EFFECT OF PROCESSING ON PROTEIN SOLUBILITY AND DIGESTIBILITY OF SORGHUM

by

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ABSTRACT

A metabolism trial was conducted using eight cross-bred Brahman steers to determine the effect of grain processing on protein solubility and digestibility of sorghum. Sorghum grain evaluated was a commercial hybrid grown under irrigated conditions in Canyon, Texas. Processing methods evaluated were dry rolling (DR), micronizing (M), reconstitution (R) and steam-flaking (SF). The processed grains comprised 75% of the non-supplemented ration with cottonseed hulls, molasses and mineral comprising the remainder of the ration. Feed intake of the rations was recorded daily and grab samples composited for analyses. Samples of processed grain were stored separately. After a 10 day adjustment period, total fecal and urine samples were collected and composited over a 5 day collection period. Processed grain and dried fecal samples were analyzed for soluble nitrogen components by the Landry and Moureaux procedure. Apparent digestibilities of dry matter and nitrogen as well as nitrogen balance were calculated.

Apparent dry matter digestibility was highest in steers fed the reconstituted grain (75.6%) and similar among the DR (68.8%), M (66.2%) and SF (64.1%) cattle. Apparent nitrogen digestibility and nitrogen balance followed a similar pattern being highest in the cattle fed R grains (32.0% and 10.84 g/day), respectively.

Processing method affected the percentage distribution of the soluble nitrogen with micronizing decreasing the proportion of nitrogen in fraction I and increasing the proportion in fraction V. All processing methods decreased the proportion of soluble nitrogen in fraction III as compared to the DR control. Moist treatments increased and heat treatments within moisture levels decrease the digestibility of fraction I with R being more digestible than SF (59.9 vs 43.3%) and DR being more digestible than micronized grain (30.1 vs 24.2%), respectively.

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INTRODUCTION

On a world wide basis, sorghum is the third most important food crop and is the chief food staple for Asia and Africa. In the United States, approximately 90% of the production is used for livestock feed. It is the basic feed ingredient of livestock in the Southwest. The nutritive value of sorghum is consistently lower than corn as reflected by lower gains, increased feed requirements and processing costs and therefore lower returns for livestock fed sorghum based rations. Reasons for the lower nutritive value appear to be related to the availability of protein. Sorghum has the lowest protein digestibility of common cereal grains (NRC, 1975). Identification of the fraction components of sorghum would aid both the farmer and feeder in increasing the nutritive value. Since sorghum is processed before feeding, the object of this paper is to determine the effect of processing on the protein fractions of sorghum.

REVIEW OF LITERATURE

Structure and Characteristics of the Sorghum Grain Kernel

The structure of the sorghum grain caryopsis is important in determining the availability of the kernel constituents for use by the animal (Cooley, 1963). The mature kernel of sorghum grain consists of the embryo, or germ,

and the endosperm surrounded by the pericarp and, in some varieties, the testa. The peripheral endosperm layer located between the endosperm and the pericarp, consists of two to six concentric layers containing a dense protein matrix. This area is exceedingly difficult for water or digestive fluids to penetrate and may account for the relatively low digestibility reported for sorghum grain (Rooney, 1968).

The presence of water-soluble proteins in corn was first recognized by Chittenden and Osbourne (1891). The solubility of nutrients in different solvents has been used to evaluate the nutritive value of several cereal grains. The solubility of proteins depends on the ionization of the amino group which is repressed by low pH (Craine and Fahrenholtz, 1958). Nash and Wolf (1967) reported increased protein solubility in soybeans by incorporation of 2-mercaptoethanol in the solvent.

A method to classify the protein fractions of sorghum as to their availability and location within the grain would be beneficial to breeders and grain processers. The procedure employed most by other workers (Jones and Beckwith, 1970; Skoch et al., 1970; Virupaksha and Sastry, 1968) is based on the classical procedure of Osbourne and Mendel (1914). The major problem associated with this procedure is the low nitrogen recoveries (approximately 50-70%) (Skoch et al., 1970). Landry and Moureaux (1970)

developed a procedure which solubilized most of the nitrogen of sorghum and yields five different soluble fractions. Jambunathan and Mertz (1973) reported nitrogen recoveries ranging from 83 to 96% in sorghum grain, with a distinctly different distribution of proteins among the five fractions.

