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# Evaluation of Fungal or Chemical Treatments for Barley Straw in Ruminants Feeding 1-Chemical composition, *in vitro, in vivo* digestibility and voluntary intake

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## ABSTRACT

Sixty male lambs weighing 30.2± 1.43kg and 5.5±0.5 months old were used to evaluate the effect of three form of straw[ untreated barley straw (US), a 7% urea treated barley straw (UTS), and fungi (*Pleurotus ostreatus*) treated barley straw (FTS)],on chemical composition, cell wall constituents, *in vitro* and *in vivo* dry and organic matter digestibility and voluntary intake. Urea and fungal treatments significantly (P<0.05) decreased the cell wall components and increase the CP content and *in vitro* DM and OM digestibility of barley straw as compared with the US. Average daily intake and *in vivo* digestibility of DM and OM were significantly (P<0.05) higher in UTS and FTS comparing to the US. Likewise, the daily intake and digestibility of DM and OM of FTS was significantly (P<0.05) higher than those in UTS. In conclusion, treated straw by urea or fungi, improved CP content , nutrients digestibilities ,daily feed intake and nutritive value index.

**Keywords**: barley straw, urea treatment, fungi, treatment, sheep.

## INTRODUCTION

Interest in the effective utilization of agricultural and manufacture by-products is increasing all over the world due to economical factors and pollution. In Iraq agricultural and manufacture by-products are considered as stable source of ruminant feeds (Al-Ani *et al.*, 1991; Hassan *et al.*, 1998; Hassan, 2005) therefore, non traditional feed resources such as crop residues and Agro-industrial by-products must be studied in order to decrease the relay on traditional resources, to fill the gap and to decrease feeding costs. Utilization of by-product can not only be used in favor of solving feed shortage problem but also as a method to control environmental pollution (Zaza, 2004). The major limitations of using these residues as ruminant feeds are their poor in nutrients such as protein content and vitamins and they are rich in fibers with low digestibility, or law palatability and high lignin contents. The degree of lignifications is relatively more important in controlling hydrolysis rate in animal digestive tract. Therefore, chemical and biological treatments are used for many by-products to improving their nutritional value and transforming them into animal feed with high quality (Villas-Boas *et al.*, 2002; Hassan *et al.*, 2008). Many efforts have been employed to remove the lignin and/or to break up the linkages between lignin and carbohydrates and to increase their feed values by chemical and biological treatments (Abo-Eid *et al.*, 2007; El-Shafie *et al.*, 2007 and Abo-Eid, 2008). The possibility of biological methods of straw treatment has a great appeal as an alternative to the use of expensive (in terms of money and energy) chemicals. The main objective of this study was to evaluate the effect of chemical and microbial treatments of barley straws with

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urea or fungi (*Pleurotus ostreatus*, *P.O*) respectively on chemical composition, cell wall constituents, *in vitro* and *in vivo* dry and organic matter digestibility and voluntary intake.

## METRIALS AND METHODS

### Barley straw preparation

The barley straw was chopped (approximate 1-2 cm) and divided into three treatments [Untreated straw (US, control), Chemical treated straw with urea (UTS) and Fungal treated straw with *P.O.* (FTS)].

### Chemical treatment

The barley straw used in this experiment was chopped and treated with urea at rate of approximately 70 g / kg DM dissolved into 60% moisture on DM bases (Hassan and Muhamad, 2009).Urea was applied by spraying equal weight of urea solution on straw to provide a treatment level of 70 g urea per kg straw DM .The sprayed straw was mixed well to bring urea solution into contact with straw as completely as possible. The freshly-made material was covered with polyethylene nylon for approximately 4 weeks to absorb moisture and ammonia that formed during the heating process. At the end of the incubation period the polyethylene nylon was removed and sun dried (40 $C^0$) for three days then stored in nylon bags for *In vitro* and *in vivo* digestibility trail and necessary tests.

