may bind to the proteins and cause conformational changes which affect the physical properties of the isolated proteins.

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Detoxification of Jojoba Meal

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Jojoba meal, after removal of the oil from the seeds of *Simmondsia chinensis*, contains up to 30% crude protein, making it a potential animal feed ingredient. This meal is toxic to rodents and chickens and less toxic to sheep. Toxicity is due to the presence of simmondsin and as many as three other structurally related cyanomethylenecyclohexyl glycosides in the meal. Solvent extraction and heat and chemical methods were investigated for detoxifying the meal. A new method was devised to hydrate the cyano compounds in the meal rapidly to amides using ammoniacal hydrogen peroxide. This detoxified jojoba meal was well accepted by mice, chickens, and sheep at 10% additive levels in rations. Toxicity of simmondsin is apparently related to the cyano aglycon which can be split from the glucose conjugate in the gut or stomach of monogastric animals. Microorganisms in the rumen of sheep serve to detoxify simmondsin in jojoba meal.

Jojoba is an oilseed shrub that grows naturally on arid lands in the southwest United States and in Mexico. Several thousand acres have been planted in California and Arizona as the basis for a new economic crop. The principal product of this new industry is an unusual oil comprised of esters of long-chain monounsaturated acids and alcohols with structures similar to sperm oil, a commodity no longer available in the United States. After removing the jojoba oil from jojoba seeds, the remaining meal is high in protein and is a potential livestock feed ingredient (Verbiscar and Banigan, 1978a,b).

The term jojoba meal is used here to mean the plant material remaining after the seeds containing some hulls have been processed to remove jojoba oil. Jojoba seed meal is the residual material left after deoiling completely dehulled seeds. The jojoba meal that we have used throughout this study contains hull material. The presence of hulls in the seeds facilitates expression of jojoba oil but

Table I. Composition of Jojoba Seed Meal, Jojoba Meal, and Hulls[a]

component	seed meal no. 377,[b] %	meal no. 377,[c] %	meal no. 278,[d] %	seed hulls, %
crude protein, N × 6.25	29.1	24.1	20.1	7.0
moisture	8.9	5.8	5.7	10.7
crude oil (ether extract)	3.0	1.6	0.9	0.7
crude fiber	8.1	11.0	14.3	15.6
ash	3.1	4.9	3.6	4.4
total sugars	8.8		6.4	3.3
simmondsin (I)	5.2	4.2	3.6	0.2
simmondsin 2′-ferulate (II)	1.5	0.5	1.3	0

[a] Products courtesy of San Carlos Apache Jojoba Development Project, San Carlos, AZ. [b] Hexane extracted hulled seed meal. [c] Methylene chloride extracted meal from seeds with ~8.5% hulls. [d] Hexane extracted meal from seeds with ~17% hulls.

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Table II. Solvent Extraction and Heat Treatments

meal	solvent/treatment	solvent/ meal, v/w	% toxicants I	% toxicants II	% crude protein	% crude oil	% moisture
H149[a]	hexane	3:1	5.0	1.5	24.9	1.3	7.0
J1[a]	methylene chloride	2:1	4.2	0.5	24.1	1.6	5.8
J27[a]	methyl chloroform	2:1	4.6	1.3		1.5	
H26[b]	methanol, ambient	4:1	0.31	0.06	34.3	0.02	4.4
H127[c]	methanol, ambient	6:1	0.05	0.01	28.2	0.06	5.3
H35[b]	acetone, reflux	10:1	0.63	0.05	31.3	0.16	3.9
H36[b]	2-propanol, ambient	9:1	2.9	0.40	32.2	0.3	5.2
H43[b]	methylene chloride/methanol (85:15)	8:1	0.33	0.05	33.5	0.04	3.7
J31[d]	boiling water extracted	8:1	0.08	0.08	28.5	4.4	5.0
J19[d]	water, pH 3.2 (phosphoric acid)	8:1	Tr	0.43	22.5	2.1	6.9
H148[e]	100 °C, 3 h, water extracted	8:1	0.19	0.38	25.4	0.5	12.8
H76[e]	135 °C, 15 h		0.27	0.18	26.7	1.5	2.4

[a] Deoiling batches. [b] Batch extracted several times. [c] Extracted four times at solvent/meal (3:2) per batch. [d] Denatured protein to facilitate filtration. [e] Oven heated dry state.

it also lowers the percent composition of crude protein in the meal from the initial 29–30% in the seed meal. However, even the hulls contain some nutritionally useful components including about 7% crude protein and 3% sugars. The nutritionally significant composition of jojoba seed meal, jojoba meals, and hulls are summarized in Table I.

The problem with jojoba meal as an animal feed is its high content of simmondsin (I), simmondsin 2′-ferulate (II), and related cyanomethylenecyclohexyl glycosides (Elliger et al., 1973, 1974). Booth et al. (1974) reported that rats did not do well on diets containing deoiled jojoba seed meal or pure simmondsin. In their experiments, jojoba seed meal and simmondsin were added to control diets at several concentrations. As the concentration of simmondsin increased in the formulated diets, the feed intake of the rats decreased and the rats lost weight. In the treatment with 10% nondetoxified jojoba seed meal in the diet, representing about 0.75% total toxicants, three of the four rats died in 12 days. At a 15% jojoba seed meal level all four rats died in 12 days. The results with pure simmondsin added to a control diet were about the same. A 0.6% simmondsin level diet had a slightly less negative effect on rat feed intake and body weight loss than the 10% jojoba seed meal diet. Further data was developed by this USDA group which demonstrated the oral toxicity of simmondsin (Booth et al., 1974). When weanling rats were given a single oral dose of simmondsin at 4.0 g/kg body weight, no adverse effects were observed up to 14 days after dose. A single 3.6 g/kg dose of simmondsin administered intraperitoneally to mice was also nonlethal. However, when weanling rats were given oral doses of simmondsin at 750 mg/kg daily for 5 days, all rats lost weight and died within 10 days after the initial dose. These experiments demonstrate the oral toxicity of simmondsin during chronic administration to rodents. When nondetoxified jojoba meal in a basal diet was fed to chicks, growth was markedly depressed (Reid, 1977).

