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DIGESTION AND RUMEN FERMENTATION BY SHEEP FED ON RATIONS CONTAINING RICE STRAW TREATED OR NOT WITH UREA, FUNGUS AND EFFECTIVE MICRO-ORGANISM

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ABSTRACT: *Rice straw has low quality due to its low content of essential nutrients, palatability and digestibility. Therefore, the quality of rice straw needs to be improved in order to increase its digestion by ruminants. The purpose of this study was to compare untreated rice straw and those treated with urea, fungus, and effective micro-organisms in diets of sheep. Digestibility and nitrogen balance trials were carried out using twelve mature Barki rams (average body weight 48/kg) divided into 4 groups and fed on 4 diets: diet 1 untreated rice straw with concentration feed mixture (CFM), diet 2: urea ensiled rice straw with CFM, diet 3: Trichoderma ressie-treated rice straw with CFM, and diet 4: effective micro-organisms ensiled rice straw with CFM. Three female sheep fitted with permant rumen fistula were used for rumen fermentation parameters, and the nitrogen leaving the rumen was estimated from urinary purine derivatives. Results revealed that digestibility coefficient of nutrients in diet 3 differ significantly from those of other diets. The same trend was observed with the results of nitrogen balance. Values of pH and ammonia-N concentrations in the rumen liquor of sheep showed an opposite trend to those of VFAs concentration. Excretion of purine derivatives in the urine measured as an index for microbial nitrogen leaving the rumen as well as the microbial protein synthesis were enhanced by treating rice straw containing diets.*

Keywords: *Rice straw, urea, fungus, effective micro-organisms, sheep, nitrogen balance, digestion, rumen fermentation, Purine derivatives, microbial protein.*

INTRODUCTION

Rice straw is characterized by low protein, minerals, energy contents and have a poor nutritive value for ruminants (Doyle *et al.*, 1986). This low nutritive value can be improved by treating with urea, fungus and effective micro-organisms (Ørskov *et al.*, 1983; Gupta and Langer, 1988; Perdock *et al.*, 1982; Rai *et al.*, 1993; Mohamed, 1998; Ibrahim, 2001 and Khalil, 2006). Urea treatment is one of various chemical treatments which was found beneficial as it increased the nutritive value of rice straw by increasing the protein content and nutrient digestibility and palatability of rice straw (Wongsrikeao

and Wanapat, 1985). The use of micro-organisms to improve by-product quality has been considered as an alternative to chemical or physical treatments (Zadrazil and Brunnert, 1981). However, many of these so-called biological treatments require some kind of chemical /physical pre-treatments and each of these processes has different objectives, which are often not well-defined and research into issue often takes a clear objective.

The use of lignolytic micro-organisms (lignolytic fungi) is an alternative method to convert lingo-cellulosic materials to more digestible feed for animals (Karunanandaa *et al.*, 1995). Therefore, in the current experiment untreated rice straw, and these treated with urea, fungus, and effective micro-organisms were used in diets for sheep. The objectives of this study were to compare these four diets in terms of digestibility of nutrients, nitrogen balance, ruminal fermentation, urinary purine derivatives and microbial nitrogen synthesis in sheep.

MATERIALS AND METHODS

Feeds preparation and treatment:

Rice straw was chopped into 5 cm lengths prior to treatments that were:
Diet 1: Untreated rice straw + concentrate mixture (CFM).
Diet 2: Urea ensiled rice straw + (CFM).
Diet 3: *Trichoderma ressei*-treated rice straw + (CFM).
Diet 4: Effective micro-organisms ensiled rice straw + (CFM).

Urea ensiled rice straw was prepared by spraying chopped straw with urea solution to yield treated straw that contains 60% dry matter and 4% urea (William *et al.*, 1984). The treated straw was then covered with polyethylene sheets for 21 days, then sheets were opened and the treated rice straw was aerated for 24 hours prior to feeding (to reduce the smell of ammonia).

Trichoderma ressei (Long Bacterium, *Trichoderma* causing high protein yield and high degradation of cellulose and hemicelluloses, Iconomou *et al.*, 1997). The inoculums of fermented rice straw was used at 10% (w/w), mixed well and spread in the trays. The treated rice straw was shuffled upside down daily for the proper incubation period (7 days). At the end of the fermentation period, the treated rice straw was collected and sun-dried until the moisture content was reduced to less than 10% (Khalil, 2006).

