

Nutritional Value of Cottonseeds and It's Derived Products :

II. Free Gossypol, Available Lysine and *In Vitro* Protein Digestibility

A. Mujahid, M. Abdullah*, A. R. Barque and A. H. Gilani

Department of Animal Nutrition, University of Agriculture, Faisalabad, Pakistan

ABSTRACT : The study was conducted to evaluate the nutritional value of seeds and various seed fractions of different varieties of cotton (MNH 147, CIM 240, NIAB 78, FH 87, CIM 109, MNH 93, FH 682, GOHAR 87, SLS I and B 557). Linter, hull, kernel and meal were obtained from cottonseed by physical and chemical methods. Free gossypol and available lysine contents of seed and it's fractions were determined. *In vitro* protein digestibility of cottonseed meal was also determined. Free gossypol and available lysine contents ranged between 0.22-2.26% and 0.64-1.32% in seed, and 0.03-0.29% and 1.38-2.36% in meal, respectively. FH 87 was highest both in free gossypol and available lysine content, and NIAB 78 was lowest in free gossypol content and FH 862 was lowest in available lysine content. *In vitro* protein digestibility of cottonseed meal ranged between 66.02-79.96%. Statistical analysis revealed significant ($p < 0.05$) varietal differences in free gossypol, available lysine and *in vitro* protein digestibility of cottonseed and derived products. (*Asian-Aus. J. Anim. Sci.* 2000. Vol. 13, No. 3 : 356-359)

Key Words : Cottonseed, Free Gossypol, Available Lysine, *In Vitro* Protein Digestibility

INTRODUCTION

Cottonseed (CS) and it's derived products have been attractive protein sources for animal feeds (Kuiken, 1952) but have limitations due to the presence of gossypol, cyclopropene fatty acid and deficient in lysine (Altschul et al., 1958; Phelps, 1966; Rojas and Scott, 1969) due to binding of epsilon amino group with gossypol during processing (Anderson and Warnick, 1966; Phelps, 1966; Waldroup, 1981). Cottonseed meal (CSM) is first limiting in lysine and further reduction in lysine availability that occurs in normal commercial processing additionally impair utilization of cottonseed meal (CSM) protein (Lyman et al., 1953; Baliga and Lyman, 1957). The loss of available lysine in CSM, is partly due to it's reaction with the pigment gossypol present in glanded cottonseed (Lyman et al., 1959) but other compounds are also involved (Martinez et al., 1967). In cotton plant their exist almost exclusively discrete bodies called pigment glands in leaves, stem, roots and seeds (Boatner, 1948) which are source of gossypol. CS contains both intraglandular and extraglandular pigments, but intraglandular pigments are more important of these gossypol are most important as it forms about 35-50% of the total intraglandular pigments. Concentration of gossypol in CS varies with the variety, oil content, locality of growth (Schwartz and Alsberg, 1923; Smirnova, 1936; Cherry et al., 1978), rainfall during the cotton growing season (Gallup, 1927) and agronomic factors (Pons et al.,

1950). Malik and Khan (1964) worked on American varieties and reported that the concentration of gossypol ranged between 0.75-1.96%. The present study was undertaken to determine free gossypol and available lysine contents of cottonseed and it's derived products as influenced by variety and to determine *in vitro* protein digestibility of cottonseed meal prepared from different varieties.

MATERIALS AND METHODS

Certified CS of different varieties (MNH 147, CIM 240, NIAB 78, FH 87, CIM 109, MNH 93, FH 682, GOHAR 87, SLS I and B 557) were collected from Directorate of Cotton (Research), Ayub Agriculture Research Institute, Faisalabad. Various physical fractions of CS were separated by physical and chemical methods (AOAC, 1990). Free gossypol (AOCS, 1972), available lysine (Booth, 1971) true protein (Munro and Fleck, 1966) and *in vitro* protein digestibility (Hsu et al., 1977) were determined. The data thus collected were subjected to statistical analysis using analysis of variance technique according to completely randomized design (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Free gossypol

Average free gossypol content in seed of different varieties of cotton was 0.91%. Variable contents of free gossypol were obtained from seed, highest being in FH 87 (2.20%) and lowest in NIAB 78 (0.25%). The free gossypol content ranged between 0.22 to 2.26% in seeds. Statistical analysis of the data showed varietal differences ($p < 0.01$). Comparison of means revealed higher values in FH 87, MNH 93, MNH 147

* Corresponding Author: M. Abdullah. Dept. of Livestock Management, University of Agriculture, Faisalabad, Pakistan. E-mail: uafhsan@fsd.comsats.net.pk.