### Biological treatment

The barley straw was spread on a polyethylene sheet and spread with 75% water content 2% formaldehyde and 1% urea for 24 h in fermentation room .The wheat grain spawns of *P.O* was used to inoculate the straw .The pasteurized straw was spread again on a polyethylene sheet and mixed with the spawn at the rate of 3 kg spawn per 100 kg straw (on DM basis) in spawning room (Fazaeli *et. al.*,2004).Then the inoculated straw was packed in black polyethylene bags (80 cm length and 40 cm diameter and 100 gauge thickness). Each bag that contained approximately 15 kg of straw (fresh weight) was tightened up with nylon thread and transferred to the fermentation room where the temperature of 26 $\pm$3 $C^0$ and the relative humidity of 70-75 % maintained by means of heater during the 21 days of incubation, when the mycelium run started, all sides of the bags were crushed, to provide a uniform distribution of mycelium for all substrate. After 21 days of incubation, the bags were removed from the fermentation room and sun dried (3 days) then stored in cotton bags for necessary tests.

### Feeding trail

Three treatments of barley straw were tested for digestibility and voluntary feed intake, by sixteen karadi male lambs. They were approximately 5.5$\pm$0.5 months old and had an average body weight of 30$\pm$1.43 kg. The lambs were randomly allocated to three treatments to receive either US, UTS or FTS. They all received an equal daily allowance of concentrate diet (2 % of the body weight) . Formulation and chemical composition of concentrate diet are presented in table 1.The lambs were penned individually indoors on dry earth bedding and the concentrate was supplied once daily (9:00 am).Straw was given *at ad libitum* level .The daily feed intake for each treatments diet was tested for a period of 5 weeks and 1 week of collection period. Daily feed intake and refused were measured and sampled during the collection period. Feces from the individual lambs were collected and weighed every morning by fitting lambs with collection bags (Saeed, 2011).The feces were mixed thoroughly by hand and 10% sub–sample was retained and stored at -15 $C^o$. At the end of collection period, the sample of feed and refusal were dried at 65 $C^o$ for 48 h and feces were dried at 65 $C^0$ until constant weight .The dried samples were ground through 1 mm mash .Aliquots of the samples from each day were pooled and analyzed chemically .The lambs were

weighed at the beginning and the end, and once every 7 days during the experimental period. The trial was carried out at the animal production field, Dept. of Anim. Prod. College of Agric. Univ. of Sulaimani, Bakrago, Sulaimani, Iraq.

**Table 1. Formulation and chemical composition of concentrate diet.**

| | |
|---|---|
| Ingredients (g/kg DM) | |
| Barley | 490 |
| Yellow corn | 390 |
| Soybean meal | 100 |
| Salt | 10 |
| Min. & vit. mix. | 10 |
| Chemical Composition (g /kg DM) | |
| DM (g/kg fresh) | 946 |
| OM | 918 |
| TN | 21.3 |
| CF | 50.8 |
| EE | 34 |
| NFE | 700 |
| ME (MJ) * | 12.4 |

*ME (MJ/ kg DM) = 0.012 CP +0.031 EE+0.005 CF +0.014 NFE (MAFF, 1977).

**Chemical Analysis and *In Vitro* Digestibility**

Proximate chemical analysis of raw and treated barley straw, feces and concentrate samples in triplicate per each determination was carried out for dry matter (DM), crude protein (CP), ether extract (EE), crude fiber (CF) and ash content according to the AOAC (1995). Untreated, treated barley straw and feces samples were analyzed according to Goering and Van Soest (1970) to determine neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL). Hemicellulose and cellulose were determined by difference. The *In Vitro* digestibilities of DM and OM for untreated and treated barley straw were determined using the method of Telley and Terry (1963).

**Statistical Analysis**

The obtained data were analyzed according to Statistical Analysis System user's Guide (SAS, 2001) for one way analysis of variance. Separations among means were carried out by using Duncan's (1955) multiple range tests.

$Yij = \mu+ti+eij$.

Yij= Responses of animal i in treatment j, μ= Overall sample mean,

ti = Effect of treatments, eij = Experimental error

**RESULTS AND DISCUSSION**

**Effect of Treatments on Chemical Composition**

Table 2. Shows the proximate analysis, cell wall components of US, UTS and FTS. Urea and fungal treatments had significantly ($P<0.05$) decreased the OM but increase the CP content of the straw .These results are in agreement with those reported by Hassan *et .al.*, (2008).Similar observation were reported by some reports (Sing *et al.,* 1990; Fazeali *et .al.,* 2006).