Effective micro-organisms (EM)- ensiled rice straw was prepared by spraying chopped rice straw with EM solution to yield treated rice straw containing 2% EM, 5% molasses and 60% moisture. The treated straw was then covered with plastic sheet for 30 days, then the plastic sheet was removed and the treated straw was aerated for 24 hours prior to feeding. Treated rice straw or not treated was mixed with a concentrate feed mixture containing (40% yellow corn, 34% wheat bran 15% soybean, 7% molasses 2% lime stone, 1. 5% common salt and 0. 5% premix) to provide a maintenance diet for mature Barki sheep of 48 kg body weight (NRC,1994).

Digestibility and nitrogen balance trials:

Digestibilities of nutrients of the experimental diets were determined using twelve adult Barki rams (averaging 48 kg, 3 animals for each diet). During an adaptation period of 21 days, the sheep were placed in individual pens and were gradually introduced to their experimental diets. Animals were offered straw *ad-lib* twice daily (8. 00 and 16. 00 h). The concentrate feed mixture was used to feed sheep at the rate of 900 g/head/day. At the conclusion of the adaptation period, rams were transferred to metabolic cages, designed for separate collection of urine and faeces. Total fecal and urine output were collected for a seven-day period, and were subjected to chemical analysis (A. O. A. C., 1995). NDF and ADF were determined as described by Goering and Van Soest (1970), water was available at all times.

Rumen Fermentation:

Three female sheep fitted with permant rumen fistulae were used in rumen fermentation trials. Rumen samples were withdrawn before feeding and 3 and 6 h after feeding. Ammonia –N determination was carried out using MgO distillation method (Al-Rabbat *et al.*, 1971). Total VFAs were determined by stream distillation as described by Warner, (1964). The pH values of the rumen fluid were determined immediately by using a glass electrode, inserted in the rumen through the fistulae.

Measurement of Purine derivatives excretion:

Twenty four hours-collection of urine was made in the last 5 days of each period. Urine was collected in containers of 75ml H_2SO_4; (10%, v/v); (pH of the final urine <3). The collected urine was then diluted with water to a fixed volume of 5L.

One sub-sample was stored at -20°C for determination of purine derivatives (PD). The PD was determined according to the procedure of Chen and Gomes, (1990). Microbial protein synthesis in the rumen of sheep fed on the experimental diets was calculated using the purine derivatives method (Chen *et al.*, 1991).

Statistical analysis:

Data of the digestibility and nitrogen balance trials were statistically analyzed using a randomized complete design while that of rumen fermentation were analyzed using incomplete block design according to Steel and Terrie, (2000) and SAS program (SAS, 2000). Significant differences between treatments were determined using the least significant differences (LCD) tests at 0.05 level of probability.

RESULTS

Chemical composition and nutrient digestibilities

The chemical composition of concentrate mixture (CFM), untreated rice straw (RS) and rice straw treated with urea (U) with fungus *Trichoderma ressei*, (F), and with effective microorganisms (EM) are presented in Table 1. Treatments increased the protein content of rice straw by 72%, 295% and 13% for (U), (F) and (EM) over the untreated rice straw, respectively. The crude fiber became (17%) lesser for (F) treatment as compared to other treatments.

Table 1: Chemical composition (% on dry matter basis) of the concentrate feed mixture (CFM), rice straw (RS) and rice straw treated with urea (U), with fungus (F) and with effective microorganisms (EM).

Item	CM	RS	U	F	EM
Organic Matter	92. 58	84. 09	82. 04	78. 33	83. 79
Crude Protein	13. 76	3. 91	6. 73	11. 52	4. 42
Crude Fiber	7. 35	37. 62	33. 73	31. 42	33. 70
Ether Extract	2. 79	0. 95	0. 92	0. 98	1. 02
Nitrogen-free Extract	68. 68	41. 61	40. 66	34. 41	44. 65
Ash	7. 42	15. 91	17. 96	21. 67	16. 21
Neutral detergent fiber	22. 53	71. 39	69. 21	66. 82	68. 70
Acid detergent fiber	8. 72	37. 60	35. 16	33. 92	34. 22

(RS), rice straw; (U) rice straw treated with 4% urea; (F) rice straw treated with *Trichoderma ressei*; (EM) rice straw treated with 2% effective micro-organisms.

