On collection day 14, sheep receiving alfalfa as 3 or 10 percent of their diets displayed significantly higher biuretolytic activity than sheep not fed alfalfa. Data from collection days 4, 7, 10, 21 and 28 do not suggest a beneficial response from the feeding of dehydrated alfalfa. Thus, although alfalfa had a significant effect on day 14, this trend was not sufficiently consistent to conclude that dehydrated alfalfa has any substantial effect upon the rate of biuret adaptation.

The Influence of Treatment of Whole Fat Soybeans With Formaldehyde to Protect the Polyunsaturated Fatty Acids From Hydrogenation in the Ruminant.

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Story in Brief

The rumen microorganisms have the ability to biohydrogenate the polyunsaturated fatty acids (PUFA) in the diet. As a result, the highly saturated fatty acids produced are absorbed and deposited in the tissue fat. The results reported here are part of a study to determine if natural protein-fat complexes such as whole soybeans can be treated to protect the PUFA from biohydrogenation.

In trial 1, ground soybeans were treated with either 5.1 or 10.2 ml formalydehyde (HCHO) per 100 gm. of material for 30 min., 2 hr. or 6 hr. Incubation of these products in laboratory rumen fermentations showed that the treatment with HCHO markedly protected the PUFA against biohydrogenation compared to untreated soybeans in which all the PUFA were biohydrogenated.

In trial 2, larger quantities of ground soybeans were treated under the 5.1 ml/30 min. and 10.2 ml/6 hr. conditions. Incubation of these samples in the laboratory showed no protection against biohydrogenation. Growing lambs were fed rations containing: (1) soybean meal, (2)

ground soybeans, (3) ground soybeans treated at 5.1 ml/30 min. and (4) ground soybeans treated at 10.2 ml/6 hr. conditions as the protein supplement. Lambs fed soybean meal grew faster with the best feed efficiency. Formaldehyde treated soybeans were less palatable than untreated soybeans. There were no differences in the percentages of PUFA in the rump or kidney fat or in the flavor of the meat from lambs consuming treated soybeans.

Introduction

There has been much emphasis recently on the role of polyunsaturated fatty acids (PUFA) in human diets to aid in the prevention of atherosclerosis. However, ruminant meat and fat tissues are characterized by low proportions of PUFA. Although the diet of the ruminant is high in PUFA, the microbes in the rumen hydrogenate them to saturated fatty acids, which are in turn absorbed from the small intestine and deposited in the various fat depots.

Recent studies in Australia and at the U.S.D.A. laboratories in Beltsville, Md., have shown that it is possible to produce an oil supplement in which the PUFA are protected from hydrogenation in the rumen. The polyunsaturated oils are coated with protein and then the whole complex is sprayed with formaldehyde (HCHO). Upon entering the acidic conditions of the abomasum and small intestine, this HCHO-protein complex is hydrolyzed and the PUFA are released for absorption. The feeding of these protected mixtures to ruminants has resulted in significant increases in the PUFA content of milk and body tissues. In addition, there has been an increase in the milk fat content.

Since the above process of preparing the protected oil-protein complex probably would be too costly for practical purposes, it would seem conceivable that the whole soybean could be ground and treated similarly since it consists of a natural protein-oil complex. The purpose of this study was to determine by *in vitro* and *in vivo* methods if full fat soybeans could be protected with HCHO from rumen biohydrogenation when fed to growing lambs. Organoleptic studies were also conducted to determine if any differences in meat flavor could be produced by such treatments.

Experimental Procedure

In Vitro Fermentations

In trial 1, whole soybeans were ground through a 1 mm screen in a Wiley mill. Approximately 100 gm samples were treated with either

5.1 or 10.2 ml of 37 percent formaldehyde (HCHO) along with sufficient water to cover the samples for 30 min., 2 hr. or 6 hr. In trial 2, the soybeans were ground through a hammer mill and approximately 20 lb. batches were treated with either 5.1 ml HCHO/100 gm for 30 min. (5.1/30 min.) or 10.2 ml HCHO/100 gm for 6 hours (10.2/6 hr.). The soybeans were then placed in a forced draft oven until dry. After being reground, portions were taken for incubation in a laboratory rumen fermentation by standard procedures previously used in this laboratory. Twenty milliliter samples were removed at 0, 24 and 48 hours and frozen. In trial 2, additional samples were taken at 6 and 12 hours. The samples were lyophilized, extracted in chloroform: methanol (2:1) and the lipids in the extract were transferred to petroleum ether. Methyl esters of the fatty acids in this lipid extract were formed by boiling in 1 percent sulfuric acid in methanol. After extracting the methyl esters into heptane, they were separated and measured by gas-liquid chromatography. Each 18 carbon fatty acid was expressed as a percent of the total 18 carbon fatty acids in that particular sample.

