

Effects of Temperature, Addition of Soybean Meal and Treatment Period on the Nutritive Value of Urea Treated Wheat Straw

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Abstract. The effect of temperature, addition of soybean meal (SBM) and treatment period on urea treated wheat straw (60 g urea kg⁻¹ straw) was studied in a 3 × 2 × 5 factorial experiment (n = 120). Independent variables comprized temperatures of 25, 35 or 45°C; with or without SBM addition (70 g kg⁻¹ straw) and treatment periods of 1,2,4,6 or 8 weeks. The dependent variables measured were: unhydrolysed urea, N-fractions, pH, cell wall constituents and *in vitro* organic matter digestibility (IVOMD).

Unhydrolyzed urea decreased significantly ($P < 0.001$) with SBM addition, increase in the duration of the treatment period and when samples were kept at 25 and 35°C compared to samples kept at 45°C. However, without the addition of SBM, low urea was converted to ammonia only at 45°C. Urea treatment caused major changes in the chemical composition of cell walls: neutral detergent fiber and hemicellulose content decreased significantly ($P < 0.01$), while acid detergent fiber and cellulose increased significantly ($P < 0.01$), with increased temperature and treatment period. The IVOMD was improved significantly ($P < 0.01$) by all of the independent variables investigated. Mean IVOMD value of treated straw with SMB for 2 weeks was comparable to that of treated straw without SMB for 8 weeks (70.6 vs. 70.2%, respectively). Overall IVOMD mean values for treated samples with or without SMB addition were 72.8 vs. 66.0%, respectively; these values were higher than untreated (original) samples (58.8%) by 14 and 7.2% digestibility units, respectively. It is concluded that treatment with urea is an efficient means for improving the nutritive value of wheat straw especially at high temperature and with the addition of SMB as a source of urease.

Introduction

The use of straw as an animal feed is limited by its low digestibility and inadequate N content. Cereals straws contain more than 80% carbohydrate, mainly cell wall polysaccharide. Much of this carbohydrate is not utilized by microorganisms in the rumen [1] due, it is believed, to covalent bonding between the polysaccharide and lig-

nin [2, p.349]. In the Kingdom of Saudi Arabia wheat straw is one of the most widely available by-products, with an estimated annual production of 6 million tons [3], and is fed heavily to sheep.

Various treatments have been developed for improving the nutritive value of lignified plant material [4]. One of these methods is ammoniation through urea treatment, which has been shown to increase digestibility by up to 20% units [5-8]. The mode of action of alkali treatment entails the cleavage of linkages between lignin and polysaccharide, saponification of acetic acid and phenolic acids, protein and silica [9]. Ammoniation through urea depends on urease activity in plant residues to release ammonia from urea in an aqueous medium [7,10]. Jayasuriya and Pearce [11] found that the addition of urease enzyme or any of its sources could reduce the treatment time required to achieve a given level of digestibility. Cloete and Kritzing [12] reported that urease activity tended to decline at temperature of 35°C. However, high moisture level (< 50%) and temperature (60°C) are required for optimum urease activity [13]. Waagepetersen and Vestergaard Thomsen [14] found that temperature up to 45°C had a positive effect with short temperature time (3-7 d) on ammonia treated barley straw. The following study was conducted to evaluate the effects of 5 treatment periods at 3 temperatures, with or without the addition of soybean meal (SBM), on the chemical composition and *in vitro* organic matter digestibility (IVOMD) of urea treated wheat straw.

Materials and Methods

One hundred and twenty wheat straw (950 g DM kg⁻¹ straw) samples were treated in small batches of 1 kg (1-3 cm long) and each was spread on a metal tray and sprayed with 1L of 6% feed grade urea solution. Each batch was turned while being sprayed and mixed, with or without the addition of SBM (70 g kg⁻¹ straw) as a source of urease. The wet straw was sealed air tightly in double layered polyethylene bags. The straw was allowed to react for periods of 1,2,4,6 or 8 weeks at temperatures of 25,35 or 45°C. Incubators were used to maintain temperatures of 35 and 45°C, while treatment at 25°C was attained by incubating the samples at constant room temperature. Each combination of temperature, SBM addition and treatment period was performed in four replications, giving a 3 × 2 × 5 factorial design.

At the end of the periods, the bags were opened and fresh samples were taken to measure pH electrometrically. The remainder materials were exposed to ambient temperature of 25°C for 24 h before being analyzed for total N (TN), free ammonia N (NH₃-N) and unhydrolyzed urea [15]. Samples of all treatments were dried at 60°C

for 24 h, ground to pass through 1 mm screen and analyzed for ash [15], neutral (NDF) and acid (ADF) detergent fiber, cellulose (CEL), hemicellulose (HC), lignin (LIG) and acid insoluble ash (AIA) using the procedure of Goering and Van Soest [16]. Samples were also analyzed for IVOMD by the method of Tilley and Terry [17], as modified by Moore [18]. N-fractions *viz* corrected total nitrogen (CTN), corrected retained nitrogen (CRN) and bound-N were calculated (see Table 1).