The purpose of this project is to devise a practical low-cost process to detoxify jojoba meal and demonstrate the efficacy of the detoxified meal in the diets of livestock including poultry, sheep, and cattle. It was desirable to develop a detoxification process so that this economic seed crop could be fully utilized. Our specific goal is to remove simmondsin and related toxicants from the meal, or to modify their cyano groups by heat, chemical, or microbial treatments, so that the resulting compounds will be less toxic than the natural compounds. In order to monitor our detoxification processes we developed analytical methods to assay the toxicants in the meal. For this purpose, pure simmondsin and simmonsin 2′-ferulate were isolated from jojoba seed meal (Verbiscar and Banigan, 1978a). Weanling mice are used as a bioassay for toxicity in processed meals and as a general test of nutritional acceptability. Studies feeding the nondetoxified and processed meals to poultry and sheep are reported here. Additional studies with poultry, sheep, and cattle are in progress, including the feeding of microbially treated meals to these livestock.

EXPERIMENTAL SECTION

Analyses. Test meals received proximate analyses for crude protein, crude fat, crude fiber, moisture, and ash. They were also assayed for the major toxicants I and II, using thin-layer chromatography (TLC) and high-performance liquid chromatography (LC) as described earlier (Verbiscar and Banigan, 1978a). The LC analysis has now been improved using Porasil A 37-75μ column packing and acetonitrile/water eluant (Verbiscar et al., 1978). Typical LC conditions are as follows: precolumn, Porasil A, 3.2 × 40 mm; column, Porasil A, 3.2 × 500 mm; eluant, acetonitrile/water (9:1); flow rate, 1.0 mL/min, <500 psi; chart speed, 0.5 cm/min; detector, UV at 220 nm; temperature, ambient; retention, simmondsin (I), 8.0 min and simmondsin 2′-ferulate (II), 5.5 min. Infrared spectrophotometry was used to confirm the presence or absence of the cyano group in analytical extracts of toxicants from the test results. The spectra were obtained by allowing ten drops of a 10-mL concentrated extract to evaporate completely on a silver chloride planchet forming a film. An estimate of CN concentration in the test meal was made by comparing CN absorption at 2217–2200 cm^{-1} with that from a standard extract of nondetoxified jojoba meal.

Solvent Extraction of Oil. Detoxification studies were carried out on deoiled jojoba meals. Solvent extraction of oil was undertaken, using screw-pressed jojoba meal with ~16% residual oil, obtained through the courtesy of Miller et al. (1979), USDA, Albany, CA. The jojoba seeds processed here contained 8.5% hulls and were supplied by the San Carlos Apache Tribe.

Larger batch deoiling was conducted in a 115-gal stainless steel kettle with agitator, using 12 gal of solvent/100 lb of meal in two steps (Table II). Although hexane is the preferred solvent for commerical oilseed extractions, it was employed here only when meal requirements for feeding tests were in the 1–25-lb range. For scale-up extractions of 200–300 lb of jojoba meal, methylene chloride (J1) and methyl chloroform (J27) were used at ambient temperatures. Extraction slurries were filtered by basket centrifugation to separate the miscella. After two extractions, solvent removal from the meal was completed by tray drying at 60–70 °C in a circulating air oven.

Table III. Jojoba Meal Detoxification by Treatment with Ammonium Hydroxide and Hydrogen Peroxide

	meal					
	H63	H64	J13	J29	H112	J21
material						
meal, kg	0.80	0.72	13.62	13.62	0.60	13.62
water, L	0.15	0.15	3.6	3.6	0.35	7.94
NH_4OH, mol	2.4	2.4	14	14	1.2	20
H_2O_2, mol	0	0	0	0	0.5	10
time, days	14	40	20	40	8	7
temp, °C	50	amb[a]	55	amb	amb	amb
yield, kg	0.75	0.73	13.62	13.69	0.62	13.35
assay, %						
moisture	6.8	9.0	7.0	8.7	9.5	7.9
crude protein, N × 6.25	40.9	37.0	30.4	29.7	32.4	32.8
crude oil	0.5	0.6	1.3	2.8	0.9	0.7
crude fiber	9.5	10.4	9.1	9.4	8.9	10.3
ash	4.7	4.9	4.5	3.9	4.1	3.5
simmondsin	0.19	0.16	0.05	0.05	<0.01	0.05
simmondsin 2′-ferulate	0.03	0.08	0.01	0.06	0.03	Tr

[a] Ambient.

Solvent Extraction of Toxicants. Experimental solvent extractions were carried out on 1 kg or less of deoiled meal, using two or three changes of solvent (Table II). Total solvent-to-meal ratios ranged from 4:1 for methanol (H26) to 10:1 for acetone (H35). The small-scale extractions were generally done in glass flasks with stirring at ambient temperatures. Exceptions were the acetone and boiling water (J31) extractions which were done at or near reflux. Simultaneous solvent deoiling with detoxification was attempted with 2-propanol (H36) and with methylene chloride/methanol (H43). Several larger scale water extractions were performed on 50-lb batches of meal where the aqueous slurries were first treated with phosphoric acid (J19) or boiled briefly (J31), to precipitate water-soluble protein, thus rendering the meal filterable. Without protein coagulation, water suspensions of the meal are extremely difficult to filter or centrifuge.

