Journal of Zhejiang University-SCIENCE B (Biomedicine & Biotechnology)
ISSN 1673-1581 (Print); ISSN 1862-1783 (Online)
www.jzus.zju.edu.cn; www.springerlink.com
E-mail: jzus@zju.edu.cn

Pharmacokinetic comparison of gossypol isomers in cattle: transfer from diet to plasma and degradation by rumen microbes*#

Chao-hua TANG[1,2], Jia LIU[1,3], Qing-yu ZHAO[1,2], Jun-min ZHANG†‡[1,2]

[1]*State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing 100193, China*

[2]*Scientific Observing and Experiment Station of Animal Genetic Resources and Nutrition in North China, Ministry of Agriculture and Rural Affairs, Beijing 100193, China*

[3]*College of Pastoral Agriculture Science and Technology, Lanzhou University, Lanzhou 730000, China*

†E-mail: zhjmxms@sina.com

Received June 1, 2017; Revision accepted Sept. 14, 2017; Crosschecked May 18, 2018

Abstract: A pharmacokinetic comparison of gossypol isomers in cattle was made by investigating their transfer from ingested food to plasma and their degradation by rumen microbes. The gossypol isomers in whole cottonseed, plasma, and rumen fluid were determined by high-performance liquid chromatography (HPLC). The intakes of (+)- and (−)-gossypol by cows on three farms were about 5.6–8.5 and 3.8–5.9 g/(d·herd), respectively. The plasma gossypol concentrations increased as ingestion increased and ranged from 0.31 to 0.48 μg/ml for the (+) form and from 0.39 to 0.59 μg/ml for the (−) form. The (+) form was slightly predominant (58.8%–59.8%) in the gossypol ingested by the cows, whereas the (−) form predominated (54.6%–55.9%) in the plasma. An in vitro study showed that at 6 h, 67.4% and 85.7% of the (−)-gossypol were degraded in 500 and 1000 μg/g treatment groups, respectively, and these increased to 83.6% and 92.5%, respectively, at 12 h. The regularity of the degradation of (+)-gossypol was similar to that of (−)-gossypol. These results showed that (−)-gossypol may be more persistent than (+)-gossypol in plasma, and that the degradation of the gossypol isomers in the rumen is rapid and not enantioselective in cattle.

Key words: Pharmacokinetics; Gossypol isomers; Cattle
https://doi.org/10.1631/jzus.B1700289 **CLC number:** S816.2

1 Introduction

Gossypol ($C_{30}H_{30}O_8$), a polyphenolic compound isolated mainly from cottonseed, is one of the factors that limit the application of cottonseed. A number of investigators have indicated that gossypol is toxic to monogastric animals as well as young ruminants (Wang et al., 2009). Generally, toxicological doses of gossypol are classified into three levels: (1) acute doses causing circulatory failure, (2) sub-chronic doses causing pulmonary edema, and (3) chronic doses causing symptoms of ill health and malnutrition (Abou-Donia, 1976). Mature ruminants are thought to be more tolerant of gossypol than monogastric animals. The high tolerance of gossypol by ruminants may be attributable to its detoxification in the rumen, which involves its binding to soluble proteins and degradation by rumen microbes (Reiser and Fu, 1962; Feng and Wang, 2011). It has been reported that 95.2% of the gossypol in cottonseed is degraded by rumen microbes during fermentation in vitro for 24 h (Feng and Wang, 2011). However, if the gossypol content in the diet is too high, it will surpass the rumen's

‡ Corresponding author

* Project supported by the National Natural Science Foundation of China (No. 31472123)

Electronic supplementary materials: The online version of this article (https://doi.org/10.1631/jzus.B1700289) contains supplementary materials, which are available to authorized users

ORCID: Jun-min ZHANG, https://orcid.org/0000-0002-8405-0536

ability to detoxify and cause clinical symptoms of poisoning (Wang et al., 2009).

