IN VITRO EVALUATION OF FIBROLYTIC ENZYMES TO INCREASE DIGESTION OF FIBROUS FEEDSTUFFS

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Summary

Fermentations were conducted to identify enzyme activities and amounts that would optimize digestion of high-fiber feed ingredients (soybean hulls, alfalfa, corn silage, and corn gluten feed). In general, adding enzymes increased in vitro dry matter disappearance, but total volatile fatty acid concentrations were not improved by enzyme treatments. The response to enzymes was similar across substrate, suggesting that substrate specificity of the enzymes is not important. The most effective enzyme preparation had greater cellulase activity than the other enzyme preparations, suggesting that cellulase might be the most important enzymatic activity for improving digestion of fibrous feedstuffs.

Introduction

Soybean hulls are a feedstuff that has excellent digestibility when measured in vitro, but this often does not translate to high digestibilities when the product is fed to cattle. This discrepancy between in vitro and in vivo observations probably is caused by the relatively rapid rate of passage of soybean hulls from the rumen. Thus, this feedstuff would likely benefit from treatments that would increase the fermentation rate. One such product would be enzyme treatment.

A previous digestion trial demonstrated that we could improve in vivo digestion of soybean hulls by adding fibrolytic enzymes to the diet. The goals of the present study were to more exactly identify the enzyme activities and amounts that are needed to optimize digestion of soybean hulls and to expand this work to encompass several other high-fiber feed ingredients (alfalfa, corn silage, corn gluten feed) that are available for use in the cattle industry.

Experimental Procedures

Fermentations were conducted in 50-mL centrifuge tubes fitted with rubber stoppers containing gas-release valves. The substrate weight was 0.30 g, suspended in a mixture of McDougall's buffer (20 mL) and strained rumen fluid (10 mL). Rumen fluid was collected from two animals fed mixed diets and pooled together before conducting the experiment. After fermentation at 39°C, the tubes were centrifuged (20,000 × g) and the liquid portion decanted. A sample of the liquid was mixed with meta-phosphoric acid and prepared for analysis of volatile fatty acids (VFA) by gas chromatography. The pellet was solubilized in an acid/pepsin solution and incubated at 39°C. The residue was then filtered through Whatman 541 filter paper, dried, and weighed to determine the undigested residue. In vitro dry matter disappearance (IVDMD; an estimate of digestibility) was then calculated. Each treatment was run in duplicate tubes, and the "no enzyme" controls were run in quadruplicate to ensure that we had an accurate value for the negative control.

Experiment 1. Seven enzyme preparations were provided by Saf Agri. The activities of the enzymes, as provided by Saf Agri,

were: **FP800** (cellulase = 25 units; xylanase = 700 units, ß-glucanase = 1400 units), **XP500** (high xylanase activity), **Mix A** (cellulase = 8 units; xylanase = 700 units, ß-glucanase = 1400 units), **Mix B** (same as Mix A, plus 250 units pectinase), **Mix C** (same as Mix A, plus 1400 units galactomannase), **Mix D** (same as Mix C, plus 1300 units papain), and **Mix E** (same as Mix C, plus 44 units fradiase).

For this study, all seven enzyme products were tested at four different inclusion levels. A control treatment with no enzyme addition also was evaluated. The enzyme levels were determined on the basis of amounts that would be provided to a lactating dairy cow and consisted of 1, 5, 15, or 30 g/day. The amounts used for the fermentations were scaled by calculating the substrate provided to each in vitro tube (0.30 grams) relative to daily feed intake by a dairy cow (55 pounds/day). The amounts required for application to beef cattle diets would be less, likely in proportion to feed intake. This study used soybean hulls and alfalfa as substrates. The fermentations were conducted for 24 and 48 hours, and data in Table 1 represents an average from these two fermentation times.

Experiments 2 and 3. Experiments 2 and 3 were identical except for the substrates (feedstuffs) that were tested. Exp. 2 used alfalfa and soybean hulls as substrates, whereas Exp. 3 used corn silage and corn gluten feed as substrates. These experiments evaluated the effects of the enzymes at different inclusion levels. Enzyme levels were selected on the basis of data from Exp. 1. For all products, we tested 5 g/d, but we also tested additional levels that showed promise in Exp. 1. Each enzyme amount was incubated with the substrate for 1 or 18 hours before starting the fermentation. The presented data is the average of these two pre-incubation times. Fermentations were conducted for 24 or 48 hours, and the presented data is the average of these two incubation times.

Results and Discussion

Experiment 1. In general, enzyme treatments increased in vitro dry matter disappearance (IVDMD), and there were differences among the enzymes and enzyme levels. In some instances, the lesser amounts of the enzymes were more effective than the greater amounts in increasing IVDMD. This was evident for FP800 and Mix C, in which the 1 and 5 g/d treatments yielded better IVDMD than any other of the enzyme treatments. There were several enzymes for which the response was the same for all of the levels tested (XP500, Mix A, B, and D), and for Mix E the response seemed to be better for the higher levels (15 and 30 g/d) than for the lower levels (1 and 5 g/d). Although enzyme treatments significantly increased IVDMD, total VFA concentrations were not affected by enzyme treatment in this experiment.