The daily dry matter intake and nutrient digestibilities of the experimental diets are shown in Table 2. Intake of dry matter from treated rice straw varied from the control group which recorded the lowest of 1249. 3 g/h/day for group 1. Dry matter intake increased by inclusion of treated rice straw with urea, fungal and effective micro-organism in diets by 4, 9 and 4%, respectively, which was pronounced for the fungal treated diet. Digestibility data are presented in Table 2. DM, CP, CF, NFE and EE digestibility values were higher ($P<0.05$) in sheep receiving diets incorporated with *T. ressei* - treated rice straw. In general, the digestibility values for the nutrients increased with treated rice straw as compared to the untreated one.

Feeding values of the four experimental diets expressed as TDN and DCP are shown in Table 3. The data indicated that the diet containing rice straw treated with fungus had the highest ($P< 0. 05$) feeding value as compared to the control one. Utilization of nitrogen in the four experimental diets is shown in Table 3. The intake of nitrogen in sheep fed on experimental diets 2,3 and 4 were significantly higher compared to that of diet 1, where diet 3 had the highest values.

Table 2: Dry matter intake (g/d) and nutrient digestibilities of the experimental diets fed to sheep.

Item	Experimental diets*			
	1	2	3	4
Dry matter Intake (g/day)				
Concentrate feed Mixture(CFM)	756. 8	743. 4	730. 1	747. 9
Rice straw	492.5	563.3	628.7	551.2
Total intake	1249.3	1306.7	1358.8	1299.1
Digestibility Coefficients (%)				
Dry matter	58.46±0.43^{c}	63.91±0.18^{b}	67.89±0.05^{a}	64.90±0.43^{b}
Organic matter	64.57±0.55^{c}	68.70±0.20^{b}	72.34±0.05^{a}	68.98±0.40^{b}
Crude protein	57.93±1.08^{c}	65.27±0.42^{b}	69.58±0.12^{a}	65.21±0.47^{b}
Crude fat	82.55±0.34^{c}	85.42±0.12^{b}	87.38±0.21^{a}	58.91±0.45^{b}
Nitrogen free extract	70.48±0.89^{b}	72.18±0.48^{b}	74.58±0.11^{a}	72.09±0.41^{b}

^{abc} Means within rows with different superscript letters differ significantly (P<0. 05).
* Each diet contained (CFM+untreated or treated rice straw)

Table 3: Nutritive values and nitrogen balance of the experimental diets.

Item	Experimental diets*			
	1	2	3	4
Nutritive value (%)				
TDN	59.74±0.49^{c}	62.67±0.21^{b}	64.34±0.01^{a}	63.44±0.35^{ab}
DCP	5.71±0.15^{d}	7.04±0.01^{b}	8.85±0.02^{a}	6.37±0.04^{c}
Nitrogen utilization (g/d)				
NI	19.69±0.03^{d}	22.44±0.07^{b}	27.58±0.04^{a}	20.22±0.14^{c}
NA	11.40±0.23^{d}	14.65±0.05^{b}	19.19±0.02^{a}	13.18±0.16^{c}
NB	2.89±0.22^{d}	5.28±0.06^{c}	8.62±0.13^{a}	6.20±0.21^{b}

^{abcd} Means within rows with different superscript letters differ significantly (P<0.05)
NI= Nitrogen intake (g/h/day)
NA= Nitrogen absorbed (g/h/day)
NB= Nitrogen balance (g/h/day)
* Each diet contained (CFM+ untreated or treated rice straw).

The effect of feeding sheep on the four experimental diets on ruminal pH, ammonia –N and VFAs concentrations are presented in Figs (1,2 and 3), respectively. Ruminal pH values decreased up to 3h after feeding, then it slightly increased up to 6h- after feeding for all diets. Data of ammonia–N and total VFA concentrations increased after feeding reaching their peak at 3h, then decreased at 6h post-feeding.