Data were subjected to statistical analysis of variance using the general linear model (GLM) procedure of the statistical analysis system [19] and least squares means were used to compare treatment means.

Results and Discussion

Urea treated straw had a strong ammonia smell at the time the bags were opened while no visible mould growth was observed on the surface of any sample.

Urea, N-fractions and pH

The effects of temperature, addition of SBM and treatment periods on urea breakdown, N fractions and pH are presented in Table 1. The table also summarizes the significance of relevant interactions. Results of unhydrolyzed urea, $\text{NH}_3\text{-N}$, pH and CRN as three factor interactions between temperature, SBM addition and treatment period are shown in Figs. 1-4, respectively.

The results indicated that temperature, urease source and treatment period significantly ($P < 0.001$) affected all the dependent variables investigated. Unhydrolyzed urea was decreased significantly ($P < 0.001$) with SBM addition, increase in the duration of the treatment period or maintenance of the samples at 25 and 35°C (Table 1). On the other hand, treatment period had little effect on urea breakdown for the treated straw, at 45°C without SBM addition. This could be due to a decline in urease activity at that temperature (Fig. 1). However, the addition of SBM to the treated straw at 45°C substantially increased urea breakdown in the samples (Fig. 1).

Treatment with urea increased the alkalinity of the treated samples and this was expected in view of the occurrence of extensive urea breakdown. Maintaining the treated samples at 25 and 35°C increased pH significantly ($P < 0.001$) by comparison to the samples kept at 45°C and this effect was significantly ($P < 0.001$) enhanced by adding SBM and by increasing the duration of the treatment period (Table 1). The slow increase in pH at 45°C (without SBM), on the other hand, reflected very low urea breakdown to ammonia at that temperature (Fig. 3).

Table 1. The effects of temperature, soybean meal (SBM) and treatment period on pH total nitrogen (TN) corrected TN (CTN), corrected retained-N (CRN), CRN, % of added-N (CRN/A), unhydrolyzed urea (urea), free NH₃-N and bound-N of urea ammoniated wheat straw

Dependent Variables	Temperature (T)			SBM (S)		Periods, wks (P)					Level of significance				
	25°C	35°C	45°C	(-)	(+)	1	2	4	6	8	SEM	T×S	T×P	S×P	T×S×P
pH	9.11 ^A	9.09 ^A	8.82 ^B	8.89 ^B	9.13 ^A	8.84 ^D	8.96 ^C	8.99 ^C	9.10 ^{Bb}	9.16 ^{Ab}	0.028	***	***	***	***
g/100g ⁻¹ DM															
TN	2.04 ^C	2.27 ^B	2.86 ^A	2.33 ^B	2.45 ^A	2.74 ^A	2.46 ^B	2.39 ^B	2.19 ^C	2.17 ^C	0.045	***	***	***	**
CTN	1.79 ^C	2.02 ^B	2.61 ^A	2.33 ^A	1.94 ^B	2.49 ^A	2.20 ^B	2.14 ^B	1.93 ^C	1.92 ^C	0.048	***	***	***	**
CRN	1.19 ^C	1.42 ^B	2.01 ^A	1.73 ^A	1.34 ^B	1.89 ^A	1.61 ^B	1.54 ^B	1.34 ^C	1.32 ^C	0.048	***	***	***	**
CRN/A	41.9 ^C	50.5 ^B	70.8 ^A	61.1 ^A	47.4 ^B	66.6 ^A	56.7 ^B	54.3 ^B	47.1 ^C	46.5 ^C	1.680	***	***	***	**
UREA	0.96 ^B	0.99 ^B	2.52 ^A	2.23 ^A	0.75 ^B	2.44 ^A	1.58 ^B	1.15 ^C	1.10 ^C	1.20 ^C	0.123	***	***	***	***
UREA-N	0.43 ^B	0.45 ^B	1.14 ^A	1.01 ^A	0.34 ^B	1.10 ^A	0.71 ^B	0.52 ^C	0.50 ^C	0.54 ^C	0.055	***	***	***	***
NH ₃ -N	0.54 ^B	0.62 ^A	0.51 ^B	0.52 ^B	0.60 ^A	0.54 ^{Ba}	0.66 ^A	0.64 ^A	0.50 ^{Bcb}	0.45 ^{Cc}	0.013	***	***	***	NS
BOUND-N	0.22 ^B	0.35 ^A	0.36 ^A	0.21 ^B	0.41 ^A	0.25 ^{Bcc}	0.24 ^C	0.38 ^A	0.34 ^{ABa}	0.33 ^{ABa}	0.019	*	***	***	***

^{A, B, C, D, a, b, c} Means within a temperature, SBM and periods with different lower case superscripts differ significantly at ($P < 0.05$) and those with different capital superscripts differ significantly at ($P < 0.001$). Except in bound-N the significant at ($P < 0.05$) and ($P < 0.01$).

