

## GOSSYPOL CONTENTS IN COTTONSEED CAKES COLLECTED FROM DIFFERENT DISTRICTS OF THE PUNJAB PROVINCE OF PAKISTAN

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### ABSTRACT

In the present study, gossypol contents of cottonseed cakes (CSC), prepared from different varieties of cotton grown in the Punjab province of Pakistan, were determined. For this purpose, cottonseed cake samples were collected from 14 districts of the Punjab including Bahawalnagar, Bahawalpur, Dera Ghazi Khan, Faisalabad, Jhang, Kasur, Khanewal, Lahore, Multan, Okara, Sahiwal, Sargodha, Toba Tek Singh and Vehari. These samples were analyzed for the free and the total gossypol contents applying American Oil Chemist Society Official Methods, Ba 7-58 and Ba 8-78, respectively. The results showed that the free and the total gossypol contents of cottonseed cakes averaged  $0.28 \pm 0.02$  and  $1.44 \pm 0.04$  per cent, respectively. The highest values of the free and the total gossypol contents,  $0.36 \pm 0.02$  and  $1.59 \pm 0.03$  per cent respectively, were recorded in cottonseed cake samples collected from Vehari district, while the lowest values,  $0.21 \pm 0.01$  and  $1.29 \pm 0.01$  per cent respectively, were found in those collected from Lahore district. Analysis of variance revealed significant differences ( $P < 0.05$ ) in the free and the total gossypol contents of cottonseed cakes among districts. It was concluded that the free and the total gossypol contents of cottonseed cakes available in different parts of the province differed significantly ( $P < 0.05$ ).

**Key words:** Gossypol, cottonseed cakes, Punjab.

### INTRODUCTION

Gossypol is a yellow pigment found in various parts of the cotton plants, including seeds, of the genus *Gossypium* (Adams *et al.*, 1960). Chemically, it is 1,1', 6,6',7,7'- hexahydroxy- 5,5'- diisopropyl- 3,3'- dimethyl (2,2'-binaphthalene) -8,8' dicarboxaldehyde with the empirical/molecular formula of  $C_{30}H_{30}O_8$  (Abou-Donia, 1976) and a molecular weight of 518.54 (Adams *et al.*, 1960). The free gossypol is biologically active and all the gossypol present in the whole cottonseeds is in this form. Processing whole cottonseeds into meal converts varying amounts of free gossypol to the bound form by binding of varying amounts of free gossypol to the proteins present in the seed. This results in a reduction of the biological activity of the compound. The total gossypol content of cottonseed meal is not affected by the process used in oil extraction because total gossypol is equal to the free plus bound amounts. Bound gossypol is found by subtraction of the free value from the total value. Discussions of gossypol usually revolve around these three terms, "total", "free", and "bound".

Gossypol may be toxic to animals particularly if the compound is in the free state, and is known to cause toxicity in monogastric (Haschek *et al.*, 1989) as well as

ruminant animals (Holmberg and Kutches, 1991). This compound has been shown to exert antifertility effects in males (Randel *et al.*, 1992) and females (Gu *et al.*, 1990). It has direct damaging effects on the epididymides, testes and the developing germ cells (Frick and Danner, 1985).

Cottonseeds and their by-products, e.g., cottonseed cakes and cottonseed hulls, have been used extensively as protein supplements in dairy animals' rations to increase fat and milk production in these animals (Ahmad, 1993). As a cheaper but rich source of high quality protein for supplementing animal diets, the use of cottonseed products has been continued over the last many decades. This indiscriminate use of cottonseeds or their by-products can adversely affect the reproductive performance of dairy animals. Gossypol contents of cottonseeds have been shown to vary between different varieties (Malik and Khan, 1964). Similarly, processing of seeds and methods applied for the preparation of cottonseed cakes can also influence the gossypol contents of the cakes (Calhoun *et al.*, 1991). However, there is little information in the literature regarding the free and the total gossypol contents of cottonseed cakes prepared from different varieties of cotton grown in the Punjab Province of Pakistan. The present study was, therefore, carried out to determine the free and the total



gossypol contents of cottonseed cakes prepared from different varieties of cotton grown at different localities of the Punjab province.

## MATERIALS AND METHODS

### Collection of samples:

A total of 56 samples of cottonseed cakes, prepared from various varieties of cotton grown at 14 districts of the Punjab province, including Bahawalnagar, Bahawalpur, Dera Ghazi Khan, Faisalabad, Jhang, Kasur, Khanewal, Lahore, Multan, Okara, Sahiwal, Sargodha, Toba Tek Singh and Vehari were used. Four points in the local market of each district headquarter were selected at random for the collection of samples and one sample of about 0.5 kg of cottonseed cakes was collected from each point.