Received August 7, 1998; Accepted May 10, 1999

and CIM 109.

The findings of present study were in accordance with previous workers who reported significant differences in free gossypol content in seeds of different cotton varieties (Almashouley and Khan, 1990; Malek and Zandi, 1990; Singh et al., 1991), while Malik and Khan (1964) reported non-significant differences due to variety.

Gossypol contents vary with the variety (Taneja et al., 1993), geographic and species variation as well as isomeric contents (Percy et al., 1996) and shown polygenic inheritance (Singh et al., 1991). Seed gossypol contents are also affected by environmental conditions (Smimova, 1936; Aras and Hernandez, 1977). Gossypol glands differed significantly due to genotype in gland distribution (Mohan et al., 1991) and number of seed gossypol glands are highly associated with seed free gossypol contents (Mohan et al., 1995). Gossypol contribute 88-90% of total CS phenolic contents (Tchiegang and Bourley, 1990) and it's concentration increased with increasing soil zinc levels (Kashyap et al., 1988).

Average free gossypol content in hulls was 0.047%. Considerable variations were found in free gossypol contents in hulls from different varieties of cottonseed, highest being in case of MNH 93 and FH 87 (0.068%) and lowest in case of NIAB 78 (0.016%). The free gossypol ranged from 0.011-0.089% in hulls of cottonseed varieties under study. The statistical analysis of the data showed varietal differences. The concentration of free gossypol in CS and CSM influenced the concentration in hull (table 1) as higher value in seed and meal reflected the same trend in hull and vice versa.

Table 1. Free gossypol content (%) in seed and it's derived products of different cotton varieties (on dry matter basis)

Variety	Seed	Meal	Hull
MNH 147	1.63 ± 0.08 ^c	0.208 ± 0.002 ^a	0.061 ± 0.007 ^{ab}
CIM 240	0.38 ± 0.03 ^c	0.041 ± 0.004 ^{cd}	0.044 ± 0.003 ^c
NIAB 78	0.25 ± 0.01 ^f	0.034 ± 0.003 ^d	0.016 ± 0.002 ^e
FH 87	2.21 ± 0.02 ^o	0.212 ± 0.004 ^a	0.068 ± 0.004 ^a
CIM 109	1.19 ± 0.03 ^d	0.120 ± 0.008 ^b	0.046 ± 0.006 ^c
MNH 93	2.04 ± 0.05 ^b	0.193 ± 0.026 ^o	0.068 ± 0.005 ^a
FH 682	0.37 ± 0.02 ^e	0.057 ± 0.001 ^{cd}	0.051 ± 0.005 ^{bc}
GOHAR 87	0.36 ± 0.03 ^{ef}	0.072 ± 0.004 ^c	0.061 ± 0.006 ^{ab}
SLS I	0.32 ± 0.02 ^{ef}	0.052 ± 0.005 ^{cd}	0.028 ± 0.004 ^{de}
B 557	0.36 ± 0.03 ^{ef}	0.047 ± 0.005 ^{cd}	0.030 ± 0.002 ^d
Mean ± SEM	0.91 ± 0.10	0.103 ± 0.010	0.047 ± 0.003

Same letters on means in a column show non-significant differences.

Average free gossypol content in meal of different varieties of cotton was 0.103%. Highest percentage of

free gossypol in meal was found in FH 87 (0.212%) and lowest in case of NIAB 78 (0.034%). The free gossypol contents ranged from 0.026-0.289% in meal from cottonseed varieties under study. Statistical analysis of the data showed varietal differences ($p < 0.01$). FH 87, MNH 147 and MNH 93 differed among themselves and also had higher levels with regards to other varieties.

