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## Effects of Treated Wheat Straw with Exogenous Fibre-Degrading Enzymes on Wool Characteristics of Ewe Lambs

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**Abstract:** Twenty Naieni breed replacement ewe lambs were used to determine the substitution effects of treated wheat straw with exogenous fiber-degrading enzymes for alfalfa hay on wool characteristics. Five treatment diets were included: 1) control (100% alfalfa hay without wheat straw), 2) 10% enzyme treated wheat straw replaced for alfalfa hay, 3) 20% enzyme treated wheat straw replaced for alfalfa hay, 4) 30% enzyme treated wheat straw replaced for alfalfa hay and 5) 30% untreated wheat straw replaced for alfalfa hay. Final fleece weight, and fleece weight index; wool production per day, feed conversion to wool; final staple length, wool and hair crimp per centimeter of length, percent of heterotype, coronal, kemp, reticulate and imbricate fibers and accumulation of scales in 100 µm fiber lengths were not affected by replacing treated wheat straw for alfalfa hay, but feeding untreated wheat straw tended to decrease final fleece weight, wool production, and final staple length. Replacing 20%, and 30% treated, and 30% untreated wheat straw for alfalfa hay numerically increased mean final diameter of fiber. The results of this experiment indicated that treated wheat straw with exogenous fiber-degrading enzymes can be replaced for alfalfa hay in Naieni replacement ewe lamb diets.

**Key words:** Exogenous fiber-degrading enzymes, wheat straw, wool, lamb

### Introduction

The main fraction of wheat straw is structural complex fibrous compounds. In ruminant, partial digestion of fibrous compounds of plant cell walls is performed through the ruminal bacteria, protozoa and fungi enzymes. Considerable research has been performed for improving fibrous feedstuff digestibility in ruminant livestock by several biotechnological products; ionophores, direct fed microbes and cell wall degrading enzymes. Currently, research has been focused on using exogenous fiber-degrading enzymes (EFDE) to stimulate rumen digestive activity.

The main components affecting efficiency of wool production are feed intake, the digestibility of diet, body weight, season (Butler and Maxwell, 1984; Kott *et al.*, 1999), and the metabolic efficiency of the individual animals (Kott *et al.*, 1999). Some studies, using fibrolytic enzymes in ruminant nutrition have shown improvement of milk yield of dairy cows (Beauchemin *et al.*, 2000), average daily gain of steers (Beauchemin *et al.*, 1995; 2003; Beauchemin and Rode, 1996) and lambs (Alikhani *et al.*, 2004; Jafari *et al.*, 2004a), and wool characteristics of lambs (Jafari *et al.*, 2004b). Titi and Tabbaa (2004) reported improvements in the digestibility of dry matter (DM), organic matter (OM), neutral detergent fiber (NDF), and acid detergent fiber (ADF) in Awassi lambs fed a concentrate based diet supplemented with fibrolytic enzymes. Titi and Lubbadah (2004) have reported an increase in lambs and kids weaning weights when their mother were fed a diet

supplemented with cellulase enzyme during the last 2 months of pregnancy and the first 2 months of lactation. Jafari *et al.* (2004b) tested two type of EFDE; which showed that one type of enzymes tended to improve fleece weight, fleece weight index (FWI), feed conversion to wool (FCW), and wool production. They also reported that ADF, NDF and crude protein (CP) digestibility are increased by treating wheat straw with EFDE (Jafari *et al.*, 2004a). Suzuki *et al.* (1994) used a fungal enzyme preparation in sheep fed hay or straw diet, and they reported that enzyme had no effects on DM or fiber digestibility. The changes in wool growth and characteristics are associated with quality and relative mixture of absorbed amino acids (Ferguson, 1967; Reis and Colebrook, 1972). Adding fibrolytic enzymes to the diets, increased viable rumen bacteria (Nsereko *et al.*, 2002), increased rate of ruminal bacterial growth and flow, and hence a greater absorption of amino acids in small intestine, which would have significant impact on the supply of metabolizable protein to the animal. Reis *et al.* (1992) provided sheep with most of their nutrients as abomasal infusions, and they found that the rate of wool growth was markedly altered by protein supply. The objective of this study was to examine the effects of treated wheat straw with EFDE on wool characteristics of Naieni breed replacement ewe-lambs.

