Effect of Gossypol Bound to Cottonseed Protein on Growth of Weanling Rats

Frank H. Smith

A study of the effect of varying levels of gossypol bound to cottonseed protein fed to weanling rats at the 10% dietary level showed that the effects were slight in meals containing less than approximately 0.75% bound gossypol. Gains were markedly depressed with levels of bound gossypol above 0.75%. Using diets ranging from 10 to 20% dietary protein, it was shown that increasing the level of bound gossypol in the cottonseed meal from 0.45 to 1.3% required approximately a 4% increase in the level of dietary protein of the latter to produce approximately the same rate of gain in weanling rats.

The limited supply of protein for man and animals is a major concern of nutritionists. Efforts are being directed toward the most efficient use of the available protein. Cottonseed is an important source of protein. Unfortunately, it contains a toxic phenol, gossypol (1,1′,6,6′,7,7′-hexahydroxyl-3,3′-dimethyl-5,5′-diisopropyl-2,2′-binaphthyl-8,8′-dicarboxaldehyde) (Withers and Carruth, 1915), which is largely detoxified during processing by a condensation of the aldehyde groups of gossypol with the free amino groups of the protein (Clark, 1928). This combination of gossypol with the free amino groups lowers the nutritive properties of the protein (Young, 1955; Baliga and Lyman, 1957; Smith *et al.*, 1958). These earlier studies were carried out with cottonseed protein to which relatively high levels of gossypol were bound. It is desirable to know the effect of a wider range of gossypol bound to protein.

A study was made to determine the effect of varying the levels of gossypol bound to cottonseed protein which was incorporated at a 10% level in diets for weanling rats, and to determine the effect of gossypol bound to cottonseed protein which was fed to rats at varying dietary protein levels.

MATERIALS AND METHODS

Preparation of Cottonseed Protein. Oil and free gossypol were removed from decorticated and flaked cottonseed by percolation with ethyl ether (Smith, 1960). The percolators were covered with aluminum foil to prevent evaporation of the ether. The extraction was continued until the effluent was very light yellow in color. The extracted residue was dried on a large tray placed in a hood which had a high-capacity, explosion-proof fan to remove ether vapors. Ether was recovered from the extract by distillation for reuse. Gossypol was precipitated from the oil by addition of an equal volume of hexane and approximately 1% of aniline based on oil volume.

The air-dried, extracted cottonseed flakes were ground in a burr cereal mill set for a medium-coarse grind; some of the cottonseed hulls unavoidably included were removed by passing the product through a 20-mesh sieve. All of the protein preparations were thoroughly mixed and analyzed for protein, crude fat, and gossypol.

Binding Gossypol to Protein. Gossypol was dissolved in 2560 ml of ethyl ether, after which 1140 ml of 95% ethanol and 385 ml of water were added. This mixture was poured over 1600 g of the ether-extracted cottonseed protein in the mixing bowl of a Hobart mixer. The mixing bowl was placed in a water bath heated by a copper coil, through which passed steam generated by immersion of an electric heating coil in a 5-l. flask of water. The mixture was stirred at low speed for about 20 min before heat was applied. The heating was regulated to bring the water bath to approximately 85°C and the meal to 72 to 75°C after 4 hr. The process should be performed outside the building or in an area provided with a high-capacity exhaust fan which will prevent flammable solvent vapor accumulation.

Four preparations containing 0.23, 0.71, 1.20, and 1.77% total gossypol and 0.01, 0.03, 0.07, and 0.12% free gossypol, respectively, were prepared and used to study the effect of varying levels of bound gossypol (total minus free) in 10% protein diets. Preparations containing 0.445 and 1.300% bound gossypol were prepared and used to study the effect of dietary protein level. Free and total gossypol were determined according to Smith (1968 and 1958, respectively).

Animals. Eight weanling rats, four males and four females weighing 40–50 g, were randomly assigned to each of the four diets prepared from cottonseed protein preparations containing the four levels of bound gossypol. The rats were housed in individual cages and had free access to food and water; they were fed for 4 weeks and were weighed weekly. Eight weanling male rats were randomly assigned to each of eight diets to study the effect of varying dietary protein level when it contained bound gossypol. The management was the same as described for the first study except that the animals were fed for 3 weeks.

Diets. The diets for the first study contained 10% protein (% N × 6.25), 5% fat supplied by the cottonseed protein and Wesson oil, 0.5% cod liver oil to supply vitamins A and D, 3% Wesson salt, 2% alphacel and starch to bring the total dietary ingredients to 100%. In addition, the diets contained the following vitamins expressed in mg/kg of diet: thiamine hydrochloride, 5; riboflavin, 5; pyridoxine hydrochloride, 5; calcium pantothenate, 20; niacin, 30; *p*-aminobenzoic acid, 30; biotin, 0.4; folic acid, 2; inositol, 10; B_{12}, 0.03; and choline chloride, 1000. The diets for the second study were similar except that protein level was varied by the substitution of cottonseed meal for starch.

