Detoxification of Jojoba Meal by Lactobacilli

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Selected strains of *Lactobacillus acidophilus* and *Lactobacillus bulgaricus* were found to grow well on jojoba seed meal and reduce the levels of simmondsin and other cyano toxicants. After standing for 21 days at 26 °C on a 30% moist jojoba meal, *L. acidophilus* 629 lowered total toxicant levels 95–98%. Ammonia used in the process facilitated the detoxification. The Lactobacilli apparently modify the cyano groups of the toxicants during their growth, thereby detoxifying the meal. This is the first time that Lactobacilli of any species or strain have been reported to act on cyano groups, indicating the possible presence of a nitrilase in this food grade microorganism. In addition to rendering jojoba meal nontoxic to mice, poultry, sheep and cattle, the Lactobacillus treatment increases palatability of deoiled jojoba meal, which is otherwise poorly accepted in animal rations. The treatment of jojoba meal with a Lactobacillus resembles an ensilage process.

Jojoba meal is the high protein material remaining after jojoba seeds containing some hulls are deoiled. Jojoba seeds contain simmondsin, a cyanomethylenecyclohexyl glucoside that is toxic to rats (Booth et al., 1974) and some animals. This principal toxicant is present at levels as high as 6% in deoiled jojoba meals. In addition, simmondsin 2′-ferulate and two other structurally related cyano glucosides are present in the meal at high levels (Elliger et al., 1974; Verbiscar and Banigan, 1978). We have investigated a number of solvent extraction, heat, and chemical methods to detoxify jojoba for use as a livestock feed ingredient (Verbiscar et al., 1980). Microbial detoxification methods and feeding studies of the treated meals in animal diets are reported here.

There were several factors in our rationale for using microorganisms to detoxify jojoba meal. We reasoned that the glucoside toxicants could be a source of carbon and energy to support microorganism growth. That is, because of the glucose moiety of the toxicants, these could be utilized by the microorganism as a nutrient. This would result in an increased protein level in the treated meal. We did not want the microorganism to merely cleave glucose from the CN-containing aglycons, which could be inherently toxic or rearrange to toxic phenylacetonitriles (Elliger et al., 1973). Microbial modification of the CN groups of the toxicants was also necessary.

The use of enzymes to detoxify food and feed plant materials have been reviewed recently (Liener, 1977), but no microbial methods were mentioned. Many plants including their edible parts contain cyano compounds that are toxic to animals and humans (Montgomery, 1965; Conn, 1973). Nitrilase enzymes that hydrate CN groups have been identified in a number of plants, (Thimann and Mahadevan, 1964), fungi, and bacteria (Jallageas et al., 1980).

It was apparent that microorganisms already in the food chain would be most desirable for animal feeding, although not necessarily for CN group modification. Accordingly, we screened 15 microorganisms for their ability to grow in jojoba meal and modify the toxicants. These included one *Saccharomyces cerevisiae*, nine strains of *Lactobacillus acidophilus*, and five strains of *Lactobacillus bulgaricus*. The *S. cerevisiae* was Fleischmann's Active Dry Yeast obtained in a grocery store. *L. bulgaricus* Alta was an unflavored yogurt obtained from a local dairy. *L. acidophilus* Knudson was a sweet acidophilus milk obtained from another dairy. The other 12 Lactobacilli strains were provided through the courtesy of Dr. C. W. Hesseltine, Culture Collection, U.S. Department of Agriculture, Peoria, IL. Growth of these latter bacteria in soybean milk has been reported (Wang et al., 1974).

Advantages in using the lactic acid bacteria are that these micoorganisms are notably nontoxic themselves and can be the basis for an ensilage process. The Lactobacilli can be adapted to use in a silo, and such a process can conceivably be used by small jojoba oil processors. However, there are no prior examples demonstrating nitrilase activity in Lactobacilli.

Our purpose was to reduce toxicity of deoiled jojoba meal for use as a livestock feed. The detoxification process needed to be practical and provide a meal that was acceptable as a feed ingredient in poultry, sheep, or cattle diets. An improvement in palatability was also necessary because deoiled meal is somewhat bitter for humans and has low acceptability in animal diets. A successful process would conserve this half of the annual jojoba oilseed harvest, a crop being cultivated and developed in the southwestern United States, in Mexico, and in other semiarid lands.

EXPERIMENTAL SECTION

Solutions. *Medium Z* is 10% solution of Carnation Instant Non-Fat Dry Milk in distilled water. *Solution X* is solution of 0.1 g of $MgSO_4 \cdot 7H_2O$, 0.2 g of $K_2HPO_4 \cdot 3H_2O$, 0.4 g of Difco yeast extract, 1.2 g of Sheffield NZ amine, and 20 mL of distilled water.

