Table IV shows eggshell thickness and egg weights of the quail hens fed the seleniferous, fly ash grown wheat. In agreement with our previous observations (Stoewsand et al., 1977), selenium-containing wheat or sodium selenite fed to Japanese quail hens significantly increased eggshell thickness, as compared to eggs produced by hens consuming low selenium diets. It should be noted that in both of these studies the dietary level of selenium was high, i.e., between 1.8 or 3.4 ppm, in great excess of an avian species nutritional requirement (Scott et al., 1969). Dietary calcium was relatively low for optimal egg production and quality (Table I).

Japanese quail body and liver weights, as well as hepatic microsomal enzyme activity, were unaltered in the high selenium, fly ash grown, wheat-fed group (Table V). It has been reported that dietary selenium may alter hepatic microsomal enzyme activities in the rat (Burk et al., 1974). These tissue–enzyme systems, so important in the metabolism of foreign compounds, may interact with a myriad of dietary elements (Becking, 1976).

These data show that fly ash, a waste material from coal-burning electric power plants, may contain selenium readily available to crops. Uptake of this element by wheat can occur, as it does in wheat grown in high selenium soils (Olson et al., 1970). Diets containing large amounts of this wheat produce some biological changes in Japanese quail, such as enhanced tissue storage of selenium, with dietary selenium levels as high as 3.4 ppm. Indeed, if further studies confirm that selenium additions to deficient diets increases eggshell thickness, this could add support to the argument for adding this element to layer diets. Fly ash high in selenium and low in toxic elements may be a desirable amendment to low selenium soils and a practical source of this element in grains and other plant foods.

ACKNOWLEDGMENT

The authors thank R. S. Murphy, J. L. Anderson, C. A. Bache, M. Gilbert, C. L. Heffron, W. A. English, A. Bunk, R. L. Bowman, M. Loveless, I. S. Pakkala, and H. J. Arnold for their assistance in this investigation. The Japanese quail were kindly furnished by the Department of Poultry Science, Cornell University.

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Received for review November 16, 1977. Accepted February 10, 1978.

Removal of the Growth Inhibitor(s) from Acid and Pressure Hydrolyzed Sawdust

Robert Britton

Sawdust hydrolyzed (HSD) with 2.3% H_2SO_4 at 42.2 kg/cm^2 pressure for 40 s caused reduced growth when included in the diets of rats. Furfural produced by the acid hydrolysis of the sawdust did not appear to cause the growth depression in rats. Extraction of the HSD with 95 and 80% ethanol removed substance(s) which inhibited cellulose digestion by rumen microorganisms. Ninety-five percent ethanol extraction of HSD was also effective in removing the compound(s) inhibiting growth in rats.

Sawdust offers great potential as an energy source for ruminants, but wood polysaccharides usually are not sufficiently degraded by rumen microorganisms to provide energy unless some manner of delignification is used. Several methods of improving wood cellulose degradation are: irradiation with high-energy electrons (Lawton et al., 1951), reduction in physical size to micron-sized particles (Stranks, 1959; Dehority and Johnson, 1961), and heating in dilute alkali (Wilson and Pigden, 1964; Mellenberger et al., 1971). These processes increased the utilization by bacteria or isolated enzymes.

Sawdust has also been subjected to modification by acid and pressure hydrolysis. Inclusion of this modified sawdust in cattle, sheep, or rat diets reduced performance in all cases (Butterbaugh and Johnson, 1974; Erlinger and Klopfenstein, 1975; Hudson, 1971). The present experiments were designed to determine the factor(s) in the hydrolyzed sawdust (HSD) responsible for the reduced performance.

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MATERIALS AND METHODS

Sawdust (80% hardwood and 20% pine) was hydrolyzed with 2.3% H_2SO_4 at 42.2 kg/cm^2 pressure for 40 s. The HSD was neutralized to pH 7 with NaOH.

