Effects of Gossypol and Raffinose on Lysine Content and Nutritive Quality of Proteins in Meals from Glandless Cottonseed

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The relative importance of gossypol and raffinose in binding and destruction of lysine and in impairing the nutritive value of cottonseed meals was studied with meals from glandless cottonseed. Nutritional evaluations with protein-depleted rats showed that: raffinose in cottonseed reduces lysine content and nutritive quality of the proteins when heat is applied; 1% concentration of gossypol is not as effective as 10% of raffinose in destroying lysine in cottonseed meal; gossypol and raffinose at these same concentrations are comparable in reducing the level of free ε-aminolysine in cottonseed proteins; and the nutritive index of cottonseed meals as determined by the rat repletion method is highly correlated with the free ε-amino groups of lysine of the protein, and poorly correlated with total lysine.

THE nutritive quality of cottonseed meal is impaired when it is heated (*5*), partly because of the destruction of a portion of the lysine in the proteins (*7, 11*). The mechanism of this destruction is not known. It is possible that both carbohydrates and gossypol are involved, as amino acids are deaminated and decarboxylated when heated in the presence of either of these two carbonyl or potential carbonyl-containing substances (*8*).

A part of the impairment may also be due to the unavailability of lysine in the meal to the animal because of the reaction in situ of gossypol, sugar, or other constituents with the free epsilon groups of lysine in the proteins (*6*).

The relative importance of raffinose and gossypol in the destruction of lysine, the binding of the ε-amino group of lysine, and the impairment of the nutritive quality of cottonseed meal was studied through the use of gossypol-free glandless cottonseed made available by the U. S. Cotton Field Station, Shafter, Calif. (*12*). This cottonseed provided proteins that had not been exposed to reaction with gossypol.

Experimental

Glandless cottonseed was dehulled, flaked, and extracted with hexane. The resulting meal was dried at room temperature, and most of the remaining hull fragments were removed on a 30-mesh screen. The stock supply of cottonseed meal prepared in this manner was designated as CM-71.

Portions of CM-71 (225 grams each) were extracted successively with 20 1-liter quantities of 80% aqueous ethyl alcohol, followed by three 1-liter quantities of diethyl ether, to remove sugars and phosphatide materials. The several lots of alcohol- and ether-extracted meal were desolventized at room temperature, combined, and mixed thoroughly. This stock meal was designated as CM-71-A.

The other meals used were prepared from CM-71 and CM-71-A.

CM-71-AR. Prepared by adding 10% by weight of twice-crystallized raffinose hydrate to CM-71-A and grinding in a ball mill for 1 hour for complete mixing.

CM-71-AG. Prepared by adding 1% gossypol in acetone solution to CM-71-A. Sufficient acetone was added to the mixture to form a slurry. This was mixed thoroughly and then the acetone was evaporated at room temperature. The dry residue was then ground in a ball mill for 1 hour.

CM-71-AGR. Prepared by adding both raffinose and gossypol to CM-71-A. Raffinose was added first as for CM-71-AR, followed by the addition of gossypol, as for CM-71-AG.

CM-71-AS. The residue obtained when CM-71-A was extracted with 1.5*M* NaCl in 50% aqueous ethyl alcohol. Ten grams of CM-71-A were suspended in 100 ml. of the salt solution in a 250-ml. bottle and shaken mechanically for 1 hour. The mixture was then centrifuged and the supernatant liquid was decanted. The residue was extracted a second time in the same manner. The residue remaining after the second extraction was suspended in water and dialyzed against running distilled water at 5° C. for 72 hours. The material was then frozen and lyophilized.

Appropriate quantities of each meal preparation were heated at 121° C. for 20 and 60 minutes in a steam-jacketed autoclave. All meals to which gossypol was added were extracted, after heating for 0, 20, and 60 minutes, with diethyl ether for 16 hours in a Soxhlet extraction apparatus to remove free gossypol.