The response to enzymes was similar between alfalfa and soybean hulls (data not shown). Thus, within the limits of the two substrates and the range of enzymes evaluated, the best choice of an enzyme did not seem to be dependent upon dietary ingredients.

Experiments 2 and 3. As in Exp. 1, the response to enzymes was similar among the substrates (data not shown). Thus, there was no evidence to suggest that different enzymes would be needed for each feedstuff. Effects of enzyme additions on IVDMD and VFA concentrations are presented in Table 2. The FP800 enzyme increased IVDMD to a greater extent than did the other enzymes. Responses to FP800 were achieved with lesser amounts (0.3 or 1.0 g/day), with no further response to the greater amounts in either experiment. The enzyme XP500 (at 5 g/day) seemed to be nearly as efficacious as FP800 in Exp. 3, but response to it was somewhat less than FP800 in Exp. 2.

Among the products Mix A, B, C, D, and E, comparisons can be made of the 5 g/d

treatments to assess the advantages of adding additional enzyme activities. Mix A represents the basic activities, with the other mixes representing addition of different activities. In general, Mix A did not improve IVDMD. However, addition of pectinase activity (Mix B) or galactomannase activity (Mix C) seemed to improve IVDMD. Interestingly, the addition of papain activity to Mix C (in creating Mix D) or the addition of fradiase activity to Mix C (in creating Mix E) resulted in less IVDMD than the Mix C alone (5 g/d). Responses to the addition of pectinase and galactomannase are a little surprising because the substrates for these enzyme activities (pectins, nonlignified hemicelluloses) are readily degraded by ruminal microbes.

Changes in VFA production in response to enzyme treatment were not related to the changes in IVDMD. This was particularly evident in Exp. 3 in which there was a negative relationship between IVDMD and VFA across the enzyme treatments. We would expect that, as digestion of a feedstuff increases (as indicated by IVDMD), there would be a concomitant increase in the end-products of that fermentation (i.e., VFA). We do not have an explanation for the lack of a relationship between these two responses in these experiments.

It is unknown if the same amounts of enzymes would be effective in production settings. However, the response to small amounts of the FP800 certainly provides us with optimism about its potential effectiveness. The greater activity of cellulase in the FP800 than in the other enzyme mixes suggests that cellulase might be the most important enzyme activity.

Table 1. Effect of Enzyme Treatment on In Vitro Dry Matter Disappearance (IVDMD) from Alfalfa and Soybean Hulls and Subsequent Volatile Fatty Acid (VFA) Concentration (Exp. 1)

Enzyme	Amount	IVDMD[a]	VFA
	g/day*	----- % -----	----- m*M* -----
None	0	68.7	93.4
FP800	1	75.4	90.1
	5	75.2	89.9
	15	71.6	93.0
	30	71.1	91.8
XP500	1	71.6	92.9
	5	71.6	92.3
	15	71.6	94.0
	30	71.7	90.1
Mix A	1	70.1	92.9
	5	69.4	91.2
	15	71.4	90.1
	30	71.6	90.8
Mix B	1	68.5	91.2
	5	70.3	90.2
	15	73.0	92.7
	30	72.5	92.9
Mix C	1	74.8	93.5
	5	73.9	91.8
	15	69.0	92.8
	30	68.7	92.0
Mix D	1	71.9	91.2
	5	72.0	90.9
	15	72.8	91.9
	30	73.6	91.0
Mix E	1	70.0	95.7
	5	70.0	93.3
	15	73.5	93.8
	30	72.7	94.1
SEM		0.88	1.5

[a]Significant effect of enzyme treatment ($P<0.0001$).

*Amount relative to a dairy cow consuming 55 pounds of feed daily. Required amounts would be less, in proportion to feed intake, for beef cattle.

Table 2. Effect of Enzyme Addition on In Vitro Dry Matter Disappearance (IVDMD) and Total Volatile Fatty Acid (VFA) Concentrations from Fermentation of Alfalfa and Soybean Hulls (Exp. 2) or Corn Silage and Corn Gluten Feed (Exp. 3)

		Experiment 2		Experiment 3	
Enzyme	Amount	IVDMD [a]	VFA [a]	IVDMD [a]	VFA [b]
	g/day*	-- % --	-- m*M* --	-- % --	-- m*M* --
None	0	65.9	83.0	71.3	75.3
FP800	0.3	67.7	83.0	74.0	73.6
FP800	1	69.3	84.3	75.1	72.0
FP800	3	67.5	80.5	72.0	73.2
FP800	5	69.9	82.3	75.3	70.9
XP500	5	67.0	78.2	74.6	74.5
Mix A	5	64.4	77.5	72.2	74.7
Mix B	5	68.6	77.3	73.9	75.2
Mix C	1	66.4	84.8	71.8	74.1
Mix C	5	67.9	79.1	76.7	75.2
Mix D	5	65.7	84.0	73.5	73.8
Mix D	15	66.8	83.1	74.7	70.3
Mix E	5	65.3	82.1	70.9	75.3
SEM		0.9	1.5	1.2	1.3

[a]Significant effect of enzyme treatment, $P<0.01$.
[b]Tendency for an effect of enzyme treatment, $P=0.07$.
*Amount relative to a dairy cow consuming 55 pounds of feed daily. Required amounts would be less, in proportion to feed intake, for beef cattle.