### Materials and Methods

**Enzyme assay:** The enzyme mixture used in this study (Pro-Mote<sup>®</sup> Biovance Technologies, Inc., Omaha, NE),

Table 1: Ingredient and chemical composition of treatment diets<sup>1</sup> (Percent, DM basis)

	Treatment <sup>2</sup>				
	1	2	3	4	5
Wheat straw	0.00	5.00	10.00	15.00	15.00
Alfalfa hay	50.00	45.00	40.00	35.00	35.00
Barley	41.98	38.49	35.17	31.92	31.92
Cotton seed meal	3.12	6.66	10.13	13.58	13.58
Calcium salt soap	3.00	3.00	3.00	3.00	3.00
Acid phosphoric	0.70	0.65	0.50	0.30	0.30
Mineral vitamin supplement <sup>3</sup>	0.70	0.70	0.70	0.70	0.70
Salt	0.50	0.50	0.50	0.50	0.50
Chemical composition					
Dry matter	91.82	92.02	92.23	92.45	92.45
CP	12.02	12.02	12.02	12.02	12.02
ADF	23.96	26.25	28.51	37.78	37.78
NDF	35.15	38.25	41.35	44.46	44.46
Ash	12.50	11.90	11.54	10.98	10.98
Metabolizable energy (Mcal/ kg) <sup>4</sup>	2.53	2.48	2.44	2.40	2.40
Ca	0.82	0.75	0.65	0.63	0.63
P	0.46	0.45	0.41	0.36	0.36
Ca:P	1.78	1.67	1.68	1.75	1.75

<sup>1</sup>Enzyme activity was 1437, 788, and 7479  $\mu\text{mol}$  per min. per ml for exoglucanase, endoglucanase, and xylanase, respectively. Concentrate enzymes was diluted and sprayed on the wheat straw 1 hour prefeeding. <sup>2</sup>Treatment diets including: 1) control (100% alfalfa hay without wheat straw), 2) 10% enzyme treated wheat straw replaced for alfalfa hay, 3) 0% enzyme treated wheat straw replaced for alfalfa hay, 4) 30% enzyme treated wheat straw replaced for alfalfa hay, and 5) 30% untreated wheat straw replaced for alfalfa hay. <sup>3</sup>Supplied per kilogram: Vitamin A 250000 IU, vitamin D<sub>3</sub> 5000 IU, vitamin E 500 IU, sodium 186 g, calcium 12 g, phosphorous 20 g, magnesium 20.5 g, sulfur 3 g, manganese 2.25 g, zinc 7.7 g, iron 1.25 g, copper 1.25 g, cobalt 14 mg, iodine 56 mg, and selenium 10 mg. Calculated metabolizable energy based on ADF by using equation (Khalil *et al.*, 1986)

contained mainly xylanase, cellulase and endoglucanase. Exoglucanase activity in enzyme solution was assayed by the filter paper test. Filter paper is hydrolyzed by adding 0.5 ml diluted enzyme in distilled water (1/400) with 1.5 ml citrate buffer 50 mM (pH, 4.8) (when incubated 60 min at 50°C. Endoglucanase activity was determined by its activity against carboxymethyl cellulose (CMC, low viscosity). This was assayed by mixing 1 ml of diluted enzyme (1/400) with 4 ml CMC (10 g/l) in 50 mM citrate buffer (pH, 4.8), and the mixture incubated at 50°C for 60 min. Xylanase activity was assayed by measuring the reducing sugar liberated from oat spelt xylan. Assay was conducted by adding 0.2 ml of diluted enzyme in distilled water (1/400) to a tube containing 1.8 ml 50 mM citrate-phosphate buffer (pH, 4.8) and 10 mg xylan spelt. The mixture was incubated at 50°C for 30 min. The reducing sugar (glucose) produced, was measured using dinitrosalicylate as described by Mandel and Weber (1969). The reactions were stopped by adding 2 ml of dinitrosalicylate and boiling the mixture solution for 15 min. The reducing sugar content was measured by absorbance at 575 nm for exoglucanase and endoglucanase and at 540 nm for xylanase as described by Mandel and Weber (1969). Units of enzyme activity were measured as  $\mu\text{mol}$  reducing sugars production per min. per ml of enzyme, where they were 1437, 788, and 7479 for exoglucanase, endoglucanase, and xylanase, respectively.