The first 3 liters of the 80% alcohol extract of each portion of CM-71 were combined and dialyzed against distilled water at 5° C. for 72 hours. The dialyzate was concentrated under vacuum to a minimum volume for paper chromatographic analysis. The inner liquor or dialyzed material was frozen and lyophilized for identification.

Alcohol-salt extracts isolated during the preparation of CM-71-AS were combined and dialyzed against 0.01*M* acetic acid at 5° C. for 72 hours with three changes of acid. Nondialyzable material was frozen and lyophilized for analysis.

Determination	Method
Total N, free gossypol, moisture	(*1*)
Total gossypol	(*14*)
Phosphorus derivatives	(*15*)
Nitrogen solubility	(*10*)
Total and reducing sugars	(*2*)
Basic amino acids (modification in sample preparation and column size)	(*13*) (*9*)
Free ε-amino groups	(*6*)
Nutritive indices	(*3, 4*)

Results and Discussion

Although CM-71-AG, CM-71-AG-20, CM-71-AG-60, CM-71-ARG-20, and CM-71-ARG-60 were exhaustively extracted with diethyl ether, analyses for free gossypol by the American Oil Chemists' Society method indicated the presence of free gossypol. The data for free gossypol recorded in Table I represent gossypol derivatives that are soluble in aqueous acetone and are probably converted to dianilinogossypol in the analytical determination.

The percentage of gossypol bound to the meal was increased by heating; however, the amount bound was less in the presence of raffinose (Table I).

Twenty-one per cent by weight of CM-71 was removed on extraction with 80% ethyl alcohol in the preparation of CM-71-A. This included 99% of the raffinose (11% of the meal) and 4% of the total nitrogen of the meal. Data recorded in Table II indicate that 96% of the phosphatide and 43% of the inorganic phosphorus were also removed. One- and two-dimensional paper chromatographic analyses of the concentrated dialyzate of the alcohol extract indicated the presence of at least five flavonoid-like

Table I. Properties of Glandless Cottonseed Meal and Meal Fractions

Sample	Time Heated, Min.	Nutritive Index	Lysine, G./16 G. N			Arginine Ion Exchange, G./16 G. N	Nitrogen, %		Gossypol, %		Sugars, %	
			DNFB	Ion exchange	Bound		Total	Soluble	Free	Total	Reducing	Total
CM-71	0	101	4.1	4.2	0.1	11.4	8.5	97	0.00	0.00	0.37	10.39
	20	76	3.7	3.7	0.0	11.6	8.5	44	0.00	0.00	0.52	11.46
	60	68	3.1	3.5	0.4	10.2	8.6	29	0.00	0.00	0.62	10.49
CM-71-A	0	96	4.6	4.6	0.0	11.4	10.3	86	0.00	0.00	0.05	0.09
	20	101	4.3	4.3	0.0	11.3	10.4	42	0.00	0.00	0.02	0.06
	60	86	4.1	4.2	0.1	11.4	10.5	41	0.00	0.00	0.02	0.06
CM-71-AR	0	93	4.4	4.4	0.0	11.5	9.4	90	0.00	0.00	0.17	7.03
	20	86	4.2	4.3	0.1	11.5	9.6	45	0.00	0.00	0.21	7.05
	60	71	3.6	4.0	0.4	11.3	9.6	36	0.00	0.00	0.42	6.91
CM-71-AG	0	90	4.2	4.4	0.2	11.6	10.1	79	0.06	0.55	...	...
	20	75	3.9	4.4	0.5	11.6	10.3	39	0.02	0.84	...	...
	60	61	3.5	4.3	0.8	11.2	10.4	41	0.02	0.82	...	...
CM-71-ARG	20	85	4.1	4.2	0.1	11.1	9.5	46	0.03	0.63	0.23	7.33
	60	70	3.5	4.1	0.6	10.9	9.5	32	0.01	0.75	0.39	6.75
CM-71-AS	0	85	4.1	4.2	0.1	10.5	10.3	80	0.00	0.00	0.02	0.04
	20	85	4.1	4.2	0.1	10.5	10.3	45	0.00	0.00	0.02	0.15
	60	75	3.7	4.2	0.5	10.5	10.3	47	0.00	0.00	0.03	0.33

