EFFECT OF MOLDY AND NONMOLDY WHEAT STRAW TREATED WITH OR WITHOUT AMMONIA ON PERFORMANCE AND BLOOD SERUM CONSTITUENTS IN STEERS

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Summary.

Mold growth decreased nutritive value of wheat straw (WS). Mold increased DM (94 vs 98%) and ADF (51 vs 56%) contents and had no effect on natural detergent fiber (NDF). Crude protein and N values were decreased in moldy wheat straw. Mold increased insoluble N content of wheat straw (WS) from 21 to 27%. Ammoniation increased the CP of nonmoldy straw from 3.8 to 8.3% and moldy straw from (3.3 to 6.2%). Aspergillus and zygomycetes fungal species were most prevalent and total numbers were higher on moldy WS increased (p < 0.10) feed intake (1.8%) as compared with nonmoldy, ammoniated, nonmoldy and moldy WS. Steers fed moldy WS had lowest (p < 0.10) feed intake (1.8%) of BW daily) compared with other dict. There was little difference (p < 0.10) in intake of nonammoniated vs. ammoniated WS. Steers fed moldy straw lost 6 kg BW. Ammoniated, nonmoldy straw elevated Blood Urea Nitrogen (BUN) (10.5 mg/dl). Alkaline Phosphatase (ALK) was greater in steers fed moldy VS nonmoldy straw (148 VS 95 U/liter, p < 0.10)

(Key Words: Steers, Wheat Straw, Mold, Ammoniation, Serum Enzymes)

Introduction

In most developing countries and particularly in Asia, large quantities of low-quality straws and other crop by-products are produced annually. Straws and other low-quality roughages are basic feed ingredients in livestock diets and may account for up to 70 to 80% of total feed. In most underdeveloped countries, these residues are often contaminated with mold because of inadequate storage facilities and insufficient protection from weather. Most of these low-quality roughages are characterized by low-protein, highly indigestible cell wall content and low available energy content, resulting in depressed feed intake as a result of increased ruminal retention time. Intake of such roughages connot supply sufficient nutrients to meet the needs of growing ruminants and may not meet maintenance requirement (Males, 1987). Several chemicals such as hydroxides of sodium, potassium, calcium and ammonium or urea have been used

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frequently to improve the feeding value of lowquality roughages (Klopfenstein, 1978). Treatment of low-quality straws with hydroxide or anhydrous ammonia consistently improved intake, digestibility and overall performance by cattle and sheep (Gates et al., 1987).

Anhydrous ammonia is more attractive for chemical treatment because of its low cost, lack of residual effect on soil and case of application to large quantities of roughages under farm conditions (Sundstol et al., 1978; Gates et al., 1987). Anhydrous animonia has been evaluated as a preservative for hay baled at high moisture levels (Knapp et al., 1974; Moore et al., 1985). Ammoniation has been extensively studied as a mean to reduce toxicity of grains and protein supplement such as cottonseed meal that have experienced growth of molds (Park et al., 1988), but similar research has not been done to determine whether ammoniation might alleviate adverse effects of moldy roughage feeds intended for use by ruminants. Objectives of the present study were to determine the effect of moldy and nonmoldy roughages on animal performance, Intake and serum metabolites and to evaluate the efficacy of ammoniation for alleviating detrimental effects associated with moldy straw.

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Materials and Methods

Wheat straw

Three hundred thirty bales weighing 1,350 kilograms of (WS) were divided into two equal stacks. One stack was saturated with water for 4 to 5 days by sprinkling water and mold growth was allowed (late October). After 15 d molds were present. Moldy WS was allowed to dry for 8 to 10 d. After drying of moldy WS, both moldy and nonmoldy WS were subdivided into two stacks. Both moldy and nonmoldy WS were placed in single stacks in the field under ambient temperature (mid-November). Covered with 2 mm block plastic sheat. All sides were covered with soil at ground level. Anhydrous ammonia (containig about 81 (10 N) was incorporated via a perforated 5 cm diameter iron pipe. Quantity of ammonia administered was calculated by flow rate at pressure indicated by pressure gauge fitted on the ammonia tank. Straw was left covered for 2 week, after which it was uncovered and unstacked for three days to allow excess free ammonia to escape. Samples of the resulting straws (nonmoldy straw; moldy straw: ammoniated nonmoldy resulting straws; ammoniated nonmoldystraw; and ammoniated, moldy straw) were analyzed for DM, N (AOAC, 1984), ADIN, ADF and NDF (Goering and Van Soest, 1975).