### Determination of gossypol:

All the samples were ground separately with a grinding mill having 1 mm mesh sieve. American Oil Chemist Society Official Methods, Ba 7-58 (Anonymous, 1988) and Ba 8-78 (Anonymous, 1988a) were applied to determine the free and the total gossypol contents of cottonseed cakes, respectively.

### Statistical analysis:

Statistical Analysis System (SAS) was used to analyze the free and the total gossypol contents. Analysis was performed as a completely randomized design of the analysis of variance technique (Steel and Torrie, 1984). For this purpose, general linear model (GLM) procedure under SAS computer program (SAS, 1990) was adopted. Whenever there was a statistical difference, least significant difference (LSD) test (Steel and Torrie, 1984) was used for comparison of means.

## RESULTS AND DISCUSSION

In this study, the free and the total gossypol contents of cottonseed cakes (CSC), prepared from different varieties of cotton grown in the Punjab province of Pakistan, were investigated. The mean values ( $\pm$  SEM) of the free and the total gossypol contents in CSC samples collected from 14 districts are presented in Table 1. The results of multiple means comparison obtained through LSD test were complex and have also been shown in the same table by superscripts.

In these studies the free and the total gossypol contents of CSC averaged  $0.28 \pm 0.02$  and  $1.44 \pm 0.04$  per cent, respectively; the range being  $0.21 \pm 0.01$  to

$0.36 \pm 0.02$  per cent for the free gossypol and  $1.29 \pm 0.01$  to  $1.59 \pm 0.03$  per cent for the total gossypol. Many workers, (Gallup 1927; Smirnova 1936; Pons *et al.* 1950; Pons *et al.* 1953; Pons and Haffpaur 1957; Frampton *et al.* 1960; Malik and Khan 1964; Ala-ud-Din 1995 and Zahid 2002) reported significant ( $P < 0.05$ ) variation in gossypol contents of cottonseeds and their by-products. Gallup (1927) reported that rainfall during the cotton growing season decreased the gossypol contents of cottonseeds. It was also concluded that different varieties of the same species may differ with respect to the contents of gossypol pigments when the seeds were grown under different conditions. These findings are in agreement with the results of present study. Smirnova (1936) studied the gossypol contents of a large number of varieties of cotton grown at different locations in USSR and found that locality of cotton growth significantly ( $P < 0.05$ ) affected the gossypol contents. Pons *et al.* (1950) reported free gossypol contents of 0.129 and 0.013 per cent and total gossypol contents of 1.57 and 0.521 per cent in two types of cottonseed meals prepared by hydraulic and screw pressed methods, respectively. The results of present study are partially in agreement with the findings of this worker, as the gossypol contents of cottonseed cakes prepared by hydraulic and screw pressed methods in this study also showed the similar trend of variation. Pons *et al.* (1953) reported a range of the free gossypol contents from 0.19 to 0.83 per cent for whole cottonseeds sampled from 8 commercial varieties of cotton grown at 13 locations throughout the cotton belt in USA and showed a significant ( $P < 0.05$ ) difference in the contents of gossypol among different locations as well as varieties.

Pons and Haffpaur (1957) studied the free gossypol contents in cottonseed cakes prepared by different methods of oil extraction and reported 0.025-0.041 per cent in screw pressed, 0.107 per cent in hydraulic pressed, 0.042-0.074 per cent in pre-press solvent and 0.026 per cent in butanone extracted raw flakes. The results of present study are not in line with the findings of these workers as the screw pressed and hydraulic pressed methods used for the preparation of cottonseed cakes in Pakistan showed a large range of the free gossypol contents in cottonseed cakes. The results of present study are in line with the findings of Frampton *et al.* (1960) who reported a great variation in the total gossypol contents of seeds from several species of *Gossypium* ranged from 0.13 to 6.64 per cent, with a mean value of 3.38 per cent. Malik and Khan (1964) analyzed four American varieties of cotton grown at



**Table I. Free and the total Gossypol contents (mean  $\pm$  SEM) in cottonseed cake samples collected from different districts of the Punjab province.**