Table II. Phosphorus Compounds in Glandless Cottonseed Meal and Meal Fractions

Sample	Time Heated, Min.	Moisture, %	Phosphorus, %						
			Total	Phytin	Acid-soluble	Phosphatide	Inorganic	Nucleic	Ester type
CM-71	0	10.2	1.85	1.59	1.68	0.102	0.06	0.078	0.02
	20	10.3	1.81	1.51	1.76	0.080	0.02	0.000	0.05
	60	10.1	1.78	1.42	1.74	0.070	0.31	0.000	0.01
CM-71-A	0	13.5	2.15	1.96	2.06	0.004	0.08	0.096	0.06
	20	12.1	2.11	1.91	2.11	0.000	0.17	0.000	0.02
	60	11.8	2.13	1.83	2.16	0.000	0.32	0.000	0.00
CM-71-AR	0	13.4	1.91	1.71	1.88	0.000	0.04	0.030	0.13
	20	11.6	1.97	1.75	1.95	0.000	0.14	0.010	0.06
	60	11.5	1.97	1.63	1.96	0.000	0.35	0.020	0.00
CM-71-AG	0	12.7	2.07	1.80	2.00	0.000	0.05	0.070	0.13
	20	11.6	2.11	1.82	2.05	0.000	0.17	0.070	0.05
	60	11.5	2.14	1.82	2.10	0.000	0.34	0.040	0.00
CM-71-ARG	20	10.9	1.95	1.65	1.92	0.000	0.16	0.040	0.11
	60	11.2	1.94	1.68	1.94	0.000	0.28	0.000	0.00
CM-71-AS	0	10.9	2.13	1.92	2.14	0.002	0.03	0.000	0.19
	20	11.5	2.13	1.97	2.15	0.015	0.16	0.000	0.02
	60	11.2	2.13	1.97	2.16	0.016	0.28	0.000	0.00

pigments and 14 ninhydrin-positive components. No basic amino acids were noted on these chromatograms. The dialyzed material obtained on lyophilization was pale yellow in color and browned rapidly on exposure to moisture and air. Qualitative tests indicated the presence of phosphatide.

The further extraction of the alcohol-extracted meal with the salt-alcohol solution removed 21% of the material by weight and 21% of the phosphorus, which included all of the nucleic acid phosphorus. Dialysis and lyophilization of the extract yielded a protein material which represented about half of the nitrogen and phosphorus content of the extract. This material on further purification yielded a protein containing 15% nitrogen, 2% sulfur, 0.1% phosphorus, and 0.25% ash. The protein also gave a strong Molisch reaction.

Nutritive indices and chemical properties of the several meal preparations are recorded in Table I. Analyses of the data from the rat repletion experiments indicated odds of 20 to 1 that differences between meals of more than 10% in the nutritive index are real.

Several significant relationships suggested by the data may be pointed out. Heating cottonseed meal (CM-71) in the total absence of gossypol reduced the nutritive value, even when the treatment was limited to 20 minutes. When the carbohydrates (and other alcohol-soluble materials) were removed prior to autoclaving, a 60-minute autoclaving period yielded a product with a nutritive index significantly higher than was obtained on heating CM-71 for 20 minutes, while a 20-minute heating period for CM-71-A did not impair nutritive value. Furthermore, a substantial part of the lysine in the meal proteins was bound and destroyed by heating CM-71, whereas neither was extensive when raffinose and other soluble substances were removed (CM-71-A) prior to heating. The addition of neither raffinose or gossypol or both to CM-71-A caused as great a destruction of lysine when the meal was heated as that encountered with CM-71. Obviously this marked difference between CM-71 and CM-71-A in the quantity of lysine destroyed cannot all be attributed exclusively to the raffinose present in CM-71.

