

Significance of Intestinal Digestion of Dietary Protein

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Introduction

In the last twenty years, elaborate models have been developed to formulate diets with greater accuracy in meeting the protein and amino acid requirements of lactating dairy cattle. Models for feeding protein to dairy cattle in the USA evolved from basic crude protein (NRC, 1978) to more complex systems based on rumen undegraded protein (RUP) and intestinal digestion of RUP (NRC, 2001). The NRC (1989) recognized that intestinal digestion of protein supplements differs; however, empirical data were lacking and as a result, a constant value of 80% was used for all feeds. The main problem was the lack of reliable techniques for estimating intestinal digestion of proteins. With improved techniques, the NRC (2001) assigned estimates of intestinal digestion to RUP fractions for each feedstuff. Data were obtained mainly from literature using the mobile bag technique and the three-step in situ/in vitro procedure of Calsamiglia and Stern (1995) using pepsin-pancreatin to predict digestibility of RUP. Values used in the French Protein System were adopted for feeds with limited or no data. Because a considerable amount of variation in intestinal protein digestion has been observed among and within feedstuffs using the three-step in situ/in vitro procedure, there is a need to improve the procedure so that a greater number of samples can be analyzed more rapidly.

Feeding proteins to ruminants that are resistant to microbial degradation in the rumen can provide a practical way to increase dietary protein and alter the amino acid profile of the protein reaching the small intestine for digestion and absorption. However, the effects of feeding high RUP on intestinal amino acid supply and animal performance have been inconsistent. Lack of response has been attributed to various factors including depression in ruminal microbial protein synthesis and factors related to quality of the dietary protein such as inadequate or overprotection of protein, reduced intestinal availability of amino acid or inherent amino acid limitations of the dietary protein. A considerable amount of variation among and within feeds in ruminal degradation and intestinal digestion of protein has been reported (Calsamiglia and Stern, 1995; Howie et al., 1996; Yoon et al., 1996). It is important that this variation be considered when determining the value of feeds as sources of protein for the ruminant animal.

Various criteria can be used to select the protein supplement for lactating dairy cows including palatability, RUP, protein quality, intestinal absorption of protein, availability and consistency of product, cost of protein and impact on animal performance. If RUP and intestinal protein/amino acid digestion data were to be readily available, an alternative method could be to determine cost of intestinally absorbable dietary protein (IADP) or amino acids.

Intestinal Protein Digestion

Methods for Measuring Intestinal Protein Digestion

The total amount of protein available for absorption from the small intestine depends on the flow of microbial and dietary protein to the duodenum and their respective intestinal digestion. Digestion of protein that leaves the rumen starts in the abomasum with acid-pepsin digestion and is completed in the small intestine with pancreatic and intestinal proteases. In vivo estimation of intestinal protein digestion involves expensive and labor-intensive experiments and requires the use of animals surgically prepared with cannulae in the proximal duodenum and terminal ileum. Apparent digestion of protein is calculated as the disappearance of CP or amino acids between the duodenum and ileum, which is subject to considerable

Therefore, several alternative procedures for estimation of intestinal protein digestion in ruminants have been developed. An in situ mobile bag technique was devised to determine intestinal protein digestion in ruminants (Hvelplund, 1985).

In Situ Mobile-Bag Technique. The mobile-bag technique (MBT) was originally introduced to measure protein digestibility in pigs (Sauer et al., 1983), but has been modified to study postruminal digestion of feedstuffs by ruminants (Kirkpatrick and Kennelly, 1984; Hvelplund, 1985). Using this method, a small amount of intact feed or feed that has been preincubated in the rumen is placed in bags. The bags are then preincubated in a pepsin-HCl solution or directly introduced into the duodenum and subsequently collected from the ileum, or more typically from the feces. Bags are then washed to remove endogenous and other contaminating proteins. Therefore, total tract measurements using this technique can be considered as estimates of true, rather than apparent digestibility (Robinson et al., 1992). However, Vanhatalo (1995) showed that contamination of feed residues by non-feed N, determined using a ^{15}N -dilution technique, resulted in somewhat lower digestion values with concentrates (barley) and forages (ryegrass), but the effect of contamination was negligible with protein concentrates (rapeseed meal). Forage rich in fiber and low in protein (straw) was markedly contaminated by non-feed N. Similar to observations with in situ measurements for estimating ruminal protein degradation, the MBT is affected by several potential sources of variation. Factors that have been addressed in recent research include porosity of bag material, sample-to-surface ratio, animal and diet effects, retention time, site of bag recovery, and microbial contamination (Hvelplund, 1985; Rooke, 1985; Voigt et al., 1985).