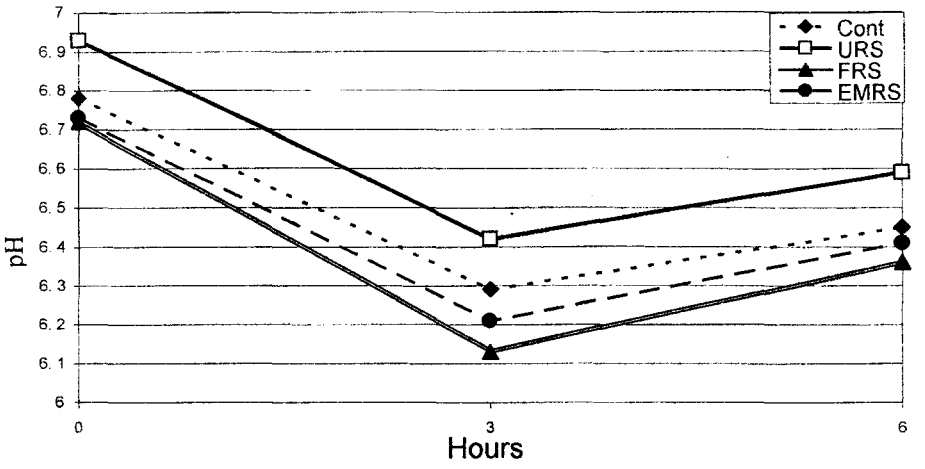

Fig. 1. Variations of pH in the rumen of sheep fed on the experimental diets.