***Significant at 0.1%. **Significant at 1%. *Significant at 5% and ^{NS}Non Significant at 5% level.

CTN = TN - N of SBM added; CRN = CTN - N of original straw; CRN/A = CRN / urea-N added × 100;

Bound-N = CRN - (urea N + NH₃-N); Urea-N = Assuming a 45% nitrogen content of urea as declared by the manufacturer.

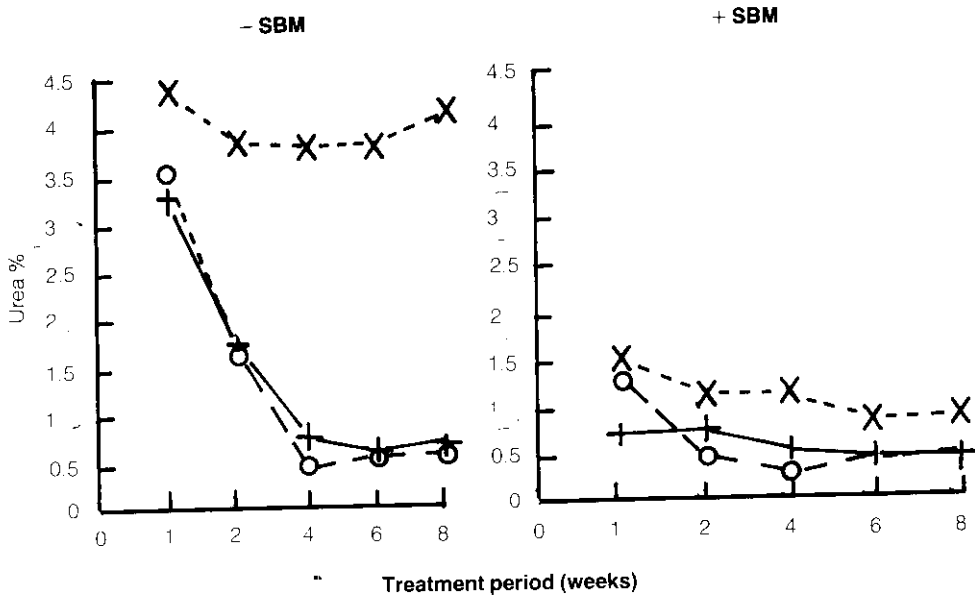


Fig. 1. Urea content amoniated wheat straw after various treatment periods at three temperatures 25°C (—○—), 35°C (—+—) and 45°C (... × ...) with or without SBM.

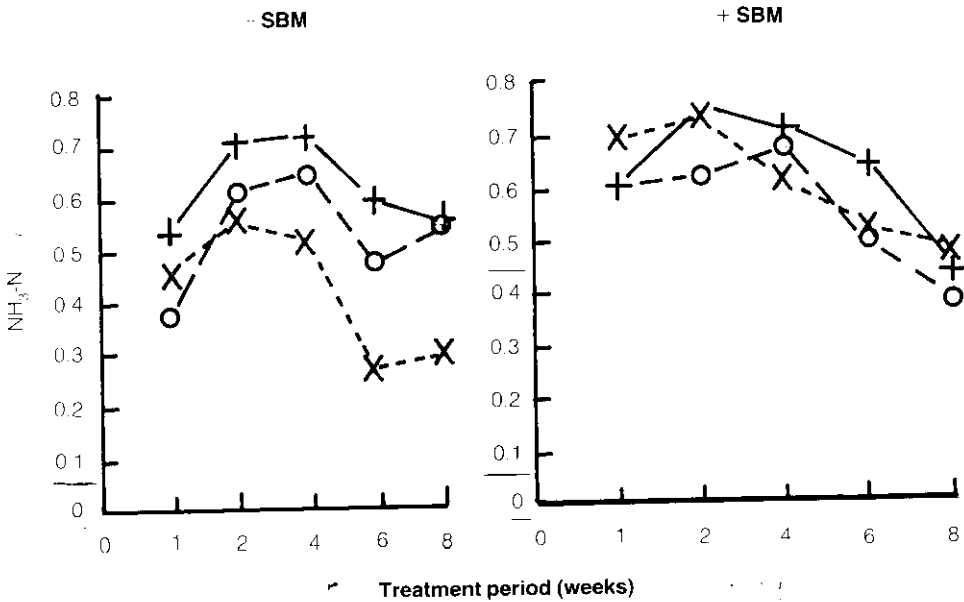


Fig. 2. NH₃-N content of urea amoniated wheat straw after various treatment periods at three temperatures 25°C (—○—), 35°C (—+—) and 45°C (... × ...) with or without SBM.