Animals

Sixteen Hereford and Hereford \times Angus steers (241 \pm 7 kg) were divided randomly into four groups and penned in four separate pens with four animals per pen. Steers were tethered individually and offered straw for 12 h, after which feed was removed and animals were released within their respective pens.

Treatments

Individual steers were allotted randomly to diets. Four animals received wheat straw (WS), four were fed moldy wheat straw (mold), four were fed ammoniated wheat straw (WS + NH₂) and the remaining four were fed ammoniated, moldy wheat straw (mold + NH₂). Feed was offered twice daily *ad libitum* at 06:00 and 14:00. Daily feed intake and orts were recorded. Animals were weighed individually before and after the experimental period (19 d).

Blood

Blood was collected from each steer via jugular venipuncture on d 0 and 19. Blood was collected into sterile serum separator tubes and serum was obtained. Serum samples were analyzed for constitutents.

Statistical analysis

Analyses of variance were conducted using GCM procedures of SAS (1982). Diet effects (moldy vs nonmoldy; ammoniated vs nonammoniated) were analyzed in 2×2 factorial arrangement. Feed intake expressed as percentage of BW and daily feed intake (kg) were subjected to split-plot analysis of variance for repeated measurements over time (Gill and Hafs, 1971). The 2 \times 2 factorial arrangement of treatments was included in the main plot and main effect treatment responses were tested using animal within mold \times NH_a as the testing term. When significant treatment interactions (mold \times NH₃) were not detected (p > 0.10), main effect means were evaluated. Serum constitutents and BW were analyzed by day as a completely random design with 2×2 factorial arrangement of treatments. When significant overall effects were detected, means were separated using the least significant difference method (Steel and Torrie, 1980).

Results and Discussion

Chemical composition

Ammonia effect

Chemical composition of WS is shown in table 1. Ammoniation did not change DM, OM and ADF contents of nonmoldy WS. Neutral detergent fiber content of WS was decreased by ammonia treatment as compared to nonammoniated WS. Most of the decrease of NDF content was seen in moldy WS (76 vs 68%) as compared to nonmoldy WS. Others have also reported similar results (Gates et al., 1987 and Mandell et al., 1988). Decreased NDF content is primarily due to solubilization of hemicullulose (Grother et al., 1985).

Crude protein and N contents of nonmoldy WS doubled as result of ammoniation. Increase in N content as result of ammoniation has been reported in previous studies by Moore et al. (1985), and Gates et al. (1987).

	Wheat straw						
Item ^a	Nonm	oldy	Moldy				
	Non-ammoniated	Ammoniated	Non-ammoniated	Ammoniated			
Dry matter ^b (%)	94	93	98	97			
Organic matter (%)	89	90	88	89			
Acid dergent fiber (%)	51	52	56	56			
Netral detergent fiber (%)	77	76	76	68			
Crude protein (%)	3.8	8.8	3.3	6.2			
Nitrogen (%)	0.6	1.4	0.5	1.0			
Acid detergent insoluble N	(%) 0.12	0.20	0.13	0.19			
ADIN ^c	21	14	27	19			

TABLE 1. CHEMICAL COMPOSITION OF MOLDY AND NONMOLDY WHEAT STRAW TREATED WITH OR WITHOUT AMMONIA

Presented on dry matter basis.

Percentage of air-dry straw.

Percentage of total N that is acid detergent insoluble.

Mold effect

Mold had profound effects on nutritive content of WS. Mold increased DM (94 vs 98%) and ADF (51 vs 56%) content of moldy WS but it had no effect on NDF content of nonmoldy WS (table 1). Gregory et al. (1963) and Tindall (1983) reported that crude fiber content of moldy materials was increased by mold growth. Mold decreased N (0.6 vs 0.5%) of WS but it was increased (0.5 vs 1.0%) by ammonia treatment (table f). Similar results have also been reported by Gregory et al. (1963) and Johnson et al. (1987) whereby mold growth decreased N content. These workers reported that higher proteolytic activity of microorganism in moldy materials resulted in low CP content. Ammoniation of moldy WS doubled the N (0.5 vs 1.0%) content (table 1). Ammoniation decreased acid detergent insoluble nitrogen (ADIN) content of moldy WS from 27 to 19%, which is mainly due to solublization of cell wall contents.