In Vitro Experiments and HSD Extractions. Two in vitro dry matter disappearance (IVDMD) experiments were utilized to determine whether extraction procedures would remove materials inhibiting cellulose digestion by rumen microorganisms. Initially, petroleum ether was used for extraction, but negligible amounts of dry matter were extracted and it was not investigated further. A sequential ethanol (ETOH) extraction (Figure 1) of the HSD was performed starting with absolute ETOH, then 95% ETOH, and finally 80% ETOH. Upon refrigeration of the extracts, precipitates formed in each extract. The precipitates were resolubilized in 0.2 N NaOH. All extracts

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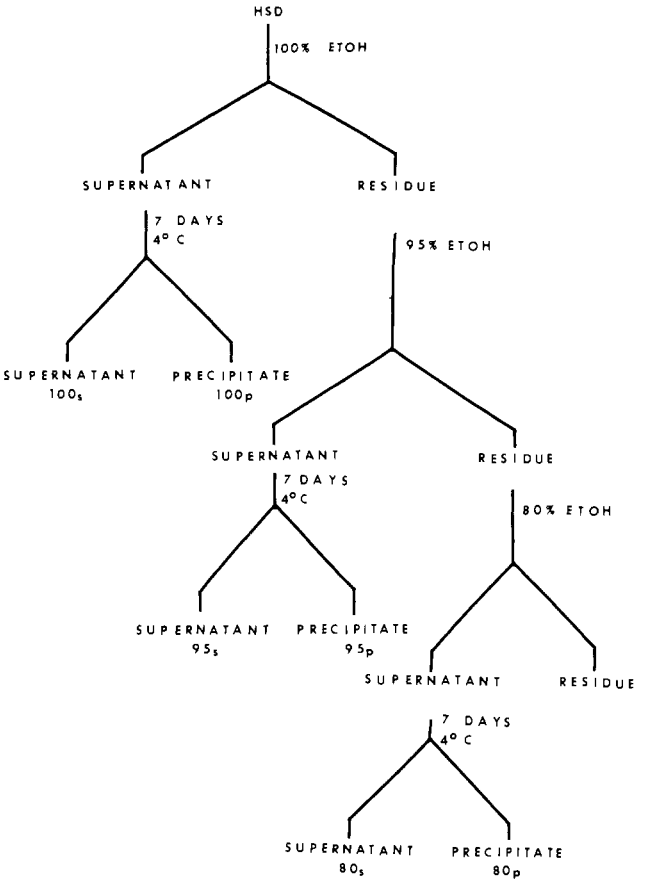

Figure 1. Flow diagram for HSD extractions for in vitro experiments.

and precipitates were dark reddish brown. The first in vitro experiment tested the effects of adding the ETOH extracts or the precipitates from the ETOH extracts on IVDMD (Tilley and Terry, 1963) of solka floc (cellulose).

The second in vitro experiment was conducted to estimate the IVDMD of HSD, a 1:1 mixture of HSD and solka floc, HSD residue after 95% ETOH extraction and solka floc. In both in vitro experiments, 30 mL of half rumen fluid and half McDougall's artificial saliva (1948) were incubated for 48 h with a measured amount of solka floc (about-0.3 g) with the respective treatments.

Rat Trial 1. A 28-day growth trial with eight male Long-Evans weanling rats per treatment was initiated to test what effects increasing HSD levels or increasing furfural levels within HSD levels had on performance. Three levels of HSD (10, 20, and 30% of the diet DM) were fed. Within each level of HSD were three levels of furfural (high, medium, and low). The control diet contained no furfural or HSD. The high furfural (3.5%) HSD (HFHSD) was prepared as stated above. Low furfural (0.66%) HSD (LFHSD) was prepared by treating HFHSD with steam and removing most of the furfural. Medium furfural HSD (MFHSD) consisted of equal quantities of HFHSD and LFHSD. As the HSD level in the diet increased, equal amounts of solka floc and cerelose were replaced (i.e., 10% HSD replaced 5% solka floc and 5% cerelose). HSD was usually 50% moisture and caused problems with mixing, so these diets were dried at 65 °C in a forced air oven. Some furfural was lost in drying but enough remained so the relative furfural levels between HSD levels were as planned. Final diet furfural concentration was analyzed by Dierks Division, Weyerhauser Company who supplied the HSD (Table II). Diet compositions were listed in Table I. The rats were caged individually. Water and diets were provided ad libitum. Feed consumption was recorded daily and body weights were taken at 28 days.