Growth and Tissue Trial

Twenty Western wether lambs were randomly assigned to individual pens. They were then randomly allotted to one of the four rations shown in Table 1 and gradually brought to full feed. The lambs were fed until they reached approximately market weight of 100 pounds. Water was available at all times. The rations were 70 percent concentrate with 8 percent digestible protein. Two control rations were used — soybean meal (SBM) and ground soybeans (GSB). The treated ground soybeans were substituted for the untreated ground soybeans on an equal weight basis.

Table 1. Ration Composition (As-Is Basis)

Ingredient	Soybean meal	Ground soybeans	Ground soybeans (5.1/30 min)[1]	Ground soybeans (10.2/6 hr)[2]
Corn	59.3	56.9	56.9	56.9
Soybean meal	9.2	—	—	—
Ground whole soybeans	—	11.6	11.6	11.6
Cottonseed hulls	30.0	30.0	30.0	30.0
Salt	.6	.6	.6	.6
Calcium carbonate	.9	.9	.9	.9
Vitamin A	+	+	+	+
Vitamin D	+	+	+	+

[1] 5.1 ml formaldehyde (HCHO) per 100 gm of soybeans, 30 min. exposure.
[2] 10.2 ml HCHO per 100 gm of soybeans, 6 hr. exposure.

At the end of the trial all lambs were slaughtered at which time omental fat samples were taken and frozen. The carcasses were chilled and after several days samples were taken of the shoulder, rump and kidney fat. The loins were removed and stored in a freezer for organoleptic analysis. Fat samples were extracted and analyzed for fatty acids in the same manner as described for rumen samples.

Organoleptic

The loins were cut in half and one of the halves was returned to storage. The other half was boned and all the excess fat was removed. All the loins from sheep on the same treatment were composited and ground twice—first coarsely and then finely. The ground lamb was frozen until ready for use.

A taste panel was organized consisting of six members — three females and three males. The treatment comparisons were SBM/GSB, SBM/30 min., SBM/6 hr., GSB/30 min., and GSB/6 hr. with 4 replications per treatment comparison.

The ground lamb was baked in ¼ lb. loaves wrapped in aluminum foil to an internal temperature of 150°C in a 325°F oven. The triangle taste test was used with 2 samples per sitting. The sittings were held either mid-morning or mid-afternoon when taste buds should be the keenest. A room with low intensity, uniform lighting was used so evaluation would be based on flavor only and not color.

Results and Discussion

Trial 1.

A summary of the *in vitro* data from trial 1 is shown in Figure 1. Linoleic acid, the major 18 carbon PUFA in soybeans, decreased from 48 percent in the untreated GSB to 0 percent during the 48 hr. incubation period while the saturated 18 carbon fatty acid, stearic, increased from 8 to 91 percent. Such a pattern is expected as the linoleic acid is being hydrogenated to the more saturated form of stearic acid.

The graphs representing the GSB treated with 5.1 or 10.2 ml HCHO show substantial protection of the linoleic acid from the biohydrogenation process. The 5.1/30 min. treatment showed a slight decrease in linoelic from 52 to 30 percent while stearic increased from 7 to 21 percent. Oleic acid, the logical intermediate in this conversion, increased only slightly. The 5.1/2 hr. and 5.1/6 hr. treatments showed stearic, oleic and linoleic acids remaining fairly stable during the incubation period. No particular advantage was observed in using the higher concentration of formaldehyde. The 10.2/30 min. graph shows linoleic, oleic and

