January 2003

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Influence of Rinsing Technique and Sample Size on *In situ* Protein Degradation

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Use of machine rinsing or increasing sample size does not change *in situ* dry matter disappearance or undegradable intake protein values of soybean meal or Soypass.

Summary

Four experiments were conducted to evaluate effects of in situ *bag rinsing technique and sample size on the variation of undegradable intake protein (UIP) and dry matter disappearance (DMD) of soybean meal (SBM) and Soypass, a heat-treated soybean meal. Five rinsing techniques and five sample sizes were used to test effects. Soybean meal had higher DMD, lower UIP and higher variance for UIP than Soypass. A steer difference was noted for experiments with steer as a replication and also contributed a larger effect than day and run within day. Rinsing technique and sample size were not significant in concentrate fed steers but were in mixed diet steers. There was a rinsing difference with highest machine rinses having higher DMD and lower UIP values. A size difference was noted with largest sample size having lowest DMD and highest UIP. No difference was found between hand and machine rinsing and no evidence was found to eliminate the use of an increased sample size.*

Introduction

Over the past twenty-five years, *in situ* digestion techniques have been used extensively for measuring ruminal degradation of feedstuffs. Moreover, *in situ* digestion techniques are commonly used to predict undegradable intake protein (UIP) value of protein sources. However, *in situ* techniques suffer from variation involving rinsing techniques and sample sizes. If incubated samples are washed too thoroughly, undigested sample may be lost. If sample size is increased too much, dry matter disappearance (DMD) may be inhibited. Assays of rapidly degradable protein sources are influenced both by variation in DMD and UIP. Also, rapidly degradable feedstuffs incubated with small initial sample sizes leave minimal residue for further analysis. If the initial sample size can be increased, more residue will be remaining for subsequent analysis. Error across technicians may further contribute to the variation of *in situ* digestion techniques. Therefore, the objectives of this study were to evaluate the effect of *in situ* bag rinsing techniques and sample size on the variation of UIP and DMD of soybean meal (SBM) and Soypass, a heat-treated soybean meal.

Procedure

Four experiments were conducted to evaluate effects of *in situ* bag rinsing technique and sample size on UIP and DMD of SBM and Soypass. All four experiments were conducted under similar conditions. Samples were weighed as

received (unground) into 10 x 20 cm dacron bags with 50-µm pore size (Ankom Technology, Inc., Fairport, NY). Steer, day and run were used as replications: Exp. 1 day and run; Exp. 2 steer and run; Exp. 3 steer and day; and Exp. 4 day and run. Run was a duplicated rinse within day or steer. Three replicate bags within steer within day within run were used. Twenty *in situ* bags were placed in a larger mesh bag. All mesh bags were incubated for one 16-hour incubation period in a ruminally fistulated steer fed either a concentrate or mixed diet. Experiments 1 and 3 used a feedlot diet with 7.5% roughage, while Exp. 2 and 4 used a mixed diet (70% forage:30% concentrate). After bags were rinsed, all bags were dried overnight at 60°C and allowed to air equilibrate for two hours before the bag and residue were weighed. Protein analysis was conducted on the residue contained in each bag by weighing a sub-sample for nitrogen analysis using the combustion method (LECO, Inc., St. Joseph, MI). The UIP (% of CP) and DMD then were calculated. Each experiment was analyzed separately as a 2x2x2x5 factorial design.

Bag Rinsing

Experiments 1 and 2 evaluated the effect of five rinsing techniques. Experiment 1 used the concentrate diet, while Exp. 2 used the mixed diet. Two hand-rinsing techniques and three machine-rinsing techniques were tested. The first hand-rinsing technique consisted of rinsing the mesh bags containing the *in situ* bags in 39°C water until color could not be distinguished in the rinse water. Bags then were rinsed individually to remove any particles from the outsides of the bags. The second hand-rinsing technique consisted of removing all the *in situ* bags from the mesh bags and rinsing in a five-gallon bucket in 39°C water. Bags were agitated for one minute, water was drained and the procedure was repeated two additional times. The three machine rinses used a commercial clothes washing machine with 3, 5 or 8 rinses. A rinse consisted of a one-minute agitation in 45 liters (250 mL/bag) of 39°C water and a two-minute spin. All bags contained 5 g of sample per bag.

Table 1. Undegradable intake protein values and their corresponding coefficients of variation for Experiments 1 and 2.

	Treatment					
Item	Hand 1[a]	Hand 2[b]	Machine 3[c]	Machine 5[d]	Machine 8[e]	SEM
Experiment 1 – Concentrate Diet						
Soybean meal						
UIP[f], %CP	70.7	71.0	74.1	68.8	68.9	2.0
CV	8.1	14.3	4.1	12.5	13.6	3.9
Soypass						
UIP, %CP	92.3	93.2	93.6	93.5	92.7	2.0
CV	1.9	1.8	1.5	1.4	1.5	3.9
Experiment 2 – Mixed Diet						
Soybean Meal						
UIP, %CP	19.4[g]	22.9[g]	21.8[g]	23.5[g]	4.3[h]	2.1
CV	30.2	68.7	39.6	38.3	44.1	9.1
Soypass						
UIP, %CP	73.4[g]	71.1[g]	72.9[g]	70.2[g]	55.5[h]	2.1
CV	9.6	4.9	10.2	5.8	4.3	9.1

[a]Hand 1 rinse consisted of multiple rinses with an individual rinse.
[b]Hand 2 rinse consisted of multiple rinses without an individual rinse.
[c]Machine 3 consisted of 3 machine rinses (rinse = 1 min agitation, 2 min spin).
[d]Machine 5 consisted of 5 machine rinses.
[e]Machine 8 consisted of 8 machine rinses.
[f]UIP = undegradable intake protein.
[gh]Means within row with unlike superscripts differ ($P < 0.05$).