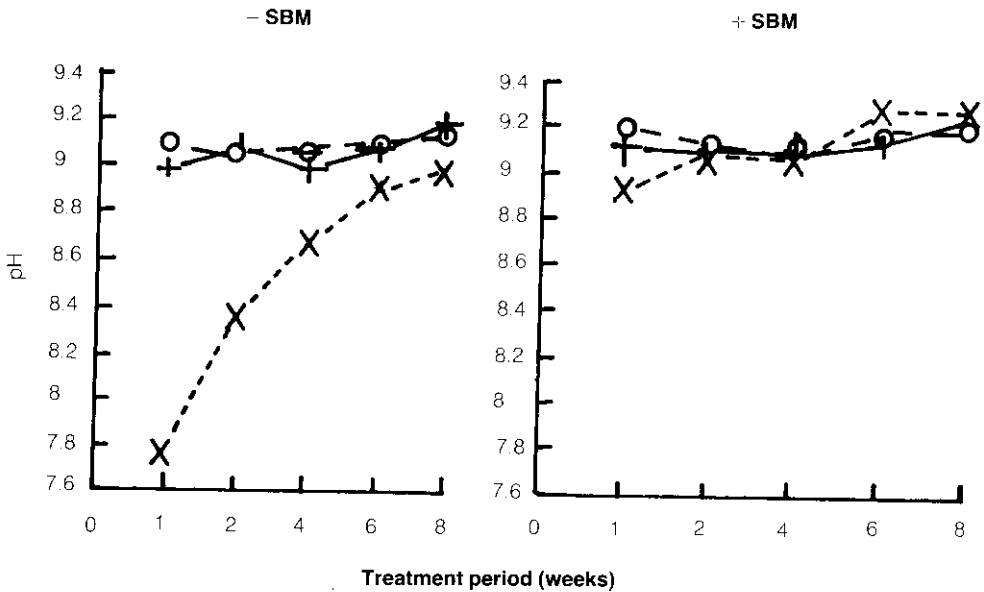


Fig. 3. pH of urea ammoniated wheat straw after various treatment periods at three temperatures 25°C (—○—), 35°C (—+—) and 45°C (... × ...) with or without SBM.

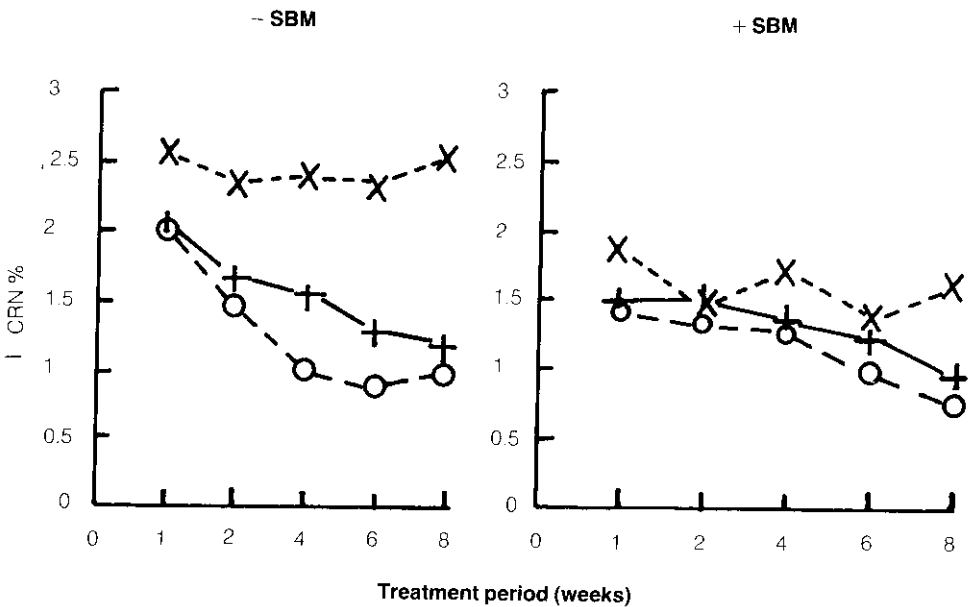


Fig. 4. CRN content of urea ammoniated wheat straw after various treatment periods at three temperatures 25°C (—○—), 35°C (—+—) and 45°C (... × ...) with or without SBM.