Table I. Diet Composition for Trials 1, 2, and 3

Ingredient	Control, %
Soybean meal	26.4
Ground corn	25.6
Vitamin premix[a]	10.0
Corn oil	5.0
Trace minerals[b]	0.1
NaCl	0.5
Dicalcium phosphate	1.9
$CaCO_3$	0.5
Solka floc[c]	15.0
Cerelose[c]	15.0

[a] Vitamins were mixed in soybean meal and the premix contained 550 IU vitamin A, 39.6 IU vitamin D_3, 282 μg of riboflavin, 991 μg of panthothenic acid, 1.76 mg of niacin, 11 mg of choline chloride, 2.2 μg of vitamin B_{12}, 139 μg of menadione sodium bisulfite, and 440 μg of ethoxyquin. [b] Trace mineral premix contained 10% Mn, 10% Fe, 10% Zn, 1% Cu, and 0.1% Co. [c] Amount varies according to treatment and trial.

Table II. Treatment Means, Standard Errors, and Statistical Comparisons of HSD and Furfural Levels in in Vivo Trial 1

HSD,[a] %	Furfural,[b] %	Daily gain, g	Feed intake, g	Gain/feed
0	0.00	4.89[c]	16.2	0.30
10 L	0.03	4.65	16.6	0.28
M	0.08	4.27	15.9	0.25
H	0.20	4.72	17.0	0.31
20 L	0.06	3.60	14.9	0.24
M	0.15	3.33	14.2	0.23
H	0.26	3.34	13.9	0.25
30 L	0.07	1.30	10.8	0.12
M	0.26	1.45	11.0	0.13
H	0.43	2.87	12.8	0.22
Standard error		±0.29	±0.64	±0.01
Statistical comparisons[d]				
0 vs. all others		**	**	**
HSD linear		**	**	**
HSD quadratic		NS	NS	*
Furfural linear within 10% HSD		NS	NS	NS
Furfural quadratic within 10% HSD		*	NS	**
Furfural linear within 20% HSD		NS	NS	NS
Furfural quadratic within 20% HSD		NS	NS	NS
Furfural linear within 30% HSD		*	*	**
Furfural quadratic within 30% HSD		NS	NS	*

[a] Percent of diet dry matter. [b] Analyzed furfural levels in the diet dry matter. [c] Each mean is the average of eight male rats. [d] Probabilities are: ** = $P < 0.01$, * = $P < 0.05$, NS = not significant.

Rat Trial 2. A second 28-day rat growth trial using seven weanling, male Long-Evans rats per treatment, was conducted to determine if extraction of the HSD with 95% ethanol (ETOH) would reduce the toxic effects. Four treatments were compared in this trial. Treatment 1, a control, was the same as trial 1 and treatment 2 was 30% HSD, also as in trial 1. The third treatment was the 95% ETOH extract of an amount of HSD equal to that used in treatment 2. Appropriate amounts of dry matter were removed from equal portions of cerelose and solka floc for all HSD treatments. The fourth treatment consisted of the HSD residue after 95% ethanol extraction incorporated as 30% of the diet dry matter. The HSD was extracted with 95% ETOH in a soxhlet extractor until no

Table III. Effect of Adding the Ethanol Extracts of HSD on in Vitro Dry Matter Disappearance (IVDMD) of Solka Floc by Rumen Microorganisms

