	40x10 ⁵	31x10°	69x10 ⁵
i f	3	7.00	16x10 ⁵	32x10 ⁵	44x10 ⁵
Raw Cotton	6	6.91	11x10 ⁵	40x10 ⁵	18x10 ⁵
Stalks	9	7.57	31x10⁴	26x10⁴	36x10⁴
1	24	6.82	17x10 ⁴	43x10⁴	20x10⁴
} [48	6.97	10x10 ⁴	60x10⁴	70x10⁴
	0	7.04	40×10 ⁵	21x10 ⁵	21x10 ⁵
l	3	7.04	12x10 ⁵	21x10 ⁵	77×10 ⁵
Treated	6	7.02	16x10 ⁵	16x10 ⁵	64x10 ⁵
Cotton Stalks	9	7.66	73x10 ⁴	85x10 ⁴	10x10 ⁵
Staiks	24	7.72	10x10⁴	55x10⁴	70x10⁴
	48	6.66	83x10 ⁴	10x10 ⁴	22x10 ⁴

Discussion of ruminal microorganisms:

No effect of yeast treatment was observed on the pH of the rumenal fluid allover the time of the experiment. This result agreed with that stated by NewBold et al., 1995, Sullivan and Martin 1999 and Lynch and Martin 2002 who concluded that, usage of Saccharomyces cerevisiae in in-vitro trials did not modify the mean pH. Martin and Nisbet 1992 reported that, the utilization of lactic acid by rumenal bacteria is enhanced by yeast culture which consequently maintains a constant pH. This result also was supported by that obtained by Putnam et al., 1997, Williams et al., 1991, Lila et al., 2004 and Newbold et al., 1995.

Table (11) Behavior of microbial flora in Blank samples

Item	Intervals/ hours	рН	Enterococci count	Lactic acid bacteria count	Total anaerobic bacteria count
	0	7.14	46x10⁵	30x10 ⁵	34x10 ⁵
	3	6.96	41x10 ⁴	30x10⁴	40x10⁴
Blank (raw	6	7.04	40x10⁴	15x10⁴	57x10⁴
rumenai fluid)	9	7.20	70x10 ³	86x10 ³	62x10 ³
	24	7.26	43x10 ³	57x10 ³	32x10 ³
	48	7.26	30x10 ³	30x10 ³	20x10 ³
	0	6.97	24x10 ⁵	42x10 ⁵	43x10 ⁵
	3	6.95	75x10 ⁵	34x10 ⁵	31x10 ⁵
Rumenal fluid-	- 6	7.01	34x10⁴	37x10⁴	21x10⁴
Saccharomyce s mixture	9	7.21	45x10⁴	25x10⁴	44x10⁴
	24	7.18	36x10 ³	76x10 ³	20x10 ³
	48	7.11	56x10 ³	55x10 ³	52x10 ³

Total anaerobic bacterial count was linearly increased throughout the whole time of the experiment. This result is supported by the findings of NewBold et al., 1995 who reported that, Saccharomyces cerevisiae increased the number of total viable bacteria in in-vitro studies. Increased bacterial numbers in the rumen have been one of the most constantly reported effect in animal fed another yeast culture, Wiedmeier et al., 1987, Lila et al., 2004, Harrison et al., 1988. It has been suggested that, increased bacterial flora in animals fed Saccharomyces cerevisiae is central to the action of yeast in the rumen. (Lila et al., 2004, Newbold et al., 1995). Recent research (Sullivan and Martin 1999) demonstrated that, Saccharomyces cerevisiae culture stimulated the growth of rumenal bacteria which consequently had a positive effect on total fiber digestion particularly cellulose and also on the microbial protein supply (Newbold et al., 1995). A wide range of mechanisms by which Saccharomyces cerevisiae might stimulate bacterial growth in the rumen have been proposed by Rose 1987, Wallace and Newbold 1992.