However, the CP content of UST was significantly (P<0.05) higher than the CP content in the FTS. So it was expected that the treated straw with high level of urea (7.5 %) to have a higher concentration of CP as compared with straw treated with *P.O.* (Hassan *et al.,* 2008) or wheat straw treated with another *Pleurotus species* (Sing *et al.,* 1990). The protein content of mycelium was reported relatively high (Ragunathan *et al.,* 1996), which make it expected that straw treated with fungal mycelium would have higher CP. Both urea and fugal treatments significantly (P<0.05) reduced NDF, ADF and ADL contents of the straw and no differences was observed among urea and fungi to degrade these components. Urea treatment can chemically break the ester bonds between lignin and hemi-cellulose and cellulose, and physically make structural fibers swollen (Chenost and Kayouli, 1997). These effects enables rumen microbes to attack the structural carbohydrates more easily, increasing digestibility, and at the same time increasing palatability of the treated straw (Bod'a, 1990). However ,in fungi treatment the reduction in NDF, ADF and ADL contents of the straw was due to the natural habitats of *P.O.* fungi that largely depend on organic carbon (for their energy requirement) including carbon in the form of structural material such as lignocelluloses (Jennings and Lysek,1996). The losses of NDF and ADF from the barley straw suggested that these fungi could solubilize and utilize the cell wall as carbon source and thus changed the ratio of soluble to un-soluble carbohydrates in the straw (Taniguch *et al.,* 2005). The decrease in NDF and ADF contents of the treated straw has been supported by other reports (Singh *et al.,* 1990;Okanon *et al.,* 2005). Decrease of ADL fungal treated straw had been reported when it was treated with urea or *P.O.* (Hassan *et al.,* 2008). It could be as a result of lignin degradation enzymes, produced by *Pleurotus* fungi during the fermentation period (Singh *et al.,* 1990; Boyle *et al.,* 1992).

Urea and fungal treatments also significantly (P<0.05) reduced the concentration of cellulose and hemicellulose. Among the treatments Barley straw treated with Fungal had the lowest hemicellulose content while the cellulose content was similar for both treatments. These reduction in hemicellulose was explained that the fungi depends on lignocelluloses materials, mostly release and utilized the hemicellulose and cellulose as carbohydrate sources , and able to produce laccase, cellulose, xylanase and glycosidase enzymes to degrade lignocelluloses compounds and utilize the releasing sugars(Zadrazil *et al.,* 1996). This degradation was dependent on the substrate used (Abo-Eid *et al.,* 2007), the species of microorganism (Mahrous *et al.,* 2005) and incubation period (Zaza *et al.,* 2008).

**Effect of Treatments on the *in vitro* Digestibility**

As it is shown in Table 2, urea and fungal treated barley straw were significantly (P<0.05) increased the digestibility of DM and OM .However, Duncan comparison test indicated that barley straw treated with fungi had significantly (P<0.05) higher DM and OM digestibility than urea treated barley straw. The lignin binds with hemi-cellulosic components of cell wall and through covalent linkages and physical binding, can not prevent accessibility and biodegradation of straw carbohydrate by cellulose and hemicellulytic microorganisms (Karunanandaa and Varga, 1996). Improvement the digestibility of treated straw could be as a result of solubilization of structural polymers by urea or fungi (Boyle et al., 1992), which made it more accessible to the rumen micro organisms. Similar results were reported by (Zadrazil *et al.,* 1996). Beside the culturing conditions, the ability of various strains of fungi in cell wall degradation and digestibility improvement may be different (Tripathi and Yadav, 1992).

**Table 2. Proximate analysis, cell wall components and *in vitro* digestibility in untreated, urea and fungi treated barley straw (% of DM basis).**

| *Treatments* Items % | T1 US | T2 UTS | T3 FTS | SEM |
|---|---|---|---|---|
| DM | 96.2 | 94.5 | 95.1 | 2.40 |
| OM | 87.8[a] | 84.7[b] | 82.7[b] | 0.32 |
| CP | 4.1[c] | 19.9[a] | 12.4[b] | 0.32 |
| $NH_3$-N | 0.082[b] | 0.716[a] | 0.079[b] | 0.032 |
| Ash | 8.4[b] | 9.8[b] | 11.9[a] | 0.34 |
| NDF | 81.3[a] | 75.8[b] | 74.16[b] | 0.81 |
| ADF | 54.3[a] | 50.1[b] | 50.2[b] | 1.01 |
| Lignin | 11.1[a] | 9.2[b] | 9.13[b] | 0.039 |
| Hemicellulose | 27.0[a] | 25.7[b] | 23.96[c] | 0.85 |
| Cellulose | 43.2[a] | 40.9[b] | 41.07[b] | 0.92 |
| IVDMD | 45[c] | 53.8[b] | 57.5[a] | 1.53 |
| IVOMD | 44[c] | 51.3[b] | 56.9[a] | 1.51 |
| ME MJ/ kg DM [#] | 6.6[c] | 7.7[b] | 8.54[a] | 0.123 |