Fraction[a]	Amount of dry matter added from extract,[b] mg	IVDMD, %	Regression equation[c]	Correlation[d]
Control	0	75.98		
100s	33.1	71.92	$y = -0.26x + 78.58$	−0.96
100p	28.0	75.66	$y = 0.05x + 75.53$	+0.88
95s	32.6	65.82	$y = -0.37x + 76.52$	−0.99
95p	20.6	12.13	$y = -0.85x + 55.84$	−0.73
80s	21.9	60.37	$y = -0.61x + 77.70$	−0.80
80p	26.1	44.79	$y = -0.65x + 70.58$	−0.96

[a] Each extraction treatment described in Figure 1. [b] Each fraction was added at three levels. These levels were depicted because each had similar amounts of dry matter added from each fraction. All levels were included in the regression equation. [c] Regression of y (IVDMD) on x (mg of extract dry matter). [d] Correlation between IVDMD and extract dry matter.

further color was removed. Rats were cared for and data collected as in trial 1. After final weights were taken, the rats were sacrificed and liver, kidneys, and adrenals immediately excised and weighed.

Rat Trial 3. The third rat trial investigated the effects of the treatments imposed in trial 2 on nitrogen balance. Twenty-four male Long-Evans rats were randomly allotted to the four treatments and fed their respective diets for a 10-day preliminary period followed by a 7-day collection period. Urine was acidified with 5 mL of 6 N HCl daily, composited, and frozen until analyses. Fecal collections were collected daily, composited, and frozen until analyses. All nitrogen analyses were conducted according to AOAC (1960). Rat urines were scanned in the UV region from 340 to 220 nm. Optical density of the peaks were recorded and compared according to treatment.

Data from the three animal trials were studied using analysis of variance techniques, and differences in treatment means were tested using orthogonal comparisons (Steel and Torrie, 1960).

RESULTS AND DISCUSSION

Rat Trial 1. No consistent effects of furfural on rat growth were observed within HSD levels (Table II). The furfural levels were not equally spaced between HSD treatments because of the problems encountered in mixing the diets. Therefore, linear and quadratic effects of furfural across all levels of HSD were not statistically compared. However, the effects of furfural content within the three HSD levels were compared. No consistent negative linear or quadratic ($P > 0.05$) trends associated with increasing furfural were observed in performance. In fact, increasing furfural levels in the 30% HSD was associated with a linear increase in gain ($P < 0.05$), feed intake ($P < 0.05$), and feed conversion ($P < 0.01$). Therefore, it seems unlikely that furfural present in the HSD is responsible for the reduced growth.

Furfural is produced by the action of strong mineral acids on pentose sugars (White et al., 1964). The combination of 2.3% H_2SO_4 and 42.2 kg/cm^2 pressure for 40 s is an efficient means of converting the sawdust hemicelluloses to furfural. The hydrolysis conditions used to produce the HSD were also the optimum conditions for furfural production (Butterbaugh and Johnson, 1974). Levels of furfural present in these diets may not have been high enough to cause toxicity problems, but the reduced growth in the rats fed HSD was similar to that noted in other rat experiments (Hudson, 1971) and suggests factor(s) in the HSD other than furfural were responsible for the negative effects.

Inclusion of all HSD levels reduced ($P < 0.01$) gain, feed, and feed efficiency compared to the control (Table II). The 30% HSD fed rats had a greater depression ($P < 0.01$) in growth, feed intake, and feed efficiency compared to the 10% HSD treatment. The 20% HSD fed rats were not significantly different from either the 10 or 30% HSD groups in gain or feed intake, which suggests a linear effect of HSD level on these measures in the range studied.

