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Effects of Source of Gossypol and Supplemental Iron on Plasma Gossypol in Holstein Steers

J. E. P. Santos,[1] H. Mena,[2] J. T. Huber,[2] and M. Tarazon[2,*]
[1]Veterinary Medicine Teaching and Research Center, University of California-Davis, Tulare 93274
[2]Department of Animal Science, University of Arizona–Tucson 85721-0038

ABSTRACT

Four experiments were conducted to evaluate factors influencing concentrations of plasma total gossypol (TG) in 30 Holstein steers fed cottonseed products. At the end of each 28-d experiment, steers were weighed and blood samples were collected and analyzed for plasma TG concentrations. During the entire study, steers did not show any overt signs of gossypol toxicity. In the 28 d before experiment 1, 30 steers with a body weight (BW) of 273 kg were fed a standardization diet with 15.0% Upland whole cottonseed (WCS) that resulted in a mean intake of 9.08 g/d of TG per steer/d and a plasma TG of 1.66 μg/mL. In experiment 1, 30 steers were fed 1 of 5 diets with 15.0% Upland WCS, but different levels of supplemental Fe [0, 150, 300, 450, and 600 mg/kg of diet dry matter (DM)]. Average daily gain was not affected by level of Fe in the diet, but DM intake, plasma TG, and plasma TG response decreased linearly as Fe in diets increased. In experiment 2, steers were fed diets with 15.0% Upland cottonseed as whole, cracked, roasted, cracked-roasted, or extruded. Analysis of the seed revealed that roasting or extrusion markedly reduced free gossypol (FG) content. Minor effects on animal performance were observed, but plasma TG decreased with roasting or extrusion of seeds, with the greatest reduction when the seed was cracked and then roasted. In experiment 3, steers were fed 2 levels of WCS (7.0 or 14.0% of DM) with 3 levels of cottonseed meal (2.8, 5.5, or 8.5% of DM) in the diet. Animal performance was not altered by diet, but plasma gossypol concentrations and responses were greater in steers fed diets with more WCS, because of the greater FG intake. In experiment 4, 24 steers were fed diets with 15.0% cottonseed (Upland or Pima) either as whole or cracked. Pima cottonseed increased TG and FG intakes, which resulted in greater plasma TG concentration and response. Animal response to processing of cottonseed tended to differ according to type of cottonseed. However, feeding Pima and cracking of cottonseed increased gossypol availability and plasma TG concentrations.
(**Key words:** gossypol, whole cottonseed, cottonseed meal, iron sulfate)

Abbreviation key: CSM = cottonseed meal, **FG** = free gossypol, **PG** = plasma gossypol, **TG** = total gossypol, **WCS** = whole cottonseed.

INTRODUCTION

Cotton is a major crop throughout the Southern United States and by-products of the cotton industry such as linted whole cottonseed (**WCS**) and cottonseed meal (**CSM**) are extensively used as dietary ingredients for dairy and beef cattle (Arieli, 1998). Upland cotton (*Gossypium hirsutum*) is the predominant type of cotton grown in many areas of the world, but Pima cotton (*Gossypium barbedense*) production is increasing in the Southwestern United States and other parts of the world (Lewkowitz, 2004). Upland cottonseed is generally fed unprocessed, but Pima cottonseed is naturally devoid of linters and is generally processed by cracking or grinding to increase digestion and nutrient use (Sullivan et al., 1993a,b), thus preventing the appearance of whole seeds in the feces.

Gossypol is a naturally occurring toxin produced by the pigment glands found throughout the cotton. However, gossypol concentration is greater in the kernels of cottonseed than other parts of the plant. Because of gossypol, the amount of cotton products fed to cattle has to be limited to avoid risk of toxicity (Coppock et al., 1987; Calhoun et al., 1995; Arieli, 1998). Gossypol exists in both the free and the bound forms. Most of the gossypol found in WCS is in the free form, whereas most of the gossypol in products of cottonseed treated with temperature and pressure, such as CSM, is in the bound form (Mena et al., 2001, 2004), which results in lower plasma gossypol (**PG**) concentrations (Mena et al., 2001, 2004). Gossypol also exists as a mixture of (+) and (–) enantiomers, with the (–) isomer having the higher biological activity (Joseph et al., 1986).

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Corresponding author: J. E. P. Santos; e-mail: Jsantos@vmtrc.ucdavis.edu.
*Present address: Universidad de Sonora, Santa Ana, Sonora, 84600, Mexico.