Effects of Processing on Sorghum Grain

Hale et al., (1966) determined that steam flaking of sorghum improved gain and feed efficiency compared to dry rolled processing. The improved efficiency of feed conversion with moist grain is due to increased digestibility of organic matter (15-30%) and protein (15-23%) (Riggs, 1970).

Studies by Schake, et al., (1970) and Potter, McNeil and Riggs (1971) have shown that steers fed micronized grain rations consumed less grain, thus improving feed efficiency. Gains and carcass grade from steers fed micronized and dry rolled grain were not significantly ($P < .05$) different than thos of steers fed reconstituted and steam flaked rations (Potter et al., 1971).

Potter et al., (1971) has also suggested that, since more protein is absorbed from reconstitued and steam flaked grains than from micronized and dry rolled, micronizing may lower biological value through decreased ruminal conversion of grain protein to microbial protein. They also

reported that abomasal proteins from steers fed micronized and dry rolled processed grains were lower in lysine than those fed steam flaked and reconstituted grains. Sorenson et al., (1971) induced that popped grain can be converted more efficiently by cattle than dry, ground grain.

EXPERIMENTAL PROCEDURE

Metabolism Trial

Eight Brahman-cross half-sib steers were blocked by weight and steers within each block were fed one of 4 processes of a hetero-yellow sorghum grain dry rolled, steam flaked, micronized or reconstituted. Rations consisted of 80% grain, 15% cottonseed hulls, 2% mineral mix and 4% molasses. The steers were fed to appetite twice daily and refusals collected, weighed, and subtracted from the day's total feed intake. After a 10 day adjustment period feces and urine were collected from all steers during a 5 day collection period. Total amounts were recorded, and representative samples were composited for later analyses. Apparent digestibilities of dry matter and nitrogen as well as nitrogen balance were calculated by difference between intake and excretion of nutrient.

Protein Fractionation

Protein extraction was carried out on samples of each processing method and on feces samples from all steers. To

facilitate grinding, all samples were dried at 55°C before grinding through a udy sample mill.

The techniques described by Landry and Moreaux (1970) which separates the proteins into 5 soluble fractions, was followed in the protein fractionation. Two gram portions of each sample were extracted with 20 ml of the solvents successively and for the specified times to yield the fractions shown in table 1.

The nitrogen analysis was accomplished by Kjeldahl digestion of 5 ml of each fraction followed by colorimetric determination of nitrogen.

RESULTS

The effect of grain processing method on apparent digestibilities and nitrogen balance is shown in table 2. Steers fed reconstituted grain digested the most ration dry matter (75.6%) followed by dry-rolling (68.6%). Heat treated grains were the least digestible with values of 64.1% and 66.2%, for steam flaking and micronizing, respectively. Thus, the heat treatments, steam flaking and micronizing, appeared to reduce digestibility while reconstitution increased digestibility. Nitrogen digestibility data followed that of apparent dry matter digestibility as the greatest nitrogen digestibility occurred in steers fed the reconstituted grain while the lowest nitrogen digesti-

TABLE 1. LANDRY AND MORGAN PROTEIN FRACTIONATION SEQUENCES

Fraction	Solvent	Time (Min.)	Temp.
I	5 M NaCl:H_2O	60,30,30,15,15	4°C
II	70% Isopropyl Alcohol	30,30,30	Room
III	70% Isopropyl Alcohol and 2-Mercaptoethanol (.6%)	30,30	Room
IV	Borate Buffer With NaCl (pH 10, .5 M) and 2-Mercaptoethanol (.6%)	60,30,15	Room
V	Borate Buffer with NaCl (pH 10, .5 M) and 2-Mercaptoethanol (.6%) and Sodium Lauryl Sulphate (.5%)	60,30,15	Room

Fractions were centrifuged after shaking and supernatants were stored for nitrogen analysis.

