The Effect of Heat Treatment of Forages on Degradation Kinetics and Escape Protein Concentration

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Summary

An *in situ* study was conducted to evaluate the effects of heat treatments on the degradation kinetics and escape protein concentrations of forages (alfalfa and berseem clover). Alfalfa collected at 4 and 7 weeks post-harvest and berseem clover collected at 5 and 7 weeks post-harvest were freeze-dried and then heated to 100, 125, and 150° C for 2 hours. Heat treatment effects were determined by placing two bags of sample (for each treatment, maturity, and forage species for a given incubation times) into the rumen of one fistulated steer fed alfalfa hay. Bags were incubated for periods of 0 to 48 hours. Increasing levels of heat treatments of forages increased concentrations of neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent insoluble nitrogen (ADIN) and non-degradable protein (NDP), potentially degradable protein proportion (PDP), and protein escaping rumen degradation (PEP) while decreasing water soluble protein (WSP) and the rates of crude protein (CP), except immature berseem clover and cell wall (CW) degradation. PEP was greater and rate of CP degradation was lower at 100 and 150° C compared to 125° C in immature berseem clover.

Introduction

Forage protein serves as a source of metabolizable protein to the ruminant by providing both ruminally degradable protein for microbial growth and some ruminally undegradable protein for intestinal digestion. The crude protein content of forages often exceeds animal requirements in many production systems. The high solubility--mainly due to non-protein nitrogen--and rate of ruminal degradation of forages result in excessive amounts of ammonia production in the rumen. Some of the ammonia produced in the rumen is utilized by rumen bacteria for microbial protein synthesis; the remainder of the ammonia is either recycled and/or excreted as waste. Because of rapid and extensive degradation of forage in the rumen, bypass protein concentrations of forages are usually low. Because young, growing beef and lactating dairy cattle can meet their protein requirements only partially with microbial protein synthesized in the rumen, the remainder of the required protein must be supplied by dietary protein that escapes ruminal degradation.

Several chemical and physical treatments of feedstuffs have been described in the literature to reduce the rate of ruminal protein degradation and increase the proportion of protein that escapes degradation in the rumen. One of the common physical techniques used is heat treatment. However, exposing feedstuffs to high temperatures may cause overheating, which results in decreased digestibility of feedstuffs.

In this study, our objectives were to evaluate the effects of heat treatments on the degradation kinetics and escape protein concentrations of forages; and secondly, to compare to responses of two forage species to heat treatments.

Materials and Methods

Alfalfa and berseem clover used in this study were second-cut materials harvested at two stages of maturity: 5 and 9 wk for berseem clover, and 4 and 9 wk for alfalfa. Both alfalfa and berseem clover were harvested at the same time to obtain the same maturity at three locations; then those locations were protected with cages for sample collections. Forages were collected from three locations giving a total of three samples. One sample, representing one location, was divided in half with one half being added to each of the other two locations. This was done to increase sample size for analysis. Then samples were heated at 100, 125, and 150° C for 2 h.

To determine the effects of heat treatments on chemical compositions of forages, heat-treated samples of alfalfa and berseem clover were ground through a 1-mm screen and then analyzed for NDF, ADF, CP, and ADIN concentrations.