The CRN for treated straw was significantly higher ($P < 0.001$) at 35 and 45°C than at 25°C (Table 1, Fig. 4). This was mainly due to low urea hydrolysis to ammonia at 45°C as well as the increase of $\text{NH}_3\text{-N}$ and bound-N in samples treated at 35°C. Addition of SBM decreased CRN and urea content of the treated straw significantly ($P < 0.001$) due to rapid release of ammonia from urea (Table 1). Values of $\text{NH}_3\text{-N}$ for straw treated at 35 and 45°C with SBM, and at 35°C without SBM, tended to be higher than that of the straw kept at 25°C (Fig. 2). The depression of $\text{NH}_3\text{-N}$ content of the samples treated at 45°C, without SBM, was apparently due to low urea hydrolysis at this temperature. Values of bound-N were significantly higher ($P < 0.01$) for straw treated at higher temperature (35 and 45°C) than at 25°C, while no significant ($P < 0.05$) difference was observed between samples kept at 35 and 45°C (Table 1).

The above results are concordant with the findings of Cloete and Kritzing [12] who reported that high temperature (35°C) decreased the hydrolysis of urea to ammonia, and that free $\text{NH}_3\text{-N}$ content of samples treated at 35°C tended to be higher than that of samples treated at 24°C. These authors suggested that the decrease in urea hydrolysis for samples ammoniated at 35°C was caused by a decline in urease activity, and that higher temperature catalyzed the binding of extractable ammonia to straw despite the release of less ammonia from urea. In our experiment, it appeared that high temperatures (35 and 45°C) also catalyzed the binding of $\text{NH}_3\text{-N}$ and bound N to straw.

The results obtained at 25°C (without SBM) agree with the findings of Solaiman *et al.* [20] on straw treated with ammonium hydroxide, and with those of Ibrahim and Pearce [21], Cloete and Kritzing [12] and Dias-da-Silva and Sundstol [6] on straw treated with urea. Total N content of 1.57% obtained in the present study after 4 weeks of treatment also agreed closely with the results of Ibrahim and Pearce [21] who reported a TN value of 1.60% in barley straw after 28 days of treatment with urea (80 g kg^{-1}) at a moisture level of 1000 g kg^{-1} straw. In the present study, $\text{NH}_3\text{-N}$ content increased from 0.01 in the original samples to 0.37 and 0.54% in the treated samples after 1 and 8 weeks of treatment period, respectively. Similar results have been reported previously by Cloete and Kritzing [12] who recorded an increase in free $\text{NH}_3\text{-N}$ content of wheat straw treated with 7.5% urea at a moisture level of 375 g kg^{-1} straw from 0.03 to 0.37 and 0.44% after 0.1 and 8 weeks of treatment period, respectively. Dias-da-Silva and Sundstol [6] found that ammoniation of wheat straw by 4% urea at a moisture level of 400 g kg^{-1} straw gave TN and $\text{NH}_3\text{-N}$ values of 1.45 and 0.58%, respectively, after a treatment period of 60 days. Corresponding values in the present study were 1.55 and 0.54%, respectively, after an 8 weeks treatment period.

Cell wall constituents and IVOMD

The effect of temperature, addition of SBM and treatment periods on cell wall constituents and IVOMD of urea treated wheat straw are shown in Table 2. The table also summarizes the significance of relevant interactions. Results of HC, CEL and IVOMD as three factor interactions between temperature, SBM addition and treatment period are presented in Figs. 5-7, respectively.

The results indicated that all of the dependent variables were affected significantly ($P < 0.01$) by temperature, SBM addition and treatment period, except that of AIA was non significantly ($P > 0.05$) affected by SBM addition.

Ammoniation is known to produce a marked improvement in the nutritional value of treated material by solubilizing the hemicellulose fraction as well as by swelling the cellulose moiety, thus improving fiber flexibility and dry matter digestibility [22]. In the present study, NDF content decreased significantly ($P < 0.01$) with increased temperature and treatment period. This decrease was mainly due to a significant ($P < 0.01$) decrease in HC content (Table 2, Fig. 5), which, along with the reduction in NDF, produced a proportional increase ($P < 0.01$) in ADF, and CEL contents (Table 2, Fig. 6). On the other hand, lignin was increased significantly ($P < 0.01$) with increased temperature, and decreased significantly ($P < 0.01$) with increased treatment period, while all cell wall constituents except AIA were decreased significantly ($P < 0.01$) with SBM addition (Table 2). The changes observed in cell wall composition in this study agree with the findings of Dias-da-Silva and Sundstol [6] and Mascarenhas-Ferrera *et al.* [8] for urea treated straw and the findings of Given *et al.* [23] and Mason *et al.* [24] for straw treated with ammonia.

The changes in cell wall structure and N retained in treated straw in this study provide further support for the increased IVOMD values. The latter increased significantly ($P < 0.01$) with increased temperature, SBM addition and longer treatment period (Table 2). Overall IVOMD mean values for treated straw with or without SBM addition were 72.8 vs 66.0%, respectively. These values were higher than untreated (original) straw (58.8%) by 14.0 and 7.2% digestibility units, respectively.