Jonston and Watts (1964) reported that free gossypol content of commercial graded cottonseed meal was 0.05%, which was in agreement with the findings of present study. Bressani et al. (1975) reported lower free gossypol content (0.017-1.76%) in American cottonseed meal. Similarly the value for Yugoslav CSM (Rajic and Kordic, 1976) was also lower (0.025-0.063%) than the findings of present study. The difference in free gossypol content might be due to different environmental conditions (Smimova, 1936; Arias and Hernandez, 1977), varieties (Taneja et al., 1993), geographic and species variation (Percy et al., 1996). The free and total gossypol contents of Turkish CSM (Tuncer and Yalcin, 1986) were 0.057 and 0.418% in extracted, and 0.069 and 0.565% in expeller meal, respectively, which was in agreement with the results of present study. Greek CSM also showed almost the same trend (0.034-0.128%) of free gossypol as reported by Papadopoulos and Ziras (1987). The free gossypol contents of meal from Pars and Margarin (0.026%) and from Varamin (0.16%) were in agreement with the findings of the present study (Malek and zandi, 1990).

Available lysine

Average percentage of available lysine in seed and its derived products of different varieties of cotton is presented in table 2.

Table 2. Available lysine content (%) in seed and it's derived products of different cotton varieties (on dry matter basis)

Variety	Seed	Meal	Hull
MNH 147	0.977 ± 0.060 ^{cd}	1.79 ± 0.04 ^{bc}	1.35 ± 0.03 ^{ab}
CIM 240	0.830 ± 0.029 ^{gef}	1.67 ± 0.05 ^{bc}	1.27 ± 0.03 ^{bc}
NIAB 78	0.832 ± 0.042 ^{gef}	1.72 ± 0.08 ^{bc}	1.31 ± 0.07 ^b
FH 87	1.197 ± 0.032 ^a	2.07 ± 0.07 ^a	1.52 ± 0.04 ^a
CIM 109	1.107 ± 0.040 ^{ab}	2.04 ± 0.06 ^a	1.43 ± 0.04 ^{ab}
MNH 93	1.070 ± 0.022 ^{bc}	1.90 ± 0.08 ^{ab}	1.42 ± 0.05 ^{ab}
FH 682	0.765 ± 0.012 ^g	1.58 ± 0.05 ^c	1.11 ± 0.03 ^c
GOHAR 87	0.925 ± 0.032 ^{de}	1.81 ± 0.13 ^{bc}	1.36 ± 0.09 ^{ab}
SLS I	0.783 ± 0.056 ^{gf}	1.70 ± 0.07 ^{bc}	1.26 ± 0.05 ^{bc}
B 557	0.900 ± 0.035 ^{def}	1.79 ± 0.11 ^{bc}	1.36 ± 0.08 ^{ab}
Mean ± SEM	0.939 ± 0.094	1.81 ± 0.03	1.34 ± 0.02

Same letters on means in a column show non-significant differences.

Average content of available lysine in different varieties of CS was 0.939%. Variations in available lysine content in seed of different varieties of cotton were observed, highest (1.197%) in case of FH 87 and lowest (0.765%) in case of FH 682. The available lysine ranged between 0.640-1.320% in CS. The statistical analysis of the data revealed varietal differences ($p < 0.01$).

Average content of available lysine in kernels of different varieties of cottonseed was 1.34%. Variable amount of available lysine was recovered (0.98-1.69%), highest in case of FH 87 (1.52%) and lowest in case of FH 682 (1.11%). Comparison of means revealed no difference among means of varieties FH 87, CIM 109, MNH 93, GOHAR 87, B 557 and MNH 147; NIAB 78, CIM 240 and SLS 1 and between SLS 1 and FH 682. Nwokolo et al. (1976) reported 89% lysine availability in CSM. Tchiegang and Bourelly (1990) reported that chemical reaction between gossypol and kernel constituents especially lysine, resulted in decreased availability and protein quality while decreasing gossypol toxicity. Eskin et al. (1995) developed model system in which time temperature and level of gossypol used produced a 33-38% decrease in lysine availability. CSM heated with gossypol at a ratio of 16:1 at 90°C for 30 min reduced the available lysine from 21-22 to 12-14 mmol/100 mg protein. A significant ($p < 0.05$) increase in available lysine was evident in the presence of 0.8 and 1.6×10^{-1} M choline and ethanolamine.