Heat Treatments. Heat treatments of deoiled meals included dry heating in open trays in an oven at 135 °C (H76, Table II) and higher, with major toxicant destruction monitored over the treatment period. Moist heat treatments were carried out in an autoclave at 100 °C for 3 h and at 125 °C for 12 h. It was thought possible that the autoclaved meals might detoxify with heat alone at the higher temperatures, but this did not prove to be the case. Even when simmondsin levels dropped, toxicity remained high for the heat-treated meals, dry or moist. The 100 °C treatment (H148, Table II), designed to denature water-soluble protein, required a subsequent water extraction step to lower toxicant levels. At 175 °C, dry or moist, the meal decomposes.

Ammonia Treatments. The ammonia-treated meals were prepared following a patented method (Elliger et al., 1975) and are summarized in Table III. For H63 and H64, deoiled meal was distributed among several mason jars and wetted with ammonium hydroxide. In the 30-lb batches, J13 and J29, treatments were carried out in three 5-gal bottles. The sealed containers were allowed to stand at ambient or higher temperatures for various periods of time. The meals were then oven dried to moisture levels <10% and assayed.

Ammoniacal Hydrogen Peroxide Treatments. In a typical small batch, a solution of 0.5 mol of hydrogen peroxide and 1.2 mol of ammonium hydroxide in 350 mL total water was sprayed on 600 of jojoba meal in a rotating 1-gal bottle. The temperature was kept below 35 °C by periodic external cooling. After standing at laboratory temperature for 40 h, an assay showed simmondsin to be low and simmondsin 2′-ferulate about 50% gone. After 8 days both toxicants were at very low levels (H112, Table III).

In a scaled-up example, a solution of 10 mol of hydrogen peroxide and 20 mol of ammonia in water was sprayed onto 30 lb (13.62 kg) of deoiled jojoba meal. The meal was divided into 3 × 10 lb portions, each contained in a 5-gal bottle. As above, the reagent was sprayed evenly over the surface of the meal in rotating bottles over several hours with external cooling. The stoppered bottles were then allowed to stand undisturbed at room temperature for 7 days, and the meal dried at 75–80 °C and assayed, J21.

In a still larger batch, 20 mol each of hydrogen peroxide and ammonium hydroxide in a total of 12.72 L of water were sprayed onto 60 lb of meal in a 50-gal rotating drum. The reagent solution was applied with an electric airless spray unit. The exotherm in the meal was controlled only by the reagent addition rate, as the temperature rose to 50 °C or higher, undoubtedly with some loss of reagents. After 11 days the meal was oven-dried to yield 60.8 lb of semidetoxified jojoba meal, J49. An assay showed 0.77% simmondsin, 0.09% simmondsin 2′-ferulate, 30.5% crude protein, 1.7% crude oil, and 8.7% moisture. This meal was fed to sheep.

Amide Analogue of Simmondsin. A solution of 500 mg of simmondsin hydrate in 10 mL of distilled water was treated with 1.5 mL of 30% hydrogen peroxide and 1.25 mL of 29% ammonia solution. After 1 h at room temperature, a TLC monitor of the reaction solution showed a trace of simmondsin, and after 2 h all of the simmondsin had reacted. Water was removed under vacuum on a rotary evaporator and the residue was triturated with methyl ethyl ketone to yield 440 mg of a white, crystalline, hygroscopic solid. An infrared spectrum confirmed the absence of CN and showed strong amide absorption at 1620 and 1670 cm^{-1} and medium absorption at 1600 cm^{-1} probably due to C=C. A TLC on Merck silica gel G using ethyl acetate/ethanol (7:5) developer indicated this amide was ~95% pure with an R_f 0.31 compared to R_f 0.60 for simmondsin. A LC assay on the Lichrosorb Si 60 column and acetonitrile/2-methoxyethanol (97:3) eluant system (Verbiscar and Banigan, 1978a) showed simmondsin amide to have good purity and a retention of 32 min, compared with 6.3 min for simmondsin.

Animal Feeding Studies. *Mice.* Mouse testing was carried out by Professor Charles W. Weber at the University of Arizona. Twenty Charles River CD-1 mice, 10 male and 10 female per treatment, were housed one pair per cage in suspended stainless steel cages. Feed and water were supplied ad libidium to the animals for a 3-week test

Table IV. Three-Week Values from Mouse Feeding Studies

meal	% jojoba meal in diet	% toxicants I + II in diets	change in body wt/ mouse, g	total feed intake, g	% mortality
control[a]	0		15.1	68.4	0
J13[b]	10	0.006	5.6	70.8	0
J19[b]	10	0.043	8.7	78.8	0
J21[b]	10	0.005	2.7	56.8	0
J31[b,f]	10	0.016	13.6	93.0	0
H127[c]	24.8	0.014	−2.5	30.4	50
H127 + AA[d]	24.8	0.014	−1.9	34.9	0
H112[c]	21.6	0.017	−3.0	28.0	0
H63[c]	17.1	0.038	−2.4	35.7	0
H64[c]	18.9	0.045	−3.0	28.7	0
H26[c]	20.4	0.075	−1.3	46.9	30
H43[c]	22.4	0.085	−1.7	34.3	10
H35[c]	22.4	0.150	−2.9	24.5	60
H148[c]	27.6	0.160	−2.8	23.5	95
H76[c]	26.2	0.120	−2.4	25.2	95
H36[c,e]	21.8	0.720	−2.6	9.1	100