Inoculum Preparation. Inocula were prepared by suspending lyophilized pellets of sterile bovine serum containing the dormant bacteria as obtained from the Culture Collection, U.S. Department of Agriculture, Peoria, IL, in 10 mL of autoclaved medium Z. The culture tubes were incubated at 37 °C until curdling occurred, usually in 1–3 days. A 1-mL transfer of this culture was made to a second 10 mL of medium Z which was incubated to the curdling point and then stored at 8 °C until used in a detoxification experiment. Cultures of the Lactobacilli were routinely maintained in this manner.

Culture Protocol for Screening Microorganisms. A 125-mL Erlenmeyer flask with a cotton plug closure was charged with 2.0 g of deoiled jojoba meal, 18 mL of distilled

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Table I. Microorganism Screening

organism	toxicants as % meal[a] simmondsin	sim. 2′-ferulate	culture conditions pH	growth	odor
control[b]	4.9	2.3	6.2		
L. acidophilus[c]					
B-629	1.1	0.25	6.8	heavy	musty
B-1833	1.5	1.3	7.6	heavy	sour
B-1868	2.5	1.3	7.4	light	fresh
B-1910	2.8	0.39	6.9	light	sour
B-1911	0.58	0.33	7.0	heavy	fresh
B-1912	2.1	0.90	7.1	light	fresh
B-2178	3.8	1.5	5.5	light	fresh
B-2092	3.7	1.6	5.6	light	fresh
Knudsen[d]	2.1	1.0	6.2	heavy	sour
L. bulgaricus[c]					
B-548	4.1	1.3	4.6	light	fresh
B-734	1.9	1.5	4.1	medium	sour
B-1909	2.8	1.5	4.7	heavy	fresh
B-1918	3.5	1.1	7.0	light	fresh
Alta[e]	3.5	1.5	4.4	light	fresh
S. cerevisiae[f]	3.7	1.5	5.9	heavy	yeasty

[a] After 10 days, submerged cultures were assayed by high-performance LC. sim. = simmondsin. [b] Meal suspended in medium and autoclaved. [c] All B strains were supplied by the Northern Regional Research Center, U.S. Department of Agriculture. [d] Obtained from Knudsen Creamery Co. [e] Obtained from Alta-Dena Dairy. [f] Fleischmann's dry yeast powder.

water, and 1.0 mL of solution X and then autoclaved at 121 °C for 15 min. The resulting suspensions were inoculated with 2 mL of the prepared bacterial inoculum and placed on a rotary shaker. For the *S. cerevisiae* experiment, a small amount of dry yeast powder was added directly to the jojoba meal suspension. The cultures were shaken at 200 rpm for 30 min at 2-day intervals. After 10 days the flasks were removed, the contents were inspected, the pH was measured, and the contents were assayed for toxicants (Table I).

Analytical Sample Preparation. *Screening Study.* Each of the 10-day-old cultures and the control were diluted with 25 mL of acetone, mixed well, and vacuum filtered, washing the insoluble residue twice with (2:1) acetone–water. The combined filtrates were concentrated to near dryness on a rotary vacuum evaporator and then transferred to a 10-mL volumetric flask by using acetone. A 5-mL aliquot of this solution was concentrated to dryness on the rotary evaporator, and the residue was redissolved in 1 mL of methanol. The methanol solution was diluted with 3 mL of ethyl acetate and passed through a column containing 0.5 g of Merck silica gel G. The column was washed with 10 mL of (7:3) ethyl acetate–ethanol, the eluates were concentrated, and the residue was redissolved in methanol to a volume of 10 mL for assay.

Treated Meals. Microbially treated meals were dried in an oven at 75 °C to a moisture level of <10%. A 10-g sample was ground to a fine powder and Soxhlet extracted with acetone for 7–8 h. Extractions for 20 h increased the percent toxicant analysis, but for detoxified meals the total toxicant difference between the 8- and 20-h extractions is small. The acetone extract was evaporated to dryness, and the sample reconstituted to 10 mL with methanol. These solutions were assayed by high-performance LC (Verbiscar et al., 1980).

Jojoba Meal. The jojoba meal used in the following experiments was from the 1978 harvest of seeds by the San Carlos Apache Tribe, San Carlos, AZ. The oil was expressed from the seeds containing some hulls by using a Hander press at that location. The resulting meal was further deoiled with hexane in a Blaw-Knox Rotocell extractor at the Angola Soya Co., Angola, IN. The deoiled meal was dried for 45 min in three stages at 90–120 °C by using a steam-jacketed desolventizer. The meal obtained from this process consisted in a mixture of small flakes and powder sized at 30% < 35 mesh, 20% < 16 mesh, and 50% > 16 mesh. Analysis showed 25.6% crude protein, 4.8% moisture, 1.3% crude fat, 8.5% crude fiber, 3.6% ash, 6.2% simmondsin, 1.5% simmondsin 2′-ferulate, and as much as 2% toxicants III + IV.