**Lambs and treatments:** Twenty Naieni breed replacement ewe lambs with an average body weight of  $24.88 \pm 1.74$  (kg) and age of  $270 \pm 12$  days were randomly allotted to individual pens. In order to adapt lambs to the pens and respective diets, lambs were transported to individual pens two weeks prior to the start of experiment. Lambs were treated one time for internal parasites, and given regular intra-muscular injection of vitamins A, D<sub>3</sub>, and E before starting experiment. Every day the concentrate enzyme was diluted in water (1:10) and sprayed on wheat straw 1 hour prefeeding. The enzyme concentrate was 0.6 liter per ton of wheat straw on DM basis. Lambs were fed by treatment diets including, 1) control (100% alfalfa hay without wheat straw), 2) 10% enzyme treated wheat straw replaced for alfalfa hay, 3) 20% enzyme treated wheat straw replaced for alfalfa hay, 4) 30% enzyme treated wheat straw replaced for alfalfa hay, and 5) 30% untreated wheat straw replaced by alfalfa hay. All diets were isonitrogenous (12.02%, DM basis). Forage to concentrate ratio was 50:50 in all diets. The criteria used to formulate diets were based on NRC (1989) nutrient requirements for replacement ewe lambs. Nutrient ingredient and chemical composition of experimental diets are shown in Table 1. Feed ingredients were analyzed for DM (48 h at 60°C), ash, ADF (AOAC, 1990), and NDF (Van Soest *et al.*, 1991).

Table 2: Effect of treated wheat straw with exogenous fiber-degrading enzymes<sup>1</sup> on wool quality characteristics of ewe lambs

Item	Treatment <sup>2</sup>				
	1	2	3	4	5
Fleece weight, g					
Initial	820±68	891±68	708±68	783±68	762±79
Final	510±33	500±35	487±35	517±33	445±38
FWI <sup>3</sup> , g/kg					
Initial	32.92 <sup>ab</sup> ±3.04	36.50 <sup>a</sup> ±3.04	26.96 <sup>b</sup> ±3.04	32.25 <sup>ab</sup> ±3.04	30.67 <sup>ab</sup> ±3.51
Final	15.36±1.06	15.21±1.14	15.40±1.16	15.40±1.06	14.06±1.22
FCW <sup>4</sup> , g	2.53±0.19	5.50±0.20	2.65±0.20	2.40±0.19	2.75±0.22
Wool production, g/d	6.08±0.40	5.96±0.42	5.80±0.42	6.16±0.39	5.31±0.46

<sup>1</sup>Enzyme activity was 1437, 788, and 7479  $\mu\text{mol}$  per min. per ml for exoglucanase, endoglucanase, and xylanase, respectively. Concentrate enzymes was diluted and sprayed on the wheat straw 1 hour prefeeding. <sup>2</sup>Treatment diet including: 1) control (100% alfalfa hay without wheat straw), 2) 10% enzyme treated wheat straw replaced for alfalfa hay, 3) 20% enzyme treated wheat straw replaced for alfalfa hay, 4) 30% enzyme treated wheat straw replaced for alfalfa hay, and 5) 30% untreated wheat straw replaced for alfalfa hay <sup>3</sup>Fleece weight index. <sup>4</sup>Feed conversion to wool. <sup>ab</sup>Within row, means that do not have a common superscript differ ( $P<0.05$ ).

