

Elimination of Free Gossypol in Cotton Seed Meal (*Gossypium herbaceum*) - A Preliminary Study

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Analysis of mechanically extracted cotton seed meal (*Gossypium herbaceum*) showed that it was good source of crude protein (23.9%). It contains 0.5% crude fat, 21.76% crude fiber, 9.28% moisture and 4.64% ash. Gossypol content in the meal was found to be 0.54%. Among the various methods tested for the elimination of gossypol, water treatment following solvent extraction was found to eliminate 98-99% of this anti-nutritional substance. Autoclaving at 10 and 15 lb. for 15 min removed 20-25% of gossypol, whereas autoclaving at the same pressure for 10 min removed 10-12% of gossypol. Heating the meal in hot air oven at 60, 80, 100°C removed 9, 10 and 12% of gossypol, respectively while microwave roasting at full and high power for 2 min removed only 5-8% of gossypol.

Key words: Gossypol, cotton seed meal, fish feed

Most fish species, depending on their physiological needs, require 20-40% crude protein in their diets (Hastings, 1979). Fish meal has been the most promising protein supplement in formulated fish diets. However, due to its increasing cost and inadequate supply, several workers have successfully replaced fish meal with other cost-effective and easily available materials (Bergot & Berque, 1983; Inaba *et al.*, 1963). Recently, a wide variety of low-cost material of plant and animal origin were evaluated for their nutritive richness and possible use in formulated fish ration (Jafri *et al.*, 1992; Khan & Jafri, 1994)

Although easy availability, low cost and high nutritive value of a wide variety of agro-based industrial wastes such as oil cakes, bran and polish etc. facilitate fish feed formulation, the presence of certain anti-metabolites and

toxins in feed ingredients of plant origin may limit or depress fish growth. A major disadvantage of using cotton seed meal as a dietary ingredient in fish ration is the presence of anti-nutritional factors, mainly gossypol, that can exert a deleterious effect on fish (Hendricks & Bailey, 1989; Lovell, 1989). Heating and moisture treatment, solvent extraction and addition of iron salts have been used, with varying degrees of success, to reduce the effect of such substances (Singleton & Kratzer, 1973; De Silva & Anderson, 1995).

In the present study an attempt has been made to evaluate the nutritional value of cotton seed meal for its use in fish ration, quantify the free gossypol content in the meal, and examine the effectiveness of several methods to achieve the elimination of this substance from this raw material. The methods attempted include heating and moisture

treatment, autoclaving and microwave roasting.

Materials and Methods

Free gossypol in cotton seed meal (*Gossypium herbaceum*) was quantified by the method described by Raj & Ali (1982). Sample of cotton seed meal (mechanically extracted) was defatted using petroleum ether (40-60°C), dried and used for the estimation of free gossypol.

Finely ground samples of solvent extracted cotton seed meal were subjected to water treatment, heating, autoclaving and microwave roasting for the elimination of free gossypol. The method of water treatment involved continuous washing of the meal samples with water for various duration (60, 90, 120 and 180 min), with the meal water ratio adjusted

to 1:5. For heat treatment, the samples were kept in hot air oven at various temperatures (60, 80 and 100°C). During autoclaving the samples were subjected to different pressure (10 lb and 15 lb.) for 10 and 15 min. Microwave roasting was carried out for 1-2 min at high and full power in a Batliboi Eddy microwave oven (2450 MHz).

Proximate composition of treated and untreated cotton seed meal was analysed by the AOAC (1984) methods. Nitrogen free extract (NFE) was calculated by difference.

Results and Discussion

Solvent extracted cotton seed meal contained 0.54% of free gossypol. Among the various methods tested for the elimination of gossypol from the cotton seed meal (Table 1), washing for

Table 1. Elimination of gossypol from cotton seed meal during different treatments (% of original)

Duration (min)	Treatment							
	Water treatment	Autoclaving		Heating (°C)			Microwave roasting	
		10 lb	15lb	60	80	100	High	Full
1	-	-	-	-	-	-	0.9±0.1 (0.5-1.0)	1±0.54 (0.8-1.1)
2	-	-	-	-	-	-	5±0.44 (3-6)	8±0.57 (6-10)
10	-	10±0.47 (9.0-10.5)	12±0.36 (11-13)	-	-	-	-	-
15	-	20±0.44 (18-21)	25±0.40 (24-27)	-	-	8±0.44 (6-9)	-	-
30	50±0.72 (47-52)			9±0.40 (7-10)	10±0.40 (9-12)	12±0.46 (10-13)	-	-
60	90±0.78 (89-93)	-	-	-	-	-	-	-
120	98±0.40 (96-99)	-	-	-	-	-	-	-
180	99±0.56 (96-100)	-	-	-	-	-	-	-

Values are mean ± SEM of replicate sample treatments (n=6); figures within parenthesis show the range.