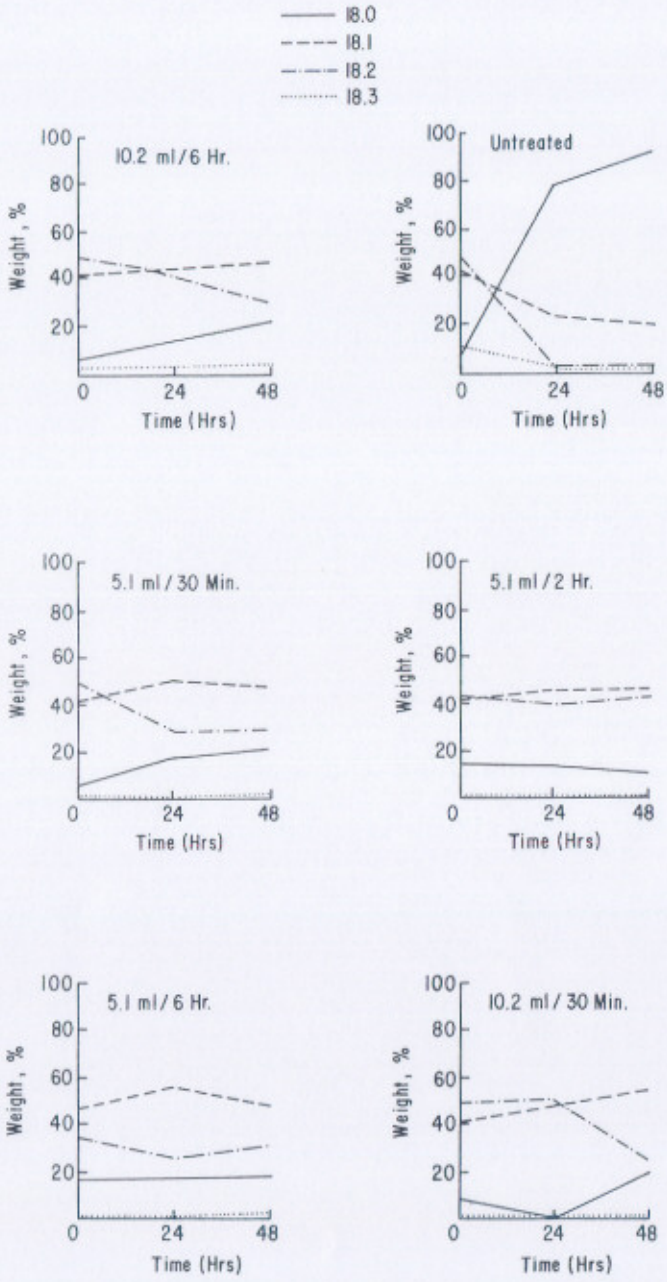

Figure 1. **Biohydrogenation of the 18-carbon fatty acids in treated and untreated ground soybeans during incubation for trial 1. Stearic is represented by 18:0; oleic, 18:1; linoleic, 18:2 and linolenic, 18:3.**

stearic remaining fairly constant during the first 24 hrs. of incubation after which there was a sharp decrease in linoleic and a sharp increase in stearic acid suggesting loss of protection. Improved protection over the whole incubation period was obtained under the 10.2/6 hr. condition. In all of the treatments linolenic was present in too small of a quantity to obtain meaningful information. Neither the length of exposure nor the level of formaldehyde seemed to have any effect on the changes in linoleic acid.

Trial 2.

Figure 2 shows a summary of the *in vitro* data obtained from trial 2. Protection of the PUFA against biohydrogenation was not so obvious as noted in trial 1. For the untreated GSB, linoleic acid decreased from 46 to 7 percent during incubation while stearic increased from 29 to 72

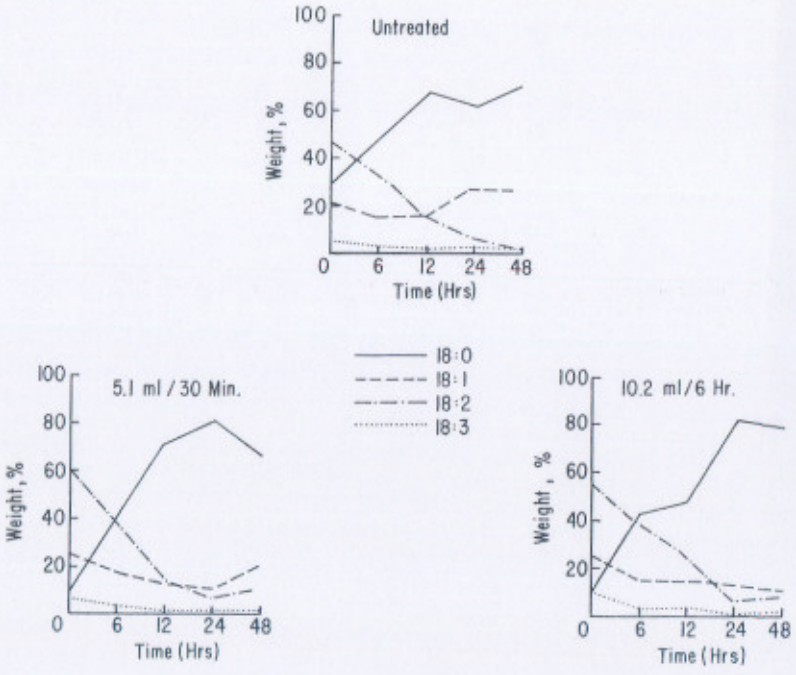

Figure 2. Biohydrogenation of the 18-carbon fatty acids in treated and untreated ground soybeans during incubations for trial 2. Designation of fatty acids is same as in Figure 1.

percent. There was only a slight increase in the oleic acid. With both the 5.1/30 min. and 10.2/6 hr. treatments, linoleic also decreased sharply while stearic increased. Oleic remained relatively stable. Again linolenic was present only in small quantities.