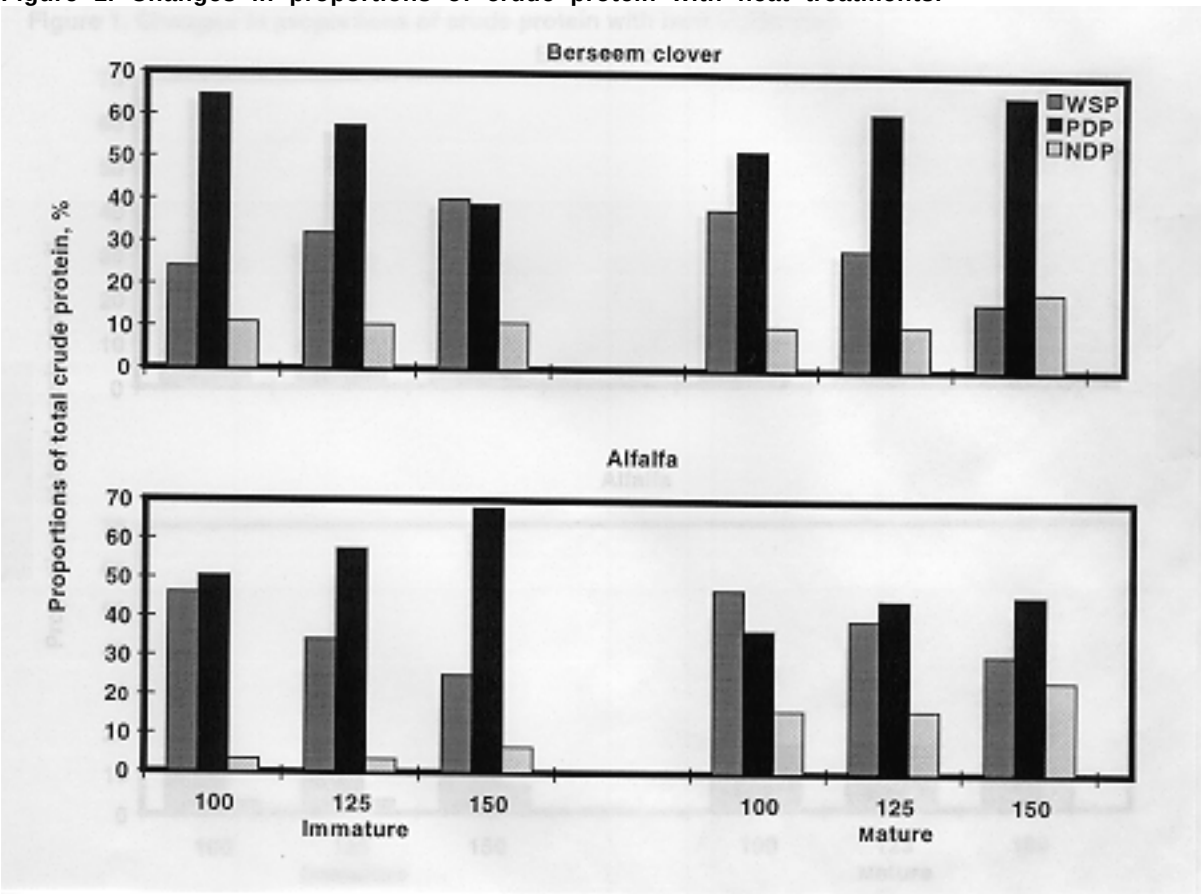
To estimate *in situ* degradation kinetics and fractions of N and cell wall (CW), heat-treated samples of alfalfa and berseem clover forages were ground through a 2-mm screen. Approximately 3 g of one of the forage samples was weighed into a bag with internal dimensions of 12 x 10 cm; therefore, the ratio of sample weight to exposed bag surface area was approximately 12.5 mg/cm2. Bags used were constructed of Dacron® polyester having an average pore size of 50 microns. Bags were suspended in the rumen by stringing the looped portion of the bag onto a ring of tygon tubing filled with .63 cm steel hunting shot. Four bags were affixed to each ring.

One mature fistulated steer fed alfalfa hay twice a day was used for incubation of samples. However, this experiment was repeated twice to obtain replication for laboratory analysis. Dacron® bags were placed in the ventral portion of the rumen and incubated for periods of 0, 3, 6, 12, 24, 32, and 48 h. Two bags of sample for each maturity and forage species were inserted for each incubation time except at 12 h. At 12 h, four bags of samples--two for estimation of *in situ* N degradation and two for estimation of the percentage of escape protein--were inserted. Insertion of bags into the rumen was done in reverse order of incubation times, allowing all bags to be removed simultaneously and minimizing variation associated with the washing process that followed. Bags were washed under running water three times and placed in ice water overnight. The following morning, bags were washed under running water until rinsates were clear. After washing, all bags were dried for 48 h at 65° C, and DM recovery was determined. Undigested forage residues were analyzed for nitrogen by the micro-Kjeldahl procedure (AOAC, 1980) and NDF (Goering and Van Soest, 1970).