Porosity of bag material used in the MBT has ranged from 9 Φm (Hvelplund, 1985) to 80 Φm (Todorov and Girginov, 1991). Comparative data from these studies indicate that differences in protein digestion using the MBT resulting from bag pore size were rather small and of minor importance with both concentrates and forages. Vanhatalo (1995) found that with several feeds, the free surface area (percentage of total cloth area) rather than the pore size of the bag material had a significant effect on N disappearance from the mobile bags. Bag cloth with a small free surface area (2%) gave lower digestion values than cloth with a large surface area (35%), especially with forages. Reduction in surface area obviously restricted digestion because the difference between the cloths was not associated with digestion in the large intestine or particle loss from the bags.

The effect of ruminal preincubation on intestinal protein digestion using the MBT seems to be important only for certain feeds. Hvelplund et al. (1992) reported negligible effect of ruminal preincubation on estimates of intestinal protein digestion for soybean meal, cottonseed cake, coconut cake, peas, grass silage, and whole-crop barley silage. In contrast, ruminal preincubation had important effects on intestinal protein digestion of heat-processed canola seed (Deacon et al., 1988), rapeseed meal and meat and bone meal (Rooke, 1985), and some forages (De Boer et al., 1987). Using the MBT, Volden and Harstad (1995) concluded that ruminal preincubation is not required for soybean meal, fish meal, extracted rapeseed meal, corn gluten meal and guar meal; however, peas, rapeseeds, lupin seeds, barley, and oats should be evaluated after ruminal preincubation. With forages, preincubation in the rumen was necessary to predict intestinal protein digestion from the indigestible CP fraction determined with the MBT (Mgheni et al., 1994), and at least 48 h of ruminal incubation were needed (Vanhatalo et al., 1996). Consequently, it seems that ruminal preincubation should be routinely included in the MBT protocol.

Cherian et al. (1988) reported a dramatic decrease in intestinal digestion of soybean meal, meat and bone meal, and canola meal protein for pigs when pepsin-HCl predigestion was not included in the protocol. Finlayson and Armstrong (1986) indicated that in formaldehyde- or heat-processed proteins, the acidic environment in the stomach may be important for predicting intestinal protein digestion. In contrast, the effect of pepsin-HCl preincubation on intestinal digestion of protein has been considered negligible (Graham et al., 1985; Voigt et al., 1985). Vanhatalo et al. (1995) observed that pepsin-HCl preincubation did not affect intestinal CP digestion values, and pepsin-HCl preincubation is not necessary when the MBT includes preincubation of feeds in the rumen.

Average retention time of bags in the digestive tract values vary widely within and among experiments,

(Graham et al., 1985; Hvelplund, 1985; Voight et al., 1985; van Straalen et al., 1993). The proteolytic activity of the small intestine is substantial and ideally, bags should be recovered at the end of the ileum using re-entrant cannulas. However, for practical purposes, fecal collection is more convenient. Estimates obtained from fecal collection of bags assume that bags and feed residues are not contaminated with microbial protein from large intestinal fermentation and that protein leaving the ileum is not further digested by microbes in the large intestine. Because remaining N after digestion is often very small, the contribution of potentially contaminating microbial protein may be of greater concern. Hvelplund (1985) and Kohn and Allen (1992) suggested that microbial contamination was small enough to be ignored. In contrast, Varvikko and Vanhatalo (1990) reported that microbial contamination decreased estimates of intestinal digestion between six and 38 percentage units for ryegrass and rapeseed straw, respectively. Voigt et al. (1985) found a correlation of .92 between ileal and fecal collection of bags, and concluded that fecal collection of bags was appropriate. Jarosz et al. (1994) used the MBT to estimate intestinal digestibility in 10 samples of whole plant and straw from pea, barley, and ryegrass hay at different stages of maturity. Nitrogen disappearance was greater in bags recovered from feces than from the ileum for most feeds tested; however, differences were significant only in pea and barley straw. Collecting bags from feces instead of the ileum led to a slight increase in the CP digestion values of vegetable concentrates (rapeseed meals, soybean meal), but those of animal origin (meat and bone meal) or forages (grass silages) were usually not affected by bag recovery site (Vanhatalo and Ketoja, 1995).