Treatment of Meal with Lactobacilli. *Small-Scale Batches.* Typical small-scale batches designed to provide a quantity of detoxified jojoba meal for mouse feeding tests are summarized in Table II. In J103, for example, 400 g of sterile medium containing 2.5% milk solids was inoculated with 10 mL of *L. acidophilus* 629 and allowed to curdle. The resulting culture was sprayed on 780 g of jojoba meal rotating in a 1-gal bottle. After the batch was allowed to stand in an incubator at 30 °C for 8 days, a high-performance LC assay showed simmondsin (I) to be low, but simmondsin 2′-ferulate (II) was nearly unchanged. After 21 days toxicants I and II were both at low levels. Results for the *L. acidophilus* 1911 (J104) and *L. bulgaricus* 734 (J105) strains were similar. In a modification of this procedure, an 800-g batch of meal was first spray treated with 12 g of concentrated ammonium hydroxide in 50 mL of water. A 400-g culture of the Lactobacillus was then sprayed on the meal as above. Detoxification at 30 °C proceeded more rapidly in these treatments, noted as J110, J111, and J112 in Table II. After 4 days toxicants I and II were low, and after 13 days they were lower than the corresponding meals not pretreated with ammonia.

Scaled-Up Batches. Typical large-scale batches designed to provide quantities of detoxified meal for cattle, sheep, and poultry studies are summarized in Table III. The J71 and J87 meals were prepared in a 50-gal polyethylene tank with a clamp-on lid fitted with a 4-in. opening. The inoculum was applied to the 32.7 kg of the meal through the opening by using a Wagner airless electric sprayer while the tank was rotated on a drum roller. In these two batches, 163 g of lactic acid in 4 L of water was sprayed on the meals 1 day prior to inoculation in order to suppress growth of random microorganisms, a procedure that was discontinued for the 100-kg batches.

A typical improved process using ammonia is summarized as meal J176-15 (Table III). Thirty-eight liters of a 3.8% solution of milk solids was inoculated with 2 L of standard *L. acidophilus* 629 inoculum and incubated at 37 °C to the curdling point. Just prior to use, 1.2 L of concentrated ammonium hydroxide was added with stir-

Table II. Treatment of Jojoba Meal with Lactobacilli

	J105 (B-734)[d]	J111 (B-734)[d]	J103 (B-629)[d]	J110 (B-629)[d]	J104 (B-1911)[d]	J112 (B-1911)[d]
meal charged,[a] g	780	800	780	800	780	800
inoculum preparation[b]						
milk solids, g	10	10	10	10	10	10
water, mL	390	440	390	440	390	440
std inoculum, mL	10	10	10	10	10	10
NH_4OH, mol		0.24		0.24		0.24
culture conditions						
time, days	21	13	21	13	21	13
temperature, °C	30	30	30	30	30	30
meal produced, g	682	729	682	732	699	719
assay, %						
moisture	5.6	5.0	6.8	5.2	6.5	4.8
protein, $N \times 6.25$	28.2	31.5	28.0	30.2	28.2	30.9
protein, dry basis[c]	29.9	33.3	30.0	31.9	30.2	32.5
fat	1.0	0.9	1.3	0.9	1.1	0.9
fiber	10.1	9.7	9.2	10.6	8.9	9.8
ash	4.0	3.9	3.9	4.0	4.0	4.0
simmondsin	0.11	0.04	0.13	0.04	0.10	0.05
simmondsin 2′-ferulate	0.19	0.14	0.25	0.11	0.23	0.15

[a] None of these meals were sterilized prior to treatment. [b] Inoculum was applied to the meal evenly by using a Wagner airless electric sprayer as the container was rotated. [c] Charged meal protein, dry basis, 25.5%. [d] Meal number; strain is indicated in parentheses.

Table III. Scaled-Up Treatment of Jojoba Meal with *L. acidophilus*

	J71 (B-629)[e]	J87 (B-1911)[e]	J176-15 (B-629)[e]
meal charged, kg	32.7[a]	32.7[a]	100
inoculum preparation			
milk solids, g	600	600	1450
water, L	8	8	38
std inoculum, mL	200	200	2000
NH_4OH, mol			23.5
culture conditions			
container, gal	50[b]	50[b]	100[c]
time, days	47	41	21
temperature, °C	~26	~26	~26
meal produced, kg	32.5	31.5	102
assay, %			
moisture	11.4	10.2	6.0
protein, $N \times 6.25$	26.1	25.7	28.5
protein, dry basis[d]	29.5	28.6	30.3
fat	2.3	2.3	1.2
fiber	7.9	9.1	8.2
ash	4.0	3.7	3.8
simmondsin	0.13	0.20	0.07
simmondsin 2′-ferulate	0.24	0.74	0.27

[a] A solution of 163 g of lactic acid in 4.0 L of water was sprayed on the meal 1 day prior to inoculation. [b] Inoculum was sprayed on the meal as the tank was rotated on a drum rotator. [c] Inoculum and meal were simultaneously sprayed into the tank. [d] Charged meal protein, dry basis, 25.5%. [e] Meal number; strain is indicated in parentheses.