Tissue Data

Table 2 shows the results from the tissue analysis of the rump and kidney fat. The mean values for lambs fed the SBM ration are: stearic, 17.9 percent; oleic, 38.2 percent and linoleic, 5.8 percent.

The lambs on the GSB ration had 10.4 percent linoleic, but the other fatty acids were similar to the levels observed in lambs fed SBM. Rump fatty acid compositions from lambs on the two formaldehyde treatments were comparable to the results obtained on the GSB ration.

The same general results were obtained in the long chain fatty acid analysis of kidney fat. The formaldehyde treatments did not result in increased PUFA in kidney fat over the SBM and GSB rations. These results would be expected as protection of the PUFA was not observed in the *in vitro* analysis.

Performance Data

The lambs on the SBM ration gained the fastest and had the best feed efficiency (Table 3). They were followed closely by the lambs on the GSB ration. Lambs fed either of the two formaldehyde treatments performed similarly but had a slower daily gain and poorer feed efficiency than either of the control groups. The lambs on the 10.2/6 hr. ration gained the slowest, .31 lbs. per day, and had the poorest feed conversion with 7.32 lbs. feed per lb. of gain. The lambs on the SBM and GSB rations consumed the most feed per day suggesting that the formaldehyde rations were less palatable. However, greater number of lambs are needed to give more meaningful performance data.

Table 2. Weight Percents of the 18-Carbon Fatty Acids in Fat Depots.

	Rump				Kidney			
Rations	18:0[1]	18:1	18:2	18:3	18:0	18:1	18:2	18:3
SBM	17.9	38.2	5.8	1.3	27.7	31.1	5.4	3.4
GSB[2]	19.1	36.2	10.4	1.2	30.7	30.0	9.7	.9
GSB (5.1 ml/30 min)	16.5	38.1	8.2	2.3	23.8	31.7	8.6	2.0
GSB (10.2 ml/6 hr)	18.2	35.5	8.8	2.6	28.9	30.2	8.0	2.2

[1] Stearic is designated by 18:0; oleic, 18:1; linoleic, 18:2 and linolenic, 18:3.
[2] Means of 4 animals, all other figures represent 5 animals.

Table 3. Performance Data of Lambs Fed Treated and Untreated Soybeans

	Rations			
	SBM	GSB	5.1/30 min.	10.2/6 hr.
No. of lambs	5	4	5	5
Days on trial	84	91	84	91
Average daily gain, lbs.	.52	.45	.35	.31
Daily feed consumption/ head, lbs.	3.03	2.87	2.32	2.29
Feed required per lb. gain, lb.	5.86	6.38	6.58	7.32

Organoleptic Data

No significant differences in flavor ($P<.01$) were found among any of the treatment comparisons (table 4). Analysis of variance showed no flavor preference for any particular treatment over another (see Table 5).

Table 4. Organoleptic Analysis for Flavor of Ground Lamb

Comparison	Number of comparisons	Number of correct responses
SBM/GSB	22	10
SBM/30 min.	22	8
SBM/6 hr.	24	11
GSB/30 min.	24	11
GSB/6 hr.	24	6

No significant differences in flavor ($P<.01$) among any of the comparisons.

Table 5. Analysis of Variance to Determine Meat Flavor Preference Due to Treatment of Soybeans

Source of Variation	df	Mean Square Variance	Mean Variance	Variance Ratio
Total	91	110.5543	–	–
Treatments	3	3.9900	1.33	1.099*
Residual	88	106.5643	1.21	–

*No significant difference in preference for flavor of meat from any particular treatment ($P>.05$).

Conclusions

The results of trial 1 show that the PUFA in whole fat soybeans can be protected from biohydrogenation by rumen microorganisms. Results reported by other workers (Ohio) have shown that formaldehyde treatment of whole fat soyflour will protect the PUFA when fed to dairy cows and allow passage into the milk. Apparently, the particle size of the material ground through the hammer mill in trial 1 was too coarse to allow proper contact with the formaldehyde. Thus, protection was not afforded to the PUFA. Additional trials with soyflour are now underway.