In spite of the potential sources of variation that have been observed, several studies have shown that the MBT may be useful in predicting intestinal digestibility of protein. Hvelplund (1985) regressed *in vivo* intestinal protein digestion values for seven feeds measured in intestinally cannulated cows on estimates of the same feeds obtained using the MBT with fecal collection of bags and found a correlation of .81. DeBoever et al. (1995) concluded that a reasonable relationship existed between intestinal protein digestibility of compound feeds based on tabular values for the ingredients and those calculated using the mobile-bag technique. Masoero et al. (1994) tested intestinal CP disappearance using the MBT and compared these values with true intestinal digestibility of proteins according to tabular values for 29 feeds. Tabular values were found to be generally higher than those estimated using the MBT (89.8 vs 84.4%). With both evaluation methods, feeds of plant origin seemed more digestible than those of animal origin. For 18 of the 29 feeds tested, intestinal protein digestibilities were similar between the two procedures, whereas differences were observed in feeds with high fiber content. Some differences could be attributed to non-feed contamination, which is consistent with Vanhatalo and Varvikko (1995) who reported that fibrous feeds with low N content, such as rapeseed straw, can be seriously contaminated by non-feed N, which affects CP digestion values using the mobile-bag method.

Intestinal digestibility of ruminally undegraded feed CP varies considerably, depending on the feed type (Vanhatalo, 1995). Protein-rich concentrates (soybean meal) were characterized by high undegradable CP digestibility, whereas protein concentrates relatively high in fiber (rapeseed meal) or ash (meat and bone meal) were characterized by lower digestibility. The undegradable CP digestibility of concentrates was usually greater than that of forages. However, the ranking of forages is likely to be affected by non-feed N. As digestibility of undegraded CP is affected by both the extent of ruminal degradation and feed type, measured values rather than a constant factor should be preferred in modern protein evaluation systems.

The MBT provides an easy and fast way to determine intestinal digestion of proteins compared with using cannulae in the proximal duodenum and the terminal ileum. However, it is necessary to standardize and validate the procedure to provide consistent and reliable results so that a database can be generated for intestinal CP digestibility of supplements commonly fed to ruminants. A summary of intestinal protein digestion data adapted from Stern and Bach (1996) for various feed ingredients derived from 21 references using the mobile bag technique is presented in Table 1. Mean values ranged from 50.0 to 98.2% for lupine seeds and wheat flour middling, respectively.

Three-Step In Situ/In Vitro Procedure. The TSP (TSP) of Calsamiglia and Stern (1995) was developed to: 1) closely simulate physiological conditions of ruminants, including potential effects of ruminal fermentation; 2) be rapid, reliable, and inexpensive; 3) be applicable to a wide variety of protein supplements; and 4) accurately reflect differences in protein digestion. Dacron bags containing feed

containing 1 g/L of pepsin. After incubation, pH was neutralized with 1 N NaOH and a pH 7.8 phosphate buffer containing 3 g/L of pancreatin was added to the solution followed by incubation at 38°C. After a 24-h incubation, a 100% (wt/vol) trichloroacetic acid solution was added to precipitate undigested proteins. Preincubation of samples in the rumen did not affect pepsin-pancreatin digestion of residual CP in soybean meal, corn gluten meal, and blood meal, but decreased pepsin-pancreatin digestion of residual CP in hydrolyzed feather meal, fish meal, and meat and bone meal (80 vs 70, 88 vs 81, and 82 vs 56%, respectively, for nonruminant vs ruminant preincubation). Pepsin digestion before pancreatin digestion increased CP digestion of all proteins tested by a mean of 23 percentage units. The pancreatin digestion step was validated using 34 duodenal samples from which small intestinal CP digestion was determined *in vivo*. The regression equation of *in vivo* estimates on pancreatin digestion had a coefficient of determination of 0.91.