Gossypol exists as (+) and (−) optical isomers because the rotation about its internaphthyl bond is restricted. The biological properties of the gossypol isomers differ markedly. Generally, the (−)-enantiomer is considered more toxic than (+)-gossypol (Lordelo et al., 2005) as it binds more strongly to proteins (Wang et al., 1992) and is an active antispermatogenic agent. Pharmaceutical comparisons of the gossypol isomers have been made in humans and monogastric animals (Chen et al., 1987; Wu, 1989; Gamboa et al., 2001; Jia et al., 2008). A study of the pharmacokinetics of racemic gossypol in cattle showed that the half-lives of racemic gossypol were 67.0, 67.5, and 40.0 h, respectively, in three dairy cows administered 450 mg/kg (feed intake) gossypol for 7 d (Lin et al., 1991). Previous studies have shown that plasma gossypol isomer concentrations correlated positively with the dietary ingestion of free gossypol and that plasma (+)-gossypol concentrations were always lower than (−)-gossypol concentrations, even though the cows were fed a diet containing a higher proportion of the (+) isomer (Noftsger et al., 2000; Mena et al., 2001, 2004; Santos et al., 2002, 2003). However, many questions remain unanswered, such as whether the degradation of gossypol isomers by rumen microbes is enantioselective. Furthermore, the simultaneous determination of the gossypol isomers in the plasma and rumen fluid has been reported (Mena et al., 2001; Schneider et al., 2002; Bullock et al., 2010), but not validated.

Therefore, we first validated a high-performance liquid chromatography (HPLC) method for simultaneously determining the gossypol isomers in plasma and rumen fluid. The concentrations of gossypol isomers in ingested food and plasma in dairy cattle were then estimated on three farms, and the degradation of the isomers by rumen microbes was determined in vitro, to compare the pharmaceutical kinetics of the two isomers.

2 Materials and methods

2.1 Reagents and chemicals

The (±)-gossypol acetic acid standard (95%) was purchased from Sigma-Aldrich (Berlin, Germany). D-Alaninol was obtained from Aladdin® Industrial Corporation (Shanghai, China). The (±)-gossypol acetic acid used for the in vitro study and (−)-gossypol acetic acid were provided by Yangling Ciyuan Biotechnology Co., Ltd. (Shaanxi, China). *N*,*N*-Dimethylformamide (DMF), acetonitrile, KH_2PO_4, acetic acid, and the other chemicals used were of chromatographic grade or higher. Complex reagent (CR) was prepared as a mixture of 2% D-alaninol, 10% acetic acid, and 88% DMF (v/v).

2.2 Farm selection and sampling

Three dairy farms located in Shandong (Farm 1), Beijing (Farm 2), and Heilongjiang (Farm 3) Provinces, China, in which cottonseed was used as a feedstuff, were selected for sampling. The regular diets fed on the three farms, as well as the average daily feed intake and the respective average daily milk yield, are presented in Table S1. Representative cottonseed from each farm was collected and the intake of cottonseed per animal per day was recorded. Ten dairy cows from each farm were chosen for blood sampling. Blood was sampled from the jugular vein into a Vacutainer tube (BD Biosciences, San Jose, CA, USA) containing heparin anticoagulant, and centrifuged at 1500*g* for 10 min to separate the plasma, which was then stored at −20 °C until assayed. Animal care and procedures were approved and conducted under established standards of the Institute of Animal Science, Chinese Academy of Agricultural Sciences (Beijing, China).

2.3 In vitro fermentation

Gossypol-free total mixed ration (sampled from Farm 2 with cottonseed excluded) was weighed into 75 glass bottles (0.5 g per bottle), which were divided into three groups: a control group, a 500 μg/g gossypol group (racemic gossypol added at 500 μg/g), and a 1000 μg/g gossypol group (racemic gossypol added at 1000 μg/g). The degradation of the gossypol isomers by rumen microbes was assessed in vitro after incubation for 6, 12, 18, 24, and 48 h. To prepare the samples, buffer solution (pH 6.85) was formulated as described in Menke and Steingass (1988) and 50 ml was added to each bottle. Gossypol was dissolved in ethanol, and 1 ml of 250 or 500 μg/ml gossypol-ethanol solution was pipetted into the bottles, to final gossypol concentrations of 500 and 1000 μg/g.

Only ethanol (1 ml) was added to the bottles in the control group.