### Measurement of wool characteristics and sample analyses:

Lambs were weighed (12 hour feed removal) at the beginning (d 0) and at the end (d 84) of the trail. Diets were fed as a total mixed ration twice daily at 0730 and 1630. Feed and water were available *ad libitum* during the experimental period. Refused feed was weighed and recorded daily during the experiment for each lamb.

Lambs were sheared and greasy fleece weights were measured. Left mid-side wool sample of each lamb was taken at the beginning and at the end of the trail, prior to shearing. Wool samples were cleaned in non-ionic organic soap (fleuzan) at 40°C for 20 min. and dried at room temperature, prior to measuring wool characteristics. Wool samples were analyzed for staple length, wool crimp and hair crimp per centimeter, accumulation of scales in 100  $\mu\text{m}$  fiber length, proportions of heterotype, reticulate, imbricate, coronal and kemp fibers (ASTM, 1991), and average fiber diameter ( $\mu\text{m}$ , Lynch and Miche, 1976), for each lamb.

In order to calculate wool production per day and fleece weight index (FWI), fleece weight was divided by 84 and body weight at 84 day, respectively. Also feed intake was divided by final fleece weight to calculate the ratio of feed conversion to wool (FCW).

**Statistical analysis:** One of the lambs of treatment 5 was sick and was removed from experiment. The unbalanced data were analyzed as a completely randomized design using the GLM procedures of SAS (SAS Institute Inc., Cary, NC, 2001). The main effect was treatment. Some variables used as a covariable in the least squares model were initial fleece weight for final fleece weight, FCW and wool production, and initial body weight for final FWI. Differences among Lsmeans were tested by using the PDIFF option of SAS (SAS Institute Inc., Cary, NC, 2001), at  $P<0.05$ , otherwise noted.

### Results

Replacing treated wheat straw with EDFE for alfalfa hay had no effect on final fleece weight and wool production (Table 2), but untreated wheat straw replaced for alfalfa hay tended to decrease ( $P<0.23$ ) final fleece weight (445 vs. 510, g), and wool production (5.31 vs. 6.08, g/day). The difference between initial fleece weights was not significant (Table 2).

Initial and final FWI were not affected by treatment diets, except in treatment 3 which initial FWI was significantly ( $P<0.05$ ) different from other diets (Table 2). Final staple length was not affected by treatment diets, but feeding untreated wheat straw compared to alfalfa hay tended to decrease ( $P<0.22$ ) final staple length (3.80 vs. 4.79, cm; Table 3).

Replacing 20%, and 30% treated wheat straw with EFDE for alfalfa hay caused numerical increase ( $P<0.20$ ) final wool diameter (34.40, and 34.89, respectively, vs. 30.49,  $\mu\text{m}$ ; Table 2). Also, feeding untreated wheat straw compared to alfalfa hay, tended to increase ( $P<0.17$ ) wool diameter (34.97 vs. 30.49,  $\mu\text{m}$ ; Table 3).

Final accumulation scales were not different for treatment diets, but they were numerically lower for lambs fed untreated wheat straw compared to diets 4 and 1 (Table 3).

Heterotype, reticulate, imbricate, coronal and kemp fiber, initial and final wool crimp per centimeter and final hair crimp per centimeter were not affected by treatment diets, but initial hair crimp per centimeter for diet 1 was lower than the other treatment diets (Table 3).

### Discussion

The results of this experiment showed that replacing treated wheat straw with fibrolytic enzymes for alfalfa hay tended to improve fleece weight, wool production, FWI and FCW. These positive results are in agreement with the studies that treating forage portion with EFDE used

Table 3: Effect of treated wheat straw with exogenous fiber-degrading enzymes<sup>1</sup> on wool quality characteristics of ewe lambs