[#] ME=IVOMD × 0.15 (MAFF,1977) ; [abc] Means with the different superscripts within row are significantly (P<0.05) different ; SEM=Standard Error of Mean

**Feeding Trail**

***In vivo* Digestibility**

The effect of urea and fungi treatments on the *In vivo* digestibility's of the nutrients of straw and concentrate diets are shown in Table 3. The total tract digestibilities were significantly affected by urea and fungi treatments. Urea and fungal treated barley straw were significantly (P<0.05) increased the *in vivo* digestibility of DM, Similar findings were observed for DM and OM digestibilities. However, barley straw treated with fungi had significantly (P<0.05) higher OM digestibility than the straw treated with urea. The digestibility of CP, CF,

NDF, ADF, hemicellulose and cellulose were also significantly (P<0.05) affected by urea and fungi treatments. When the barley straw was treated with urea and fungi, the digestibility's of the most components were increased. However, lambs fed fungi treated straw showed the higher amounts of digestibility than those lambs fed urea treated straw .These results are supported by the findings of the *in vitro* digestibility of this study and other reports (Zadrazil *et al.,* 1996 ; Fazeali *et .al.,* 2006; Hassan *et al.,* 2008 ). A few reports in which digestibility of fungal treated straw were evaluated *in vivo*. However, Marwaha *et al. (*1990),and Fazeali *et .al.* (2006), found that treatment of wheat straw by *P. sajor-caiuled* and two species of *P fungi* led to an increase (P<0.05) in the digestibility of DM,CP,CF and ADF in Jersey calves and sheep respectively . Yoshida et al.,(1993)found an increase (by 11%)in the DM digestibility of straw cultivated with *P.O.*

**Daily intake of barley straw**

The effect of treatments on daily feed intake and nutritive value index of barley straw for 6 weeks period are presented in Table 3. Urea and fungal treated barley straw significantly (P<0.05) increased the daily DM intake. At the same time differences in daily DM and OM intake between urea and fungi treatments were significantly (P<0.05) different .The Duncan comparison test indicated that barley straw treated with fungi had significantly (P<0.05) higher DM intake than the straw treated with urea. The digestible DM and OM intake were also significantly (P<0.05) increased for the urea and fungi treatments. Improvement of intake could be due to the physical (softness of the straw structure) and chemical (cell wall degradation) changes of barley straw through solubilization of structural polymers by urea (Boyle *et al.,* 1992), which made it more accessible to the rumen micro organisms or by solid state fermentation process by fungi .In addition urea and fungal treatment significantly increased the DM and OM digestibility of straw, which increased the voluntary intake. Yamakawa *et al.* (1992) reported an increase in DM intake of *P.O.* treated rice straw from12-13 in normal straw to about 20 g/ $kgW^{0.75}$ in treated straw by sheep. The nutritive value index (NVI) according to (Fazaeli *et al.,* 2006) calculated as follows: (NVI) = Relative intake x DM digestibility (Relative intake = amount of DM intake from treated straw /amount of DM intake from initial straw).In general digestible OM intake and NVI were the highest for fungi treated straw and the lowest for untreated straw .while digestible OM intake and NVI for urea treated straw were in between .In conclusion, Treatment of barley straw by *P.O.* fungi or urea resulted in a reduction of cell wall and its components and increasing of CP, *in vitro* and *in vivo* digestibility's, daily feed intake and nutritive value index by lambs.

