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Note

Nutritional metabolism of hydrogen peroxide/ anhydrous ammonia-treated barley straw in ewe lambs

Mohammed DIOURI*, Randall D. WIEDMEIER

Skaggs Nutrition Research Laboratory, USU, Logan, UT 84322-4815, USA

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Abstract — A control and three treatments of barley straw were compared: NH_3 (3% of dry matter (DM)), 3% NH_3 after rehydration of the straw to 15% moisture with water, and 3% NH_3 after rehydration to 15% moisture with a H_2O_2 solution (0.32% of DM). Forages were fed to fistulated ewe lambs, with a supplement, at two levels of intake (ad libitum and 75% ad libitum). At the ad libitum intake, the treatments mainly improved the intake; and digestible DM intake was 336, 455, 501, and 552 grams per head per day for untreated, NH_3, $NH_3 + H_2O$, and $NH_3 + H_2O_2$-treated straws, respectively. The first, second, and fourth values were different from each other ($P < 0.01$), but the third one was not different from the fourth ($P = 0.09$). A similar trend was observed in most other nutrient utilization parameters and fermentation characteristics. Intake restriction raised DM digestibility from 56.9% to 58.2% ($P = 0.04$). Anhydrous ammonia cannot provide alkalinity for the optimal action of hydrogen peroxide.

straw / treatment / ammonia / hydrogen peroxide

Résumé — **Métabolisme alimentaire de la paille d'orge traitée à l'ammoniac anhydre et/ou à l'eau oxygénée chez les agnelles.** Un témoin et trois traitements de paille d'orge : NH_3 (3 % MS), 3 % NH_3 après réhydratation de la paille à 15 % d'humidité avec de l'eau, et 3 % NH_3 après réhydratation à 15 % d'humidité avec une solution de H_2O_2 (0,32 % MS) ont été comparés. Ces pailles ont été distribuées, à 8 agnelles fistulées, à deux niveaux d'ingestion (ad libitum et 75 % ad libitum), selon un dispositif en carré latin. Lorsque l'ingestion était à volonté, les traitements ont agi principalement sur l'ingestibilité, et la MS digestible a été de 336, 455, 501, et 552 grammes par tête par jour pour le témoin, les pailles traitées à NH_3, $NH_3 + H_2O$, et $NH_3 + H_2O_2$ respectivement. Les première, deuxième, et quatrième valeurs ont été significativement différentes l'une de l'autre ($P < 0{,}01$) ; alors que la troisième ne l'a pas été de la quatrième ($P = 0{,}09$). Une tendance semblable a été observée pour la plupart des autres paramètres de digestion. La digestibilité de la MS est passée, en moyenne, de 56,9 % à 58,2 % avec la restriction de l'ingestion ($P = 0{,}04$). L'ammoniac ne peut pas fournir une alcalinité suffisante pour une action optimale de l'eau oxygénée.

paille / traitement / ammoniac / eau oxygénée

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* Correspondence and reprints
Université Moulay Ismaïl, Faculté des sciences, Département de Biologie, BP 4010, Béni M'hamed Meknès, Morocco.
Tel.: 212 5 53 88 70; fax: 212 5 53 68 08; e-mail: diouri@fsmek.ac.ma

1. INTRODUCTION

Anhydrous ammonia (NH_3) treatment of cereal straws has proven to be successful for several reasons. Its alkalinity makes cellulose and hemicellulose more available by partially dissolving them and by breaking the bonds between them and lignin. NH_3 also supplements the straw with nitrogen, although only part of this added nitrogen is available to the animal [12].

Hydrogen peroxide (H_2O_2) is another chemical compound that has greatly improved the utilization of different low-quality forages. This oxidizing agent acts optimally in alkaline medium [4].

Sodium hydroxide (NaOH) has been used to provide alkalinity for H_2O_2 [7], but NaOH has the potential of sodium toxicity. Replacing it by NH_3 was expected not only to remedy this disadvantage but also to enhance the beneficial effects of NH_3. The main objective of this study was to determine the impact of the combined NH_3/H_2O_2 treatment on straw nutritive value and the possible interaction or additivity of the two treatment components.

2. MATERIALS AND METHODS

2.1. Treatments and animals

Four different treatments were compared:

(1) Control, (2) 3% NH_3, (3) 3% NH_3 after rehydration of straw to 15% moisture with water, (4) 3% NH_3 after rehydration of straw to 15% moisture with a H_2O_2 solution (to reach a level of 0.32% DM of H_2O_2).

Treatment 3 was used to isolate the effect of H_2O_2 and to confirm the results of former studies reporting the positive effect of moisture on straw ammoniation [8].