Intestinal CP digestion of solvent-extracted soybean meal and non-enzymatically browned soybean meal was similar within method using either the *in situ* MBT or the *in situ/in vitro* TSP (Mansfield and Stern, unpublished data). However, there was a substantial difference between the two procedures for estimating intestinal digestion (Table 2). Estimates differed by almost 22 percentage units, 99.4 vs 77.8% between the *in situ* MBT and the *in situ/in vitro* TSP, respectively, with almost complete disappearance of CP from the mobile-bags. Differences between methods may be a result of protein digestion that occurs in the large intestine. Hvelplund (1985) indicated that 50% of soybean meal protein that left the ileum was digested in the large intestine and observed a significant site of collection (ileum vs feces) × feed (soybean meal vs canola meal) interaction using the *in situ* mobile bag technique.

More recently, McNiven et al. (2002) modified the TSP by replacing the ruminal *in situ* step with an *in vitro* method involving a pre-incubation with a protease enzyme followed by incubations in pepsin and pancreatin. If successful, the *in situ* rumen fermentation step could be excluded, thereby eliminating the use of ruminal cannulated cows. McNiven et al. (2002) concluded that their method could predict intestinal protein digestion of heat damaged soybeans. However, recent evaluation of this modified procedure (Stern et al., unpublished data) compared with the original TSP procedure using 36 different feedstuffs, resulted in a poor relationship ($R^2 = 0.26$) between procedures.

Estimates of intestinal protein digestion of different protein supplements using the original TSP (Table 3) indicate that large variation exists among and within protein supplements. Low intestinal protein digestion was found for meat and bone meal (55%), batch-dried blood meal (63%), and hydrolyzed feather meal (67%), whereas all remaining protein supplements averaged greater than 70%. To evaluate the effects of different processing procedures on intestinal protein digestion of various animal proteins, Howie et al. (1996) obtained seven samples each of several animal byproducts. Estimates of intestinal digestion of RUP, determined using the TSP, ranged from 40.9 to 70.1% ($\sqrt{V} = 56.0\% \pm 4.0$), 59.2 to 75.2% ($\sqrt{V} = 65.3\% \pm 2.1$), 72.0 to 90.3% ($\sqrt{V} = 79.6\% \pm 2.5$) and 28.8 to 79.2% ($\sqrt{V} = 61.4\% \pm 6.8$) for meat and bone meal, hydrolyzed feather meal, ring-dried blood meal, and batch-dried blood meal, respectively. Yoon et al. (1996) used 18 Menhaden fish meal samples from various processing plants to evaluate the effects of processing on intestinal digestion of protein and noted values ranging from 72.8 to 86.4% ($\sqrt{V} = 77.7\% \pm 3.5$). These results demonstrate that considerable variation exists in intestinal digestion of protein among and within different protein sources, and this variation can possibly be influenced by source of raw material, quality of raw material (storage time and temperature), drying conditions, and various other factors.

Stern et al. (2005) used the *in situ* Dacron polyester bag technique and the TSP to evaluate various processing methods of soybean meal. Rumen undegradable protein (Figure 1a) ranged from 23.2 to 68.3% for solvent-extracted soybean meal and non-enzymatically browned soybean meal, respectively. Intestinal CP digestion (Figure 1b) ranged from 57.7% to 83.8% for non-enzymatically browned soybean meal and mechanically extracted soybean meal with fresh soygums, respectively. Ruiz Moreno and Stern (2005, unpublished data) used the TSP to evaluate consistency in processing procedures. Intestinal protein digestion of three carloads for each of four ruminal protected soybean products [solvent extracted SBM (SE) heat treated (SOLH); SE SBM nonenzymatically browned (SOLNEB); mechanically-extracted (ME)

distillers dried grains with solubles (DDG-A, DDG-B and DDG-C) were evaluated. Means and standard deviation (sd) for SOLH, SOLNEB, MEC1G and MEC2 were 70.8, \pm 2.1; 68.2, \pm 1.0; 83.0, \pm 1.55; and 81.5, \pm 2.5, respectively (Figure 2a). Variation in processing of each protected soybean product was not great; however the mean intestinal protein digestion was fairly large ranging from 68.2 to 83.0% among the four soybean products. Means and standard deviations for DDG-A, DDG-B and DDG-C were 71.9, \pm 3.2; 72.0, \pm 0.5; and 80.6, \pm 4.2, respectively (Figure 2b). Variation in processing was low for DDG-B (range of 71.7 to 72.6%), but there was a fairly large variation in processing of DDG-A (range of 69.6 to 75.5%), and DDG-C (range of 77.2 to 85.3%). Another noteworthy point is that the mean intestinal protein digestion of the three distillers dried grains products ranged from 71.9 to 80.6%. From these types of observations, it appears that the TSP can be a useful method of evaluating quality control of protein within and among processing procedures.