Item	Treatment <sup>2</sup>				
	1	2	3	4	5
Staple length, cm					
Initial	4.41 <sup>b</sup> ±0.36	5.41 <sup>ab</sup> ±0.36	5.40 <sup>ab</sup> ±0.36	5.15 <sup>b</sup> ±0.36	6.34 <sup>a</sup> ±0.41
Final	4.79±0.50	3.96±0.50	4.21±0.50	4.07±0.50	3.80±0.58
Diameter, µm					
Initial	26.48±1.39	27.41±1.39	28.62±1.39	27.53±1.39	27.99±1.60
Final	30.49±2.02	30.94±2.02	34.40±2.02	34.89±2.02	34.97±2.23
Wool crimp per cm					
Initial	17.72±1.12	17.95±1.12	17.33±1.12	17.83±1.12	15.73±1.29
Final	13.24±1.40	12.71±1.40	14.48±1.40	13.44±1.40	13.81±1.62
Hair crimp per cm					
Initial	3.48 <sup>b</sup> ±0.49	3.68 <sup>ab</sup> ±0.49	5.60 <sup>a</sup> ±0.49	5.90 <sup>a</sup> ±0.49	5.13 <sup>a</sup> ±0.50
Final	5.96±0.16	5.68±0.16	8.12±0.16	7.15±0.16	8.4±0.34
Accumulation scales in 100 µm fiber length					
Initial	7.88±0.58	7.69±0.58	7.52±0.58	7.63±0.58	7.44±0.67
Final	7.61±0.55	6.66±0.55	7.29±0.55	7.49±0.55	6.19±0.63
Fiber percent, %					
Heterotype	1.25±1.00	2.09±1.00	2.08±1.00	1.65±1.00	1.66±1.00
Reticulate	14.17±3.59	17.08±3.59	17.50±3.59	14.95±3.59	12.22±4.15
Imbricate	45.00±5.09	50.00±5.09	39.17±5.09	47.31±5.09	48.89±5.09
Coronal	34.42±5.34	27.08±5.34	30.42±5.34	26.99±5.34	29.44±6.16
Kemp	5.16±2.20	3.75±2.20	10.83±2.20	9.10±2.20	7.78±2.54

<sup>1</sup>Enzyme activity was 1437, 788, and 7479 µmol per min. per ml for exoglucanase, endoglucanase, and xylanase, respectively. Concentrate enzymes was diluted and sprayed on the wheat straw 1 hour prefeeding

<sup>2</sup>Treatment diet including: 1) control (100% alfalfa hay without wheat straw), 2) 10% enzyme treated wheat straw replaced for alfalfa hay, 3) 20% enzyme treated wheat straw replaced for alfalfa hay, 4) 30% enzyme treated wheat straw replaced for alfalfa hay, and 5) 30% untreated wheat straw replaced for alfalfa hay. <sup>a,b</sup>Within row, means that do not have a common superscript differ ( $P < 0.05$ )

in diet of dairy (Beauchemin *et al.*, 2000), growing cows (Beauchemin 1995; 1996; 2003; Beauchemin and Rode, 1996) and lambs (Alikhani *et al.*, 2004; Jafari *et al.*, 2004a; b; Titi and Tabbaal, 2003). Cows fed the enzyme-treated forage produced 1.3 kg per day milk more than cows fed control diet (Lewis *et al.*, 1995). Beauchemin *et al.*, (1995) reported that the average daily gain of steers fed alfalfa cubes was increased by the addition of up to four times the amount of enzyme, but the average daily gain of steers fed timothy hay was increased by the addition of 16 times the amount of enzymes. Titi and Tabbaa (2004) reported improvement in the digestibility of DM, OM, NDF, and ADF in Awassi lambs fed a concentrate based diet supplemented with enzymes. Titi and Lubbadah (2004) have reported increase in lambs and kids weaning weights when their mother were fed a diet supplemented with cellulase enzyme during the last 2 months of pregnancy and the first 2 months of lactation. Jafari *et al.*, (2004b) tested two types of EFDE, they found that one types of enzyme tended to improve fleece weight, FWI, FCW, and wool production. Also, they have shown that ADF, NDF and CP digestibility increased by treating wheat straw with EFDE (Jafari *et al.*, 2004a).