[a] Whole egg used as protein source at 7% protein in total diet. [b] Jojoba meals supplemented by whole egg to equal 7% dietary protein. [c] Jojoba meals as sole source of protein in diet. [d] H127 diet supplemented with L-lysine (0.81%), L-histidine hydrochloride (0.15%), DL-Methionine (0.55%), DL-isoleucine (0.64%), and L-tryptophan (0.12%). [e] Data for 1 week only. [f] Controls gained 17.2 g and ate 84.9 g feed total.

period. Preliminary screening was undertaken to explore the toxicity of various processed jojoba meals. In the first feeding system, the jojoba meals were incorporated into the diet at 7–8% crude protein levels as the sole source of protein. A typical formulation consisted in the basal diet of corn oil 3%, sucrose 56.69%, bentonite 4.3%, cellulose 3%, vitamin mix 4%, dicalcium phosphate 3%, sodium chloride 0.4%, potassium chloride 0.2%, trace minerals 0.2%, chromic oxide 0.2%, and choline chloride 0.2%, plus the processed jojoba meal 24.81% in this case (H127, Table IV). The control diet contained 15.97% whole egg, representing a 7% crude protein level. In the second feeding system, jojoba meal was incorporated into the diet at 10% level to supplement whole egg to bring the crude protein level to 8% (J13, J19, J21, and J31, Table IV). In the third system, jojoba oil was fed at 1% and 2% levels, a proportional amount of corn oil was removed from the above basal diet which used a whole egg protein source (Table V).

Poultry. Poultry feeding studies on several of the more advanced meal treatments have been carried out by Professor B. L. Reid at the University of Arizona. Broiler chicks, Hubbard variety, nine males and nine females, housed six chicks per cage, were used per treatment. Jojoba meals were added to a basal control diet at 5% and 10% levels and these formulations were fed for 4 weeks. The basal control diet consisted of milo 56.25%, soybean meal 31.25%, dehydrated alfalfa meal 3%, meat scraps 2.5%, dicalcium phosphate 1%, limestone 1%, fat 3%, sodium chloride 0.4%, trace minerals 0.1%, Pr-9 vitamins 1%, chromic oxide 0.2%, and DL-methionine 0.2%. Results are given in Table VI.

Sheep. Sheep feeding studies were undertaken by Professor John E. Trei and Edward A. Nelson at California State Polytechnic University, Pomona. Various feeding trials were conducted with lambs on detoxified and nondetoxified jojoba meals. A basal diet consisted in oat hay 60%, rolled corn 22%, cottonseed meal 10%, liquid molasses 5–7%, dicalcium phosphate 0.5%, trace minerals

Table V. Jojoba Oil Toxicity in Mice

oil in diet	week	body wt, g	change in body wt, g	feed intake/ day/ mouse, g	mortality, %
control[a,b]	1	13.9	+4.9	2.67	0
	2	19.0	+5.1	3.34	0
	3	24.1	+5.1	3.77	0
1%[a,c]	1	13.7	+4.7	2.91	0
	2	19.8	+6.1	4.28	0
	3	24.4	+4.6	4.74	0
2%[a,d]	1	10.8	+1.8	3.11	0
	2	13.0	+2.2	3.46	0
	3	16.9	+3.9	4.44	0
10%[e]	1	7.9	−1.0	1.06	10
	2	9.3	+1.4	1.94	30
	3	12.4	+3.1	1.87	30
10%[f]	1	20.5	−2.3[g]	2.67	0

[a] 10 M and 10 F weanling mice per treatment. [b] Basal diet, whole egg at 7% crude protein, corn oil control. [c] Normal feces. [d] Soft feces. [e] 5 M and 5 F weanling mice in treatment. [f] Adult mice. [g] Range in change in body weight for 1 week −6.9 to 0.1 g.

Table VI. Poultry Feeding Studies[a]

meal	% meal in diet[c]	% toxicants I + II in diet	av body wt at 4 weeks, g	g of feed/ g gain	feed consumed/ bird, g	mortality, %
control A[b]			720	1.55	1055	0
J1	5	0.24	574	1.73	921	5.6
J1	10	0.47	140	2.73	270	55.6
J21	5	0.003	720	1.59	1078	0
J21	10	0.005	536	1.93	954	0
J31	5	0.008	654	1.69	1033	0
J31	10	0.016	554	1.90	959	0
control B[b]			731	1.70	1169	0
J29	5	0.006	643	1.86	1115	0
J29	10	0.011	617	1.86	1070	0

[a] Broiler chicks, 9 M and 9 F per treatment. [b] Commercial basal diet. [c] Jojoba meal as percent of total diet with substitutions to maintain diet isonitrogenous.

0.5%, and vitamins A, D, and E. The lambs were adapted to the basal ration for a minimum of 7 days before introducing them to the experimental diets. For each treatment, jojoba meal was substituted by weight for cottonseed meal. The concentrate to roughage ratio was maintained across the rations, but no attempt was made to achieve isonitrogenous diets. The rations were pelleted. The lambs were fed ad libidium and feed records were taken daily. Lamb weights were taken weekly. Results are summarized in Table VII.

Simmondsin Administration to Mice. Simmondsin was administered to mice in various dosage forms by Professor Robert F. Raffauf and Donald Kosersky at Northeastern University.

Intraperitoneal. Simmondsin hydrate at 188, 375, and 750 mg/kg dose levels in 0.25% agar was administered intraperitoneally at 24-h intervals to groups of six male Charles River CD-1 mice. Control mice were given the vehicle alone. All drug and vehicle control injections were made in a constant dosage volume of 10 mL/kg. Each animal received injections daily for 9 days beginning on day 2. Body weights were monitored over an 11-day period. Body weights of the mice treated at all three dose levels were essentially identical with those of the vehicle control throughout the 11-day period.