**Table 3. Effect of treatments on the *In vivo* digestibility of the nutrients Daily intake of barley straw.**

| Items % *Treatments* | T1 US | T2 UTS | T3 FTS | SEM |
|---|---|---|---|---|
| DM | 59[b] | 69[a] | 73[a] | 2.39 |
| OM | 63[c] | 73[b] | 77[a] | 2.1 |
| CP | 39[b] | 61[a] | 63[a] | 1.33 |

| | | | | |
|---|---|---|---|---|
| CF | $37^{c}$ | $64^{b}$ | $67^{a}$ | 1.36 |
| NDF | $71^{b}$ | $78^{a}$ | $82^{a}$ | 2.3 |
| ADF | $55^{c}$ | $61^{b}$ | $65^{a}$ | 1.04 |
| Hemi-cellulose | $76^{b}$ | $95^{a}$ | $95^{a}$ | 3.2 |
| Cellulose | $63^{c}$ | $73^{b}$ | $79^{a}$ | 236 |

$^{abc}$ Means with the different superscripts within row are significantly (P<0.05)different, SEM=Standard Error of Mean

**Table 4. Effect of treatments on daily nutrients intake of barley straw during the digestibility trail.**

| Items / *Treatments* | T1 US | T2 UTS | T3 FTS | SEM |
|---|---|---|---|---|
| DMI (g /day ) | $444^{c}$ | $505^{b}$ | $610^{a}$ | 12.3 |
| OMI(g /day ) | $390^{c}$ | $428^{b}$ | $504^{a}$ | 10.4 |
| DMI (g/ kg $W^{0.75}$) | $34.7^{c}$ | $39.5^{b}$ | $47.7^{a}$ | 0.43 |
| OMI(g /kg $W^{0.75}$) | $30.5^{c}$ | $33.0^{b}$ | $39.4^{a}$ | 0.39 |
| Digestible DMI (g /day ) | $262^{c}$ | $348.5^{b}$ | $445.3^{a}$ | 18.5 |
| Digestible OMI(g /day ) | $245.7^{c}$ | $308.1^{b}$ | $388.1^{a}$ | 16.8 |
| Digestible DMI (g/ kg $W^{0.75}$) | $20.5^{c}$ | $27.2^{b}$ | $35.6^{a}$ | 0.32 |
| Digestible OMI(g/ kg $W^{0.75}$) | $19.2^{c}$ | $24.1^{b}$ | $30.3^{a}$ | 0.31 |
| Nutritive value index (NVI) | $100^{c}$ | $125^{b}$ | $157^{a}$ | 5.2 |

$^{abc}$ Means with the different superscripts within row are significantly (P<0.05)different,

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# تقيم المعاملة الفطرية أو الكيميائية لتبن الشعير في تغذية المجترات

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## ملخـص

استخدم في هذه الدراسة ستة عشر حمل ذكر بمتوسط وزن 1.43 +30 كغم وبعمر 0.5+5.5 شهراً لتقيم تأثير استخدام ثلاثة أشكال من التبن [تبن شعير غير معامل، تبن معامل باليوريا، تبن معامل بالفطر Pleurotus ostreatus ] في التركيب الكيميائي، مكونات جدار الخلية، معامل الهضم المختبري In vitro وفي الحيوان In vivo للمادة الجافة والعضوية وكمية المتناول. أظهرت النتائج ان المعاملة باليوريا وبالفطر أدت الى انخفاض معنوي ($P<0.05$) في مكونات جدار الخلية وزيادة معنوية ($P<0.05$)في محتوى البروتين الخام و معامل الهضم المختبري للمادة الجافة والعضوية مقارنة بالتبن غير المعامل. كذلك اظهرت النتائج ان المعاملة باليوريا والفطر أدت الى زيادة معنوية ($P<0.05$)في كمية المتناول اليومي ومعامل هضم المادة الجافة والعضوية In vivo مقارنة بالحملان المغذات على التبن غير المعامل . مع ذلك ،فأن كمية المتناول اليومي ومعامل هضم المادة الجافة والعضوية كانت اكثر معنويا ($P<0.05$) في التبن المعامل بالفطر مقارنة بالتبن المعامل باليوريا. نستنتج من ذلك، أن معاملة تبن الشعير باليوريا أو الفطر أدت الى تحسن في محتوى البروتين الخام، معامل هضم العناصر الغذائية، والمتناول اليومي ودليل القيمة الغذائية .

**الكلمات الدالة**: تبن الشعير، المعاملة باليوريا، المعاملة بالفطر، اغنام.

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