Ten 23 kg straw bales were randomly assigned to each of the four treatments. Ten bales were treated with enough water to bring the moisture level to 15%. Ten bales were treated with a H_2O_2 solution to rehydrate to 15% moisture. All four stacks were then placed in separately sealed 6 mm plastic bags. NH_3 was then introduced in the gaseous form into the appropriate bags through a perforated pipe at 3% DM. After ammoniation, the bags were sealed for approximately 21 days (during a moderately cold autumn) and then opened to allow excess ammonia to escape. Straw was then ground and mixed.

Eight yearling ewe lambs (30 to 51 kg), fitted with ruminal fistulae, were used. The ewes were from three different breeds (4 Navajos, 2 Columbias, and 2 Blackfaced).

After proper recovery from fistulation and adaptation to a straw-based diet, sheep were placed in elevated metabolism crates.

Treatments were administered in a split plot with a 4*4 Latin square design with repeated measures. Each of the 4 trial periods was composed of two subperiods. In the first subperiod, sheep had ad libitum access to straw in order to determine the intake. A supplement (whose nutrient composition is presented in Tab. I), composed of ground faba bean (*Vicia faba*) and fortified with vitamins and minerals as needed to meet nutrient requirements [13], was top dressed. The supplement intake was gradually adjusted to represent 25% of the ration in order to eliminate the possible negative associative effect between concentrate and roughage. Daily rations were given in 2 equal portions at 08.00 and 16.00 h. Diets were fed for a 14 d adaptation period followed by a 7 d collection period.

In the second subperiod, consisting of 5 d adaptation and 5 d collection periods, the sheep received the same diet but their ration was limited to 75% of each animal's ad libitum intake.

2.2. Measurements and analyses

During d 1 to 5 of the collection periods, total fecal output was collected and weighed

Table I. Chemical composition of different treated straws and of the faba bean supplement (at the time of feeding).

Nutrients	Control	NH_3	$NH_3 + H_2O$	$NH_3 + H_2O_2$	Suppl.
DM	93.2	92.7	92.3	92.1	90.3
	% DM basis				
OM	92.4	92.1	92.5	92.3	96
CP	3	7.2	7.8	8.1	21.1
Cellulose[1]	45.6	45.9	46.3	45.9	11.2
Hemicellulose[2]	27.8	24.9	23.4	23.5	19
Lignin	8.4	7.7	7.6	7.2	2.2

[1] ADF-ADL.
[2] NDF-ADF.

twice daily at the normal feeding times. Fecal samples were dried at 60 °C for 72 h and then ground and passed through a 2 mm screen. Composites of fecal samples, within ewe and period, were then made.

Feed (straw and supplement) and refusal were weighed at each feeding. Starting 1 d before the beginning of the collection periods, samples of feed and refusals were taken at each feeding. These samples were ground and passed through a 1 mm screen and proportional composites were made.

All feed, ort, and fecal composite samples were analyzed for laboratory dry matter (DM) (105 °C for 8 h), OM [1], CP [5], ADF, NDF, and ADL [14].

Rumen fluid samples were taken on days 6 and 7 of the ad libitum collection periods at 0, 2, 4, 6, 8, 12, and 16 h after the 16.00 h feeding of day 6. Volatile fatty acids (VFA), ammonia nitrogen (NH_3-N), and pH were measured. pH was taken immediately. Ammonia nitrogen was determined spectrophotometrically using Nessler's reagent [5]. Volatile fatty acids were determined by gas chromatography.

Data were analyzed using a general linear model including period, straw treatment, and sheep. Subperiod was later included in the model to test the intake level effect. The least significant difference (LSD) multiple mean comparison was applied to the variables studied.

3. RESULTS AND DISCUSSION

The nutrient composition of the four straws and of the supplement is presented in Table I. Crude protein was drastically increased by ammoniation, agreeing with Chestnut et al. [2]. The alkaline attack of ammonia on the ester bonds, linking lignin with cellulose and hemicellulose, supplied the straw with nitrogen. This alkaline attack also affected lignin and hemicellulose by partially dissolving them. The positive effects of ammoniation were further increased by rehydration of the straw. This was probably due to the phenomenon of fiber swelling [3], making the fibers more available to NH_3. The presence of water increases the homogeneity of the reaction and provides an aqueous solution required by most chemical reactions. Another positive effect, (namely a decrease in forage lignin content) was added by H_2O_2 treatment probably because of the oxidizing ability of this component.

Cellulose content was not affected by the treatments. Nevertheless, digestible cellulose intake was increased by each additional treatment component. This may mean that cellulose became more available to the rumen microorganisms.