Table VII. Sheep Feeding Studies

meal	J27[a]	J1-a[b]	J1-b[c]	J1-c[d]	J13[e]	J19[f]	J49[g]
days of study	49	35	35	63	42	42	35
lambs/group	4	4	4	4	2	2	4
av/starting wt, kg							
controls	31.9	24.3	24.3	31.3	18.8	18.8	31.4
treatment	30.6	24.6	24.1	23.1	23.1	16.8	32.6
kg of feed lamb^{-1} day^{-1}							
controls	1.75	1.41	1.41	1.83	1.11	1.11	1.68
treatment	1.62	0.75	0.84	1.16	1.12	1.03	1.59
av daily gain, kg							
controls	0.24	0.22	0.22	0.30	0.20	0.20	0.16
treatment	0.20	0.06	0.06	0.17	0.13	0.19	0.12
kg of feed/kg gain							
controls	3.3	2.9	2.9	3.3	2.4	2.4	4.7
treatment	3.7	5.4	6.0	3.9	3.9	2.5	5.9

[a] J27, nondetoxified meal at 10% level. [b] J1-a, nondetoxified meal at 20% level. [c] J1-b, nondetoxified meal at 30% level. [d] J1-c, continuation feeding of the J1 treatment lambs on the nondetoxified meals at the 30% level for 18 days, then the 20% level for 36 days, and lastly a 10% level for 8 days. Only three control lambs were continued in the basal ration during this time. [e] J13, ammonia detoxified meal at 10% level. [f] J19, denatured protein, water extracted meal at 10% level. [g] J49, ammoniacal hydrogen peroxide detoxified meal at 10% level.

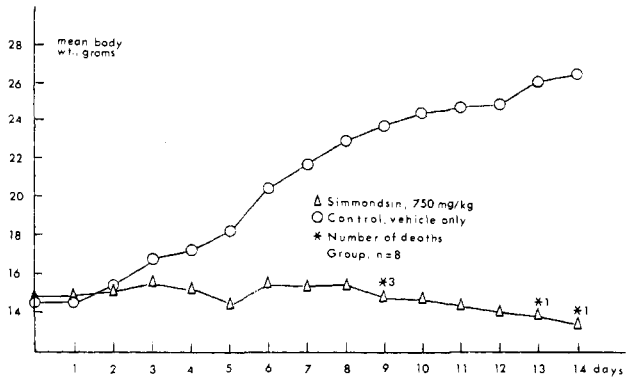

Figure 1. Oral administration of simmondsin to mice.

Oral. Simmondsin hydrate at 750 mg kg^{-1} day^{-1} in water was administered to eight mice by gastric intubation, 10 mL/kg, for 14 days. The mice sustained weight losses and three mice died on day 10, one on day 13, and one on day 14 (Figure 1).

RESULTS AND DISCUSSIONS

Deoiling to 1–2% levels is viewed as the initial step in any jojoba meal detoxification process. Table V summarizes experiments on the effects of jojoba oil in mice under chronic administration conditions. In these experiments jojoba oil was added to a basal diet at several concentrations. At a 1% level the mice ate well with good growth and normal feces. At a 2% level, feces were soft and growth was less than controls. At 10% levels mice did poorly and some died. Jojoba oil seems to act as an intestinal lubricant similar to mineral oil which is known to cause the elimination of vitamins and other essential nutrients when ingested by humans. Deaths among the mice at 10% levels were probably due to antinutritional rather than toxic factors. Only low doses can be tolerated without inducing a diarrhea effect. Moreover, the oil is far too valuable a commodity to feed to livestock.

Hexane is the preferred solvent for commercial oil seed extractions and is recommended for removing residual jojoba oil from pressed jojoba meal. For safety reasons, methylene chloride and methyl chloroform were used here to deoil larger batches of jojoba meal for detoxification studies. These two low hazard commercial solvents removed some of the simmondsin and simmondsin 2′-ferulate from the meal as well as the oil. Typical deoiling experiments with these three solvents and toxicant extractions with various solvents are summarized in Table II. Solvent extraction was carried out mainly for experimental purposes such as the isolation of toxicants and other components in the meal and the preparation of low toxicant meals for animal feeding studies.

Methanol ranks high among solvents investigated for toxicant extraction. In addition to the toxicants I and II, methanol also removes other materials including carbohydrates. Acetone is less effective but more selective than methanol for extracting simmondsin and related toxicants. This led to its selection as the solvent of choice for Soxhlet extraction in our method for toxicant assay of jojoba meals. 2-Propanol efficiently deoiled a pressed oily meal but proved deficient as a solvent for simultaneously extracting toxicants at ambient temperature. Methylene chloride/methanol (85:15) was also effective for simultaneously deoiling and lowering toxicant level. Methylene chloride/methanol (75:25) was even more effective in removing oil and toxicants at the same time. In general, the alcohol solvents and acetone are not suitable for use in a commercial process to remove the toxicants from the meal. These solvents become wetter as they are used, requiring a recovery unit for drying them. A disadvantage in simultaneous deoiling with toxicant extraction is that the recovered jojoba oil contains residual trace amounts of toxicants, especially simmondsin 2′-ferulate.