TABLE 2. EFFECT OF PROCESSING ON APPARENT DIGESTIBILITIES AND NITROGEN BALANCE

Item	Process			
	Dry	Reconstituted	Micronized	Steam flaked
Apparent digestibility, % dry matter	68.8	75.8	66.2	64.1
Nitrogen, %	22.4	32.0	17.9	19.8
Nitrogen balance, g/day	- .99	+10.84	+ 4.33	± 1.95

bility occurred in the micronized steam flaking and dry rolling gave close values of 22.39 and 19.79, respectively. Thus the heat treatments also appeared to decrease nitrogen digestibility, while the non-heat processes tended to increase it.

Nitrogen balance data ranged from -.99 g/day for dry rolled to 10.84 g/day for reconstitution. Micronized and steam flaked grains produced close values of 4.33 g/day and 4.95 g/day, respectively. Therefore, the wet processes, reconstitution and steam flaking, increased the nitrogen balance while dry rolling and micronizing tended to decrease nitrogen balance.

The effect of processing of sorghum on protein solubility is given in table 3. Total soluble nitrogen was quite variable because of several problems which are mentioned in the discussion. Steam flaking produced the highest value for Fraction I, while micronized gave the lowest value. This fraction is one of the most soluble and digestible fractions and thus increased recovery of this fraction is beneficial.

Fractions II and II and IV yielded fairly consistent data throughout the four processes. Reconstituted and steam flaked had the highest solubilities in fraction II, while dry rolling had the lowest solubility. The highest solubility in fraction III was produced by dry rolling, while

TABLE 3. EFFECT OF PROCESSING OF SORGHUM ON PROTEIN SOLUBILITY

Item	Process			
	Dry	Reconstituted	Micronized	Steam flaked
Crude protein, % (DM)	8.8	8.9	9.0	9.0
Total soluble nitrogen, % of CP	115.0	100.2	116.6	79.0
Soluble fractions, % of total soluble nitrogen				
Fraction I	23.1	22.8	16.0	24.0
Fraction II	11.5	15.0	11.7	15.0
Fraction III	33.0	24.4	24.3	24.2
Fraction IV	7.1	6.1	7.8	7.0
Fraction V	25.2	31.8	37.5	29.7

the other three processes gave nearly equal data. Micronized yielded the highest value for fraction IV, while the data for the other processes of the fraction were very similar.

Micronized produced the highest solubility of fraction V while dry rolling produced the lowest solubility. Steam flaked and reconstitution gave intermediate values. There was no relationship between wet, dry, or heat treatments within fraction V.

The results of protein fractionation on feces from steers fed differently processed grains are summarized in table 4. Total fecal soluble nitrogen had the same problems as soluble feed nitrogen which confounds interpretation of data. However, feces from steers fed reconstituted grain had the lowest soluble nitrogen and the moist treatments had less fraction I nitrogen than the dry treatments. Thus moist processing appears to increase the digestibility of fraction I.

Fraction II yielded like data for all methods.

Steam flaked gave the highest value (30.6) for fraction III and micronized gave a markedly lower value (20.2). Dry rolling produced the second highest solubility in this fraction while reconstitution produced the second lowest. There was no relationship between dry or wet treatments and solubility of fraction III.

Steam flaking and reconstitution produced the greatest

TABLE 4. EFFECT OF PROCESSING OF SORGHUM ON PROTEIN FRACTIONATION OF FECES

Item	Dry	Reconstituted	Micronized	Steam flaked
Total nitrogen, %	1.76	1.67	2.11	2.1
Soluble fractions, % of total soluble nitrogen				
Fraction I	24.3	11.9	14.9	8.6
Fraction II	6.9	6.9	5.9	5.3
Fraction III	30.1	22.6	9.0	30.8
Fraction IV	13.2	18.7	17.3	20.0
Fraction V	26.5	31.6	39.6	30.7
Total soluble nitrogen, % of total nitrogen	116.8	65.2	94.2	157.6

recovery from fraction IV while micronized and dry rolling decreased solubility of this fraction. Therefore, moist processing seems to increase recovery of this fraction while dry treatments reduce it.