Modified Three-Step In Situ/In Vitro Procedure. An in vitro batch incubator (DaisyII) was used to simplify the TSP to reduce the cost and labor involved in the determination of intestinal digestion of proteins (Gargallo et al., 2006). Use of the Daisy II incubator eliminates the need to use trichloroacetic acid (TCA), an acid that is classified as highly corrosive, toxic and environmentally unfriendly, while also improving the ability of the procedure to estimate intestinal digestion of individual amino acids.

Four tests were conducted to study the effects of the type of pepsin (Sigma P-7012 vs Sigma P-7000), the type of bags used for the incubation of samples (nylon bags, Ankom R510, vs bags for fiber analysis, Ankom F57), the amount of sample per bag (0.5, 1, 2 or 5 g) and the number of bags per incubation bottle (5, 15, 20 or 30 bags) on the estimated intestinal digestion of protein. A soybean meal sample heated at 170°C for 0, 0.5, 1, 2, 4, 6 and 8 h was used in all preliminary tests to determine the optimum conditions of the technique. The SBM samples processed at 170°C for different times provided a wide range of pepsin-pancreatin digestion (0 h = 82.2%, 0.5 h = 78.5%, 1 h = 78.4%, 2 h = 57.4%, 4 h = 29.7%, 6 h = 23.9%, and 8 h = 10.7%) using the procedure of Calsamiglia and Stern (1995). Intestinal digestion of 12 protein supplements was determined using the proposed DaisyII and the TSP techniques. The protein supplements used for this test were: blood meal, fish meal, green peas, lupin seeds, whole cottonseed, corn gluten meal, alfalfa pellets, heat-processed SBM, sunflower seeds, barley dried distillers grains, corn dried distillers grains and corn gluten feed.

Results using the two types of pepsin were related ($P-7012 = (0.99 (\pm 0.04) * P-7000) - 0.29 (\pm 2.33)$; $r^2 = 0.99$, $P < 0.001$, $n = 14$). Intestinal digestion of soybean meal samples obtained from the TSP assay were highly related with those obtained using the DaisyII incubator with Ankom R510 bags ($DaisyR510 = (1.37 (\pm 0.06) * TSP) - 15.45 (\pm 3.85)$; $r^2 = 0.98$, $P < 0.001$, $n = 14$) and Ankom F57 ($DaisyF57 = (1.33 (\pm 0.06) * TSP) - 15.76 (\pm 3.87)$; $r^2 = 0.98$, $P < 0.001$, $n = 14$). The amount of sample per bag and the number of bags per incubation bottle did not affect estimates of intestinal digestion of protein. These results indicate that the use of up to 30 nylon bags (Ankom R510) with 5 g of sample in each DaisyII incubation bottle could be used to estimate intestinal digestion of protein in ruminants.

Average estimates of intestinal CP digestion using the Daisy II incubator (71.2%) were similar to the TSP (69.2%), and were related ($TSP = (0.905 (\pm 0.12) * Daisy II) + 4.75 (\pm 9.09)$; $r^2 = 0.84$, $P < 0.001$, $n = 12$), with the slope not different from 1 and the intercept not different from 0 (Figure 3). This relationship indicates that the Daisy II technique can be used to predict the intestinal digestion of proteins, reducing the cost and labor involved in the process.

Intestinally Absorbable Dietary Protein

Intestinally absorbable dietary protein (IADP) is defined as the amount of protein from a specific feed that is available for absorption in the small intestine and is calculated as RUP multiplied by intestinal protein digestion. The IADP value provides an index of the quality of protein supplements as sources of RUP for ruminants. Because there is a large amount of variation associated with measurements of ruminal degradability and intestinal digestibility among and within protein supplements, and there seems to be no correlation between RUP and intestinal protein digestibility (Yoon et al., 1996), the use of published values for RUP and intestinal digestion to calculate IADP is not adequate. Therefore, only data from experiments

Results indicate that corn gluten meal (74%) and ring-dried blood meal (67%) provided the largest amount of IADP, followed by non-enzymatically browned soybean meal, batch-dried blood meal, Menhaden fish meal and hydrolyzed feather meal (51 to 58%). Because corn gluten meal tends to stick together when wet, exposure of the surface area in Dacron polyester bags decreases as previously described (Stern et al., 1983a). Using an RUP value of 57%, determined in vivo by Stern et al. (1983b), instead of 83%, as determined in situ, and assuming that intestinal digestion is 89%, the IADP for corn gluten meal would be approximately 51%. Using IADP to evaluate the various processing methods of soybean meal (Figure 1c), it should be noted that non-enzymatically browned soybean meal had an IADP of 39.4%, similar to mechanical extracted soybean meal with fresh soygums at 41.3%, emphasizing the importance of accounting for both RUP and intestinal digestion of protein (Stern et al., 2005).