Kinetic parameters associated with the disappearance of N from bags were estimated from a one-pool version of Mertens' (1977) discrete lag model of CW digestion. Modifications of the model by Wechsler (1981), which allow estimation of both digestion and lag functions from a single formula, were also incorporated. Model estimates of the pool size, rate constant (k), and discrete lag time of the potentially digestible N in each sample were obtained by fitting recovery data to the model using nonlinear regression analysis (SAS, 1982).

Loss of DM from bags caused by exposure of substrates to the digestive action of the rumen and the washing process that followed resulted in the partitioning of CW and N in each of the maturity-treatment combinations into three fractions: 1) soluble fractions of CW (WSCW) and N (WSN) were determined as the differences between initial CW and N content and amounts of CW and N recovered in 0 time-incubation; 2) potentially digestible fractions of CW and N were determined as 100 - (non-digestible fraction and water soluble fractions of CW and N); 3) non-digestible fractions of CW (NDCW) and N (NDN) were determined as the differences between initial CW and N content and amounts of CW and N recovered after 48 h incubations of samples in the rumen.

Figure 1. Changes in proportions of crude protein with heat treatments.



A modified technique reported by Mullahey et al. (1992), as adapted from the *in situ* technique reported by Anderson et al. (1988), was used to determine the percentage of forage protein that escaped ruminal degradation.

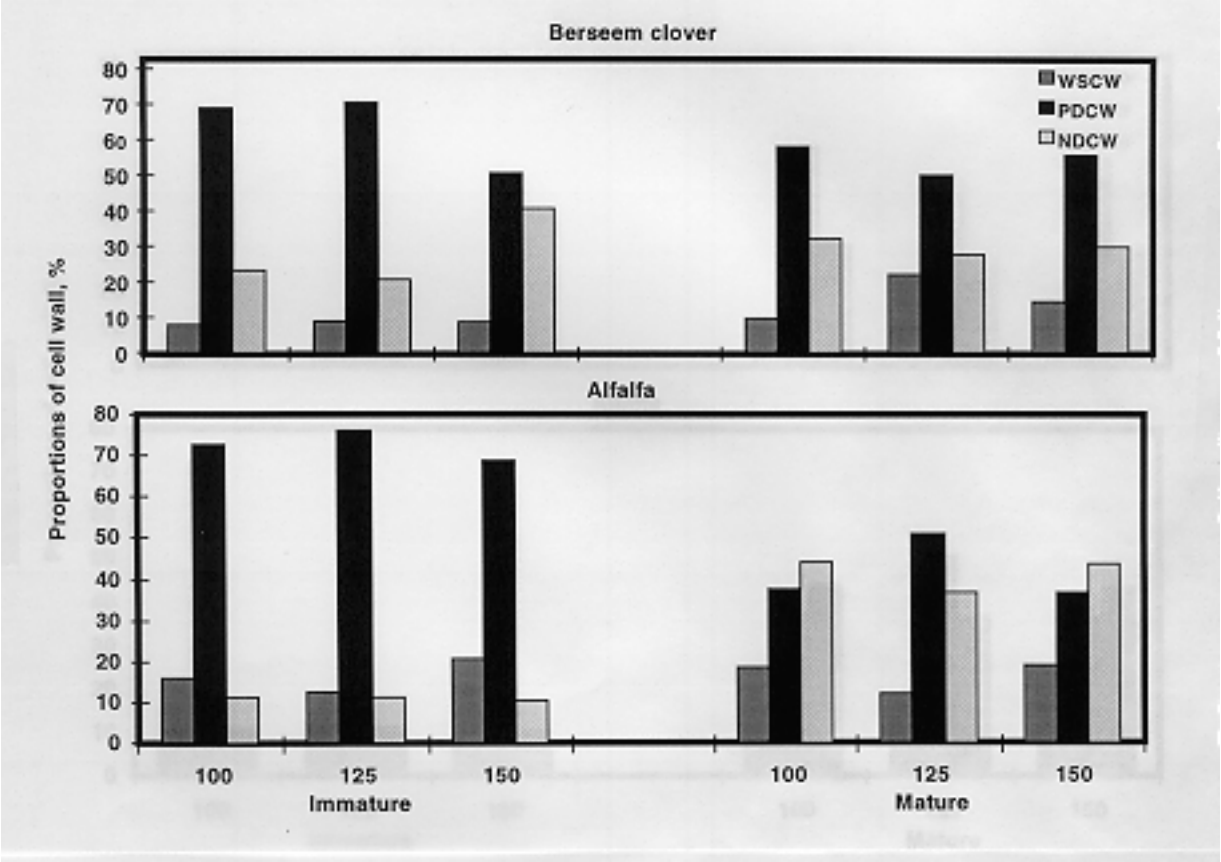
The proportion and concentration of total protein which would escape ruminal digestion were calculated as total residual N remaining following 12-h incubation, adjusted for the indigestible N (ADIN) using the following equations:
Escape Protein Percentage, % of total protein = (Total residual N - ADIN of total residue)/ (Total plant-N - ADIN of total plant) x 100
Escape protein concentrations, % of DM = 6.25 x (Total residual N - ADIN of total residue)