Average content of available lysine in meal of different varieties of cottonseed was 1.81%. Variable amount of available lysine was recovered in meals highest in case of FH 87 (2.07%) and lowest in case of FH 682 (1.58%). The available lysine ranged from 1.38 to 2.36% in meals. The statistical analysis of the data showed varietal differences ($p < 0.01$). Comparison of means revealed no differences among FH 87, CIM 109, and MNH 93; MNH 93, GOHAR 87, MNH 147, B 557, NIAB 78, SLS 1 and CIM 240 and between CIM 240 and FH 682.

True protein

Average content of true protein in seed, kernel and meal of different varieties of cotton was 20.42, 30.21, and 43.65%, respectively. Variable content of true protein in seed kernel and meal was observed in different varieties of cotton, highest in case of FH 87 and lowest in case of FH 682. The true protein in seed kernel and meal ranged between 13.77-28.06, 22.83-37.57, and 31.96-55.23%, respectively. Statistical analysis of the data revealed varietal differences ($p < 0.05$).

In vitro protein digestibility (IVPD)

Average IVPD of meal of different cottonseed

varieties is presented in table 3. Considerable variation in IVPD of meal was observed from seeds of different varieties of cotton, highest being in case of FH 87 (78.15%) and lowest in case of GOHAR 87 (66.78%). The IVPD of meals ranged from 66.02 to 79.96% in CS. The statistical analysis of the data revealed varietal differences ($p < 0.01$). Comparison of means showed higher values in FH 87 and CIM 109 while no differences among means of varieties SLS 1, MNH 147 and MNH 93 and NIAB 78, among B 557, CIM 240, FH 682 and GOHAR 87.

Table 3. Crude protein, true protein and *in vitro* protein digestibility (IVPD) of meal of different cottonseed varieties (on dry matter basis)

Variety	Crude protein (%)	True protein (%)	IVPD (%)
MNH 147	46.54 ± 1.06 ^{bc}	43.40 ± 1.41 ^{bc}	70.54 ± 0.85 ^{cd}
CIM 240	43.73 ± 1.15 ^c	41.48 ± 1.18 ^{bc}	67.14 ± 0.21 ^e
NIAB 78	44.10 ± 2.38 ^c	40.91 ± 3.01 ^{bc}	69.31 ± 0.43 ^d
FH 87	55.65 ± 1.60 ^a	51.37 ± 1.39 ^a	78.15 ± 0.61 ^a
CIM 109	53.37 ± 1.45 ^a	47.65 ± 1.63 ^{ab}	76.16 ± 0.97 ^b
MNH 93	50.43 ± 1.87 ^{ab}	45.85 ± 1.77 ^{ab}	69.43 ± 0.51 ^{cd}
FH 682	41.97 ± 1.24 ^c	38.48 ± 0.90 ^c	66.96 ± 0.30 ^e
GOHAR 87	46.43 ± 3.27 ^{bc}	42.87 ± 3.61 ^{bc}	66.78 ± 0.23 ^e
SLS I	43.33 ± 1.77 ^c	41.15 ± 2.14 ^{bc}	71.33 ± 1.09 ^c
B 557	46.03 ± 2.69 ^{bc}	43.34 ± 3.09 ^{bc}	67.23 ± 0.46 ^e
Mean ± SEM	47.15 ± 0.80	43.65 ± 0.79	70.30 ± 0.52

Same letters on means in a column show non-significant differences.

El-Refai et al. (1987) reported that digestibility of glandless CS flour was slightly higher than that of casein. Craig and Broderick (1981) reported true digestibility as 91%, in unheated meal, 84% after 60 min and 71% after 120 min of autoclaving. They also reported IVPD of solvent extracted and screw pressed meals to be 80-85% which was slightly higher than the findings of the present study, while contradictory results with cottonseed flour were due to extensive dehulling, low fiber content and heat treatments by Craig and Broderick (1981), which effected the true digestibility of protein by reducing free gossypol toxicity.

