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## CHARACTERIZATION OF FROST-DAMAGED IMMATURE SOYBEANS FOR ALFALFA AND ALFALFA-BROME HAY, CORN SILAGE OR CORN-BASED DIETS

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#### Summary

One compositional study and three single stage in vitro rumen fermentation experiments were conducted to characterize the feeding profile of frost-damaged immature soybeans (FDIS). Dawson and Dassel soybean plants were harvested at 86, 93, 100, 107 days postplanting and frozen at -5 cto contrast with natural frozen soybeans of the same varieties that were serially planted and harvested after the first killing frost. No differences between soybean variety were detected for crude protein (CP), ether extract (EE) or dry matter content (DM). Crude protein and EE content differed (P<.05) between maturities of artificially frozen (AF) soybeans. Only the EE content differed (P<.01) with maturity of naturally frozen (NF) soybeans. Planting date had an effect (P<.001) on composition of mature soybeans primarily due to increasing EE component. Exp. 1. Five levels of FDIS (0, 5, 10, 15, 20%), three substrates (alfalfa hay, ALF), corn silage (CS), ground corn (WSC)) and two fermentation times (24 or 48 hours) were evaluated in single stage in vitro fermentations. FDIS supplementation had no effect on in vitro dry matter disappearance (IVDMD) of ALF or WSC, indicating that the upper limit of FDIS supplementation had not been reached. IVDMD of corn silage was depressed (P<.01) when FDIS exceeded 10%. Increasing levels of FDIS increased NH\_-N (P<.01) but had no effect on VFA concentration in fermentation liquor. Alfalfa-brome hay, ALF and CS were supplemented with FDIS for 48-hour IVDMD comparisons. IVDMD was not affected by substrate, but FDIS increased IVDMD of CS (P<.001). Exp. 3. Four treatments, soybean meal (SBM), heated mature raw soybeans (HMB), heated FDIS (HFDIS) and FDIS, were used to differentiate oil content and trypsin inhibitor effects on roughage fermentation. Only SBM increased IVDMD (P<.05) of forages tested. FDIS included at 20% of the DM did not significantly decrease IVDMD of forages in most instances but may not stimulate digestion in the same manner as SBM.

(Key Words: Soybean, Frost-Damaged, Immature, Composition, In Vitro Fermentation).

### Introduction

In northern regions of soybean production and southern regions where double cropping is practiced, killing frosts frequently occur before the crop has matured. Green, freeze damaged soybeans have little market value due to poor storage and processing characteristics arising from fat rancidity and presence of antinutritional factors in the damaged soybean. The inability to market frost-damaged soybeans leaves producers exposed to economic losses.

Frost-damaged soybeans have high crude protein and energy density. If livestock feeding is practical, it could provide an alternative marketing avenue for the producer. Little research has been done with frost-damaged immature soybeans (FDIS), but suspected high oil content and the presence of trypsin inhibitor cause skepticism about the use of this product for livestock feeding.

The purpose of this study was to characterize frost-damaged immature soybeans as a supplement to ruminant diets.

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#### Materials and Methods

Two systems were employed to obtain FDIS of varying maturity to determine effect of harvesting method and stage of maturity on chemical composition. To evaluate artificial freezing of soybeans planted on a common date, two 00 maturity varieties of soybeans (Dawson and Dassel) were planted on June 3, 1987. Soybeans were harvested at 86, 93, 100, 107 days postplanting and at full maturity to provide a wide range of maturities. Whole plants were cut, bagged and placed in a cooler to be chilled at  $5\,^{\circ}$ C for 4 hours and  $-5\,^{\circ}$ C for 4 additional hours. Plants were then allowed to wilt 24 hours at 32  $\,^{\circ}$ C before soybeans were separated and frozen for subsequent analysis. Natural freezing was done using Dawson and Dassel soybean varieties planted at weekly intervals from June 29 to August 3, 1987. A killing frost occurred on October 21 providing soybeans from plants 84, 91 and 98 days postplanting and mature. Soybeans were analyzed for dry matter content, crude protein, ether extract and ash. Comparisons of procedure and maturity were made using analysis of variance and least squares means for a 4 x 2 randomized complete block design.