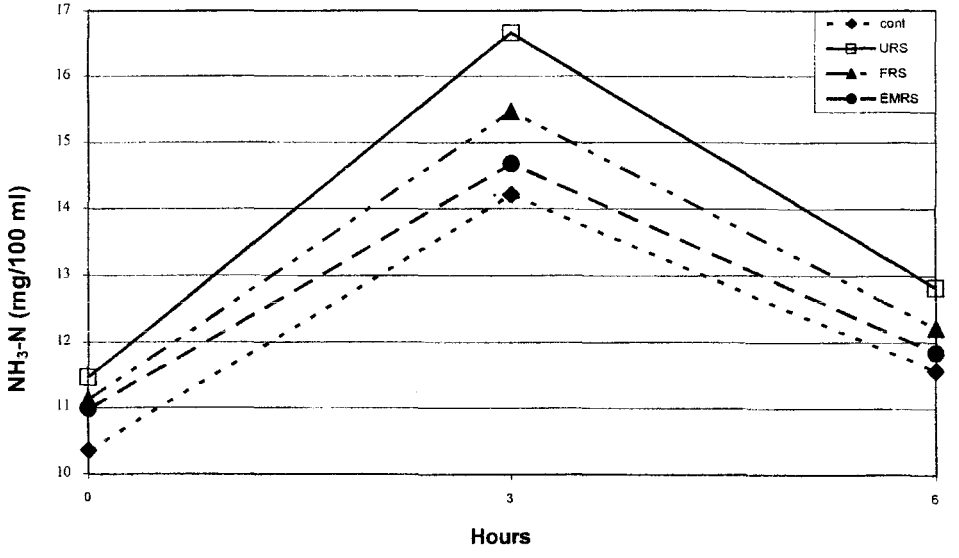

Fig. 2. Variations of NH_3-N in the rumen of sheep fed on the experimental diets

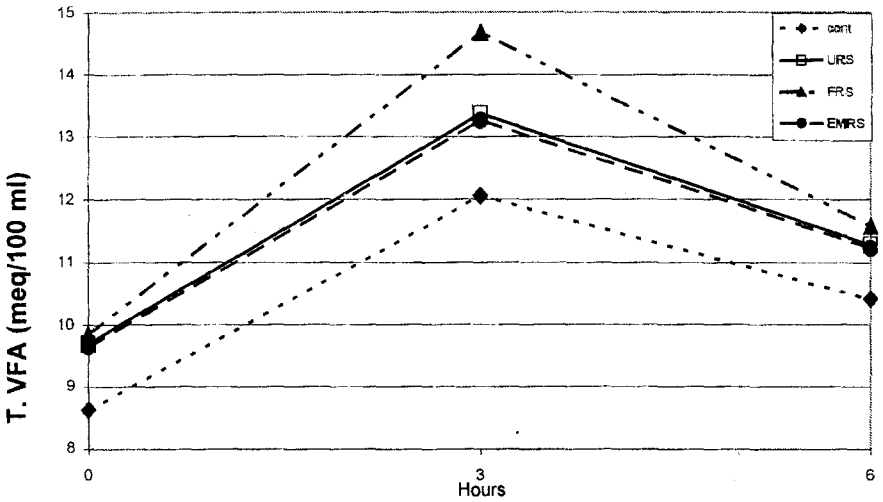

Fig. 3. Variations of total VFA (meq/100 ml) in the rumen of sheep fed on the experimental diets

Total purine derivatives excretion (PDE mmol/day) and microbial protein synthesis (MP, g/day) are presented in Table 4. Microbial protein synthesis was more efficient for all treated compared to the control one, while diets containing fungus (*T. ressei*)-treated rice straw being the most efficient.

Table 4: Urinary purine derivatives and microbial nitrogen (g / day) leaving the rumen by sheep fed on the experimental diets.

item	Experimental diets[1]			
	1	2	3	4
Purine derivatives exertion (mmol/d)	17.9±0.22^{c}	18.33±0.05^{b}	19.73±0.10^{a}	18.57±0.12^{b}
Allantion exertion (mmol/d)	14.70±0.18^{c}	15.58±0.05^{b}	16.77±0.08^{a}	15.84±0.24^{b}
Uric acid exertion (mmol/day)	2.59±0.03^{c}	2.75±0.01^{b}	2.95±0.02^{a}	2.73±0.01^{b}
Microbial nitrogen[2] (g/d)	13.23±0.19^{c}	14.13±0.05^{b}	15.35±0.08^{a}	14.30±0.27^{b}
Microbial protein[3] (g/d)	82.69±1.19^{c}	88.31±0.31^{b}	95.94±0.50^{a}	89.38±1.69^{b}

abc means within rows with different superscript letters differ significantly (P<0.05)
[1]Each diet contained (CFM+ untreated or treated rice straw)
[2] Microbial nitrogen estimated according to Chen *et al.*, (1991),
[3] Microbial protein= (microbial- NX 6. 25).

DISCUSSION

The modification of the chemical composition of rice straw as a result of it exposure to urea, fungal and effective micro-organisms treatments involves primarily an increase in nitrogen content, a slight decrease in neutral detergent fiber and acid detergent fiber and softening in texture. This is in agreement with the finding of (Han, 1978; Moo-Young *et al.*,1981; Tuen *et al.*, 1991; Walli *et al.*, 1993 and Khalil, 2006). Lower fiber content may have been due to the microbial attack during the incubation period. Similar results were obtained (Langer *et al.*, 1980; Shoukry *et al.*, 1985; Langer and Bakhi, 1986; Lawrence and Abada, 1987; Ali, 1996; Salem, 2003 and Kahlil, 2006).

The increased in dry matter intake that occurred when urea, fungal and effective micro-organisms treated rice straw was incorporated into experimental diets suggests strongly that rice straw treatments especially fungal treatment (*T. ressei*) could be considered as an effective extender. Perdok *et al.* (1984) and Tuen *et al.* (1991) reported improvements in the intake of urea-treated rice straw for cattle, buffaloes and goats. The present results showed that urea and fungal treated rice straw was effective in increasing feed intake by sheep and in improving their ability to digest the diet. The positive effect of treated rice straw on dry matter intake and the observed improvement could probably be attributed to the softening of forage fiber and the reduction of the mastication load as well as fermentation enhancement in the rumen.

In this study, nutrient digestibilities were improved ($P<0.05$) by urea and fungal treatment of rice straw incorporated into experimental diet 2,3 and 4, respectively. Similarly it has been found that urea treatment of rice straw (Saadullah *et al.*, 1981 and Jayasuriya and Perera, 1982), barley straw (Kowalecrzyk, 1994) and sugar beet pulp treated with fungus (Kahlil, 2006) significantly improved organic matter digestibility.