Three rumen-cannulated lactating Holstein dairy cows were used as the donor animals for the collection of rumen fluid. Rumen fluid obtained from the animals 1 h before the morning feed was filtered through four layers of gauze, mixed in equal proportions, incubated in a water-bath at 39 °C, and continuously purged with anaerobic CO_2 for later use. Air was removed by the infusion of nitrogen gas into the bottles, which were inoculated with 25 ml of filtered rumen fluid, purged with anaerobic nitrogen gas for 5 s, and sealed with a butyl rubber stopper and a screw cap. Needles were inserted into the caps to release the gas produced during fermentation. The bottles were then incubated at 39 °C. Fermentation was terminated in five bottles from each group by placing them in iced water after 6, 12, 18, 24, or 48 h. After incubation, the mixtures were transferred to tubes and centrifuged at 3500*g* for 5 min. The supernatants were collected and stored at −20 °C before analysis.

2.4 Determination of gossypol isomers in cottonseed, plasma, and rumen fluid

2.4.1 Pretreatment

The gossypol isomer content of cottonseed was determined as described previously (AOCS, 2009). The cottonseed was dehulled manually and the kernels dried for 4 h at 82 °C, cooled to room temperature, and ground with a grinding mill equipped with a 1.5-mm screen. The cottonseed kernel powder (0.2 g) was precisely weighed into a 15-ml tube to which 10 ml CR was added and mixed, and the mixture was derivatized at 95 °C for 30 min. The derivatized mixture was centrifuged at 8000*g* for 10 min, and the supernatant was filtered through a 0.2-μm filter (Waters Corporation, USA) before analysis.

The gossypol isomer content in the plasma and rumen fluid was determined using similar protocols. Briefly, 1 ml of plasma or rumen fluid was pipetted into a 2-ml tube and freeze-dried at −20 °C with a freeze-dryer (Epsilon 1/2-4, Christ, Germany). CR (1 ml) was added to the dried sample and the mixture was derivatized at 95 °C for 30 min and then centrifuged at 8000*g* for 10 min. The supernatant was collected and filtered through a 0.2-μm filter (Waters Corporation, USA) before analysis.

2.4.2 HPLC analysis

The analysis was conducted using a 1200 Infinity Series HPLC system (Agilent Technologies, USA). The filtered liquid (20 μl) was injected into a C18 column (Agilent Eclipse XDB-C18, 5 μm, 4.6 mm× 150 mm; Agilent Technologies, USA). The HPLC analysis was performed at a flow rate of 1 ml/min at a column temperature of 30 °C with a diode array detector (DAD) at 254 nm. The solvent system used was 22% KH_2PO_4-H_3PO_4 buffer (pH 3.0, 10 mmol/L) and 78% acetonitrile (v/v).

A calibration curve was constructed from three data points. The linearity of the HPLC method for the detection of (+)- and (−)-gossypol in plasma ranged from 0.2 to 1.5 μg/ml, according to the calibration curves Y=61.03X–3.1188 (R^2=0.994) and Y=69.288X–0.6698 (R^2=0.987), respectively. The calibration curves for (+)- and (−)-gossypol in rumen fluid were Y=88.001X–4.0668 (R^2=0.999) and Y=94.502X–5.5836 (R^2=0.999), respectively. The accuracy and precision of the method were evaluated by adding different concentrations of gossypol isomer standards to the matrix. The gossypol isomer concentrations in the samples were quantified with an external standard.

2.5 Data analysis

Data are displayed as the arithmetic mean± standard error (SE). To correlate the concentrations of gossypol in rumen fluid with the corresponding time points, a non-linear regression was carried out. The obtained R^2 values were used to predict such relationships. Tukey's tests were conducted to compare the differences among gossypol levels in rumen fluid at different time points in the in vitro study. P<0.05 was considered statistically significant. Statistical analysis was carried out using GraphPad PRISM software.

3 Results and discussion

3.1 Validation of the HPLC method

The separation of (+)- and (−)-gossypol in samples raises an important issue because these two enantiomers have different biological properties. Chiral separation and precolumn derivatization have been two of the major methods used to separate gossypol

isomers in previous studies (Cass et al., 1999, 2004; Hron et al., 1999). Cellulose coated onto APS silica was used under reversed-phase conditions to measure the enantiomeric ratios of gossypol in the cottonseed, flowers, and roots of *Gossipium* species (Cass et al., 2004). The gossypol isomers were separated well with reversed-phase HPLC combined with a Chiralcel® OD-RH chiral column, with a resolution value of 5.3 (Wang et al., 2012). However, none of these studies validated the method used for quantification. The limits of detection (LOD) for (+)- and (−)-gossypol were 2.5 and 1.5 μg/ml, respectively (Wang et al., 2012). These methods are unsuitable for the quantification of gossypol in samples such as plasma, in which the gossypol isomer concentrations are low.