ring. The curds dissolved resulting in a homogeneous foggy inoculum. The ammoniated inoculum was then sprayed simultaneously along with 100 kg of jojoba meal into a 100-gal polyethylene tank with a clamp-on cover. A pump with a Viton impeller was used to spray the inoculum through a superfine Fogg-It Waterfog nozzle at a rate of ~0.3 gal/min. The charged tank was then rolled to improve the mixing of inoculum and meal. After standing for 21 days at 26 °C, the granular meal was ground further in an Alpine M25 hammer mill with a 4-mm round-hole screen. The meals was then tray dried overnight in a forced air oven at 75 °C to a moisture level of ~6%. The composition of J176-15 is given in Table III. A total of 2800 kg was processed in this manner. Seventeen consecutive 100-kg batches J176-12 to -28 had toxicant ranges of 0.07–0.27% (av 0.12%) for simmondsin, 0.14–0.26% (av 0.20%) for simmondsin 2′-ferulate, and 0.03–0.11% (av 0.04%) for the minor toxicants III + IV.

Animal Feeding Studies. *Mice.* Weanling mouse (CD-1 strain) feeding tests were carried out at the University of Arizona (C.W.W.). The detoxified jojoba meals were incorporated into a basal diet at 10% levels (Verbiscar et al., 1980) (Table IV).

Chickens. Feeding tests with Hubbard variety broiler chicks were carried out at the University of Arizona (B.L.R.). The jojoba meals were added to a basal diet at the 5 and 10% levels, maintaining the diets isonitrogenous

Table IV. Mouse Feeding Studies[a]

meal no.	% toxicants in diet[b] simmondsin	% toxicants in diet[b] sim. 2′-ferulate	change in body wt/mouse, g	total feed intake, g
control[c]			17.2	84.9
J96[d]	0.022	0.060	7.5	67.7
J97[e]	0.009	0.009	6.0	66.2
J98[f]	0.004	0.016	11.1	81.5
J103[g]	0.013	0.025	5.5	59.2
J104[g]	0.010	0.023	5.8	65.3
J105[g]	0.011	0.019	4.6	61.2
J110[h]	0.004	0.011	6.9	67.1
J111[h]	0.04	0.014	6.4	42.9
J112[h]	0.05	0.015	7.9	68.2

[a] Weanling mice, 10 males plus 10 females, 3-week feeding. [b] Jojoba meal in diet at 10% levels. sim. = simmondsin. [c] Whole egg as protein source. [d] J87 meal vacuum dried rather than oven dried, 25.8% protein. [e] J87 meal extracted with acetone, 28.0% protein. [f] J87 meal extracted with boiling water, 28.0% protein. [g] Lactobacilli-treated meals. [h] Lactobacilli- plus ammonia-treated meals.

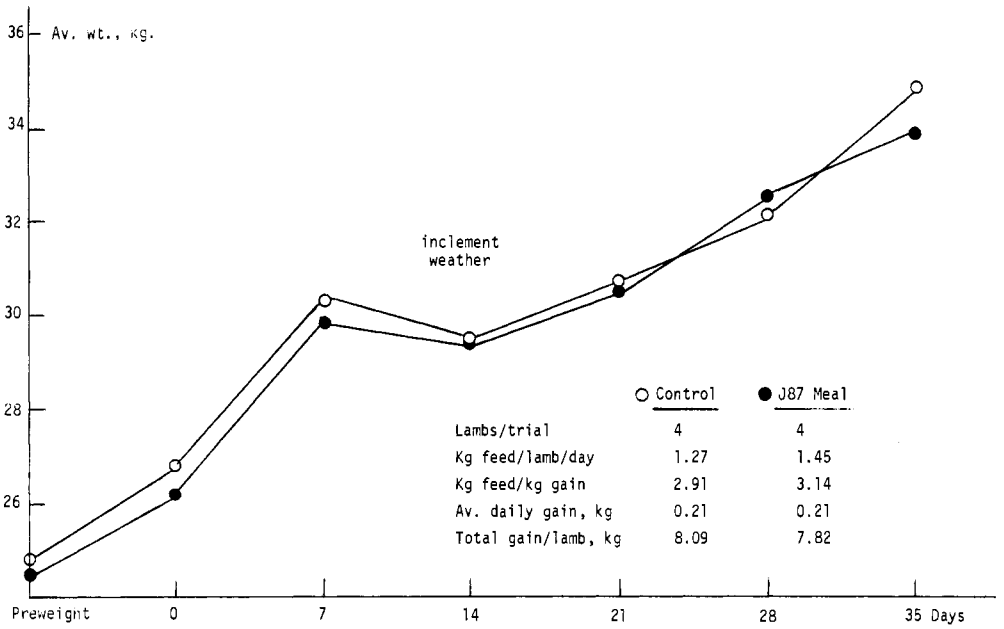

Figure 1. *Lactobacillus acidophilus* 1911: detoxified jojoba meal fed to lambs.