Sample Sizes

Experiments 3 and 4 evaluated the effect of five sample sizes. Experiment 3 utilized the concentrate diet, while Exp. 4 made use of the mixed diet. Bags contained 5, 10, 20, 30 or 50 g of sample per bag. After incubation, bags were machine rinsed with five machine rinses.

Results

There was a sample effect across all experiments ($P < 0.01$). Soybean meal had higher DMD and lower UIP values than Soypass and higher variance for UIP. Variance for DMD was also higher ($P < 0.01$) for SBM compared to the Soypass in concentrate fed steers (Exp. 1 and 3). Heat treatment of the Soypass condenses sugar residues with amino acids rendering the protein undegradable in the rumen. The heat treatment is stopped before the product becomes indigestible in the small intestine. Consequently, DMD is reduced, increasing the UIP value of the product. Previous research has shown depressed *in situ* degradabilities of protein sources when dietary forage is decreased. Slime produced in the rumen of animals fed concentrate diets could block pores of the dacron bag, thus reducing DMD. The inconsistent flow through the bag then increases the variation in DMD values.

A steer effect was noted for experiments with steer as a replication ($P < 0.01$). Steer had a larger effect than day with F-statistics of 243.5 and 8.6, respectively. In Exp. 3, the F-statistic for steer was 146.2 and day in Exp. 4 was 4.4. Additionally, steer contributed more variation than run with a F-statistic of 146.18 for steer and 10.61 for run. It was hypothesized that steer, day, and run would contribute similar amounts of variation when evaluating DMD and UIP. Since steer contributed the most variation, it is suggested that it be included in replications in *in situ* incubation.

Rinsing technique and sample size were not significant ($P = 0.85$) in concentrate fed steers (Exp. 1 and 3) but were ($P < 0.01$) in steers fed a mixed diet. There was a rinsing effect ($P < 0.01$) in Exp. 2, with 8 machine rinses having

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higher DMD and lower UIP values (Table 1). It was hypothesized that machine rinsing would eliminate technician-induced variation involved with hand-rinsing *in situ* bags. However, with increased rinsing, washout was expected to occur with over rinsing of bags. No difference was detected between hand and machine rinsing, suggesting machine rinsing is a suitable technique for rinsing bags. Reduction in time spent rinsing bags by hand makes the machine method more efficient and will likely reduce error between technicians. Five machine rinses is suggested to ensure proper rinsing and reduced washout. A sample size effect ($P < 0.01$) was noted for Exp. 4, with 50 g having the lowest DMD and highest UIP values (Table 2). The ratio of sample size to bag surface area is important in *in situ* studies. With excessive inclusion of sample in the bags, DMD can be inhibited. Previous research has shown that effective digestion of soybean meal protein is greatest at a lower sample size to bag surface area. A sample size range from 10-30 g is suggested ensure DMD is not inhibited and to also increase residue amount remaining after ruminal incubation

Table 2. Undegradable intake protein values and their corresponding coefficients of variation for Experiments 3 and 4.

	Treatment					
Item	5g	10g	20g	30g	50g	SEM
Experiment 3 – Concentrate Diet						
Soybean meal						
UIP[a], %CP	49.3	51.9	52.7	49.4	53.7	1.6
CV	12.6	12.0	8.2	12.9	8.2	3.9
Soypass						
UIP, %CP	89.8	88.2	88.6	89.3	88.9	1.6
CV	1.1	2.2	2.0	1.3	1.6	3.9
Experiment 4 – Mixed Diet						
Soybean Meal						
UIP, %CP	41.3[b]	44.5[bc]	47.6[cd]	49.7[d]	55.9[e]	2.1
CV	21.9	12.3	17.3	13.0	6.4	4.8
Soypass						
UIP, %CP	88.8[bc]	88.3[bc]	88.1[bc]	86.2[b]	91.5[c]	2.1
CV	2.3	3.6	2.7	3.9	4.1	4.8

[a]UIP = undegradable intake protein.
[bcde]Means within row with unlike superscripts differ ($P < 0.05$).

In summary, steer contributed more variation than both day and run. There is no difference between hand and machine rinsing, but with increased rinsing, washout can occur. Sample size can be increased, yet the sample size to bag surface area should be monitored due to depressed DMD at higher ratios. Based on the lack of effects and very high UIP values produced in a concentrate fed steer, a mixed diet is a better model for *in situ* incubation.

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