Sr. No.	Districts	Free gossypol (%)	Total gossypol (%)
1.	Bahawalnagar	0.26 $\pm$ 0.02ef	1.40 $\pm$ 0.03fg
2.	Bahawalpur	0.32 $\pm$ 0.02b	1.51 $\pm$ 0.03b
3.	Dera Ghazi Khan	0.25 $\pm$ 0.02ef	1.38 $\pm$ 0.03g
4.	Faisalabad	0.27 $\pm$ 0.02de	1.42 $\pm$ 0.03efg
5.	Jhang	0.24 $\pm$ 0.02fg	1.36 $\pm$ 0.03g
6.	Kasur	0.25 $\pm$ 0.02ef	1.38 $\pm$ 0.03g
7.	Khanewal	0.30 $\pm$ 0.03bcd	1.47 $\pm$ 0.04bcde
8.	Lahore	0.21 $\pm$ 0.01g	1.29 $\pm$ 0.01h
9.	Multan	0.28 $\pm$ 0.02cde	1.44 $\pm$ 0.04def
10.	Okara	0.27 $\pm$ 0.02ef	1.41 $\pm$ 0.03fg
11.	Sahiwal	0.29 $\pm$ 0.03bcde	1.45 $\pm$ 0.04cdef
12.	Sargodha	0.31 $\pm$ 0.02bcd	1.50 $\pm$ 0.03bcd
13.	Toba Tek Singh	0.31 $\pm$ 0.03bc	1.50 $\pm$ 0.05bc
14.	Vehari	0.36 $\pm$ 0.02a	1.59 $\pm$ 0.03a
	Overall mean	0.28 $\pm$ 0.02	1.44 $\pm$ 0.04

Values with different superscripts within a column differ significantly ( $P < 0.05$ ).

four places in Pakistan. According to these workers, the total gossypol contents in whole cottonseeds ranged from 0.85 to 1.96, 1.22 to 1.92, 0.75 to 1.96 and 0.75 to 1.95 per cent at Faisalabad, Jhang, Sahiwal and Sargodha, respectively. Similarly, the total gossypol contents ranged from 1.26 to 1.85, 1.20 to 1.96, 0.75 to 1.22 and 1.47 to 2.00 per cent for 387 F, AC-158, Lasani-11 and AC-134 varieties of cotton, respectively. Differences in contents of gossypol both for various locations and varieties of cotton were significant ( $P < 0.05$ ). Ala-ud-Din (1995) analyzed cottonseed cake samples collected from different places of the Punjab province in Pakistan and reported the average free gossypol contents of  $0.28 \pm 0.06$  per cent. Similarly, Zahid (2002) reported variations both in the free and the total gossypol contents of cottonseed cakes prepared from different varieties of cotton grown in various locations of Punjab province in Pakistan.

Regardless of processing methods, numerous steps are involved in conditioning cottonseed kernels (meats) prior to oil extraction. Therefore, it is important to realize that the free gossypol levels in the resulting cottonseed meals reflect not only the gossypol contents of the cottonseeds being extracted but also the total process used at a particular plant at any given time. Because of this, free gossypol levels vary not only with processing methods but also among plants using the same method and even within plants, as adjustments are

made in processing conditions and as the conditions of the seed storage change. According to Pons *et al.* (1953), agronomic factors can also affect the gossypol contents of cottonseeds. Gallup (1927) found that rainfall during the cotton growing season decreased the gossypol contents of cottonseeds. Different varieties of the same species may differ with respect to the contents of gossypol pigments when the seeds were grown under different conditions. Locality of growth also appeared to affect the gossypol contents of the cottonseeds (Smirnova, 1936).

It appears that the variations in the percentages of both the free and the total gossypol in cottonseed cakes collected from different localities were due to differences in varieties of cotton grown in different localities, agronomic and seasonal effects and different methods of oil extraction which are being applied for the preparation of cottonseed cakes in the Punjab Province. A large number of cotton varieties are grown in the Punjab, which appear to affect the gossypol contents of cottonseeds and their by-products.

Depending upon the method of oil extraction, the concentration of free gossypol contained in different feedstuffs may vary considerably. It is concluded that studies are needed to be undertaken regarding improving existing methods of preparing cottonseed cakes with a view to minimize free gossypol contents in cakes and other by-products.