Removal of the toxicants from the meal by water washing is impractical unless the water-soluble protein is denatured. Coagulation of the jojoba proteins by brief boiling or acidification to pH 3–4 by using phorphoric acid, hydrochloric acid or sulfuric acid greatly facilitates filtration or centrifugation. As applied here in meals J31, J19, and H148 in Table II, water was effective for removing simmondsin but less effective for simmondsin 2′-ferulate. The heavily caked quality of these water-washed meals required longer drying time and protection against microbial infection. Also, the water-soluble, nutritionally useful carbohydrates, representing 8–9% of the deoiled meal, are removed along with the toxicants. These disadvantages are partly offset by the low cost of processing materials and the short processing time.

Dry heating, or baking, jojoba meal at 135 °C for 15 h does have an effect on lowering levels of toxicants I and II substantially, H76. However, this meal was highly toxic to mice (Table IV), beyond expectations based on toxicant I and II levels alone. It appears that heat breaks up simmondsin and related compounds but does not destroy the toxic cyano functional group. Dry heat treatments at higher temperatures for shorter times were also ineffective detoxification processes for one reason or another, in-

cluding ignition of the meal at 170 °C. Dry or moist heating takes away the bitter taste of jojoba meal but not its toxicity, which may even be enhanced. Meal H148 which was heated for 3 h at 100 °C, followed by water extraction of toxicants also was consistant with this pattern of relatively high toxicity of heat-treated meals to mice.

The third phase of our detoxification studies was concerned with chemical treatments to modify the cyano groups of the toxicants in the meal. Hydration to an amide was one of the goals, assuming that the amides are less toxic than the corresponding cyano compounds. Indeed, when the synthetic amide analogue of simmondsin was added to a basal mouse diet at a 0.75% level, the mice exhibited normal feed intake and increases in body weight. On the adulterated diet, 9-g weanling mice grew to 13.1 g in 1 week with an average feed intake per day of 3.5 g. This represents a daily average oral dose of simmondsin amide at 2.6 g/kg body weight.

A patent (Elliger et al., 1975) reported the detoxification of jojoba meal by treatment with ammonia in a closed container and noted that amides are the products of this reaction system. This ammonia treatment process was repeated and scaled-up, and the temperature was varied (H63, H64, J13, and J29, Table III). Increasing the temperature to 50 °C increased the rate of toxicant conversion as expected for this hydration reaction. When jojoba meal was suspended in methanol and treated with ammonium hydroxide for 6 days at ambient temperature, the principal reaction was hydrolysis of simmondsin 2′-ferulate to simmondsin. In the latter system simmondsin levels increased from 5.1% to 5.5% at the expense of simmondsin 2′-ferulate which dropped from 1.5% to 0.12%. This demonstrated the rather slow hydration of the cyano group of simmondsin by ammonia at ambient temperatures.

The hydration of cyano groups to amides using alkaline hydrogen peroxide was first reported by Radziszewski (1884). Later investigations of this reaction have shown that hydration of a cyano function to an amide with hydrogen peroxide in base can be 10 000 times faster than with hydroxyl ion alone (Wiberg, 1953, 1955). Hydrogen peroxide alone is ineffective in this hydration. Cinnamonitrile (Murray and Cloke, 1934) and acrylonitrile (Payne and Williams, 1961), two C=C conjugated cyano compounds, have been converted to amides by Radziszewski reactions conditions.

The Radziszewski reaction is carried out in solution, usually water, using alkali bases such as sodium hdyroxide. Aqueous treatment of jojoba meal without denaturing the protein leads to an unfilterable mass as the meal absorbs several times its weight of water. Also, base causes hydrolysis of the glycoside linkage of simmondsin, resulting in the formation of 2-hydroxy-3-methoxybenzylcyanide (Elliger et al., 1973), a compound that is more toxic than simmondsin (Booth et al., 1974). Normal Radziszewski reaction conditions are, therefore, undesirable for treating jojoba meal.

Successful application of the Radziszewski reaction to jojoba meal requires a volatile base with hydrogen peroxide to assure an even distribution of reagents throughout the moist meal. Ammonia was selected for this purpose although it has not been reported previously in this reaction. When dilute ammonium hydroxide and hydrogen peroxide are combined there is no heat generated. The reagent then can be sprayed evenly on the meal as it is tumbled or agitated in a container. However, heat is generated during this mixing.

Ammoniacal hydrogen peroxide sprayed on jojoba meal substantially modified simmondsin within 2 days. After 40 h simmondsin levels were reduced from an initial 4.5% to 0.19%. As a comparison, treatment of jojoba meal with ammonia alone reduced simmondsin levels to 0.16% in 40 days. We found that in an aqueous solution of pure simmondsin, ammoniacal hydrogen peroxide hydrates the cyano group to an amide within 2 h (Banigan and Verbiscar, 1978).

An advantage in using hydrogen peroxide rather than ammonia alone is the much shorter treatment time required for equivalent detoxification (Table III). Ammoniacal hydrogen peroxide treatment requires about 1 week compared to 5 or 6 weeks for the ammonia process. After these treatments, excess ammonia was evaporated, leaving nutritionally acceptable meals. The nitrogen levels in these treated meals were high due to absorption of ammonia by the meals.

Ammoniacal hydrogen peroxide is a new reagent for converting the toxicants in jojoba meal, namely simmondsin and related cyano compounds, to the corresponding amides. Simmondsin amide was detected in extracts of ammoniacal hydrogen peroxide treated jojoba meal by using LC. Meals from other seeds containing cyanogenic glycosides have been treated with this reagent with good success (Banigan and Verbiscar, 1978).