Urea treated straw (without SBM) for 1,2,4 and 6 weeks at 35°C, and for 4 and 6 weeks at 45°C; and all treated samples at 35 and 45°C (with SBM), showed higher IVOMD values than samples kept at 25°C. Thus, ammoniation appeared to be faster and more effective at higher temperatures, especially with SBM addition (Fig. 7).

The present results suggest that the addition of SBM increased IVOMD significantly ($P < 0.01$) and reduced the treatment period from 8 to 2 weeks. Hence, the

Table 2. The effects of temperature, soybean meal (SBM) and treatment period on neutral detergent fiber (NDF), acid detergent fiber (ADF), hemicellulose (HC), cellulose (CEL), lignin (LIG), acid insoluble ash (AIA) and *in vitro* organic matter digestibility (IVOMD) of urea ammoniated wheat straw

Dependent Variables	Temperature (T)			SBM (S)		Periods, wks (P)					Level of significance				
	25°C	35°C	45°C	(-)	(+)	1	2	4	6	8	SEM	T×S	T×P	S×P	T×S×P
g/100g ⁻¹ DM															
NDF	71.2 ^A	69.9 ^{Ba}	68.8 ^{Ch}	72.3 ^A	67.4 ^B	70.2 ^{ABb}	71.0 ^{Aa}	70.1 ^{ABbc}	68.7 ^C	69.3 ^{BCc}	0.299	NS	**	***	*
ADF	49.7 ^C	50.8 ^B	51.9 ^A	52.6 ^A	49.0 ^B	48.7 ^D	49.3 ^C	51.6 ^B	51.8 ^B	52.6 ^A	0.253	*	***	*	NS
HC	21.5 ^A	18.8 ^B	17.0 ^C	19.7 ^A	18.5 ^B	21.5 ^A	21.7 ^A	18.5 ^B	16.9 ^C	16.6 ^C	0.309	***	***	***	***
CEL	39.5 ^B	39.9 ^{AB}	40.3 ^A	41.5 ^A	38.3 ^B	37.5 ^D	37.9 ^D	40.3 ^C	41.4 ^B	42.3 ^A	0.257	NS	***	NS	NS
LIG	6.84 ^B	7.01 ^B	7.68 ^A	7.41 ^A	6.94 ^B	7.83 ^A	7.78 ^A	7.44 ^B	6.39 ^C	6.44 ^C	0.095	NS	***	*	NS
AIA	3.57 ^B	3.97 ^A	3.89 ^A	3.82 ^A	3.80 ^A	3.45 ^{Ch}	3.68 ^{BC}	3.77 ^{BCa}	3.97 ^{AB}	4.18 ^A	0.061	NS	***	*	NS
IVOMD	66.8 ^B	71.0 ^A	70.3 ^A	66.0 ^B	72.8 ^A	64.5 ^C	66.7 ^B	71.0 ^A	72.3 ^A	72.3 ^A	0.537	***	*	*	NS

^{A,B,C,D,a,b,c} Means within a temperature, SBM and periods with different lower case superscripts differ significantly at ($P < 0.05$) and those with different capital superscripts differ significantly at ($P < 0.01$).

***Significant at 0.1%, **Significant at 1%, *Significant at 5% and ^{NS}Non Significant at 5%.

The IVOIMD of untreated (original) wheat straw, (58.8%).