Conclusion

From the above results, it could be concluded the following:

- The treatment with fungus (p. ostreatus) and the addition of yeast (S. cerviciae) could be used successfully to enrich chemical composition, well DMD and OMD of rise straw and cotton stalks.
- The better effect of the used biological treatment was with rice straw than with cotton stalks in general.
- The effect of fungus treatment was the best, in particular to cotton stalks.
- From the obtained data, it is clear that, administration of Saccharomyces cerevisiae to small ruminants stimulates the growth of the beneficial rumenal bacteria which consequently positively affects the rumenal fiber degradation.

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تأثير معاملتين بيولوجيتين على القيمة الغذائية لبعض المخلفات الزراعية (قـش الأرز وحطب القطن)

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أجريت هذه الدراسه بالمعمل المركزي للأغنية و الأعلاف لدراسة تأثير قش الأرز المعامل وحطب القطن المعامل بفطر عيش الغراب المحارى (بلوروتس أوستريتس) فقط وإضافة خميرة الخباز (سكاروميسيس سرفاسي)، تحضين الخميرة لـــ ١٥ ساعة واستخدام الفطر والخميرة معا. وذلك على التركيب الكيماوي وتحليل الألياف وهضمية المادة الجافة (IVDHD) وهضمية المادة العضوية (IVOMD) والــ pH وميكروبات سائل الكرش (انتروكوكاي – لاكتيك أسيد بكتريا – مجموع البكتريا غير الهوانية) أظهرت نتائج التركيب الكيملوى في حالة قش الأرز المحضن مع الخميرة لمدة ١٥ ساعة زيادة في محتوى البروكين (مَن ٦٫١% إلى ٦,٢%) وفي حالة حطب القطّن المحضن مع الخميرة لمدة ١٥ ساعة (من ٣٠٥% إلى ٥٠٩%) وقد ظهرت زيادة إضافية عند معاملة القش المعامل بالفطر (من ٤٠٩% إلى ٧.٦%) وحطب القطن المعامل بالفطر (من ٣.٥% إلى ١٠.٩%). أظهرت نتائج محتوى الألياف تناقصا نتيجة المعاملة على وجه العموم، ففي حالة قش الأرز المحضن بالخميرة لمدة ١٥ سَاعة حدث تناقض (من ٤٨,٢% إلى ١٦.٤%) وحطب القطن المعامل بالفطر والمحضن مع الخميرة (من ٤٨,٢% إلى ٣٠٠,٩%). في تحليل مكونات الألياف وجد تناقص كبير في محتوى اللجنين في كلا المانتين المختبرتين قد يرجع ذلك للمعاملة بالفطر أو الخميرة أو كلاهما. أظهرت نتائج هضم المادة الجافة (IV-DMD) أن أعلى قيم مضمية كانت مع الأرز المعمل بالفطر والخميرة (٢٠٤٤%) وبنفس المنوال مع هضمية المادة العضوية (IV-OMD) ٢٠٠٥٤%. مع ذلك وجد أن أعلى قيمة هضمية حقيقية (IV-True digest.) كانت مع الأرز المعامل بالمعاملتين (فطر + خميرة) ٢٦.٢٨. إضافة الخميرة لم تكن لها تأثير على الــ PH علَّى طول التجربة. استخدام االخميرة مع المجترات الصغيرة نتبه ميكروبات سائل الكرش والتي بالتالي سوف تلعب دور في هضم الألياف، ٣٠ ٢٠٠٪ مقابل ٥٦ ١٠٠٪ ٣٠ ، ١٠٠٪ مقابل ٥٥ ٢٠٠٪ ٢٠ ، ٢٠ X،۱ "مقابل ۸ × ۲ العدد بكتريا انتروكوكاي و بكتريا لاكتيك أسيد و العدد الكلي للبكتريا اللاهوانيه على التوالمي. أظهرت النتائج أن زراعة المشروم والتنضين لو الإضافة بالخميرة قد تُحسن من القيمة الغذائية لقَشَ الأرز وحطب القطن. وعليه فإن المعاملة البيولوجية فصلا عن إسهامها في رفع القيمة الغذائية لتلك المخلفات وتطويعها لتغذية الحيوان فإنها تسهم في تقليل التلوث البيئي الناشئ من سوء إستخدامها في الحريق.

