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# DETOXIFICATION OF COMMERCIALLY PRODUCED COTTONSEED MEAL

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Free gossypol content of cottonseed meal was reduced to 0.045 % by keeping it in boiling water for 15 mins. Biological evaluation of the water treated meal indicated no adverse effects on its NPU and PER. These values were comparable with the chemically treated meals and better than the meal detoxified with solvents.

*Key Words:* Cotton seed cake: detoxification; biological evaluation.

## INTRODUCTION

Cottonseed – a by-product of cotton, is abundantly available in Pakistan. Each ton of lint produces 1.7 tons of seeds. The seeds, on the average, produce 55.5 % meats [1], which contain upto 30 % each of crude protein and oil [2]. Although cottonseed meal is rich in protein, its use as a source of protein for human beings or monogastric animals is limited due to the presence of gossypol [1]. A part of the gossypol present in the seeds is in free form, while the rest is bound with the protein. It is the free form, which is toxic [3]. The physiological effect of cottonseed meal has been reviewed by Berardi and Goldblatt [4].

Organic solvents or mixture of these, e.g. acetone, acetone-hexane-water mixture and alcohol, have been employed for reduction of free gossypol from cottonseed meal [5, 6]. Treatment of the meal with ferrous salts has also been reported to reduce this toxic material [7, 9].

Present studies deal with the exploration of methods to reduce free gossypol content of commercially produced cottonseed meal with minimum adverse effect on the nutritive value of the meal. Net Protein Utilization (NPU) and Protein Efficiency Ratio (PER) were also studied.

## MATERIALS AND METHODS

Samples of cottonseed meal obtained from Messers Koh-i-Noor Oil Mills and the local market, were finely ground in a ball mill (Pascall Engineering Co. Ltd's ball grinder) to pass through 80-mesh sieve prior to the start-up of chemical analysis or detoxification treatment.

*Analytical methods.* Free gossypol was determined by A.O.C.S. methods [10]. Micro-Kjeldahl method was applied for nitrogen determination [11]. Free lysine contents of the meal were estimated by the method of Baliga *et al*, [12].

*Detoxification treatments.* (i) *Treatment with solvents:* Ground sample was separately extracted with (a) 70, 80, 90, and 100 % ethyl alcohol, (b) ethyl alcohol-hexane mixture (1:3), (c) ethyl alcohol-acetone mixture (1:1) in a Soxhlet extractor for 18 hr at 70-80°. The solvent extracted meal was dried at 70-80° for removal of the solvents.

(ii) *Treatment with different chemicals:* Cottonseed meal was treated with different concentrations of $FeSO_4$, $Ca(OH)_2$ combination of $Ca(OH)_2$ and $FeSO_4$ and $Ca_3PO_4$ (Table 2).

(iii) *Treatment with water alongwith heating.* Cottonseed meal was soaked in different meal: water ratios varying from 1:5 to 1:60 for 30 min and centrifuged. The meal was also boiled with different volumes of water for 15, 25 and 30 min and centrifuged. The water soluble protein, lost during water processing of the meal, was recovered by boiling treatment. The detoxified meal, alongwith recovered protein, was dried at 80 ± 2° and ground to 80 mesh powder in a ball mill.

*NPU and PER: Iso*nitrogenous diets containing 10 % protein were prepared from detoxified meals. The control diet contained casein as a source of protein. NPU was determined by following the method of Miller and Bender [13], while PER was calculated according to the method of Campbell [14].

## RESULTS AND DISCUSSIONS

1. *Solvent extraction of free gossypol:* Free gossypol content of the solvent extracted meal ranged from 0.020 to 0.033 % when the meal was treated with different concentrations of alcohol, alcohol and acetone, and alcohol

and hexane mixtures (Table 1). This extraction with all the solvents reduced the free gossypol content to a safe limit. Use of solvent extraction of free gossypol has also been suggested by various workers [5,15,16]. However, the product may prove carcinogenic if residual solvent is not removed [17]. Furthermore, recovery of the solvents and its losses during extraction are liable to render the product uneconomical in countries like Pakistan where the temperature goes upto 45°.

2. *Detoxification of the meal with different chemicals:* It is obvious from the results (Table 2) that heating of the meal with either ferrous sulphate or calcium hydroxide did not reduce the gossypol content to permissible limits. Treatment with 0.25 and 0.50 % tricalcium phosphate also did not produce the desired results. However, 15 min heat treatment with a mixture containing 1.0 calcium hydroxide and 0.15 % ferrous sulphate reduced the free gossypol to the recommended limit of 0.05 %, which was further reduced to 0.045 % when the meal was treated for 25 min.

3. *Detoxification of the meal with different ratios of water:* (a) The free gossypol content were reduced from 0.30 to only 0.12 % when the water : meal ratio was 60: 1. This treatment resulted in loss of protein. These losses increased almost threefold when the meal:water ratio increased from 1: 5 to 1: 60 (Table 3).