RESULTS AND DISCUSSION

The results from feeding varying levels of bound gossypol are shown as mean weight gains in Figure 1. The effects of two levels of gossypol bound to portions of the same cottonseed protein which were fed at varying protein levels are shown in Figure 2.

Each point plotted in Figure 1 represents the mean 4-week weight gain of eight rats fed diets containing 10% protein with the different levels of bound gossypol. The effect of bound gossypol was slight up to a level of 0.68%, but was marked

Department of Animal Science, North Carolina Experiment Station, Raleigh, North Carolina 27607.

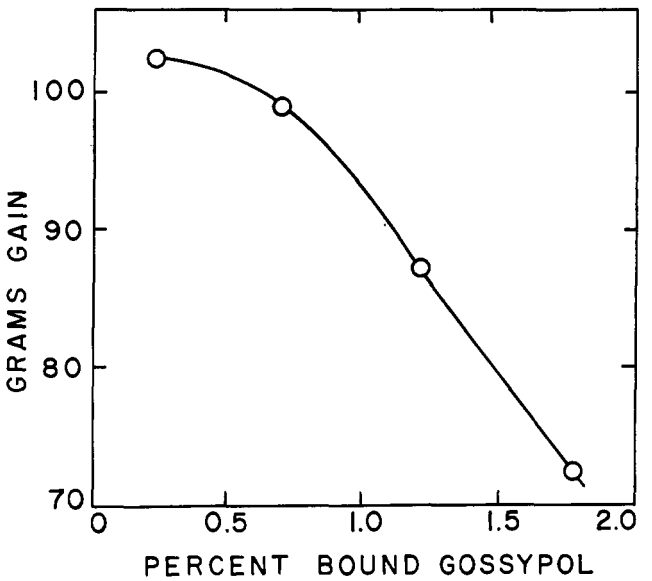

Figure 1. Effect of dietary level of bound gossypol on growth of rats fed 10% protein

above 0.75 to 0.80%, as indicated by the curve in Figure 1. Earlier studies (Smith *et al.*, 1958) had shown that levels of free gossypol similar to the levels in these preparations did not significantly affect weight gains when fed to rats at the 10% protein level; however, levels of 1.1% bound gossypol did reduce gains, $p < 0.01$. In this study, the most marked change in the rate of gain occurred at a level slightly lower than the 0.85% bound gossypol, below which Lyman *et al.* (1959) found most cottonseed meals to be of good nutritive quality.

Each point in Figure 2 represents the mean 3-week weight gain of eight weanling male rats fed at the protein level indicated. The line with the open circles represents the gains of rats fed the cottonseed meal containing 0.445% bound gossypol, and the line with the closed circles represents the gains of rats fed a portion of the same meal with the bound gossypol raised to 1.3%. The lines are essentially parallel and indicate that the protein level of diets prepared from the high-bound gossypol meal must be 4% higher than in diets prepared from low-bound gossypol meal to produce about the same weight gains in weanling rats. These data indicate that increasing the level of bound gossypol from 0.445 to 1.30% required at

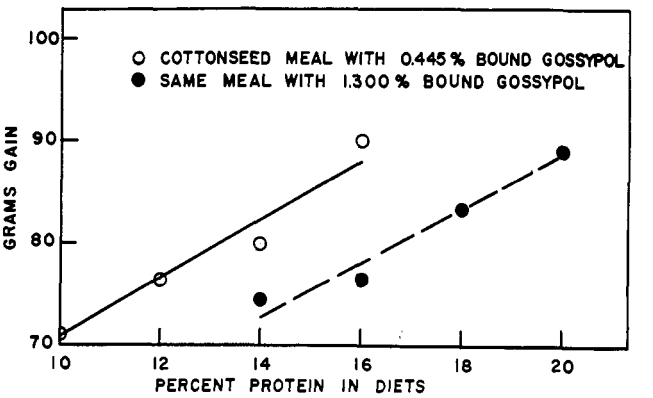

Figure 2. Effect of varying levels of bound gossypol and dietary protein on the growth of rats

least 25% more protein to produce about the same weight gains. The effect of bound gossypol on nutritive value is probably due to a reduction in availability of amino acids, as reported by Kuiken (1952), resulting from the formation of a gossypol–protein complex by the combination of the formyl group of gossypol with the free amino groups of the protein (Clark, 1928; Lyman *et al.*, 1959).

Cottonseed protein could be much more efficiently used if the bound gossypol could be eliminated or reduced to a low level. A reduction to 0.75% bound gossypol would be of considerable importance in animal production.

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