Table V. Poultry Feeding Studies[a]

meal no.	% meal in diet[c]	% toxicants I + II in diet[d]	av body wt (4 weeks), g	feed conversion, g of feed/g of gain
control[b]			833	1.79
J87	5	0.047	761	1.88
J87	10	0.094	734	2.02
control[b]			748	1.71
J71	5	0.019	689	1.79
J71	10	0.037	667	1.84
control[b]			675	1.69
J176-15	5	0.017	613	1.84
J176-15	10	0.034	569	1.94

[a] Each mean represents three or four replicate groups of Hubbard variety broiler chicks feeding for 4 weeks. [b] Commercial basal diet. [c] Jojoba meal as percent of total diet with substitutions to maintain diet isonitrogenous. [d] Simmondsin (I); simmondsin 2′-ferulate (II).

Table VI. Cattle Feeding Studies[a]

	groups		individuals	
	control diet	meal J176 mixture[b]	control diet	meal J176 mixture[b]
no. of steers	7	7	6	6
mean initial wt, kg	385	386	301	307
mean final wt, kg	488	475	406	393
feeding period, days	84	84	70	70
av daily gain, kg	1.23	1.06	1.50	1.23
av daily feed, kg	10.8	11.0	8.0	7.5
feed/100 kg of gain, kg	878	1038	533	610

[a] Preliminary performance data. [b] A mixture of sixteen batches 100 kg each of detoxified meals J176-12 to J176-28 added at a 10% level.

with a control diet (Verbiscar et al., 1980) (Table V).

Sheep. Lamb feeding studies were carried out at California State Polytechnic University (J.E.T. and E.A.N.). Jojoba meals were added to a basal diet at 10% levels, substituting for cottonseed meal. The rations contained 5% molasses and were pelleted (Verbiscar et al., 1980). In the *L. acidophilus* 629 meal test J71 (Figure 1), eight lambs were prefed to basal diet for 11 days prior to allocation to treatment and control groups. The feeding trial lasted 35 days during which time the lambs were allowed to consume all the feed they wanted each day. Feed remaining in feeders was weighed back after 24 h. The lambs readily consumed both the J71 and basal rations. In the *L. acidophilus* 1911 meal test J87 (Figure 2), eight lambs were preconditioned on the basal diet for 9 days prior to the start of feeding. This feeding trial lasted for 37 days. Again the four lambs in the control and treatment groups each began eating their respective diets immediately.

Cattle. Cattle feeding studies were carried out at the University of Arizona (R.S.S.) using crossbred steers produced at the University. Feeding trials were undertaken on deoiled but nondetoxified jojoba meal, partially treated meals, and more thoroughly detoxified meals. The control diet contained sorghum grain (66.2%), alfalfa hay (10%), cottonseed hulls (10%), cottonseed meal (5%), molasses (5%), animal fat (3%), dicalcium phosphate (0.55%), salt (0.25%), and vitamin A. Jojoba meal treated with *L. acidophilus* 629, J176 batches 12–28, similar in composition to J176 batch 15, was added at a 10% level to the control diet at the expense of the 5% cottonseed meal and 5% of the sorghum grain.

Two groups of seven steers each were placed in a pen and assigned to one of the experimental diets, which were fed ad libidum. Individual animal weights were taken every 28 days for a total of 84 days. Six additional steers were placed in individual pens and fed the additive diet, comparing performance with six steers on the control diet for a total of 70 days. Preliminary results are summarized in Table VI. Details of these experiments and related work will be reported elsewhere.

RESULTS AND DISCUSSION

Our preliminary experiments with *L. bulgaricus* Alta indicated that this strain grew on wetted jojoba meal and lowered the simmondsin level. Accordingly we obtained 12 strains of Lactobacilli that had been studied for their ability to grow in soybean milk (Wang et al., 1974), a commerical *L. acidophilus* Knudson, and a commercial baker's yeast. A goal was to identify strains that would act on both simmondsin (I) and simmondsin 2′-ferulate