Since many animals could not stand the limited intake and did not go through the second subperiod, only the ad libitum intake values were used for treatment comparison.

At the ad libitum intake, digestible DMI was increased by ammoniation ($P = 0.0006$), by rehydration ($P = 0.12$), and by H_2O_2 ($P = 0.09$). Comparing NH_3 with $NH_3 + H_2O_2$ reveals a highly significant difference ($P = 0.003$). These three treatment components had similar effects on most nutrient utilization parameters (Table II). Although the DM digestibility was slightly increased, the improvement of digestible DMI was primarily obtained by an increase of the DMI. This increase in DMI was reported to be due to an increase in the rate and extent of fiber digestion [2]. The fermentation characteristics (Tab. II) were not improved enough (especially among the three treated straws) to allow a large increase in DM digestibility. The same pattern of influence of ammoniation was reported by Llamas-Lamas and Combs [10]. These authors obtained, however, a slightly higher improvement in DM digestibility than we did. This may be because they used wheat straw, which is more responsive to ammoniation than barley straw [6].

Rumen ammonia nitrogen and total VFA (Tab. II) confirmed the effect of ammoniation and that of rehydration. Propionate concentration was not affected by any of the treatment components. This disagrees with Males and Gaskins [11], who noted a higher propionate concentration when ammoniated straw was fed.

The trend of the response of DM digestibility (Tab. III) and of other digestion variables (data not shown) to the treatments was similar at limited intake.

Overall, limiting the intake enhanced DM digestibility ($P = 0.04$) and digestibility of other nutrients. This improvement was, however, minute. Similar findings have been reported by Llamas-Lamas and Combs [10].

In our study, as well as in many others [9], forage was ammoniated for 21 days. Since ambient temperature was low, this treatment time should have been lengthened in order to get better results for ammoniation.

Table II. Effect of straw treatments on nutrient utilization and fermentation characteristics at the ad libitum intake level.

Item[1]	Control	NH_3	$NH_3 + H_2O$	$NH_3 + H_2O_2$	SE[2]
DMI ($g \cdot head^{-1} \cdot d^{-1}$)	630^b	811^c	869^{cd}	936^d	32.2
DM digestibility (%)	53.7^b	56.3^c	57.9^{cd}	58.9^d	0.64
Digestible DMI ($g \cdot head^{-1} \cdot d^{-1}$)	337^b	455^c	501^{cd}	552^d	21.3
CP digestibility (%)	57.8^b	58.5^b	59.0^b	61.2^b	1.84
Cellulose digestibility (%)	57.4^b	61.5^c	61.8^c	62.9^c	1.2
Hemicellulose digestibility (%)	52.0^b	63.7^c	70.1^d	72.8^d	1.53
pH	6.82^b	6.85^b	6.84^b	6.81^b	0.04
NH_3-N ($mg \cdot dl^{-1}$)	13.4^b	19.8^c	21.8^d	19.9^c	0.6
Total VFA (mM)	57.7^b	65^c	74.6^d	71.5^{cd}	2.5
Ac ($mol \cdot 100\ mol^{-1}$)	71.1^b	73.7^c	72.9^c	72.9^c	0.5
Pr ($mol \cdot 100\ mol^{-1}$)	17.5^b	16.7^b	16.9^b	16.7^b	0.4

[1] DMI: dry matter intake, VFA: volatile fatty acids, Ac: acetate, Pr: propionate.
[2] Standard error of the LS means.
[b, c, d] Means in a row lacking a common superscript differ ($P < 0.05$).

Table III. Effect of 75% limited intake on DM digestibility of treated straws.

	Ad-libitum intake		75% limited intake		SE[3]	$P > F$
	n[1]	DMD[2] (%)	n[1]	DMD[2] (%)		
Control	6	53.6	6	53.7	0.88	0.94
NH_3	8	56.3	8	58.5	0.74	0.04
$NH_3 + H_2O$	5	59.0	5	61.3	0.97	0.095
$NH_3 + H_2O_2$	6	59.1	6	59.7	0.88	0.63
Average	25	56.9	25	58.2	0.43	0.04

[1] Number of observations (animals who went through the two intake levels).
[2] Dry matter digestibility.
[3] Standard error of the LS means.

The effect of H_2O_2 was always numerically apparent but not significant. The treatment procedure may have been the reason for that: NH_3, which was supposed to raise the pH for an optimal action of H_2O_2, was introduced after H_2O_2.

4. CONCLUSION

Comparing our results with those of other studies using alkaline hydrogen peroxide, we can conclude that anhydrous ammonia cannot totally replace sodium hydroxide in providing alkalinity for hydrogen peroxide.

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