Table IV. In Vitro Dry Matter Disappearance (IVDMD) of HSD by Rumen Microorganisms

Treatment	IVDMD, %
HSD	40.85
Solka floc	76.07
HSD-solka floc (1:1)	60.61
HSD extracted with 95% ETOH	68.62

In Vitro Experiments I and II. The first in vitro experiment evaluated the effect of the different ETOH extracts on IVDMD of solka floc (Table III). Dry matter from each alcohol fraction was incubated at three different amounts, and the effects on IVDMD were analyzed by regression. The levels shown in Table III were chosen because they had similar amounts of dry matter added from each fraction. All levels were included in the regression. These results show that the absolute ETOH did not remove a substantial amount of inhibitor(s). The slopes of the regression equations showed that the 95 and 80% ETOH fractions contained material(s) inhibitory to cellulose digestion. This depression in IVDMD proved that a substance(s) could be removed from the HSD which had a negative effect on cellulose digestion. Reduced cellulose digestion in sheep has been reported (Butterbaugh and Johnson, 1974) fed a diet containing 35% HSD. These authors used an ammonia neutralized HSD in their experiments and felt the depressions in cellulose digestibility may have been due to the high rumen ammonia levels in the HSD fed lambs which may have caused an alteration of microbial action in the rumen. The HSD used in these experiments was neutralized with NaOH, and hence, ammonia levels were not a problem. The depressions in cellulose digestibility reported here seem attributable to the compound(s) isolated by the ETOH fractionation.

The second in vitro experiment compared the IVDMD of HSD, solka floc, a combination of HSD and solka floc (1:1) and the HSD residue after 95% ETOH extraction (Table IV). IVDMD of HSD was considerably lower than for the isolated cellulose (solka floc). Butterbaugh and Johnson (1974) reported an apparent increase in lignin from 21% in the raw wood to 60% in the HSD. They attributed this increase to synthesis of artifact lignin from carbohydrate degradation products and other compounds and concluded that these materials were largely indigestible. The data on HSD IVDMD reported here (40%) were consistent with their observations on the amount of lignin in HSD. The IVDMD of the HSD residue after 95%

Table V. Effects, Standard Errors, and Treatment Statistical Comparisons of HSD and 95% ETOH Extraction of HSD on Rat Growth (in Vivo Trial 2)

Treatment[a]	Daily gain, g	Feed intake, g	Gain/feed
Control (1)	5.26[b]	16.1	0.33
HSD (2)	3.23	12.0	0.27
HSD extract (3)	1.49	9.5	0.15
HSD residue (4)	5.19	18.5	0.28
Standard error	±0.20	±0.48	±0.01
Statistical comparisons[c]			
1, 4 vs. 2, 3	**	**	**
1 vs. 4	NS	**	**
2 vs. 3	**	**	**

[a] Numbers in parentheses used to designate treatments for statistical comparisons. [b] Each mean is the average of seven male rats. [c] Probabilities are: ** = $P < 0.01$, NS = not significant.

ETOH extraction showed a dramatic increase compared to HSD and nearly reached the IVDMD of solka floc. While these results are not directly comparable to rat growth, it was assumed that the 95% ETOH extraction which was successful in removing the inhibitor(s) of IVDMD might also remove the compound(s) which were inhibitory to growing rats.

Rat Trial 2. The second rat growth experiment was initiated to test if the 95% ETOH extraction did remove the growth inhibitory factor(s) as was indicated by the second in vitro experiment. Rats fed diets containing 30% HSD that had been 95% ETOH extracted did not exhibit growth depression ($P > 0.05$) compared to the control, while the unextracted HSD or the extract concentrated on the control did both show large depression ($P < 0.01$) in growth, feed intake, and feed efficiency (Table V). Rats fed the HSD extract showed greater ($P < 0.01$) growth depressions than those fed the unextracted HSD. Since the amount of HSD extracted was the same as would have been included in a 30% HSD diet, the growth depressing effects should have been similar. The feed efficiency of the HSD and HSD residue fed rats were not different ($P > 0.05$) which suggests that the depressed feed intake of the HSD fed rats was mainly responsible for the decreased gain. Greater effects were observed with the extract than plain HSD. This suggested that the inhibitor(s) activity was enhanced by the extraction.