Three fermentation studies were conducted to determine the effects of FDIS on ruminal fermentation. In experiment 1, five levels of FDIS (0, 5, 10, 15, 20%), three substrates (alfalfa, corn silage, ground corn) and two fermentation times (24 and 48 hours) were used in a 5 x 3 x 2 factorial designed experiment. Single stage in vitro dry matter disappearance (IVDMD), pH, NH<sub>3</sub> and VFA concentrations were measured as dependant variables. Experiment 2. Alfalfa-bromegrass hay, alfalfa and corn silage substrate fermentations were compared using the same levels of FDIS used in Exp. 1. These 48-hour single stage fermentations were conducted to determine the effect of FDIS supplementation on high fiber substrates of differing quality. Analysis of variance was performed for a 5 x 2 factorial design. Experiment 3. Four treatments were used to separate the effects of oil content and trypsin inhibitor in unprocessed soybeans on fermentation of roughages. Treatments included soybean meal (SBM), heated mature raw soybeans (HMB), heated frost-damaged immature soybeans (HFDIS) and FDIS. Roughages from Exp. 2 were used with two levels of FDIS (10 or 20%). IVDMD means were separated by orthogonal contrast to determine the effects of the high oil and antinutritional factors of FDIS on fiber digestion.

#### Results and Discussion

There were no differences in composition of the soybean varieties used. Stage of development did affect (P<.05) crude protein and fat content of artificially frozen soybeans (Table 1). Protein decreased slightly (P<.05) as fat increased in artificially frozen soybeans, suggesting a simple protein dilution effect. The EE content of naturally frozen soybeans was greater for mature soybeans (Table 1). Differences (P<.001) occurred between mature soybeans from each harvesting method, suggesting planting date had an effect on soybean composition. No difference was detected in crude protein content between maturity groups for the naturally frozen soybeans. This can be attributed to a diminishing protein dilution effect, a result of lower overall fat content of the natural frozen soybeans due to planting date. For experimental and practical purposes, harvesting methods were considered equal and immature soybeans were pooled for IVDMD studies.

Experiment 1. Increasing level of FDIS increased (P<.001) in vitro NH<sub>3</sub> concentration for all substrates (Table 3). FDIS level did not affect total VFA concentration or acetate/propjonate ratio. Substrate did affect these variables (P<.001) as would be expected. Substrate x level interaction for IVDMD, NH<sub>3</sub>, VFA and A/P was significant (P<.01) and due to depressed fermentation of corn silage at 20% FDIS. Frost-damaged immature soybean supplementation had no effect on IVDMD for alfalfa or ground corn but decreased (P<.001) IVDMD for corn silage over levels of FDIS. This indicates that the maximum level for FDIS incorporation had not been reached for alfalfa or ground corn. The interaction with corn silage is not easily explained and did not occur in Exp. 2.

IVDMD was unaffected by roughage source in Exp. 2 (Table 4). Increasing proportion of FDIS improved corn silage IVDMD in this study. Composition of corn silage (Table 2) indicated a deficiency in crude protein. FDIS increased availability of NH<sub>3</sub>·N which may have stimulated fermentation. Alfalfa and alfalfa-brome hay IVDMD were not affected by FDIS supplementation. No decrease was noted in IVDMD at the 20% level for any of the substrates, indicating again that the effective upper limit of FDIS supplementation was not reached.

No differences in IVDMD were found between mature soybeans and immature soybeans or between heated FDIS and FDIS in Exp. 3. These results indicate antinutritional factors did not have detrimental effects on fermentation of forages. IVDMD was higher (P<.05) for forages supplemented with SBM (Table 5). Based on comparisons of full fat and defatted soybeans, it appears that fat content may be the primary factor limiting increases in IVDMD

associated with SBM supplementation. Heating FDIS did allow some improvement (P<.05) in IVDMD when substrate contained 20% soybeans (Table 5).

In summary, compositional and in vitro studies indicate that FDIS is a viable feedstuff for ruminants. Upper limits of feasible FDIS incorporation in high concentrate rations were not reached with 20% supplementation. FDIS increased IVDMD and improved ruminal available NH<sub>3</sub> in forages limited in crude protein in some instances. FDIS did not significantly decrease IVDMD at the 20% level in forages.