Fraction V, quantitatively the second most important fraction was increased by micronized and decreased by dry rolling. Reconstituted and steam flaked gave similar values of 31.6 and 30.7, respectively. There was no relationship between wet, dry, or heat treatments of this fraction. Mean apparent digestibility of fraction I (table 5) was highest in the steers fed the reconstituted grain and lowest in the micronized grain treatment. Moist treatments appeared to increase digestibility and heat treatment appeared to decrease digestibility of fraction I.

Discussion

The percentage of total N in fraction II and III, total soluble N recovery and the low N recovery in fraction I shown by the data are not consistent with the data of other workers. These unusually high and low data may be a direct result of several problems encountered during the experiment namely storage problems with fraction I and extreme difficulty in Kjeldahl digestion of fractions II and III.

Fraction I, the NaCl/H_2O soluble fraction, even though stored at -^{0}C, became contaminated with various genera of

TABLE 5. THE EFFECT OF PROCESSING OF SORGHUM ON DIGESTIBILITY OF THE WATER SOLUBLE FRACTION (FRACTION I)

Treatment	Nitrogen intake, g	Fraction I, feed		Total N excreted, g	Fraction I, feces		Nitrogen loss, g	%, digestibility
		% Total nitrogen	Nitrogen intake, g		% Total nitrogen	Nitrogen excreted, g		
Dry rolled	420.5	26.91	113.2	273.2	29.0	79.0	34.3	30.1
Micronized	374.0	18.70	70.0	308.8	15.5	45.1	24.9	24.2
Reconstituted	337.9	22.84	79.7	210.3	15.4	32.0	17.7	59.9
Steam flaked	386.7	18.96	73.3	311.6	13.5	41.5	31.8	13.3

fungi and bacteria. This contamination was probably due to the water based, unbuffered solvent of the fraction, since the alcohol soluble and pH buffered soluble fractions did not become contaminated. Because of this contamination, some of the nitrogen of the fraction may have been utilized by the fungi and bacteria, therefore nitrogen values for fraction I were lower than previously reported. To overcome this problem, Kjedahl analysis could be run on fraction I as soon as its fractionation was complete. This would alleviate storage and thus, stop any possibility of bacterial contamination.

The Kjedahl digestion of the alcohol soluble (II and III) fractions was also a source of error in the data. The usual procedure, consisting of addition of 5 ml H_2SO_4 and digestion mix to the sample and then digestion was not satisfactory for these two fractions. After failure of this method, percholoric acid and a surfactant were added to the H_2SO_4 and 10 ml, acid were added to each sample. However, this method also did not succeed as the sample-acid mixture dehydrated and dried and in some instances, the distilling flasks shattered and the samples were lost.

Therefore, from 20-25 ml digestion acid, 1 gm digestion mineral mix, and 5 Monger granules were added to 5 ml samples and digestion and distillation resulted. Although many flasks still boiled dry, addition of extra acid and

digestion mineral mix alleviated the problem and these samples also continued normal digestion.

This unusual behavior of the alcohol soluble fractions may be due to formation of a complex and subsequent evaporative loss of the digestion acid and alcohol solvent.

Because of the excess amount of acid needed, both samples were diluted to 50 ml, instead of the usual dilution of fraction II to 25 ml and fraction III to 50 ml. Although the 50 ml volume is the same as that normally used for autoanalyzer analysis, there existed in this 50 ml portions, three to four times as much acid as normal. Thus, more NaOH was required to dilute the sample and thus machine error may have been induced causing above normal reading.