REFERENCES

- Almashouley, A. H. and A. Khan. 1990. Quality survey of cottonseed oil industry in Pakistan. Pak. Cott. 34:45-53.
- Altschul, A. M., C. M. Lyman and F. H. Thurber. 1958. Cottonseed meal. In: Processed Plant protein Foodstuffs (Ed. A. M. Altschul). Academic Press Inc. NY, USA.
- Anderson, J. O. and R. E. Warnick. 1966. Sequence in which essential amino acids become limiting for growth of chicks fed rations containing cottonseed meal. Poult. Sci. 45:84-89.

- AOAC. 1990. Official Methods of Analysis. Association of Official and Analytical Chemists (15th Ed.). Arlington, Virginia-22201, USA.
- AOCS. 1972. Official and Tentative Methods of the American Oil Chemists Society (3rd Ed.). AOCS, Champaign, Illinois, USA.
- Arias, F. J. and F. Hernandez. 1977. Estimation of gossypol in commercial and promising varieties of cotton in Colombia. *Revista-Instituto Colombiano Agropecuario*. 12:125-134.
- Baliga, B. P. and C. M. Lyman. 1957. Preliminary report on the nutritional significance of bound gossypol in cottonseed meal. *J. Am. Oil Chem. Soc.* 34:21.
- Boatner, C. H. 1948. Chemistry and chemical technology. In: Cottonseed Products (Ed. A. E. Bailey). Wiley Interscience, NY, USA.
- Booth, V. H. 1971. Problems in the determination of FDNB-available lysine. *J. Sci. Food. Agri.* 22:658.
- Bressanl, B. R., A. Aburto, R. Gomez-Breves and J. Braham. 1975. The effect of free gossypol from different cotton seed meals on the growth of rats and free lysine and gossypol in organs, muscle and serum. *Archivos Latinoamericanos de Nutricion*. 25:1.
- Cherry, J. P., J. G. Simians and R. J. Kohel. 1978. Nutritional Improvement of Feed and Feed Protein (Ed. M. Friedman). Plenum, NY, USA. pp. 343-363.
- Craig, W. M. and G. A. Broderick. 1981. Effect of heat treatment on true digestibility in the rat, *in vitro* proteolysis and available lysine content of cottonseed meal protein. *J. Anim. Sci.* 52:292-301.
- El-Refai, A. A., M. A. Owon, K. A. Ammar and A. M. Harras. 1987. Utilization of cottonseed protein in frankfurter. I. Functional properties and digestibility of cottonseed proteins. *Chemie Microbiologie Technologie der Lebensmittel*. 11:13-17.
- Eskin, N. A. M., O. Akomas and M. Latta. 1995. A study of gossypol reduction by choline and ethanolamine using a model system. *Food Chem.* 52:43-46.
- Gallup, W. D. 1927. The gossypol content and chemical composition of cottonseed during certain periods of development. *J. Agri. Res.* 34:987-992.
- Hsu, H. W., D. L. Vavak, L. D. Satterlee and G. A. Miller. 1977. A multienzyme technique for estimating protein digestibility. *J. Food Sci.* 4:1269-1273.
- Jonston, C. and A. B. Watts. 1964. The chick feeding value of meals prepared from glandless cottonseed. *Poult. Sci.* 43:957-963.
- Kashyap, J., J. Sharma, A. Taneja and V. Gupta. 1988. Effect of Zn on seed weight gossypol and oil content of H777 and G-27 cultivars of cotton. *J. Ind. Soc. Cott. Imp.* 13:159-162.
- Kuiken, K. A. 1952. Availability of essential amino acids in cottonseed meal. *J. Nutr.* 46:13-25.
- Lyman, C. M., B. P. Baliga and M. W. Slay. 1959. Reactions of proteins with gossypol. *Arch Biochem. Biophys.* 84:486.
- Lyman, C. M., W. Y. Chang and J. R. Couch. 1953. Evaluation of protein quality in cottonseed meals by chick growth and by a chemical index method. *J. Nutr.* 49:679.