120-180 min proved to be the most successful technique, which eliminated up to 98-99% of gossypol from the meal. Autoclaving of the solvent extracted meal at 10 and 15 lb for 15 min removed 20-25% of gossypol, whereas autoclaving at the same pressure for 10 min removed 10-12% of gossypol from the meal. Heating the meal in hot air oven at 60, 80 and 100°C removed 9, 10 and 12% of gossypol, respectively. Microwave roasting at high and full power for 2 min eliminated only 5-8% of gossypol from the meal.

Gossypol content and proximate composition of treated cotton seed meal are given in Table 2. The results indicate that water treatment of cotton seed meal eliminates gossypol content to the maximum extent and also does not alter the nutritive value of the meal.

The gross nutrient analysis of cotton seed meal carried out during the present study compares favorably with the values

reported by other workers (NAS-NRC, 1981, 1983; De Silva & Anderson, 1995). Although cotton seed meal has been used for the polyculture of the Indian major carps (Jhingran, 1983), no extensive study has so far been carried out to examine and quantify the effect of free gossypol present in cotton seed meal on growth of culturable fishes in India. The values obtained for the free gossypol in cotton seed meal during the present study was found to range between 0.50-0.58%. Jauncey & Ross (1982) observed that cotton seed meal contained 0.03-0.2% gossypol. Dorsa *et al.* (1982) reported 0.49% gossypol in solvent extracted cotton seed meal. Watts (1970) reported 0.2 - 0.4, 0.02 and 0.05% of free gossypol in direct solvent extracted cotton seed meal, screw meal and pre-pressed meal, respectively.

The presence of gossypol in cotton seed meal limits its use as ingredient in formulated feeds due to its ability to

Table 2. Gossypol content and proximate composition (g/100g) of treated and untreated treated solvent extracted cotton seed meal

	Proximate Composition						
	Moisture	Crude protein	Crude fat	Ash	Crude fibre	NFE	Gossypol
Untreated	9.28±0.24 (9.04-9.51)	23.91±0.57 (23.37-24.50)	0.53±0.03 (0.50-0.55)	4.64±0.13 (4.51-4.77)	21.76±0.72 (21.04-22.47)	39.62±1.40 (38.25-40.99)	0.54±0.04 (0.50-0.58)
Water treated (180 min)	9.00±0.06 (8.90-9.00)	23.90±0.10 (23.80-24.00)	0.48±0.03 (0.45-0.50)	4.67±0.47 (4.21-5.14)	21.00±1.00 (20.00-22.00)	40.99±1.58 (39.41-42.57)	0.0054±0.01 (0.00-0.02)
Autoclaved							
i) 10 lb/15 min	8.12±0.13 (7.99-8.25)	21.75±0.82 (21.0-22.63)	0.43±0.08 (0.35-0.50)	4.69±0.31 (4.39-5.00)	22.00±0.50 (21.50-22.50)	42.51±1.68 (40.83-44.19)	0.43±0.1 (0.43-0.44)
ii) 15 lb/15 min	8.41±0.54 (7.87-8.9)	21.37±0.09 (21.34-21.50)	0.40±0.05 (0.35-0.45)	4.99±0.30 (4.70-5.29)	20.38±0.38 (20.00-20.75)	44.01±1.25 (42.76-44.25)	0.41±0.01 (0.39-0.41)
Hot air oven (100°C, 30 min)	8.50±0.50 (8.00-9.00)	22.04±0.70 (21.63-23.00)	0.50±0.01 (0.50-0.51)	4.80±0.30 (4.51-5.10)	22.0±0.50 (21.50-22.50)	40.41±0.10 (39.41-41.40)	0.48±0.01 (0.47-0.49)
Microwave roasted (Full power, 2 min)	8.31±0.12 (8.19-8.42)	21.08±0.70 (20.63-22.00)	0.52±0.02 (0.50-0.53)	4.45±0.55 (3.90-5.00)	21.00±0.50 (20.50-21.50)	43.92±1.85 (42.07-45.76)	0.50±0.01 (0.49-0.51)

Values are mean ± SEM of replicate sample treatments (n=3); figures within parenthesis indicate the range

bind with amino group of lysine (Dorsa *et al.*, 1982; Robinson *et al.*, 1984). Several methods have been proposed to reduce the effect of free gossypol on animals (Hendricks & Bailey, 1989). Commercial processing of cotton seed meal involving heating and water treatment may disrupt its glands, converting much of the free gossypol to bound form. Bound gossypol is reported to be relatively less toxic and its presence below certain level may not produce harmful effects (Friedman & Shibko, 1972). The above treatments reportedly removed 80-99% gossypol, minimizing its effect and improving the quality of cotton seed meal. Addition of iron salt to cotton seed meal has also been found to eliminate the effect of gossypol, although it may cause discolouration of the meal, rendering it unacceptable as a feed ingredient (Robinson, 1984).

In the present study, water treatment was found to be the most effective technique to eliminate free gossypol from solvent extracted cotton seed meal, removing up to 98-99% of gossypol. Autoclaving eliminated 20-25% gossypol at 10-15 lb for 15 min. The study indicates that cotton seed meal can be used as a less expensive protein source in fish ration after proper treatment with water for elimination of gossypol.

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