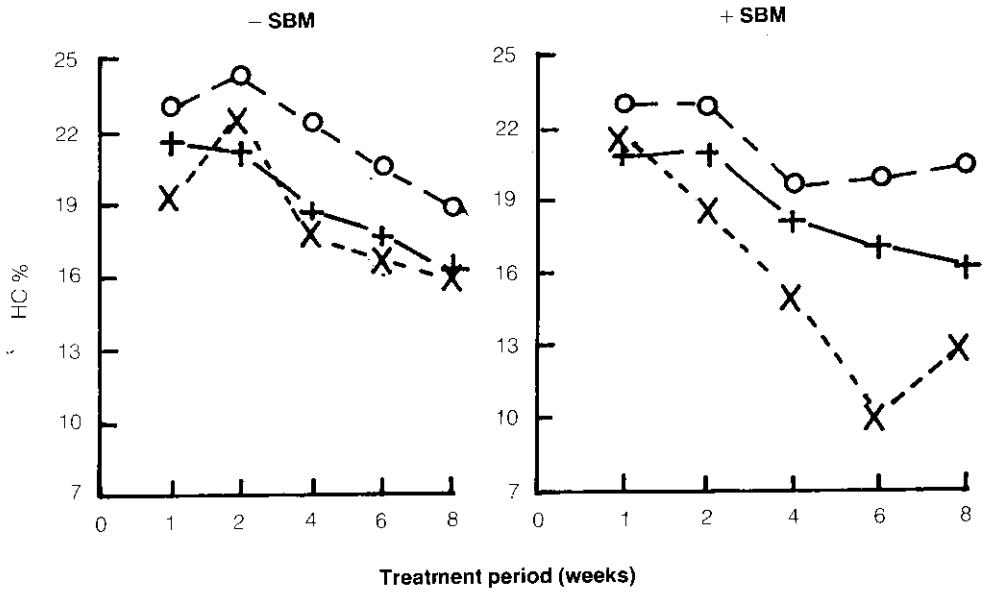


Fig. 5. Hemicellulose content of urea ammoniated wheat straw after various treatment periods at three temperatures 25°C (-o-), 35°C (-+-) and 45°C (... x ...) with or without SBM.

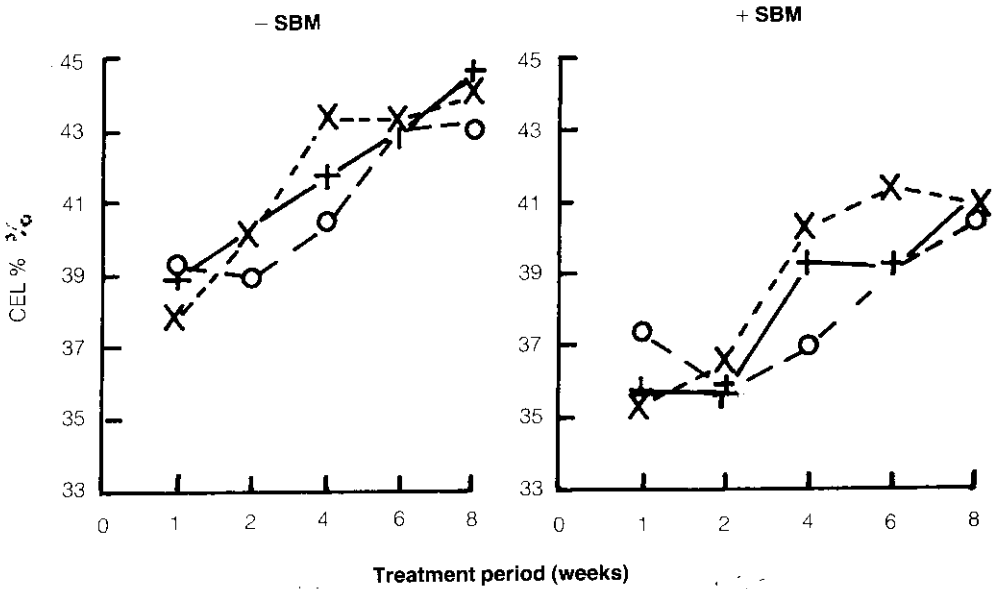


Fig. 6. Cellulose content of urea ammoniated wheat straw after various treatment periods at three temperatures 25°C (-o-), 35°C (-+-) and 45°C (... x ...) with or without SBM.

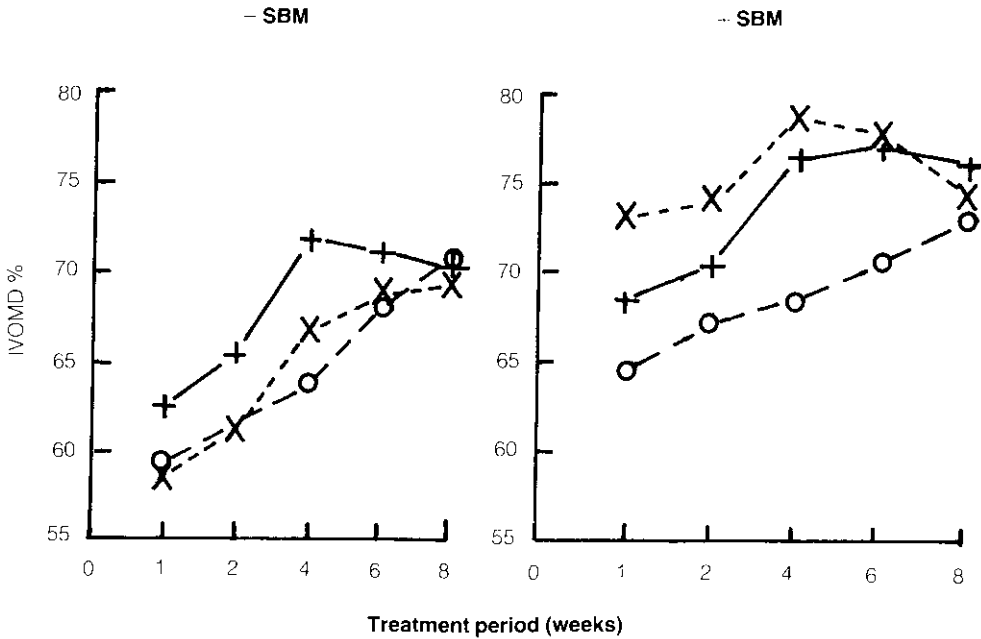


Fig. 7. IVOMD of urea ammoniated wheat straw after various treatment periods at three temperatures 25°C (-o-), 35°C (-+-) and 45°C (... × ...) with or without SBM.