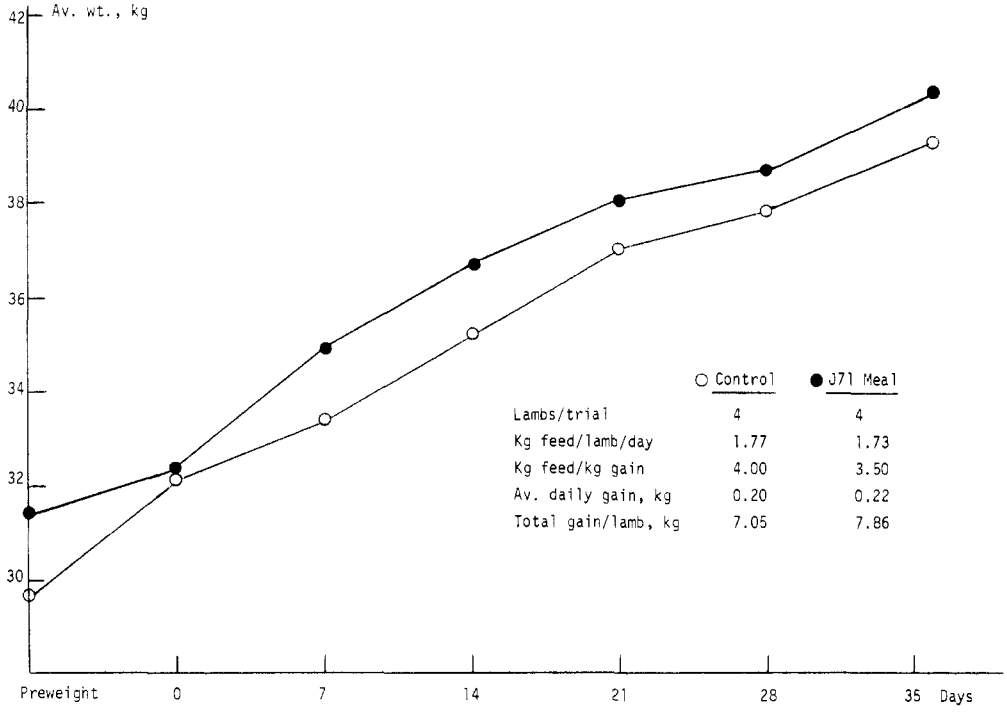

Figure 2. *Lactobacillus acidophilus* 629: detoxified jojoba meal fed to lambs.

(II). The screening and detoxification experiments were carried out by using jojoba meals with oil contents < 2%.

Screening results using the various bacteria and a yeast are summarized in Table I. Several Lactobacilli produced substantially reduced levels of I and II in these submerged cultures, especially B-629 and B-1911. In earlier work B-734 was identified as an organism that lowered both I and II levels, but this strain did not do as well in this screening project. In this experiment, B-734 showed only moderate growth and the pH of the medium dropped to 4.1 in 10 days. The two best strains, B-629 and B-1911, had heavy growth and the pH remained high at 6.8 and 7.0. Strain B-1910 lowered the level of the toxicant II nearly as well as B-629 and B-1911 but did not lower toxicant I level as well as the two best strains. It appears generally that at least in submerged cultures the effect on toxicants is less at lower pH. The yeast growth was heavy but detoxification was minimal.

Treatment of 780–800-g quantities of wetted jojoba meal with three of the Lactobacilli including B-734, B-629, and B-1911 are summarized in Table II. After 21 days at 30 °C, toxicant I + II levels in the meal dropped to 0.28–0.38% (J103–J105). When ammonium hydroxide was added to the process, toxicant I + II levels dropped to 0.15–0.20% in 13 days (J110–J112). The ammonium hydroxide was sprayed on the meal just prior to spraying the innoculum on the meal. The equivalents of added ammonia exceed the lactic acid formed in the inoculum prior to spraying onto the meal. The addition of ammonia to the treatment process results in a shorter detoxification time. This is probably due to increased growth rate of the Lactobacilli on the ammonia-treated meal. Carbon dioxide is produced by the Lactobacilli, resulting in a weight loss.

The increased crude protein ($N \times 6.25$) levels in the microbially treated meals is notable. The protein level of the charged meal on a moisture free base is 25.5%. The protein level in the meals processed with ammonia was ~30% on a dry basis (Tables II and III). This increase in the nitrogen level is due in part at least to the overall weight loss of the meals during treatment, as utilizable carbohydrate is converted to carbon dioxide by the Lactobacilli. However, the treated meals do not appear to contain ammonium salts. The added ammonia probably is utilized as a nutrient by the Lactobacilli, as indicated in the literature, or is bound in some manner.

Before we discovered the favorable results from the use of ammonia in the treatment process, two batches were scaled up to 32.7-kg levels (Table III). In these runs lactic acid was initially sprayed on the meals to suppress the growth of undesired organisms, as a substitute for sterilization. After standing at ambient temperature for 47 days, the B-629 (J71) meal had a toxicant I + II level of 0.37% whereas the B-1911 (J87) meal after 41 days had a toxicant I + II level of 0.94%. At the 10% additive level these meals were not toxic to mice (Table IV) and were acceptable for poultry (Table V) and sheep feeding (Figure 1 and 2). However, when the batch process was scaled up to 100 kg in 100-gal drums with the use of ammonia in the inoculum, the total toxicant I + II level for J176-15 was 0.34% after 21 days at 26 °C (Table III). Seventeen 100-kg batches run consecutively in this manner averaged 0.12% toxicant I and 0.20% toxicant II. Minor toxicants III + IV averaged 0.04%. The structures of these latter compounds (Elliger et al., 1974) are being clarified.

Infrared spectra of the analytical extracts from strain B-629 and strain B-1911 detoxifications showed weak CN absorption in the 2220-cm^{-1} region, indicating low toxicant and organic degradation product levels. A semiquantitative comparison made with the spectrum of extracts from nondetoxified jojoba meal agreed with high-performance LC analysis of the samples.