The most significant error resulting from the Kjeldahl procedure, however, was the addition of an excess of digestion mineral mix to the samples. This mineral mix is mostly copper and potassium sulphates. The copper sulphate imparts a blue color to the final digested sample and this color remains after dilution. Therefore, when the sample is analyzed by the colorimeter of the Autoanalyzer, the color measured is not only that of the phenol-ammonium complex, but that of the copper sulphate water complex as well. This would thus account for the unusually high readings for fractions II and III and would also cause total soluble N recovery to be too high.

To correct the above mentioned problems, 2 ml portions of fractions II and III could be used. In addition, the copper sulfate could be replaced by some other less colorful mineral.

CONCLUSIONS

Although sufficient number of animals or replicates were not evaluated to enable drawing of statistical conclusions, it would appear that apparent dry matter digestibility is increased by reconstitution but not affected by micronizing or steam flaking. Nitrogen digestibility and balance is also increased by reconstitution. Protein fractionation of sorghum grain is also affected by processing. Major differences occurred in the micronized grain which had lower proportion of soluble nitrogen in fraction I but higher proportions in fraction V.

LITERATURE CITED

A.O.A.C. 1970. Official methods of analysis (11th Edition). Association of Official Agricultural Chemists. (Washington, D.C.

Chittenden, R.H. and T.B. Osborne. 1891. A study of the corn or maize kernel. Am. Chem. J. 13:453.

Craine, E.M. and K.D. Fahrenholtz. 1958. The proteins in water extracts of corn. J. Cereal Chem. 35:453.

Hale, W.H., L. Cuitun, W.J. Saba, B. Taylor and B. Theurber. 1966. Effect of steam processing and flaking milo and barley on performance and digestion by steers. J. Anim. Sci. 25:392.

Jambunathan, R. and L.T. Hertz. 1973. Relationship between tannin levels, rat growth and distribution of proteins in sorghum. J. Agr. Food Chem. 21:692.

Jones, R.W. and A.C. Beckwith. 1970. Proximate compositions and proteins of three grain sorghum hybrids and their dry mill fractions. J. Agr. Food Chem. 18:33.

Landry, J. and T. Moureaux. 1970. Mitterogenerte des glutelines du grain de mais: extraction selective et composition en acides amines des truis fractions isolees. Bull. Soc. Chem. Biol. 52:1021.

Nash, A.M. and W.J. Wolf. 1967. Solubility and ultracentrifugae studies on soybean globulins. J. Cereal Chem. 44:183. (Abstr.).

NRC. 1975. Nutrient requirements of beef cattle. National Academy of Sciences. Washington, D.C.

Osborne, T.B. and L.B. Mendel. 1914. Nutritive properties of the maize kernel. J. of Biol. Chem. 18, 1.

Potter, G.D., J.K. McNeill and J.K. Riggs. 1971. Utilization of processed grain protein by steers. J. Anim. Sci. 32:549.

Riggs, J.K. 1970. Utilization of sorghum grain by livestock. Tex. Agr. Exp. Sta. PR-2946. College Station, Texas.

Rooney, L.W. and L.L. Clark. 1968. The chemistry and processing of sorghum grain. Cereal Sci. Today. 13:258.

Sastry, L.V.S. and T.K. Virupaksha. 1969. Alcohol soluble proteins of grain sorghum. J. Cereal Chem. 46:284.

Schake, L.M., O.D. Butler and J.K. Riggs. 1970. Commercial feedlot evaluations of four methods of sorghum grain processing. J. Anim. Sci. 31:926.

Skoch, L.V., C.M. Dyce, F.K. Shoup, J. Bathurst and D. Liang. 1970. Protein fractionation of sorghum grain. Cereal Chem. 47:472.

Sorenson, J.T., W.K. Porter, Jr., Price Hobgood, B.R. Stewart, W.C. McGuire and D.G. Wallis. 1971. Tex. Agr. Exp. Sta. PR-2944. College Station, Texas.

Virupaksha, T.K. and L.V.S. Sastry. 1968. Studies on the protein and amino acid composition of some varieties of grain sorghum. J. Agr. Food Chem. 16:199.

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