mean IVOMD value of treated straw with SBM for 2 weeks was comparable to that of treated straw without SBM for 8 weeks (70.6 vs. 70.2%, respectively). These results are in line with the findings of others [11,25], who demonstrated that the addition of exogenous sources of urease has the advantage of reducing the treatment time required to achieve a given level of digestibility in rice straw. Although the urease activity of the SBM was not determined in the present study, the results clearly indicated that SBM can serve as a source of urease for hydrolysis of urea to ammonia, which in turn improves the nutritive value of wheat straw. Additional benefits include the supply of protein and readily available carbohydrate to the animals.

From these data, it is concluded that ammoniation of straw through urea in the presence of SBM, especially at high temperatures, could well be an effective means for improving the nutritional value of wheat straw, and that SBM addition increases the IVOMD by 14% units over untreated wheat straw and decreases the treatment time from 8 to 2 weeks.

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تأثير الحرارة وإضافة مسحوق فول الصويا ومدة المعاملة على القيمة الغذائية لتبن القمح المعامل باليوريا

منصور سعد الكريديس

قسم الإنتاج الحيواني، كلية الزراعة، جامعة الملك سعود،

الرياض، المملكة العربية السعودية

ملخص البحث . تمت دراسة تأثير درجة الحرارة، إضافة مسحوق فول الصويا ومدة المعاملة على تبن القمح المعامل باليوريا (٦٠ جم يوريا/كجم تبن) في تصميم $3 \times 2 \times 5$. اشتملت العوامل المستقلة على درجات الحرارة ٢٥، ٣٥، أو ٤٥°م مع إضافة مسحوق فول الصويا (٧٠ جم/كجم تبن) أو بدونه وبلد معاملة أطوالها ١، ٢، ٤، ٦، ٨ أسابيع، أما العوامل التابعة المقاسة فاشتملت على قياس اليوريا غير المحللة، أجزاء النيتروجين، الأس الهيدروجيني، مكونات جدار الخلية ومعامل الهضم المعمل للمادة العضوية .

انخفضت اليوريا غير المحللة معنوياً ($P < 0.001$) بإضافة مسحوق فول الصويا وبطول مدة المعاملة وأيضاً في العينات المحفوظة على درجتى الحرارة ٢٥ و ٣٥°م بالمقارنة مع العينات المحفوظة على ٤٥°م، لأن هناك تحولاً قليلاً من اليوريا إلى أمونيا في العينات المعاملة على درجة حرارة ٤٥°م فقط (بدون إضافة مسحوق فول الصويا) . أدت المعاملة باليوريا إلى تغيرات كبيرة في التركيب الكيميائي لجدر الخلايا، حيث انخفضت المحتويات من الألياف NDF والهيميسليلولوز بدرجة معنوية ($P < 0.01$) في حين زاد محتوى الألياف ADF والسليولوز بدرجة معنوية ($P < 0.01$) بزيادة درجة الحرارة ومدة المعاملة . تحسن معامل الهضم المعمل للمادة العضوية بدرجة معنوية ($P < 0.01$) متأثراً بالعوامل المستقلة المبحوثة كلها . كان متوسط قيمة معامل الهضم المعمل للمادة العضوية للتبن المعامل مع إضافة مسحوق فول الصويا لمدة أسبوعين متتالاً مع التبن المعامل بدون إضافة مسحوق الصويا لمدة ٨ أسابيع (٦، ٧٠٪ مقابل ٢، ٧٠٪ على التوالي) . كان المتوسط العام لقيمة معامل الهضم المعمل للمادة العضوية للتبن المعامل مع إضافة مسحوق فول الصويا أو بدونه ٧٢، ٨ مقابل ٦٦، ٠٪ على التوالي أعلى عن عينات التبن الأصلية غير المعاملة (٨، ٥٨٪) بمقدار ١٤، ٠، ٢، ٧٪ وحدة هضمية على التوالي . نستنتج من هذه الدراسة أن معاملة تبن القمح باليوريا يمثل وسيلة فعالة لتحسين قيمته الغذائية وخصوصاً تحت درجة الحرارة العالية ومع إضافة مسحوق فول الصويا كمصدر لأنزيم اليوريز .