The detoxification of jojoba meal using a Lactobacillus resembles an ensilage process, except that a specific microorganism is used rather than the random organisms that accumlate in a silo. We used nonfat skim milk as the inoculum medium although byproduct wastes from a dairy or cheese manufacturer may be suitable. After a period of growth in the skim milk, the produced lactic acid drops the acidity to about pH 4. At this point the milk has curdled and microorganism growth is adequate for inoculation of the meal. We found that the addition of sufficient ammonium hydroxide to the inoculum caused the curds to dissolve and dispersed the microorganism growth so that the mixture could be evenly sprayed on the meal. Am-

monia added to the inoculum causes more complete detoxification in a shorter period of time. After standing for 21 days, 100-kg quantities of jojoba meal contained only 2–5% of the total toxicants originally present in the meal. These meals show acceptable palatability for feeding to certain livestock.

In a preliminary screening test for toxicity and/or nutritional quality, detoxified meals were fed to weanling mice at 10% additive levels in a basal diet which was formulated to use whole egg as the major dietary protein source to bring the total protein level to 7% (Table IV). In a prior report (Verbiscar et al., 1980), toxicity to mice was related generally to the level of simmondsin and related toxicants in the diet. When the toxicant levels are <0.05%, mice can survive for 3 weeks on the jojoba meal as the sole source of protein in their rations, but the mice lose weight. In these feeding studies toxicant levels in the diet range up to 0.082%. All of the mice gained weight but not nearly as much as the controls, with no mortality. For mice, the quality of jojoba protein does not appear to be very good.

The mice did well on *L. acidophilus* 1911 treated meal (J96) which was extracted with water, bringing the toxicant I + II level to 0.20% (J98) (Table IV). The same *L. acidophilus* 1911 treated meal was extracted with acetone to bring the toxicant I + II level to 0.18% (J97), but the mice did not do as well as on the J98 meal. This confirms earlier data that showed that mice do better on water-extracted jojoba meals (Cotageorge et al., 1978; Verbiscar et al., 1980). Water is more efficient than acetone in removing simmondsin and toxicants III and IV. It is possible that jojoba seeds contain another toxic water soluble compound such as a cyano diglycoside. A diglycoside would be highly water soluble but far less soluble in acetone than toxicants I–IV. Because mice eat more of water-washed meals and grow more, a palatability factor also seems likely. There is a palatability factor in deoiled jojoba meal that causes sheep and cattle to shun diets containing this additive. Treatment with Lactobacilli decreases toxicity and increases palatability. However, molasses was added to ruminant diets to overcome the residual palatability factor in detoxified jojoba meals.

Broiler chick feeding studies at 5 and 10% additive levels are summarized in Table V. For the chicks feeding on *L. acidophilus* 1911 detoxified meal J87, there were no statistical differences in body weights at 4 weeks of age compared to controls, although growth rate was depressed at both levels. At a 5% level *L. acidophilus* 629 detoxified meals J71 and J176-15 depressed growth rate, with an additional depression at the 10% feeding level. Feed conversion was less efficient than controls for these three meals.

In contrast to the poor performance of lambs fed nondetoxified meal (Verbiscar et al., 1980), four lambs did very well on *L. acidophilus* 1911 treated jojoba meal J87 (Figure 1). In this study the jojoba meal was added to a basal diet at a 10% level, substituting for cottonseed meal with 5% molasses added and pelleting. Lambs ate these rations as readily as the basal ration. Weight gains and feed conversion were comparable for the control and test lambs. They started quickly the first week and seemed to be consistent in their gains after the second week. There was a period of inclement weather in the second week of the feeding trial, and this is reflected by slight losses in weight during that week.

Figure 2 summarizes lamb feeding tests using *L. acidophilus* 629 detoxified jojoba meal J71 in a basal diet at a 10% level. Results with the treatment and control lambs are again parallel and comparable, with no palatability problem. Extensive performance and metabolism studies are now being undertaken on *L. acidophilus* 629 detoxified jojoba meal in sheep diets.

Preference experiments showed that steers do not like rations containing 10% deoiled (~1% oil) but nondetoxified jojoba meal, even with 6% molasses and 1% animal fat added to a pelleted diet. When four steers were given choices between the above ration and one containing 10% cottonseed meal, only 17% of the feed intake was from the jojoba meal ration. When the diet was offered in a loose nonpelleted form, even less of the jojoba meal ration was eaten, and there was considerable sorting of the diet ingredients. The low acceptability of nondetoxified jojoba meal diets by cattle indicates that a palatability factor is involved, similar to that observed for sheep and possibly mice.

Performance trials were initiated with seven cattle in a group fed a well detoxified meal J176-12 to -28. The ration contained 5% molasses plus 3% animal fat and was not pelleted. Results from the first 84 days feeding are summarized in Table VI. Six individually penned steers were also fed the same diet. Feed intake was satisfactory for both treatments in both trials, with no evidence of depressed intakes. Some sorting of the diet ingredients occurred, but it did not seem to be a serious problem. Despite the similarities in feed intake, rate of gain and efficiency of feed conversion were ~16% less for steers on the treated meal diet compared to controls. Details of these cattle experiments and a more extensive analysis of results will be reported elsewhere.