TABLE 1. COMPOSITION OF ARTIFICIALLY VS NATURALLY FROZEN IMMATURE SOYBEANS

		Artifici	a1			
Days postplanting	<u>86</u>	93	<u>100</u>	<u>107</u>	<u>Mature</u>	<u>SEM</u>
Crude protein, % a Ether extract, % a Ash, %	35.6 15.9 5.0	36.6 18.1 5.0	35.1 18.6 5.1	34.1 18.2 5.0	35.4 18.5 5.1	.93 1.03 .065
Days postplanting	<u>84</u>	Natural 91	Ъ	<u>98</u>	<u>Mature</u>	<u>SEM</u>
Crude protein, % Ether extract, % Ash, %	40.2 8.0 5.1	41.4 13.4 5.0		41.2 14.8 5.1	39.7 16.6 5.1	1.28 .60 .045

TABLE 2. COMPOSITION OF IN VITRO FERMENTATION SUBSTRATES a

	Alfalfa hay	Corn silage	Alfalfa-brome hay	Corn	FDIS
Crude protein, %	17.9	7.3	17.1	9.1	35.6
ADF, %	34.2	24.2	32.0	3.6	15.4
NDF, %	52.4	48.2	41.6	32.5	29.4
Ash, %	8.8	5.8	13.8	1.4	5.5

<sup>&</sup>lt;sup>a</sup> Percentage dry matter basis.

a Linear effect (P<.05). b Least-squares means. c,d,e,f Means with unlike superscripts differ (P<.05).

TABLE 3. EFFECT OF FDIS LEVEL ON 48-HOUR IVDMD OF DIFFERENT SUBSTRATES (EXP. 1)

-		% FDIS						
	0	_5	10	15	20	SEM_		
		<u>Alfalfa</u>	<u>Hay</u>					
IVDMD, % <sup>a</sup> NH <sub>3</sub> , mg/dl <sup>a</sup> Total VFA <sub>bc</sub> m/d A/P ratio	54.7 21.2 33.6 2.7	53.7 24.6 34.7 2.6	25.0 33.6		29.3 19.5			
		Corn Si	<u>lage</u>					
IVDMD, % NH <sub>3</sub> , mg/dl Total VFA, m/s A/P ratio		10.8 23.1	18.8 31.1	43.1 35.7 26.0 1.91	41.8 46.8	1.45 3.40		
		Ground 9	Corn					
IVDMD, % NH <sub>3</sub> , mg/dl Total VFA, m/ A/P ratio	9.3 m1 71.8			19.3	25.0	1.45		

 $<sup>^{\</sup>rm a}$  Substrate effect (P<.001), level effect (P<.001), substrate x level (P < .001).

TABLE 4. EFFECT OF FDIS LEVEL ON IVDMD OF THREE DIFFERENT FORAGE SOURCES (EXP. 2)

	% FDIS					
<u>Source</u>	0	5	10	15	20	SEM
			% IVDMD			
Alfalfa hay	50.0	51.6	51.5	51.7	53.8	1.44
Corn silage <sup>a</sup>	41.4	52.4	54.8	56.6	56.1	1.44
Alf-brome hay	50.6	51.9	51.4	51.9	54.0	1.44

a Level effect (P<.001).

Substrate effect (P<.001), substrate x level (P<.01).

Acetate to propionate ratio.

TABLE 5. EFFECTS OF FAT AND ANTINUTRITIONAL FACTORS OF FDIS ON IVDMD OF THREE SUBSTRATES (EXP. 3)

	Alfalfa hay		<u>Corn silage</u>		Alf-brome hay		
Protein	% FDIS						
source	10	20	10	20	10	20	SEM
% IVDMD							
SBM	56.3	60.7	61.0	66.0	54.9	59.4	1.21
HMB	55.3	57.3	59.9	61.0	57.5	57.8	. 53
HFDIS	55.6	56.7	58.5	62.3	56.9	58.1	.61
FDIS	53.2	53.6	59.0	58.5	57.0	59.4	.83

 $<sup>^{\</sup>mathrm{a}}$  SBM = soybean meal, HMB = heated mature soybeans, HFDIS = heated frost damaged immature soybeans.

Level effect (P<.01), substrate effect (P<.001).

SBM differs from HMB, HFDIS and FDIS.

HFDIS differs from FDIS (P<.05).