In this work, we wanted to develop a more sensitive method using ultra-HPLC-tandem mass spectrometry (UHPLC-MS/MS). We successfully separated the gossypol isomers on a Chiralcel® OD-RH column using acetonitrile and 0.2% formic acid as the mobile phase (chromatography not shown). However, the detection of the isomers was nonlinear, which may have been caused by ionization failure (with electrospray ionization in negative mode (ESI−), although we also tried ESI in positive mode (ESI+)). Therefore, we reconsidered precolumn derivatization and HPLC. Although the use of precolumn derivatization combined with HPLC to measure the gossypol isomers in plasma and rumen fluid has been reported (Mena et al., 2001, 2004; Schneider et al., 2002; Bullock et al., 2010), those studies only briefly mentioned the principle, without its validation. When different kinds of derivatization reagents, reaction temperatures, and reaction time were compared, (*R*)-(−)-2-amino-1-propanol was reported to be an efficient derivatization reagent, and its reaction with gossypol isomers at 95 °C for 30 min converted all the gossypol isomers to derivatives (He, 2013). Therefore, these optimized conditions including the derivatization reagent [(*R*)-(−)-2-amino-1-propanol], temperature (95 °C), and time (30 min) were used in our study.

Here, we showed that gossypol isomers were separated well under the conditions used (retention time of 3.35 and 5.70 min for (+)- and (−)-gossypol, respectively; Fig. 1). The LOD (signal-to-noise ratio=3) was 50 ng/ml and the limit of quantification (LOQ; signal-to-noise ratio=10) was 160 ng/ml. The LOD indicated that the method is sensitive enough to detect the gossypol isomers in both plasma and rumen fluid. The accuracy and precision of the method were assessed by spiking blank samples with different concentrations of the gossypol isomers (Table 1). The recoveries ranged from 79.66% to 105.68% (intraday) and from 71.56% to 103.54% (interday). The coefficient of variation was between 1.2% and 10.7% for the intraday variation, and between 3.8% and 7.6% for the interday variation. These values demonstrate that the method is suitable for the quantification of gossypol in plasma and rumen fluid.

3.2 Content of gossypol isomers in cottonseed and plasma of dairy cows

The gossypol isomer content in cottonseed is presented in Table 2. The (+)- and (−)-gossypol contents in cottonseed were 3725–4283 and 2495–2971 mg/kg, respectively, which are consistent with previous studies (Alexander et al., 2008). The whole cottonseed intakes on the three farms surveyed were 2.00, 1.96, and 1.30 kg/d for each animal, respectively. The amounts of gossypol isomers ingested per animal per day were calculated based on the concentrations of gossypol isomers in the cottonseed and the whole cottonseed intakes. The cows on Farm 1 ingested the largest amount of gossypol (8493 mg of (+)-gossypol, 5930 mg of (−)-gossypol), followed by the animals on Farm 2 (7286 mg of (+)-gossypol, 4880 mg of (−)-gossypol) and Farm 3 (5576 mg of (+)-gossypol, 3801 mg of (−)-gossypol).

The gossypol isomer concentrations in plasma were higher in the animals with higher gossypol intake (Table 3), which is consistent with the results reported by Mena et al. (2001, 2004). In accordance with the amounts of gossypol isomers ingested, the gossypol isomer concentrations were highest in the plasma of the cows on Farm 1 (0.48 μg/ml (+)-gossypol, 0.59 μg/ml (−)-gossypol), and lowest in the plasma of the cows on Farm 3 (0.31 μg/ml (+)-gossypol, 0.39 μg/ml (−)-gossypol). The concentrations of plasma gossypol isomers in lactating cows are directly proportional to the intake of free gossypol (Mena et al., 2001, 2004) and this relationship has been used to establish limits on the amounts of cottonseed products that can be used safely (Santos et al., 2005).