Results and Discussion

Heat treatments of forages increased the concentrations of NDF, ADF, and ADIN in both forages within both maturities. However, increasing the level of temperature did not increase ADF in immature berseem clover as much as it did in alfalfa. Even 100° C might be high enough to make the maximal change in ADF concentration; therefore, we could not see further increases in concentration of ADF with increasing temperatures in immature berseem clover. The effects of 150° C on compositions of forages were much greater than 100 and 125° C. The effects of 100 and 125° C were similar on the compositions of forages (Table 1). Heating forages over 125° C appears to affect chemical composition of forages, decreasing the overall utilization of forage by ruminant animals.

While increasing levels of heat treatments of forages increased PDP and NDP, it decreased WSP in mature forages and immature alfalfa. Immature berseem clover responded in the opposite direction. While WSP and NDP increased, PDP decreased with increasing levels of heat treatment (Figure 1). It seems that heating of forages resulted in converting WSP into PDP and NDP. While most of the WSP was converted into PDP at 100 and 125° C, most of the WSP was converted into NDP at 150° C.

Figure 2. Changes in proportions of cell wall with heat treatments



Rates of protein degradation decreased with increasing levels of heat in both mature forages and immature alfalfa. Rate of protein degradation was lower at 100° C than at 125° C in immature berseem clover. The potentially digestible nitrogen pool, as determined by a kinetic model, seems unaffected by levels of heating except in immature berseem clover. The potentially digestible nitrogen pool decreased in a linear fashion with increasing levels of heat treatment. This was an expected result, because ruminal digestibility of CP is decreased with increasing levels of heat treatment due to a decreased rate of CP degradation and increased ADIN. Percentage of protein escaping ruminal degradation increased in both mature forages and immature alfalfa with increasing levels of heating. Percentage of protein escaping ruminal degradation was the highest at 100° C compared to 125 and 150° C. Increases in PEP with heat treatments were expected, because heat treatments decrease the rates of protein degradation in the rumen; therefore, proportions and concentrations of protein escaping ruminal degradation increased. Concentrations of protein escaping ruminal degradation were higher at 100 and 125° C in both immature forages and mature berseem clover because even though PEP was higher at 150° C, higher levels of ADIN in those forages reduced the CEP. Concentration of protein escaping ruminal degradation was numerically higher at 150° C. It seems that immature forages were more sensitive to heating over 125° C than mature forages were (Table 2).

Rates of CW degradation, as with protein degradation, decreased in a linear fashion with increasing levels of heating. The potentially digestible CW pool, as determined by a kinetic model, did not show a definite trend. While the PDCW pool increased in immature and mature alfalfa and mature berseem clover with increasing levels of heating--which is the opposite of what we expected--it decreased in immature berseem clover with heat treatment of 150° C (Table 3).

Heat treatments did not affect the proportions of CW in mature forages and immature alfalfa, but some PDCW in immature berseem clover became NDCW (Figure 2). However, heating berseem clover decreased WSCW compared to untreated berseem clover. This effect was not very significant in alfalfa.