- Malek, F. and P. Zandi. 1990. Determination of free gossypol in Iranian cottonseed and cottonseed cake. *Food Chem.* 37:289-295.
- Malik, D. M. and A. H. Khan. 1964. Effect of season and location on oil protein and gossypol content of cottonseed of new long staple varieties. *Pak. Cott.* 8:163-173.
- Martinez, W. H., L. C. Berardi, V. L. Frampton, H. L. Wilcke, D. E. Greene and R. Teichman. 1967. Importance of cellular constituents to cottonseed meal protein quality. *J. Agri. Food Chem.* 15:427.
- Mohan, P., P. Singh, A. B. Dongre and S. S. Narayanan. 1995. Gossypol-gland density and free gossypol content in seed and cotyledonary leaf of upland cotton (*Gossypium hirsutum*). *Ind. J. Agri. Sci.* 65:66-68.
- Mohan, P., P. Singh and S. S. Narayanan. 1991. Variability for gossypol glands in uplands cotton (*Gossypium hirsutum* L.). *Adv. Pl. Sci.* 4:165-170.
- Munro, H. N. and A. Fleck. 1966. Recent development in the measurement of nucleic acids in biological materials. *Analyst*. 91:78-88.
- Nwokolo, E. N., D. B. Bragg and W. D. Kils. 1976. The availability of amino-acids from palm kernels, soybean, cottonseed and rapeseed meal for the growing chick. *Poult. Sci.* 55:2300-2304.
- Papadopoulos, G. and E. Ziras. 1987. Nutrient composition of Greek cottonseed meal. *Anim. Feed Sci. Tech.* 18:295-301.
- Percy, R. G., M. C. Calhoun and H. L. Kim. 1996. Seed gossypol variation within *Gossypium barbadense* L. *Cotton. Crop Sci.* 36:193-197.
- Phelps, R. A. 1966. Cottonseed meal for poultry: From research to practical application. *World Poult. Sci. J.* 22:86-112.
- Pons, W. A., C. L. Hoffpairs and R. T. O'Conner. 1950. Determination of gossypol pigment in cottonseed material. *JAOCs*. 27:300-393.
- Rajic, I. and B. Kordic. 1976. Free gossypol content in cottonseed oil meal and cottonseed of Yugoslav origin. *Veterinarski Glasnik*. 30:715-718.
- Rojas, S. W. and M. L. Scott. 1969. Factors affecting the nutritive value of cottonseed meal as protein source in chick diets. *Poult. Sci.* 48:819-835.
- Schwartz, E. W. and C. L. Alsberg. 1923. Quantitative variation of gossypol and its relation to the oil content of cottonseed. *J. Agri. Res.* 25:285-295.
- Singh, P., T. H. Singh and G. S. Chahal. 1991. Genetical control of gossypol content in intervarietal crosses of upland cotton. *Crop. Impr.* 18:141-143.
- Smirnova, M. I. 1936. Interspecific and intraspecific chemical variation of cottonseed. *Tr. Prikl. Bot. Genet. Sci. Ser.* III. 15:227-240.
- Steel, R. G. D. and J. H. Torrie. 1980. Principles and Procedures of Statistics. McGraw-Hill Book Co. Inc., New York.
- Taneja, A. D., A. P. Sharma, J. L. Sharma and D. K. Jain. 1993. Biochemical changes in cottonseeds of different *hirsutum* genotypes during development. *J. Ind. Soc. Cott. Impr.* 18:75-81.
- Tchiegang, C. and J. Bourelly. 1990. Chromatographic analysis of phenolic compounds in cotton seed kernels (*Gossypium hirsutum* L. and *G. barbadense* L.). *Cotton Fibers Tropicales*. 45:27-43.
- Tuncer, S. D. and S. Yalcin. 1986. Estimation of gossypol concentrations in cottonseed meals produced in Turkey. *Selcuk Universities Veteriner Fakultesi. Dergisi*. 2:125-134.
- Waldroup, P. W. 1981. Cottonseed meal in poultry diets. *Feedstuffs*. 53:21-24.