Treatment of jojoba meal with *L. acidophilus* 629 decreased toxicant level and increased acceptability in cattle diets. A partially treated meal was not as well accepted as a more completely treated meal. Treated jojoba meal diets were definitely preferred to untreated jojoba meal diets. For humans, deoiled nondetoxified jojoba meal is bitter, whereas *L. acidophilus* treated meal is bland. Simmondin is tasteless. For humans at least, unpalatability of jojoba meal is not related to simmondsin levels. It seems likely that this is also the case for mice and ruminants and that *L. acidophilus* modified the unpalatable compounds in jojoba meal as well as the cyano toxicants.

A statistical study of simmondsin and simmondsin 2′-ferulate levels in 30 treated meals formulated in mouse diets correlated mortality, feed intake, and body weight gain (Weber, 1979). An analysis of the data indicated that simmondsin 2′-ferulate is not significantly involved in toxicity or antinutritional aspects of jojoba meal. Only simmondsin showed statistically significant correlations.

Toxicants III and IV also probably contribute to the toxicity of jojoba meal. These "minor" toxicants are present in deoiled jojoba meal at a combined level of ~2%, compared to ~6% for simmondsin and 1.5% for simmondsin 2′-ferulate. Structural studies indicate that toxicants III and IV are monoglucosides similar to simmondsin. Microbial splitting of glucose from simmondsin in the gastrointestinal tract or stomach of animals is apparently a controlling factor that causes simmondsin to be toxic following chronic oral administration. This same enzymatic splitting to release an aglycon should occur with toxicants III and IV but not as readily with simmondsin 2′-ferulate. The latter toxicant is more slowly modified by Lactobacilli than are toxicants, I, III, and IV. Our Lactobacillus process reduced levels of simmondsin to 0.12%, toxicants III + IV to 0.04%, and simmondsin 2′-ferulate to only 0.20% in 21 days. A diglycoside toxicant

would also not be as readily split by a Lactobacillus or other gut bacteria as are the monoglycosides, although in time it too should respond to a microbial treatment process. The reduction in levels of toxicants III + IV by *L. acidophilus* 629 was proportional to the reduction in simmondsin levels.

The intertwining of toxicity and palatability factors in jojoba meal add complexity to interpretations of animal feeding data. The possibility that there is a bitter cyano polyglycoside present that is relatively intractable to solvent extraction and chemical and microbial treatment should be considered. Structural studies on the minor toxicants and other components in jojoba seeds are continuing.

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Pyrrolizidine Alkaloids of *Senecio alpinus* L. and Their Detection in Feedingstuffs

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Senecio alpinus L. was recently discovered as the cause of pyrrolizidine alkaloidosis in livestock in Switzerland. A GC–MS analysis of this plant and of hay and silage containing *S. alpinus* revealed the presence of nine different alkaloids with seneciphylline as the main constituent and senecionine, integerrimine, jacozine, jacobine, jaconine, and the unsaturated analogue of jaconine as minor constituents. The structure of the two other alkaloids are discussed on the basis of their mass spectra.

In the past 80 years poisoning of domestic animals with pyrrolizidine alkaloids (PA) have been reported from various countries all over the world, the main cause of these incidents being plants belonging to one of the three genera *Senecio*, *Crotalaria*, and *Heliotropium* (Bull et at., 1968). Besides their high acute hepatotoxicity, many PA tested so far were found to be mutagenic and carcinogenic and are therefore of considerable interest to toxicologists (IARC, 1976).

Senecio alpinus, a widespread plant in alpine meadows, was recently discovered as the cause of pyrrolizidine alkaloidosis in three herds of dairy cattle in Switzerland (Pohlenz et al., 1980). Klasek et al. (1968) were able to isolate seneciphylline and a small amount of jacozine from a sample of *S. alpinus* L. of Swiss origin. In the present paper we report a GC–MS analysis of the alkaloids derived from *S. alpinus* and the detection of such compounds in hay and silage, both having been used as feed for cattles.

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EXPERIMENTAL SECTION

Apparatus. The separation and identification of the alkaloids was achieved on a Finnigan Model 4021 GC–MS system. GC was performed on a 20-m SE 54 capillary column, with He as the carrier gas. The temperatures were injector 250 °C and column 100 °C for 1 min and 10 °C/min until 220 °C. The mass spectrometer had an electron energy of 70 eV and ion source temperature of 250 °C. CI conditions were source pressure 0.30 torr, and source temperature 200 °C. Quantitation of the alkaloids were made by peak integration.

Materials. Preblooming samples of *S. alpinus* L. were collected near Einsiedeln, Maloja, and Sörenberg (Switzerland) in June. Samples of hay and silage from the area of Einsiedeln were taken in January and stored at −24 °C until analyzed. Authentic samples of seneciphylline, senecionine, and integerrimine were kindly provided by Dr. C. C. J. Culvenor, Parkville, Australia, and Dr. M. H. Benn, Calgary, Canada.

Procedure. The samples (50–100 g wet weight) were extracted extensively with methanol in a Soxhlet apparatus, and the extracts evaporated to dryness under reduced

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