

Gossypol content of cotton free commercial feed for dairy cows

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Abstract

Gossypol is a yellow pigment occurring in all parts of cotton plants, with the highest levels found in seeds, and it exhibits a variety of toxic effects. Few data are available on the content of gossypol in the commercial complementary feed and in feed raw materials. The present study was focused on the investigation of the presence of free gossypol in commercial complementary feed not containing cotton. A total of 50 samples of commercial complementary feed for dairy cows were performed in 29 feed mills both using and not using cotton as feed material. The free gossypol contamination resulted under the detection limit of the technique (4 mg/kg) in 12 out of 50 samples analysed and ranged from 4 to 20 mg/kg in 28 samples. In 10 samples the level of free gossypol ranged from 20 to 29.5 mg/kg. Average contamination of samples was 12.2 ± 9.2 SD mg/kg. No significant difference ($P=0.571$) was shown in free gossypol concentration between feed produced in *cotton free* plants and in plants where cotton is used as feed material. Free gossypol content detected in the present study allows considering complementary feed for dairy cows not at risk. On the other hand, the detection of free gossypol in *cotton free* complementary feed, probably attributable to cross contamination of feed materials upstream of the feed mill, should be further investigated.

Introduction

Regulation 178/2002/EC (European Commission, 2002) poses the basis for food

safety along the food chain as the results of control on food, food animals and their feeding; in this context feed shall be deemed to be unsafe for its intended use if it is considered to: i) have an adverse effect on human or animal health or ii) make the food derived from food-producing animals unsafe for human consumption. Gossypol is a yellow pigment occurring in free or bound form in all parts of cotton plants, and the highest level was found in seeds (Adams *et al.*, 1960; Markman and Rzhikhin, 1969; Jaroszewski, 1998; Dodou, 2005). Cottonseed (kernels) can contain several percent (up to 10%, 0.1-100 g/kg) of gossypol. Extracted or pressed cotton kernels are protein-rich by-products of the cotton oil industry that are used for animal feeding. In addition to *Gossypium* species, gossypol is found in some other plants belonging to related genera, however, these plants do not have any industrial or agricultural importance (EFSA, 2008). Gossypol exhibits a variety of biological actions: signs of gossypol toxicity are similar in all animals and include laboured breathing and anorexia. Acute toxicity has been shown in the heart, lung, liver, and blood cells, resulting in increased erythrocyte fragility. *Post-mortem* findings include generalised oedema and congestion of lungs and liver, fluid-filled thoracic and peritoneal cavities, and degeneration of heart fibres. Reproductive toxicity is observed particularly in males, where gossypol affects sperm motility, inhibits spermatogenesis and depresses sperm counts, cause Sertoli cell toxicity and may also affect Leydig cells. Gossypol also seems to disrupt oestrus cycles, pregnancy and early embryo development, particularly in the monogastric species studied (Abou-Donia, 1976; Berardi and Goldblatt, 1980; Randel *et al.*, 1992; Dodou, 2005).

Current European legislation (EU Regulation 574/2011; European Commission, 2011) contains a list of undesirable substances in animal feed and their maximum content allowed in different feed materials, including free gossypol. The limit of free gossypol is 20 mg/kg in feed materials with the exception of cottonseed (5000 mg/kg), cottonseed cakes and cottonseed meal (1200 mg/kg). In complete feed for cattle (except calves) free gossypol limit is 500 mg/kg, in sheep and goats complete feed (except lamb and kids) the limit is 300 mg/kg.

There are many commercial varieties of cotton and, as a result, the free gossypol content in the raw cottonseed can be highly variable, but information on the level of contamination in whole cottonseed and their derivatives is very scarce. Following a call from EFSA for data on levels of gossypol in whole or processed cottonseed little information could be collected from two Member Countries that showed a gossypol content ranging from 100 to 8416 mg/kg, but it

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was reported that whole cottonseed can contain more than 14,000 mg/kg of total and free gossypol (EFSA, 2008). By now it is recognized that there is a lack of data on gossypol content (free and bound) in feed materials used for livestock in the EU. In addition, gossypol is transferred to edible tissues including muscle and offal of ruminants, poultry, and fish. It is also transferred into eggs and probably into cow's milk, as it is transferred to milk in rats. However, there is very little quantitative information on transfer rates (EFSA, 2008).