Toxicity studies with various experimental meals in mouse diets are summarized in Table IV. These data indicate that mortality to mice is related generally to the level of simmondsin (I), simmondsin 2′-ferulate (II), and related toxicants in the meals. In the table it can be seen that, when the total toxicant I + II level in the mouse ration is 0.045% or less, the mice survive for 3 weeks on a jojoba meal sole source protein diet. When the toxicant I + II level in the diet is above 0.1% many mice died after 2 or 3 weeks. The heat-treated meals H76 and H148 are more toxic than predicted by toxicant I + II levels, probably due to simmondsin degradation products that contain cyano groups.

Jojoba meal exhaustively extracted with methanol, H127, with a total dietary I + II level of only 0.014% did not support mouse growth as a sole source protein and 50% of the mice died in the third week. When amino acids were supplemented in H127-AA, the mice still lost weight but did not die. Apparently methanol removes some nutritional components as well as toxicants I and II, but probably less of two minor toxicants (Ellinger et al., 1974), which are more polar than I and II.

Jojoba meals J13, J19, J21, and J31, representing more advanced processes, were added to normal rations containing whole egg protein. The 10% level at which these meals were fed to mice approximated additive levels that may be used for livestock. The mice on these three detoxified meals generally ate well as evidenced by feed intake, but they did not grow as well as controls. J13 is an ammonia treated meal, J21 an ammoniacal hydrogen peroxide treatment, J19 a water extracted meal after protein coagulation by phosphoric acid, and J31 a boiling water extracted meal. None of these processes introduced new toxic factors in the meals.

Poultry studies on several of the more advanced meals are summarized in Table VI, where jojoba meals were added to rations at 5% and 10% levels. The chicks did not do well on nondetoxified meal J1, with high mortality at a 10% level. The ammoniacal hydrogen peroxide treated meal J21, compared well with controls at a 5% level, but performance fell off at a 10% level. Performance on the ammonia treated meal J29 was poorer than controls but not substantially so. On the water extracted meal J31, the chicks did not do well, probably due to loss of nutrients

in the water. However, mice did comparatively well on the water extracted meals J19 and J31 (Table IV). Water is an excellent solvent for simmondsin and two minor toxicants but is not especially good for simmondsin 2′-ferulate.

Deoiled jojoba meal J27 with a toxicant I + II level of 5.9% was fed as a 10% feed additive to four lambs for 49 days (Table VII). Feed consumption, rate of gain in weight, and efficiency expressed as kilograms of feed/kilogram of gain, was less than controls. The treatment lambs started with an average weight of 30.6 kg and finished at 40.1 kg for a 9.52 kg/lamb gain. The control lambs started with an average of 31.9 kg and finished at 43.7 kg, showing an 11.8 kg/lamb gain. The data indicate that lambs can utilize nondetoxified jojoba meal at a 10% level in a diet but with some loss of efficiency and average daily gain. This ration contained 7% molasses to increase palatability of the 10% nondetoxified jojoba meal diet. Other feeding trials of nondetoxified jojoba meal to lambs indicated the presence of a factor that restricts palatability for lambs (Trei et al., 1979).

Lambs fed nondetoxified jojoba meal at a 20% level (J1-a) and a 30% level (J1-b) refused to eat the first day but gradually adjusted to the rations during the first week. After adjusting, the lambs ate moderately and gained weight but not nearly as well as controls. Average feed consumption for these two groups was 0.75 and 0.84 kg of feed per lamb daily over the 35-day period, compared to 1.4 kg for the controls. Average early weight gains were 0.06 kg for the 20% and 30% treatment lambs, compared to 0.22 kg for controls. This means that these lambs ate about half as much feed as the controls and gained less than one-third as much weight.

After the initial 35 days on 20% and 30% nondetoxified jojoba meal rations, four treatment lambs were fed the remaining nondetoxified jojoba meal rations for another 63 days (J1-c). These four treatment lambs seemed to have adjusted to the nondetoxified meal diet. During this second period average feed consumption per lamb per day was 1.16 kg, with an average daily gain of 0.17 kg. Weekly respiration, pulse rates, and body temperatures taken before weighing these lambs showed no discernible pattern or treatment affects. Two control and two treatment lambs were slaughtered for histologic examination of tissues. No pathological effects or differences were noted in blood, lymph node, liver, intestine, lung, spleen, spinal cord, bone, and marrow between treatment and control lambs.

Experiments, especially in the initial days, indicated that the lambs did not like the taste or smell of nondetoxified jojoba meal rations. Preference studies were undertaken to further identify this palatability problem. Ten lambs of various breeds weighing between 20.4 and 27.2 kg were selected. Rations consisted of (1) basal mixture pellet, (2) a pellet containing 20% nondetoxified jojoba meal, and (3) a 1:1 mixture of pellets from 1 to 2. Various feeding combinations were tried with these three rations. In each case the lambs refused to eat rations containing nondetoxified jojoba meal when the basal ration was available. When there was no choice the lambs would eat the jojoba meal ration, but not in the same quantities that they would eat of a basal ration.

These studies indicate that the toxicity of deoiled but nondetoxified jojoba meal in ruminants is considerably less than in nonruminant animals. However, the utility of this meal in ruminant rations is limited due to marked reduction in feed intake and efficiency.

There were no palatability problems when three detoxified meals, J13, J19, and J49, were fed to lambs (Nelson et al., 1979). These three meals had a bland taste in contrast to the bitter taste of nondetoxified jojoba meal. The rations containing these meals at 10% levels plus 5–7% molasses were received with no adjustment period in eating behavior. The lambs on these detoxified jojoba meals ate as readily as the lambs on the control diet, and feed was accepted with little waste. Weight gains for lambs for treatment groups were relatively consistent through the feeding periods. However, the J13 and J49 treatment groups required more feed per pound of gain than did the J19 and control lambs. Caution is necessary in interpreting these results because of the small number of lambs in the studies. More extensive sheep studies are underway.