## REFERENCES

- Abou-Donia, M.B., 1976. Physiological effects and metabolism of gossypol. *Residue Rev.*, 61: 125-160.
- Adams, R., T.A. Geissman and J.D. Edwards, 1960. Gossypol, a pigment of cottonseeds. *Chem. Rev.*, 60: 555-574.
- Ahmad, M.K., 1993. Few cheaper and balanced rations for animals. *Livestock Production Research Institute, Bahadarnagar, Okara, Pakistan.*
- Ala-ud-Din, 1995. Studies on the effect of cottonseed (Gossypol) on the reproductive performance of male and female buffaloes. Final Report, Deptt. Anim. Reprod. Univ. Agric. Faisalabad, Pakistan, pp: 25-26.
- Anonymous, 1988. Sampling and analysis of oilseed by-products, determination of Free Gossypol. A.O.C.S. Official Method Ba 7-58. In: "Official Methods and Recommended Practices of the American Oil Chemists' Society", 3rd edn. Amer. Oil Chem. Soc., Champaign, Illinois, pp:1-5.
- Anonymous, 1988a. Sampling and analysis of oilseed bi-products, determination of Total Gossypol. A.O.C.S. Official Method Ba 8-78. In: "Official Methods and Recommended Practices of the American Oil Chemists' Society", 3rd edn. Amer. Oil Chem. Soc., Champaign, Illinois, pp:1-5.
- Calhoun, M.C., J.E. Huston, D.N. Uckert., B.C. Baldwin Jr., S.W. Kuhlmann and B.S. Endahl, 1991. Performance of yearling heifers on diets containing whole cottonseed. *Texas Agri. Exp. Sta. Rpt.*, 4839.
- Frampton, B.L., W.A. Pons Jr., and T. Kerr, 1960. A comparison of chemical properties of seeds of *Gossypium* species. *Econ. Botany*, 14: 197-199.
- Frick, J. and C. Danner, 1985. Effect of gossypol on human testicular function. In: "Segal, S.J. (Ed.) Gossypol: A Potential Contraceptive for Men", Plenum Press, New York, pp: 25-31.
- Gallup, W.D., 1927. The gossypol content and chemical composition of cottonseeds during certain periods of development. *J. Agric. Res.*, 34: 987-992.
- Gu, Y., C.J.G. Chang, Y. Rikihisa and Y.C. Lin, 1990. Inhibitory effect of gossypol acetic acid on human chorionic gonadotrophic hormone (hCG)-induced progesterone secretion in cultured bovine luteal cells. *Life Sci.*, 47: 407-414.
- Haschek, W.M., V.R. Beasley, W.B. Buck and J.H. Finnell, 1989. Cottonseed meal (gossypol) toxicosis in a swine herd. *J. Amer. Vet. Med. Assoc.*, 195: 613-615.
- Holmberg, C.A. and A. Kutches, 1991. Cottonseed meal feeding trials in young calves. *Proc. Ruminant Nutrition-Pharmacology and Toxicology.*
- Special Session on Effects of Gossypol on Domestic Animals. Annual Mtg. Amer. Soc. Anim. Sci. Laramie, Aug. 9.
- Malik, D.M. and A.H. Khan, 1964. Effect of season and location on oil, protein and gossypol content of cottonseeds of new long staple cottons in Punjab. *Pak. Cottons*, 8: 163-173.
- Pons, W.A. Jr., C.L. Haffpaur and R.T. O'Conner, 1950. Determination of gossypol pigments in cottonseed materials. *J. Amer. Oil Chem. Soc.*, 27: 390-393.
- Pons, W.A. Jr., C.L. Haffpaur and T.H. Hopper, 1953. Gossypol in cottonseed: Influence of variety of cottonseed and environment. *J. Agric. Food Chem.*, 1: 1115-1118.
- Pons, W.A. Jr. and C.L. Haffpaur, 1957. Determination of free and total gossypol in mixed feeds containing cottonseed meals. *J. Assoc. Official Agric. Chem.*, 40: 1068-1080.
- Randel, R.D., C.C. Chase Jr. and S.J. Wyse, 1992. Effects of gossypol and cottonseed products on reproduction of mammals. *J. Anim. Sci.*, 70: 1628-1638.
- SAS, 1990. SAS/STAT User's Guide (Release 6.04). SAS Inst., Inc., Carry, NC.
- Smirnova, M.J., 1936. Inter and intra species variations in gossypol. *Tri Priklad Botany Genet. Selkt*, 15: 227-240.
- Steel, R.G.D. and J.H. Torrie 1984. Principles and Procedures of Statistics, 2nd edition, Mc Graw Hill Co. Inc. New York, USA. pp:107-109.
- Zahid, I. A. 2002. Studies on the effects of diets containing gossypol on testes of adult teddy buck. PhD Thesis, Dept. Anim. Reprod. Univ. Agri., Faisalabad