After the final weights were taken, the rats were sacrificed and the liver, kidneys, and adrenals weighed. Both the livers and kidneys of the HSD and HSD extract fed rats were enlarged ($P < 0.01$) compared to the control and residue fed rats (Table VI). The increased size of these organs may be related to the increased metabolic strain placed on these organs to detoxify and excrete the problem compound(s) or their metabolites. The HSD residue fed rats had larger ($P < 0.01$) kidneys than the controls. This suggests that not all of the problem compounds were extracted from the HSD. As in the growth phase of the trial, the extract produced a greater increase ($P < 0.01$) in kidney size than did the HSD treatment. No effects ($P > 0.05$) were observed on adrenal weights.

Inclusion of small amounts of roughage (5 to 10% of diet DM) usually results in increased performance of ruminants and a reduction in liver abscesses. Erlinger (1974) reported HSD to be ineffective as a roughage source in two trials in which cattle were fed with high concentrate diets for cattle. In one trial, with only five steers per treatment, all the 10% HSD and the 6.67% HSD plus a 3.33% sawdust had liver abscesses while the 10% corn cob treatment had only one animal with liver abscesses. The

Table VI. Organ Weights[a] and Treatment Statistical Comparisons of Rats in in Vivo Trial 2

Treatment[b]	% body weight		
	Liver	Kidney	Adrenals
Control (1)	3.71[c]	0.622	0.014
HSD (2)	4.40	0.781	0.015
HSD extract (3)	4.53	0.874	0.017
HSD residue (4)	3.86	0.686	0.014
Standard error	±0.078	±0.015	±0.001
Statistical comparisons[d]			
1, 4 vs. 2, 3	**	**	NS
1 vs. 4	NS	**	NS
2 vs. 3	NS	**	NS

[a] Organ weights are expressed as a percent of body weight. [b] Numbers in parentheses used to designate treatments for statistical comparisons. [c] Each mean is the average of seven male rats. [d] Probabilities are: ** = $P < 0.01$, NS = not significant.

Table VII. Effect of HSD and 95% ETOH Extraction of HSD on N Metabolism in in Vivo Trial 3

Treatment[a]	ND,[b] %	NR,[b] %	ANR,[b] %	DMD,[b] %
Control (1)	80.8	37.4	46.0	66.8
HSD (2)	72.0	31.5	42.1	67.1
HSD extract (3)	70.1	29.0	41.0	68.5
HSD residue (4)	81.4	40.1	49.2	65.5
Standard error	±2.08	±1.27	±1.44	±2.36
Statistical comparisons[d]				
1, 4 vs. 2, 3	**	**	**	NS
1 vs. 4	NS	NS	NS	NS
2 vs. 3	NS	NS	NS	NS

[a] Numbers in parentheses used to designate treatments for statistical comparisons. [b] ND is nitrogen digestibility; NR is nitrogen retention expressed as a percent of N intake; ANR is nitrogen retention expressed as a percent of absorbed N; DMD is dry matter digestibility. [c] Each mean is the average of six male rats. [d] Probabilities are: ** = $P < 0.01$, NS = not significant.

increased incidence of liver abscesses might be a result of lack of a roughage character of HSD or presence of the same compounds producing enlarged livers in rats or both.

Rat Trial 3. Results from the N balance experiment confirmed the effects of 95% ETOH extraction in the growth trial (Table VII). Rats fed the HSD or HSD extract exhibited decreased ($P < 0.01$) N digestibility and N retention compared to the control and HSD residue after 95% ETOH extraction. Thus the extraction was effective in overcoming the negative effects on nitrogen metabolism caused by the HSD.