Molds

Mold species that were present on WS, ammoniated WS, moldy WS and ammoniated, moldy WS are shown in table 2. Zygomycete species (Mucor and Rhizopus) were most dominant in WS along with Aspergillus and Fusarium spp. These species were present in ammoniated WS and ammoniated, moldy WS, but were less abundant than in WS and moldy WS (table 2). Ammoniation treatment significantly decreased the spores of Mucor species in moldy WS from 119 to 12 (table 2). The second most prevalent species of fungal spores were Aspergillus. These results are similar to those of Gregory et al. (1963) and who reported that Mucor and Aspergillus species are dominant in moldy hays and grains. Many Aspergillus species were identified, but the aflatoxin forming fungus, A. flavus, was isolated only from the moldy WS (table 2). When counts of different fungi spores per g of sample were calculated, total fungi spores were 7,000, 2,600, 260,000 and 5,300 for WS, ammoniated WS, moldy WS and ammoniated, moldy WS, respectively. Ammoniation of WS decreased spore numbers by 269% and in moldy WS by 5,000%, indicating that ammoniation treatment has fungicidal effects. These results confirm previous studies (Sundstol et al., 1978 and Moore et al., 1985), in which ammonia treatment inhibited mold growth on treated materials.

Straw intake

Straw intake data for the trial are shown table 3. Split-plot analysis of variance showed a day \times treatment interaction (p > 0.10) and also analysis of variance showed mold \times ammonia interaction (p > 0.10), so simple effect means within days for each treatment were presented in table 3. Feed intake values of steers fed WS, ammoniated WS, moldy WS and ammoniated, moldy WS were 1.4, 1.5, 1.3 and 1.8% of BW, respectively.

	Wheat straw						
Name of fungi	Nonm	oldy	Moldy				
	Non-ammoniated	Ammoniated	Non-ammoniated	Ammoniated			
Aspergillus restrictus	18	6	ND	2			
Acremonium species	8	ND	ND	1			
Aspergillus terreus	5	0	22	7			
Penicillium species	4	ND	ND	I			
Alternaria species	4	1	ND	ND			
Mucor species	3	3	119	12			
Aspergillus fumigatus	3	ND	ND	ND			
Rhizopus specie	4	ND	ND	ND			
Aspergillus niger	1	ND	6	ND			
Aspergillus nudulans	0	1	11	0			
Aspergillus flavus	0	0	t	0			
Aspergillus species	ND	4	ND	4			
Scopulariopsis species	0	1	ND	5			
Total	41	16	159	32			
Total numbers/q	7,000	2,600	260,000	5,300			

TABLE 2. IDENTIFICATION AND COUNTS OF FUNG PRESENT IN MOLDY OR NONMOLDY WHEAT STRAW TREA-TED WITH OR WITHOUT AMMONIA®

ND Not detected.

TABLE 3. DAILY FEED INTAKE (PERCENTAGE OF BW) DURING 19 DAYS BY OF STEERS FED MOLDY OR NONMOLDY WHEAT STRAW TREATED WITH CR WITHOUT AMMONIA

	Straw treatment							
Item	Nonmoldy							
	Non-ammoniated	Animoniated	Non-ammoniated	Ammoniated	SE			
Number of steers	4	4	4	4				
Avg., BW (kg)	264	242	237	237	10.1			
Avg., feed intake (kg/head)	3.5 ^h	3.6 ^b	3.0 ^b	4.3°	0.12			
Percent BW, intake	1.4 ^b	1.5 ^b	1.3 ^b	1.8	0.05			

* Split-plot analyses of variance showed significant (p < 0.05) mold \times ammonia interactions, then overall means were reported.

^{bx} Values with different superscripts differ at (p < 0.10).