The present study started in 2013 within a quality control program of the Consortium of *Parmigiano Reggiano* cheese that aimed to evaluate the quality standards of feed manufactured by feed industries. Feed industries are qualified according to standards of the *List of feed and forages producers for dairy cows producing milk to Parmigiano Reggiano PDO cheese production*. One of the objective was to evaluate the efficiency of the procedures used to avoid cotton cross contamination in some industrial feed mills that produce complementary feed for lactating cows. Free gossypol presence was used at this point of the study as a marker for cotton contamination. Following the first results in which the presence of free gossypol was shown in samples collected in feed mills with very strict procedure to prevent cross contamination, the study was focused on the investigation of the presence of free gossypol in commercial complementary feed not containing cotton.

Materials and Methods

From March 2013 to January 2014 a total of

50 samples (2 kg each) of commercial complementary feed for lactating cows were collected in 29 plants; all samples were declared, as reported in the label, as cotton free; the type and the batch of the feed to be sampled were chosen random between those available at the moment of visiting the plant. The real composition of the feed was verified, in the feed mill, by comparing list of ingredients reported in the label with the production data of the batch that was sampled. On a total of 50 samples, 17 were sampled in *cotton free* plants (plants in which cotton was not used as raw material since more than one year) and 33 were sampled in plants where cotton was usually or occasionally used as ingredient. Samples were transported to the laboratory within 24 hours from sampling.

Analytical method

The samples (20 g) were extracted with acetonitrile high performance liquid chromatography (HPLC) grade (100 mL); after shaking for 60 min, the raw extracts were filtered through a folded filter paper. The solutions were diluted 1:100 with acetonitrile and transferred in a vial for HPLC. LC-MS/MS analysis was performed by a 6430 Triple Quad MS (Agilent Technologies, Santa Clara, CA, USA) equipped with an electrospray interface and a 1290 Infinity separation module. The separation was achieved on a ZORBAX SB-C18 (50 mm-2.1mm I.D.-1.8 mm) column with a pre-column filter (0.3 mm). Gradient elution was performed using eluent A (0.1% formic acid) and eluent B (0.1% formic acid in acetonitrile). The total run time was 3.5 min. The following parameters were set: flow rate 0.4 mL min⁻¹, column temperature 40°C, injection volume 10 L. Quantification was carried out by the external standard method in MRM mode (ESI negative) using the following transitions: 517.18→231.1 (Quantifier); 517.18→471.2 (I Qualifier); 517.18→489.2 (II Qualifier). MS/MS parameters were: capillary 4000 V, gas temperature 350°C, gas flow 10 L min⁻¹, nebulizer 35 psi, dwell time 200 ms, fragmentor 215 V, collision energy 40 V (Quantifier), 36 V (I Qualifier), 24 V (II Qualifier), cell acceleration 7 V. Statistical analysis to compare concentration of free gossypol in samples from *cotton free* plants and in the other plants was performed by T test.

Results

The comparison between composition declared in the label by manufacturers and the production data of the batch of feed sampled showed a clear overlapping.

Total prevalence of free gossypol positive samples resulted 76% (38 out of 50 samples):

free gossypol contamination resulted under the detection limit of the technique (4 mg/kg) in 12 out of 50 samples analyzed and ranged from 4 to 20 mg/kg in 28 samples. In 10 samples the level of free gossypol ranged from 20 mg/kg to 29.5 mg/kg. Data are shown in Figure 1. Average contamination of samples was 12.2±9.2 SD mg/kg.

Free gossypol was detected in 11 out of 17 (64.7%) feed samples collected in *cotton free* plants, and in 27 out of 33 (81.8%) feed samples collected in plants where cotton was used; in *cotton free* plants free gossypol concentration resulted in the range <4 mg/kg–29.5 mg/kg with an average concentration of 14.0±12.0 SD mg/kg. In others plants concentration of free gossypol resulted in the range <4 mg/kg–27.1 mg/kg with an average concentration of 11.3±7.4 SD mg/kg. No significant difference was shown in the prevalence of positive samples collected in feed mills that use or not use cotton as feed material.

The T test showed no significant difference (P=0.571) in free gossypol concentration between feed produced in *cotton free* plants and in plants where cotton is used as feed material.