Hudson (1971) reported decreased N digestibility and N retention in sheep fed HSD. He found that the growth depression of HSD in rat diets could be partially overcome by increasing the crude protein content of the diets from 16 to 24%. He also showed methionine (0.22%) and lysine (0.39%) additions to a 15% crude protein diet for rats partially alleviated the decreased growth of the HSD supplemented groups. Butterbaugh and Johnson (1974) reported decreased N digestibility in sheep fed 35% HSD and 64% alfalfa. Erlinger and Klopfenstein (1975) showed no differences in N digestibility of sheep fed 10 or 20% HSD but observed a linear decrease in nitrogen retention as HSD level increased. HSD effects on N metabolism appear to be twofold. One action is to decrease N digestibility. This is probably a result of the phenolic compounds in the HSD combining with dietary protein rendering it insoluble. This mechanism would be similar to that of tannin treatment of soybean meal (Driedger and

Table VIII. Absorbance of Rat Urine at 266 nm from in Vivo Trial 3

Treatment	Absorbance[a]
Control	0.176
HSD	1.362
HSD extract	0.973
HSD residue	0.153
Standard error	±0.055

[a] Each mean is the average of six male rats.

Hatfield, 1972). The second effect is on post absorptive nitrogen metabolism and may be related to the nitrogen needed to detoxify or excrete the problem compound(s). Glycine is used to detoxify benzene and the product hippuric acid is excreted (White et al., 1968).

Urine from the HSD and HSD extract fed rats was noticeably darker in color than for the control or residue treatments. All urines were scanned in the UV region. The dark urines showed an absorption peak at 266 nm. Urine from rats fed control and HSD residue diets showed little absorption at 266 nm (Table VIII). The 95% ETOH extract was also scanned and showed a typical UV spectra for lignin (Sarkanen and Ludwig, 1971) with an absorption peak near 280 nm. Syringyl alcohol and a guaiacyl derivative which are components of wood lignin show an absorption peak of 266–8 nm (Sarkanen and Ludwig, 1971). These are similar to the absorption peak in the urine of HSD and HSD extract fed rats. When the pH of these compounds is raised to 14, the absorption peak of the syringyl alcohol shifts to 245–6 nm and the guaiacyl derivative to 290 and 315 nm. The UV absorption spectrum of rat urine raised to pH 14 showed an absorption peak shift to 253 nm. It is not possible from these data to determine what compound(s) are being excreted by the rats, but we can assume these UV absorbing compound(s) are from lignin or other polyphenol degradation products present in the sawdust and released during pressure and acid hydrolysis.

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Received for review July 18, 1977. Accepted January 3, 1978. Published with the approval of the Director as Paper No. 5372 Journal Series, Nebraska Agricultural Experiment Station. Support was provided by Dierks Division, Weyerhauser Co., Hot Springs, Ark.

COMMUNICATIONS

Protein Classification and Nitrogen Extractability of Grape Seed Meal

Grape seed meal is investigated for the first time in order to evaluate its potential for food use. The amino acid composition shows that grape seed meal is deficient in sulfur-containing amino acids and in lysine, but contains the other essential amino acids in adequate levels. Protein classification based on solubility indicates that most of the protein N was residual, while glutelin, albumin, and prolamin fractions include the largest amount of simple proteins. The N extractability profiles of grape seed meal determined over the pH range 2.0–12.0 are reported.

The increasing interest for the serious food protein shortage in many parts of the world has led to intensive efforts toward exploring novel and indigenous plant protein sources at present not adequately utilized. Among the different vegetables proposed, oilseeds are the most economic, acceptable, and safe types of crops for protein production. In fact, oilseed flours as by-product of oil industry require a minimum of processing, have good biological value, and are relatively free from antinutritive factors and fermentable sugars.

Grape seed ranks as second in importance among Italian sources of vegetable oils and its production in 1976 has been of 160 000 tons (FAO, 1976). The defatted meal (10% protein) is a by-product of the oil industry, not currently marketed as animal feed because of its high crude fiber content. Grape seed meal has not yet been studied in order to obtain proteins to supplement foods. The dehulling and the presence of polyphenolic chromogens are the major problems in using grape seed meal for human diets; nevertheless, the high seed production in Italy and the very low cost of the meal lead to consider it as an available and economic protein source.

Values for proximate composition of grape seed meal have been reported (Defrancesco et al., 1976), but there

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