Economic Considerations

Determination of IADP and analysis of the amino acid profile of protein supplements make it possible to calculate the cost per kg of IADP or intestinally absorbable individual amino acids provided by each supplement. Table 4 was derived using data from Table 3, standard amino acid composition of feeds and market prices of feeds adapted from Feedstuffs. Results were calculated assuming that individual amino acids had similar ruminal degradability and intestinal digestibility as the crude protein of the original feed. In terms of IADP (\$/kg), HFM (0.43) and CGM (0.54) were the cheapest sources and SBM the most expensive source (1.89) of IADP. Ring-dried and batch-dried BM were the cheapest (\$/100 g) sources of lysine and CGM was the cheapest source of methionine. However, it should be emphasized that there is potentially large differences associated with the cost of each protein due to variation in quality within supplements. The highest variation in cost of IADP (\$/kg) was for batch-dried BM (0.73 to 2.20) and MBM (0.64 to 2.07). Although batch-dried BM on average was the second cheapest source of lysine, it is important to emphasize that it ranged from 0.86 to 2.58 for absorbable lysine (\$/100 g) due to inconsistency in processing. Selection of the best protein supplement is highly dependent on the quality of the protein. In the future, cost per kg of IADP or absorbable amino acids can be used as a guideline for selecting the appropriate protein supplement to be used in diets fed to ruminants as market prices of feedstuffs fluctuate.

Questions that Need to be Addressed

Because a considerable amount of variation in intestinal protein digestion has been observed among and within feedstuffs using the TSP procedure, it is important that several questions be addressed to help improve the quality of protein provided to dairy cattle: 1) How do we communicate to the feed industry that there is a need to improve quality control, providing consistent products to producers? 2) Can cost/IADP or individual amino acid be used as a tool for determining protein supplementation in the dairy cow diet? 3) Can we replace in situ ruminal digestion with an in vitro procedure and what can we do to expedite and improve estimation of intestinal digestion of RUP? 4) Is the modification of the three-step procedure using the Daisy II incubator the current and future method of choice for estimating intestinal protein digestion? 5) Can the modified three-step procedure using the Daisy II incubator be used to provide reliable information on individual amino acid absorption?

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Table 1. Intestinal digestion of crude protein (percentage) in ruminants using technique.^a

Feed	n	Mean
Plant Proteins		
Brewers Grains	1	76.6
Canola seed, whole	3	84.6
Canola seed, whole, heated	4	75.4
Canola meal	3	75.0
Canola meal, heated	3	80.9
Coconut, cake	5	89.6
Coconut, expeller	1	93.8
Coconut, mechanically extracted	2	88.3
Cotton seed	2	58.5
Cotton seed, meal	5	92.9
Corn gluten, meal	4	96.2
Corn gluten, feed	1	80.9
Corn, distiller	2	79.4
Groundnut meal	1	94.1
Horse beans, whole	2	88.3
Horse beans, whole extruded	1	90.7
Linseed meal	4	85.3
Lupine seed	1	50.0
Lupine seed, extruded	2	92.4
Palm kernel	7	87.8
Peanut meal	1	93.2
Peanut, mechanically extruded	1	95.9
Peas	3	87.9
Peas, extruded	1	98.0
Rapeseed meal	12	70.9
Rapeseed meal, heated	2	78.1
Soybean Hulls	1	72.4
Sesame meal	2	97.3
Soybean meal	19	96.4
Soybean meal, heated	1	96.9
Soybean meal, formaldehyde	3	66.3
Soybeans, extruded	2	96.0
Soybeans, flaked	1	87.9
Sunflower, meal	4	87.8
Wheat bran	1	70.9
Wheat flour middling	1	98.2
Wheat middling	1	78.2

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