Discussion

Free gossypol was detected in 38 out of 50 complementary feed samples analysed and in 10 samples level of free gossypol was >20 mg/kg; no maximum limit exist for free gossypol in complementary feed, but a limit of 20 mg/kg exists for feed raw materials that are used for feed manufacturing, with the exception of cottonseeds, cottonseed meal and cot-

tonseed cake in which a higher content is tolerated. Since all complementary feed collected did not contain cotton, it could be argued that in samples exceeding 20 mg/kg of free gossypol one or more than one feed raw materials used contained free gossypol at levels higher than the legal limit of 20 mg/kg reported by EC Regulation 574/2011 (European Commission, 2011), or that a source of cotton cross contaminated the sample. Moreover results showed no significant differences in *cotton free* feed mills and in other plants both in the prevalence of positive samples and in the average concentration of free gossypol indicating that, probably, feed materials are cross contaminated before entering the feed mill.

The legal limit of 500 mg/kg was fixed for complete feed for cattle. Considering that a maximum amount of 60% of complementary feed could be fed to dairy cows (10-12 kg/day), it could be deduced that maximum daily assumption of free gossypol (considering 29.5 mg/kg the highest value detected in complementary feed x 12 kg/die for cow) would be of 354 mg for a cow, corresponding to about 17.70 mg/kg of complete feed (considering a total dry matter of 20 kg).

EFSA reported that an intake of up to 40 mg/kg body weight (BW) resulted in any apparent adverse clinical effects. On the other hand, other studies showed that effects on dairy cows and heifers were found even at levels of intake below this dose: an intake of free gossypol at a level of 16-32 mg/kg BW per day, increased erythrocyte fragility. No effects on erythrocytes were observed at exposure levels of 0 and 3 mg free gossypol/kg BW per day. No other adverse effects were observed (Mena *et al.*, 2004).

Other studies showed that an intake of free

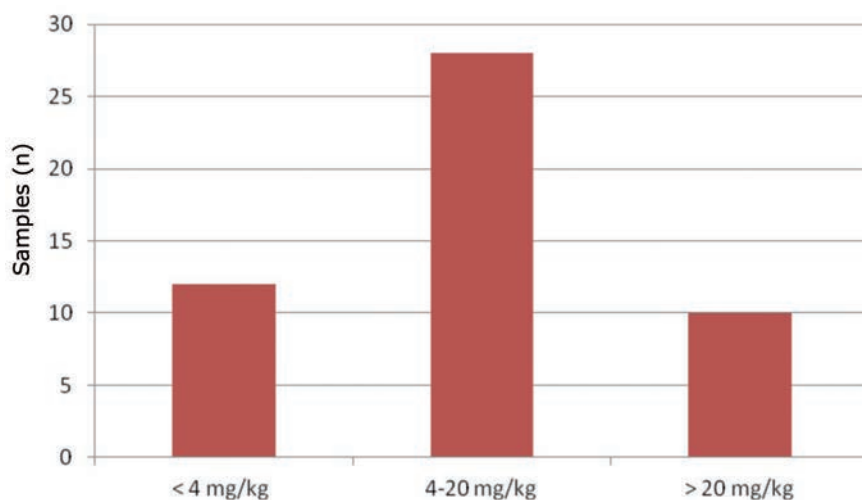


Figure 1. Distribution of free gossypol concentration in commercial complementary feed for dairy cows.

gossypol up to 40 mg/kg BW did not affect follicle and corpus luteum development in heifers (Coscioni *et al.*, 2003a, 2003b; Villaseñor *et al.*, 2003). On the contrary, heifers, exposed to 0, 17.8 or 36.8 mg free gossypol/kg BW per day fed for 70 days before superovulation and embryo collection, showed an increased number of degenerated embryos and reduced blastocyst development at the highest dosage level (Villaseñor *et al.*, 2008). Considering the average BW of a cow (about 630 kg) and considering a maximum daily intake of 354 mg of free gossypol, an intake of 0.56 mg free gossypol/kg BW can be calculated, far from the level that showed adverse effects.

Conclusions

In conclusion, free gossypol content detected in the present study allows considering the complementary feed for dairy cows collected not at risk. On the other hand, the presence of free gossypol in *cotton free* complementary feed, probably due to cross contamination of feed raw materials upstream of the feed mill, should be further investigated.

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