In a number of studies designed to evaluate the effects of feeding cottonseed products on animal performance, it was demonstrated that cattle with a well-developed rumen tolerated diets with high concentrations of total gossypol (**TG**) and free gossypol (**FG**) for extended periods (Coppock et al., 1987; Risco et al., 2002; Santos et al., 2002, 2003; Mena et al., 2004). Although ruminants with a functioning rumen detoxify gossypol to some extent (Calhoun et al., 1995), the mechanism of gossypol detoxification is not clearly understood (Coppock et al., 1987). Gossypol is thought to bind to proteins containing free amino sites, which impairs absorption of gossypol in the digestive tract (Reiser and Fu, 1962; Calhoun et al., 1995). Furthermore, it has been suggested that iron as iron sulfate binds to gossypol and reduces availability in the digestive tract for absorption (Barraza et al., 1991).

Plasma gossypol concentrations might reflect the availability of gossypol for absorption (Calhoun et al., 1995) and can be used to establish limits on amounts of cottonseed products that can be fed safely. Furthermore, type of cottonseed, concentrations of FG in cottonseed, particle size and density of cottonseed, processing method, and concentration of Fe in the diet are among the several factors that affect PG concentrations when cotton products are fed to ruminants.

The objectives of this study were to determine the effects of type and method of processing of cottonseed, supplementation with Fe from monohydrated iron sulfate ($FeSO_4{\cdot}H_2O$), and source of gossypol on PG concentrations in Holstein steers. Responses to treatments were evaluated in 4 experiments by measuring PG concentrations, PG response to gossypol intake, and animal performance. Although it was not the main objective of the study to determine animal performance, data on DM intake, BW gain, and efficiency of feed use were also evaluated.

MATERIALS AND METHODS

Animals and Feeding

Thirty Holstein steers with an initial average BW of 273 kg were selected for this study and subjected to 4 experiments of 28 d each. The University of Arizona Institutional Animal Care and Use Committee approved all procedures involving animals. Steers were housed in individual pens with shades and with free access to water and feed. Diets were offered for ad libitum intake twice daily and feed intake and refusals were recorded weekly. Diets were mixed twice for each of the experiments and samples of TMR, cottonseeds, and CSM were collected every time diets were mixed, dried at 55°C for 48 h, and ground in a Wiley mill (Arthur H. Thomas Co., Philadelphia, PA) to pass through a 2-mm screen, then in a cyclone mill (Udy Co., Fort Collins, CO) to pass through a 1-mm screen. Samples were then stored at −20°C for later analyses of DM, OM, N, ether extract (AOAC, 1990), ADF and NDF (Van Soest et al., 1991), and Fe by an inductively coupled plasma emission spectrometer (Thermo Jarrell Ash, Franklin, MA). Therefore, diets and cotton products were analyzed twice for each of the 4 experiments. Diets were kept as isonitrogenous as possible and formulated to meet nutritional requirements of Holstein steers gaining 1.5 to 1.7 kg/d (NRC, 2001). Concentrations of gossypol in diets of all experiments were calculated based on the gossypol content of cotton products and their inclusion rate in the respective diets.

During the initial standardization period of 28 d before experiment 1, all steers were fed a common standardization diet containing (DM basis) 15.0% Upland WCS, 37.0% alfalfa hay, 47.0% steam-flaked corn, and 1.0% of a mixture of minerals and vitamins. The nutritional composition of the standardization diet was: 91.8% DM, 2.78 Mcal of metabolizable energy when adjusted for 10 kg of DM intake (NRC, 2001), 14.7% CP, 6.1% ether extract, 30.3% NDF, 141 mg of Fe/kg of diet, and 960 mg of TG and FG/kg of diet. The nutrient composition of the Upland WCS was (DM basis) 19.3% CP, 18.6% crude fat, 51.3% NDF, 40.7% ADF, and 1.61% ADF insoluble CP. Concentrations of PG and animal performance during the standardization period were used for covariate adjustment during analysis of data in experiment 1.

Experimental periods lasted 28 d because we have demonstrated that this is the time required for PG to plateau after inclusion of gossypol in the diet (Mena et al., 2001, 2004). Furthermore, when the source of gossypol was removed from the diet, gossypol concentrations in plasma returned to undetected levels after 28 d (Mena et al., 2004). In all experiments, PG concentrations were used as an indicator of gossypol availability in cottonseed products.

In experiment 1, the effects of monohydrated iron sulfate supplementation on the availability of gossypol from Upland linted WCS were studied by feeding the standardization diet (960 mg/kg of TG and FG) and varying levels of Fe to 30 steers (6/treatment). Iron supplementation was designed to result in 0, 150, 300, 450, and 600 mg of Fe/kg of diet from iron sulfate, but actual concentrations of Fe in the diets after chemical analysis were 124, 240, 235, 338, and 457 mg/kg, respectively (Table 1). The difference between the planned levels and those in the actual TMR may have been caused by difficulties in obtaining representative samples of the TMR for mineral analysis.

In experiment 2, the effects of processing WCS on blood gossypol were evaluated in 30 steers (6/treat-