Table 1. Solvent extraction of the meal.

| Solvent | Free gossypol % | Protein % | Available lysine % |
|---|---|---|---|
| 100 % alcohol | 0.020 | 41.75 | 3.50 |
| 90 % alcohol | 0.022 | 41.55 | 3.60 |
| 80 % alcohol | 0.026 | 40.50 | 3.72 |
| 70 % alcohol | 0.027 | 40.26 | 3.72 |
| Alcohol + acetone (1:1) | 0.030 | 41.60 | 3.60 |
| Alcohol + hexane (1:3) | 0.033 | 42.62 | 3.68 |

Table 2. Detoxification with chemicals.

| Chemical treatment | Gossypol (%) | | Protein (%) | | Available lysine (%) | |
|---|---|---|---|---|---|---|
| | Cooking time (min) | | Cooking time (min) | | Cooking time (min) | |
| | 15 | 25 | 15 | 25 | 15 | 25 |
| 0.15 % $FeSO_4$ | 0.160 | 0.155 | 39.76 | 39.66 | 3.48 | 3.46 |
| 0.20 % $FeSO_4$ | 0.150 | 0.150 | 38.72 | 38.63 | 3.45 | 3.50 |
| 1.0 % $Ca(OH)_2$ | 0.137 | 0.132 | 39.75 | 39.75 | 3.50 | 3.49 |
| 1.0 % $Ca(OH)_2$ + 0.15 % $FeSO_4$ | 0.050 | 0.045 | 39.75 | 38.95 | 3.52 | 3.50 |
| 0.25 % $Ca_3PO_4$ | 0.080 | 0.079 | 40.26 | 39.89 | 3.57 | 3.48 |
| 0.50 % $Ca_3PO_4$ | 0.070 | 0.074 | 40.35 | 40.01 | 3.52 | 3.50 |
| Untreated | 0.270 | 0.270 | 39.72 | 39.70 | 3.81 | 3.81 |

Table 3. Effect of water treatment on gossypol and protein content of meal.

| Constituents determined | Meal: water (wt/vol.) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1:5 | 1:10 | 1:25 | 1:30 | 1:40 | 1:50 | 1:60 |
| Gossypol (%) | 0.26 | 0.22 | 0.21 | 0.18 | 0.16 | 0.14 | 0.12 |
| Protein (%) | 39.39 | 38.07 | 37.79 | 36.85 | 35.47 | 35.00 | 34.09 |
| Protein loss (%) | 5.35 | 8.57 | 9.20 | 11.46 | 14.77 | 15.90 | 18.09 |

Water soluble protein lost during water treatment was recovered by boiling or steaming. The results showed that about 64 % of the water solubilized proteins were recovered by boiling for 45 min. (Table 4). It was observed that boiling was much more effective than steam stripping where only 15 % of the lost protein could be recovered.

(b) The desired objectives were, however, achieved when the water treatment of meal was accompanied by 15 min. boiling. Free gossypol content of the meal decreased from 0.30 to 0.045 % when the meal: water ratio was 1: 10 and boiling time was 15 min. (Table 5). This treatment resulted in protein loss of 3.65 and available lysine of 0.057 %. It is evident from these results that boiling of the meal for 15 min. resulted in the reduction in free gossypol to admissible limit with a minimum loss in protein and available lysine.

Table 4. Recovery of soluble protein lost due to water treatment.

| Treatment time (min) | Recovery of protein (%) | |
|---|---|---|
| | Steaming | Boiling |
| 15 | 10.00 | 17.48 |
| 30 | 14.93 | 63.34 |
| 45 | 15.03 | 64.06 |

Table 5. Effect of boiling water treatment for 15 minutes on gossypol and protein content of the meal.

| Constituent determined | Meal: water (wt/vol.) | | |
|---|---|---|---|
| | 1:5 | 1:10 | 1:60 |
| Free gossypol (%) | 0.100 | 0.045 | 0.020 |
| Protein (%) | 39.70 | 39.51 | 38.34 |
| Loss in protein (%) | 3.16 | 3.65 | 6.48 |
| Available lysine (%) | 3.60 | 3.60 | 3.57 |

4. *Biological evaluation of the detoxified meal: Net Protein Utilization (NPU).* The results of NPU determinations showed no significant difference between untreated (40.12 %) chemically treated (39.72 %) and water boiling treatment (38.26 %). The alcohol treated meal had the lowest NPU (32.50 %). This may be attributed to protein damage caused by extraction with alcohol [15], or due to the effect of residual solvent.

*Protein efficiency ratio (PER):* Diet containing alcohol treated meal showed the lowest PER (1.81) followed by diets containing untreated (1.84), chemically treated (2.12) and boiled with water (2.03). Thus PER results are in agreement with the figures of NPU indicating detrimental effects of alcohol treatment on protein. It also indicated that water treatment of meal had the least damaging effect on the protein and available lysine.

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