Steers fed moldy WS had the lowest (p < 0.10) feed intake (1.3%) compared with all other groups (table 3), whereas ammoniated, moldy WS had the greater (1.8% WS) feed intake, which was different (p < 0.10) that all other groups. Ammoniation of moldy WS increased daily feed intake about 43 and 23% over nonammoniated, moldy WS and nonammoniated, nonmoldy WS, respectively, (table 3). However, little difference in feed intake existed between nonammoniated WS and ammoniated WS. Similar results have been reported by Horton (1979) whereby straw intakes were similar for all treatments and not improved when ammoniated straw was fed. Overall, ammoniation increased straw intake. These results are similar to previous studies (Gates et al., 1987; and Mandell et al., 1988) in which ammonia treatment increased feed intake. Mandell et al. (1988) reported that lower intake of control straw was caused by lower CP content compared with ammonia-treated straw. Grother et al. (1985) reported that increases in rate and extent of cell wall digestibility are the major factors responsible for greater intake of ammonia-treated materials.

Animal performance

Body weight of steers fed the four types of WS are shown in table 4. Analysis of variance showed no mold \times ammonia interaction (p > 0.10); therefore, only main effect means were reported. Initial BW were similar for all treatment groups (table 4). Steers fed moldy WS lost 6 kg BW; whereas, steers fed nonmoldy WS gained 5 kg during 19 d (p < 0.05; table 4). Ammoniation had no effect on BW. These steers were fed WS, ammoniated WS, moldy WS or ammoniated, moldy WS as sole diets and no concentrate was supplemented. Diets were already low in N content (0.5 to 1.4%; table 1), which amounts to only 3.3 to 8.8% CP (table 1), and all diets except ammoniated nonmoldy WS failed to meet the beef cattle requirement (8 to 9.5% CP) to maximize microbial activity and fiber digestion (Wiedmeier et al., 1983). Another important factor affecting steer BW may have been ruminal fill, because these steers were brought from native range and then fed good quality alfalfa hay for 3 wk before onset of this experiment. It seems possible that animals lost some weight during the trial because of these earlier dietary changes. Mold growth decreased the energy content of hay as much as 25 to 30% (Jaques, 1988). In spite of ammonia treatment, animals fed moldy WS lost BW because no readily available energy source in the diet was provided. These results are similar to those of Coxworth et al. (1977), who found that ammoniated WS as sole feed did not meet maintenance requirements for wintering beef cattle.

TABLE 4. BODY WEIGHTS OF STEERS FED MOLDY OR NONMOLDY WHEAT STRAW TREATED WITH OR WITHOUT AMMONIA FOR 19 DAYS (MAIN EFFECTS)[®]

Item	Straw treatment						
	Moldy						
	No	Yes	No	Yes	SEb		
Number of steers	8	8	8	8			
Initial BW (kg)	241	240	241	240	7.2		
Final BW (kg)	246	234	242	239	7.2		
Average BW (kg)	244	237	242	240	7.2		
Change in BW (kg)	$+.5^{\circ}$	- 6 ^d	+1	- 1	2.1		

• Analysis of variance showed no mold \times ammonia interaction (p < 0.10); therefore, main effect means were reported.

^b Standard error.

 cd Within main effects (moldy or ammoniated), row values with different superscripts differ (p < 0.05).