Experiments administering pure simmondsin in various dose forms to mice demonstrate the probable involvement of gut bacteria in simmondsin toxicity. A group of eight young mice were given daily doses of simmondsin at 750 mg/kg orally for 14 days (Figure 1). After this time, three mice survived compared with zero survival of five weanling rats at this dose regimen in the Booth et al. (1974) study. The three surviving mice showed signs of hepatotoxicity and possible intestinal hemmorhage.

When mice were administered simmondsin intraperitoneally at 188 and 375 mg/kg daily for 9 days, body weights of the treatment mice were essentially identical with control mice given the vehicle alone. Even at 750 mg/kg ip, there were no statistically significant differences between the body weights of treated and control mice at any time during the test period. Differences at the higher ip dose level were minimal, and there were no other drug induced effects.

These experiments demonstrate that orally administered simmondsin is toxic to rodents, whereas ip administered simmondsin shows little toxicity. This is a strong indication that intact simmondsin is not very toxic. Rather it appears that gut bacteria in rodents split simmondsin into glucose and its aglycon. The CN-containing aglycon is absorbed in the gastrointestinal tract and exerts a toxicity directly or through a rearrangement product (Booth et al. 1974; Elliger et al., 1973). In chickens, bacteria occur in the cecum where some degradation of simmondsin can be expected. A lesser degree of cecum bacterial activity could explain the lower toxicity of jojoba meal to chickens. The extensive rumen microbial system in sheep acts on simmondsin, allowing higher acceptable toxicant levels in lamb rations. An analysis of feces from lambs on a 20% nondetoxified jojoba meal diet indicated that only about 1% of the simmondsin fed was eliminated intact, and no simmondsin 2′-ferulate was detected.

Simmondsin was incubated in rumen fluid containing added amounts of cellulose (0.9%), corn starch (1.5%), and cotton seed meal (0.5%) (Temple, 1979). After 3 h, 75% of the simmondsin was metabolized, and after 6 h, 95% was metabolized. In addition, we find that certain strains of *Lactobacilli* can be used to detoxify jojoba meal. *Lactobacilli* are included among the many rumen microorganisms.

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Total Glycoalkaloids in Potatoes and Potato Chips

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The process of chipping under the conditions employed was found to be nondestructive to total glycoalkaloids (TGA) and resulted in the concentration of TGA. Removal of the potato peel from the potato slices significantly lowered the TGA content of the finished chips. Three samples of commercial chips were found to contain from 9.5 to 72 mg of TGA/100 g of chips.

Tubers of the potato plant, *Solanum tuberosum* L., contain a low level of glycoalkaloids, a class of naturally occurring toxicants. The primary constituents of this class of compounds in potatoes are α-solanine and α-chaconine, which are glycosides of the steroidal alkaloid solanidine. A total glycoalkaloid (TGA) content of 20 mg/100 g of raw potato is widely accepted as the upper limit for these compounds, both due to the potential health hazard and to the fact that they can impart an undesirable bitter flavor at higher concentrations (Jadhav and Salunkhe, 1975).

The concentration of TGA is affected by many environmental factors. Exposure to UV light, low-temperature dark storage, and mechanical damage particularly stimulate the potato to increase synthesis of TGA (Jadhav and Salunkhe, 1975; Wu and Salunkhe, 1976). Salunkhe et al. (1972) demonstrated that light and elevated temperatures dramatically increased solanine synthesis in peeled potato slices incubated for time intervals up to 48 h.

The highest concentration of TGA in the potato are found in the skin, eyes, and sprouts. Zitnak (1961) reported that the outer 3 mm of the potato contained approximately half of the TGA; however, it represented only 14% of the total weight of the potato.

Extensive literature exists concerning TGA content of raw potatoes; however, very little information is available concerning TGA in processed potato forms. Both α-solanine and α-chaconine have been reported to be heat stable (Jadhav and Salunkhe, 1975) upon instantaneous exposure to high temperature. However, the time factor was not considered. Porter (1972) has reported that these compounds decompose in the 260–270 °C area, which is 70–80 °C above normal commercial frying temperatures. Also, it is possible that potato processing involving the removal of water could elevate TGA concentrations thereby creating either a health hazard or an undesirable flavor. This project was undertaken to determine the effect of chipping on TGA concentration of potato slices as well as measure TGA levels in commercial chips.

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EXPERIMENTAL SECTION

Sampling and Potato Chipping Procedures. Norchip potatoes, harvested July 8 near Phoenix, AZ, were chipped July 11 in Fort Collins, CO. The potatoes had been shipped directly via unrefrigerated carrier and were stored in crates (2000-lb capacity) at ambient temperatures until processed.

Approximately 50 kilos of potatoes were sorted according to size and then redistributed into five groups in such a manner that each group had the same number and size distribution of potatoes. One set of potatoes was retained for compositional analysis, and the remaining four groups were chipped.

Sampling of raw unpeeled potatoes was done by the method of Fitzpatrick and Osman (1974). A portion of potatoes was manually peeled to remove the outer 1–2 mm of skin and sampled using the previously mentioned sampling procedure of Fitzpatrick and Osman (1974). The peels (~12% of the total potato) were chopped in a Waring blender and then immediately sampled. The above procedure was used to provide control TGA data on the raw whole, peeled, and peel potato.

The potatoes were chipped using a sequence designed to simulate commercial chipping operations: (1) Each group was scrubbed lightly to remove surface dirt. (2) The potatoes were peeled in a Hobart abrasion peeler (this step was omitted for sample D which was not peeled). (3) The

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