The reduction of the arginine content obtained on heating CM-71 was not noted in any of the other meals. The addition of raffinose or gossypol, or both, did not affect the arginine content. Apparently some alcohol-soluble constituent present in CM-71 is responsible for the loss of arginine during heating.

The protein obtained after alcohol-salt extraction (CM-71-AS) showed no decrease in lysine content, as measured by the ion exchange procedure, and very little decrease as measured by the dinitrofluorobenzene (DNFB) method.

The greatest binding of lysine and the lowest nutritive index were obtained when CM-71-AG was heated. Raffi-

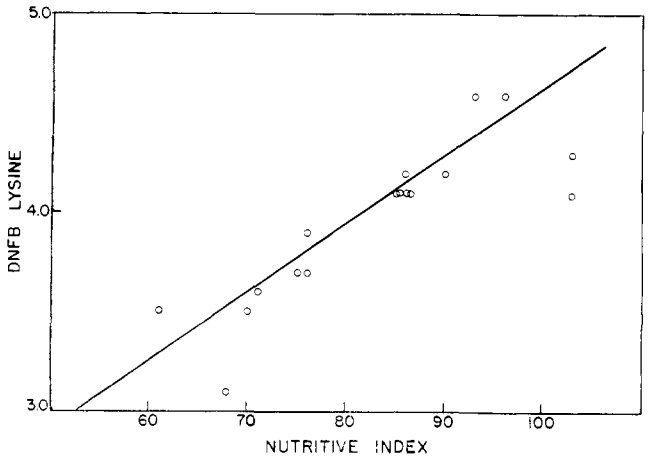

Figure 1. Regression of nutritive index on lysine with free ϵ-amino groups

Grams per 16 grams of nitrogen

nose, when added to CM-71-A, seemed to reduce the effects of gossypol, as there was no greater lowering of the nutritive index on heating CM-71-ARG than that noted for CM-71-AR (Table I).

A correlation coefficient of 0.86 (with 16 degrees of freedom) was calculated from regression analysis of the lysine data obtained by the DNFB method and the nutritive index. The curve for the regression of the nutritive index on the lysine content of the meals, as determined by the method of Conkerton and Frampton (*7*), is shown in Figure 1. The correlation between the nutritive index and the lysine content as determined by Moore and Stein (*13*) was 0.50. The lysine datum obtained by the DNFB method is interpreted here as the quantity of lysine in the meal protein with the ϵ-amino group free. The difference between this datum and the measure of lysine obtained by the ion exchange procedure apparently determines that portion of the lysine in the protein where the ϵ-amino group is not free, but can be liberated on acid hydrolysis. This is referred to as bound lysine.

The implication from the two regression analyses is that protein-depleted rats may not utilize the lysine of the protein if the ϵ-amino groups are not free. This view is supported by the results of a multiple regression analysis of the destroyed lysine and the bound lysine on the reduction in the nutritive index. The regression equation obtained from the analysis is

$$R = 9.6 + 24.9\,d + 15.1\,b$$

where R is the reduction in the nutritive value obtained with the various treatments, d is the quantity of lysine destroyed, and b is the quantity of lysine bound expressed as grams per 16 grams of nitrogen. The coefficient for b would be zero if the depleted rat utilized all of the bound lysine. The reduction in nutritive value in processed cottonseed is due, therefore, not only to lysine destruction but to lysine binding as well.

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Correction

Constitution of the Hemicellulose of Alfalfa (*Medicago sativa*). Hydrolysis of Hemicellulose and Identification of Neutral and Acidic Components

In this article by D. V. Myhre and Fred Smith [J. AGR. FOOD CHEM. **8,** 359 (1960)], the formulas, which are referred to throughout the text by Roman numerals, were inadvertently omitted. Formulas I through VII are shown below.