Implications

Heat treatment over 125° C may cause overheating in forages which results in poor utilization by ruminant animals. Heat treatment over 125° C resulted in significant increases in concentrations of NDF, ADF, ADIN and NDP. Even though there were increases in PEP, CEP decreased with heat treatment over 125° C. It seems that 100 and 125° C temperatures are sufficient to increase the CEP in forages with a little decrease in digestibility of forage.

Acknowledgments

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Table 1. Changes in composition of forages with increasing levels of heat treatment.

	Forage species																	
	Alfalfa						Berseem clover											
	Immature			Mature			Immature			Mature			Significance[b]					
Item	100	125	150	100	125	150	100	125	150	100	125	150	F	M	H	FM	MH	FMH
Proportion of DM, %																		
NDF	19.25	23.02	39.75	34.94	36.30	44.86	39.86	41.04	45.35	34.86	41.12	44.72	.01	.01	.01	.01	.01	.01
ADF	13.76	14.76	19.70	24.68	26.78	28.67	27.74	27.23	28.65	24.07	27.99	32.74	.01	.01	.01	.01	.01	.01
CP	26.95	27.50	26.83	16.31	16.02	15.90	17.33	18.50	18.48	16.75	16.20	16.70	.01	.01	.12	.01	.01	.16
Proportion of N, %																		
ADIN	0.85	0.90	9.43	1.65	1.80	5.63	1.73	1.78	7.13	1.32	1.70	5.73	.01	.01	.01	.01	.01	.01

[b]F=forage species , M=maturity, H=heating level.

Table 2. Estimates of *in situ* digestible pool size of N, rate of disappearance of potentially digestible N fractions, digestion lag times, and proportion and concentration of protein escaping rumen digestion by increasing levels of heat treatment in alfalfa and berseem clover.

	Forage species																		
	Alfalfa						Berseem clover												
	Immature			Mature			Immature			Mature			Significance[b]						
Item[c]	100	125	150	100	125	150	100	125	150	100	125	150	F	M	H	F*M	M*H	F*M*H
-k-1	.290	.140	.119	.199	.151	.129	.112	.158	.104	.329	.155	.064	.06	.08	.02	.05	.06	.02	
Lag	1.992	.579	3.611	.353	1.094	.814	1.624	5.059	1.640	3.259	2.342	.969	.03	.26	.43	.28	.41	.04	
PDNP	86.71	98.83	96.83	68.45	75.39	63.85	89.36	85.74	59.80	80.22	87.11	81.85	.27	.01	.54	.01	.31	.12	
PEP,%	66.91	71.36	84.61	54.79	55.32	62.95	85.33	68.24	81.00	54.85	61.90	86.85	.01	.01	.01	.03	.01	.01	
CEP	17.46	19.12	13.97	8.04	7.81	8.36	13.35	11.61	9.79	8.78	8.95	6.30	.01	.01	.01	.01	.01	.01	

[b]M=maturity, F=forage species, H=heating level.
[c]PDNP=digestible N pool size, PEP=proportion of protein escaping rumen digestion, CEP=concentration of protein escaping rumen digestion.

Table 3. Estimates of *in situ* rate of disappearance of potentially digestible CW fractions, digestion lag times, and digestible pool size of CW by increasing levels of heat treatment in alfalfa and berseem clover.

	Forage species																		
	Alfalfa						Berseem clover												
	Immature			Mature			Immature			Mature			Significance[b]						
Item[c]	100	125	150	100	125	150	100	125	150	100	125	150	F	M	H	F*M	M*H	F*M*H
-k-1	.474	.197	.136	.115	.141	.101	.102	.093	.087	.160	.084	.046	.13	.25	.32	.23	.61	.57	
Lag	1.69	1.69	3.15	.640	1.35	3.49	1.56	1.98	2.04	1.06	1.59	2.14	.25	.47	.04	.92	.62	.60	
PDCW	78.37	81.03	88.37	45.21	51.12	48.32	72.62	76.89	54.71	63.90	69.91	78.11	.20	.01	.54	.01	.31	.12	

[b]M=maturity, F=forage species, H=heating level.
[c]PDCW=digestible cell wall pool size.