Major determinants of wool growth are feed intake, the digestibility of the diet, body weight, season (Butler and Maxwell, 1984; Kott *et al.*, 1999), and the metabolic efficiency of the individual animals (Kott *et al.*, 1999). Probably, improvement of wool characteristics with treating wheat straw by EFDE resulted in an increase in ADF, NDF, and CP digestibility and feed intake as we reported previously (Jafari *et al.*, 2004a).

Changes in wool growth and characteristics are associated with quality and relative mixture of absorbed amino acids (Ferguson *et al.*, 1967; Reis and Colebrook, 1972), even at the same dry matter intake (DMI) level. According to McAllister *et al.*, (2001), EDFE seems to have some proteolytic activities as they facilitate degradation of cell wall bound proteins. Also cellulase has been shown to increase protein degradation of forages *in vitro* by making proteins more available to proteolytic enzymes (Kohn and Allen, 1992). There is some evidence that adding feed enzymes to the diets directly, increased the numbers of non-fibrolytic, as well as fibrolytic bacteria in the rumen (Nsereko *et al.*, 2002). The stimulation on wool growth rate resulting from treating wheat straw with EFDE can be explained by an increased growth of reticuloruminal microorganisms

and microbial flow to the small intestine, hence a greater absorption of amino acids and supply of metabolizable protein to the animal. Reis *et al.* (1992) provided sheep with most of their nutrients as abomasal infusions, and found that the rate of wool growth was markedly altered by protein supply. There is a little doubt, that wool growth was not close to the maximum of which the lambs were capable, but was limited by energy or protein supply, because the diet of lambs were formulated based on replacement ewe lamb, and the lamb had more compatibility to produce wool, and EFDE may increase energy or protein availability.

The ratio of FCW increased with feeding untreated wheat straw, with the decline in feed intake as we reported previously (Jafari *et al.*, 2004a), and this could be due to decreasing final fleece weight.

Fleece yield in relation to body weight (FWI) did not change considerably among dietary treatments. It indicates that changes in fleece yield are proportional to decreased body weight, with feeding untreated wheat straw. Thus there was considerable decrease in fleece weight per unit body weight during feeding untreated wheat straw, although, lambs fed treated wheat straw diets exhibited full potential for wool production.

An interesting result in this experiment was a decrease of the final staple length and increase in final fiber diameter in treatment diet 4 and 5 compared to other treatment diets. This finding is in contrast with one study that wool diameter and fiber length were correlated positively (Hoaglund *et al.*, 1992), but it is in agreement with a study performed in goats (Huston *et al.*, 1993). Fiber diameter and length are both associated with the size of the follicle bulb (Hynd, 1994b), and tend to change together in response to simple changes in nutrition (Hynd, 1994a). In three experiments with Merino sheep, in which wool growth was increased, by supplying amino acid or protein to the abomasums, Reis (1992b) found that both length of fiber (L) and mean fiber diameter (D) increased, but the ratio of L:D remained constant (two experiments) or varied only slightly (one experiment).

The results of this experiment indicated that feeding untreated wheat straw tended to decrease final fleece weight, wool production, and numerically increased final wool diameter. These negative responses are in agreement with Llamas-Lamas and Combs (1990) study, where they found feeding wheat straw compared to ammoniated wheat straw decreased feed intake, DM, OM, and NDF digestibility, passage rate of solid and liquid of digesta through the gut. Both roughage level and source influences DMI. Physical and chemical characteristics of roughages, such as bulk density and concentrations of fiber (e.g., NDF), and other nutrients are likely to be involved (Defoor *et al.*, 2002), and effects of roughage on DMI also seem to be associated with

differences in ruminal fermentation and digesta kinetics (Galyean and Depoor, 2003). Negative effects of untreated wheat straw on wool characteristics could be related to lower DMI as we reported previously (Jafari *et al.*, 2004a), higher NDF and ADF in diet (Table 1) and ruminal passage rate, which all of these parameters cause lower digestibility.

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