The urea and fungal treatment not only improved the dry matter intake, digestibility of nutrients and daily nitrogen intake but also nitrogen balance. Better utilization of nitrogen in group 3 receiving fungal-(*T. ressei*) treated – rice straw containing diet may reflect greater absorption of amino acids. Kahlil (2006) attributed that fungus-treated sugar beet pulp resulted in tremendous increase in some amino acid concentrations and these results could be related to the increase in true protein contents of the treated sugar beet pulp. On the other hand, Singh *et al.* (1992) reported that the amino -N content in the treated rice straw was increased more than two times after fungal treatment and the increase with the urea treatment was comparatively small.

Rumen fermentation in sheep fed on diets containing rice straw treated with fungus (diet 3) showed lower ammonia and higher VFA concentration than those of other three diets.

Microbial protein synthesis was more efficient for the diet containing fungus-treated rice straw than the other diets. This could be due to better capture of N in the rumen and increased efficiency of microbial protein synthesis (O'Mara *et al.*, 1997).

In conclusion, the positive effects of fungal treatment of rice straw were translated into significantly higher intake of dry matter, superior nutrient digestibility, significant nitrogen retention, ruminal fermentation and microbial protein synthesis by sheep over those offered untreated, urea and effective micro-organisms treated rice straw based diets. Rice straw treated with *T. ressei* could be a superior choice for use in ruminant's diets.

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معاملات الهضم ونشاط الكرش في الأغنام المغذاة على علائق تحتوى على قش الأرز الغير معامل أو المعامل باليوريا أو فطر *التريكودرما رسى* أو الأحياء الدقيقة والإنزيمات النشطة.

على محمد علام

قسم الإنتاج الحيواني- كلية الزراعة - جامعة الإسكندرية - الشاطبى

الملخص العربي

يتميز قش الأرز بإنخفاض قيمته الغذائية نتيجة لانخفاض محتواه من بعض المركبات الغذائية وانخفاض معاملات هضمها بالإضافة إلى إنخفاض استساغته. وتهدف الدراسة الحالية إلى التعرف على إمكانية رفع القيمة الغذائية لقش الأرز المقطع عن طريق المعاملة باليوريا مع السيلجة أوبإستخدام فطر *التريكودرما رسى* أو باستخدام مخلوط الأحياء الدقيقة النشطة مع السيلجة.

تم تكوين أربعة علائق كالتالي:-

١- علف مركز+ قش أرزمقطع غير معامل.

٢- علف مركز+ قش أرز مقطع معامل باليوريا ومسيلج.

٣- علف مركز +قش أرز معامل بفطر *التريكودرما رسى*.

٤- علف مركز + قش أرز معامل بمخلوط الآحياء الدقيقة النشطة مع السيلجة.

أستخدم فى تجارب الهضم والإتزان الأزوتى عدد ١٢ ذكر من الأغنام البرقى قسمت الى أربعة مجموعات متساوية وغذيت كل مجموعة على عليقة واحدة . أما تجارب نشاط الكرش فقد إستخدم فيها ثلاثة إناث أغنام مزودة بفستيولات دائمة.

وقد أظهرت النتائج ما يلى:

١- زيادة معدل إستهلاك الغذاء ومعاملات الهضم لمكونات الغذاء والإتزان الأزوتى وذلك للعلائق المحتوية على قش أرز معامل وكانت أعلى هذه الزيادات لتلك المعاملة بفطر التريكودرما رسى وكانت الزيادات معنوية.

٢_ أظهرت قيم الـ pH وتركيز الامونيا والأحماض الدهنية الطيارة فى الكرش إستجابة موجبة للمعاملات.

٣- زيادة معدل تخليق البروتين الميكروبى فى الكرش للحيوانات المغذاة على قش الأرز المعامل وكانت أعلى الإستجابات للمعاملة بأستخدام فطر *التريكودرما رسى*.

من الدراسة يمكن إستخلاص أن المعاملة لقش الأرز المقطع بأستخدام فطر التريكودرما رسى أدى إلى تحسين القيمة الغذائية للقش بشكل واضح يمكن الإعتماد عليه وبخاصة المزارع الصغير وفى حالة عدم توافر الفطر فإن المعاملة باليوريا تكون البديل.