Blood serum constituents

Pre-treatment levels of blood scrum constitutents were not substantially different among treatment groups, so average values are presented in table 5 and used as baseline. Blood serum consituents of steers after 19 d feeding also are shown in table 5. Analysis of variance showed no mold \times ammonia interactions (p < 0.10); therefore main effect means were reported. Serum glucose was increased (p < 0.10) by feeding ammoniated nonmoldy WS (71 vs 63 mg/dl), but ammoniation of moldy WS had no effect (p < 0.10) on glucose (69 vs 70 gm/dl table 5). Similarly, blood urea N and urea-N/creatinine showed a mold \times ammonia interaction (p < 0.10). Interactive means for urea N were 3.8, 10 5, 2.5 and 2.3 mg/dl for WS, ammoniated WS, moldy WS and ammoniated, moldy WS, respectively. Greater levels of blood urea N in steers fed ammoniated WS was the result of greater N intake (table 1). Ammoniation did not increase blood urea N in steers fed moldy. WS (2.3 vs 2.5 mg/dl; (p > 0.10); table 5, perhaps because of higher indigestible cell wall content in moldy WS, ammoniation could not increase N solubility of the ammoniated, moldy WS in the rumen. Albumin was higher in steers fed moldy WS (3.9 vs 3.7; p < 0.10) than in those fed nonmoldy WS. Alkaline phosphatase (ALK) was greater (p < 0.10) in steers fed moldy WS (148) vs 95 U/liter) than in those fed nonmoldy WS: but Creatine Phosphokinase Aspartate (CPK), Spartic Aminic transfer (AST) and (ALT) activities were greater in nonmoldy WS than moldy WS (table 5). Norred (1982) and Brownie and Brownie (1988) reported increased activity of ALK and AST with no change in ALT in rats experiencing aflatoxicosis. In terms of main effects means (table 5), there was no difference (p < 0.10) in gamma glutamyl transpeptidase γ GTP and electrolyte constituents for steers fed nonmoldy and moldy WS. Ammoniated WS decreased (p < 0.10) activities of CPK, Lactic dehydrogenase (LDH) and AST (170 vs 295; 1.107 vs 1.329 \pm 56 and 53 vs 71 \pm 5.3 U/liter, respectively) compared with nonammoniated WS (table 5).

Straw did not exhibit mild hepatotoxicosis related to moldy roughage; in fact, all enzyme levels were within normal ranges for cattle (Fraser, 1986). Lack of evidences of toxicosis in steers might be a result of lower straw intake by steers, as in table 3 (i.e., low consumption of mycotoxins). Another reason might be that straw was molded in late October (1987) when nighttime temperatures and humidity were low, thus resulting in decreased mold growth. Results of this trial indicate that mold decreased the nutritive quality of straw, Ammoni ation of moldy straw alleviated adverse effects of mold in terms of nutritive quality, and feed and intake. Changes in serum enzymes were observed that suggested slight mycotoxicosis; however, ammoniation of moldy roughages did not alleviate this effect of moldy roughage.

TABLE 5. BLOOD SERUM CONSTITUENTS IN STEERS BEFORE AND AFTER CONSUMING FOR 19 DAYS MOLDY CR NONMOLDY STRAW TREATED WITH OR WITHOUT AMMONIA?

Constituents	Рте-		Mold	Mold		Ammonia		
	treatment	SDb	No	Yes	No	Yes	SEc	
Number of steers	16		8	8	8	8		
Glucose (mg/dl)	74	6.6	70 ^d	63°	71 ^d	69 ^e	2.0	
Cholesterol (mg/di)	99	2.1	70	74	8 4 ^d	60 ^e	7.5	
Triglycerides (mg/dl)	24	10.4	19	21	22	18	7.5	
Urca N (mg/dl)	21	0.6	2.5 ^d	2.3 ^d	3.84	10.5 ^e	0.5	
Creatinine (mg/dl)	1.3	0.8	2.1 ^d	1.9 ^e	2.1 ^d	1.9 ^e	0.07	
Uric acid (mg/dl)	0.6	0.1	0.7	0.7	0.7	0.7	0.02	
Albumin (g/dl)	3.3	0.9	3.7ª	3.9 ^e	3.8	3.7	0.06	
Globulin (g/dl)	2.6	0.5	2.6	2.5	2.5	2.5	0.11	
Total protein (g/dl)	6.1	0.6	6.1	6.4	6.2	6.3	0.13	
Alkaline phosphatase (U/liter)	1.9	44.4	95 ^d	148 ^e	108	134	14	
Creatine phosphokinase (U/liter)	262	115	284	181	295ª	170 ^e	43	
γ-glutamyl transpeptidase (U/liter)	13	4	16	15	15	16	1.4	
Lactic dehydrogenase (U/liter)	1,097	299	1,261	1,176	1,329d	1,107°	56	
Aspartate aminotransferase (U/liter)	62	16	71ª	53e	71ª	53e	5.3	
Alanine aminotransferase (U/liter)	23	7	23 ^d	19 ^e	194	23 ^e	1.1	

ⁿ Analysis of variance showed no mold \times ammonia interactions (p > 0.10);therefore, main effect means were reported

^a Standard deviation of 16 observations.

^c Standard error (n = 8).

 $^{+*}$ Row values, within each main effect treatment, with different superscripts differ (p < 0.10).

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