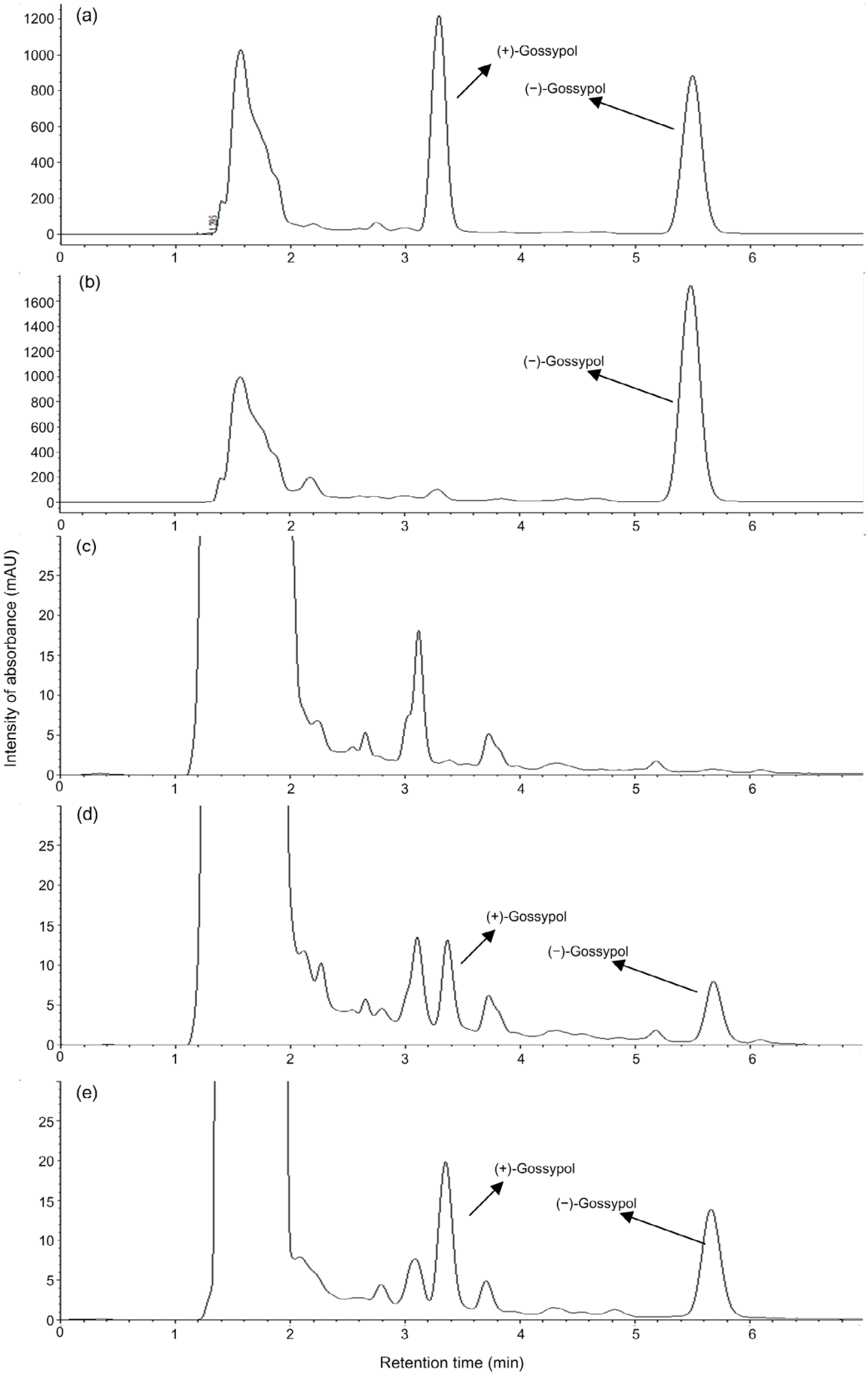

Fig. 1 Representative HPLC chromatograms

(a) (±)-Gossypol; (b) (−)-Gossypol standard; (c) Blank plasma sample; (d) Blank plasma samples fortified with 1.5 μg/ml racemic gossypol standard; (e) Positive plasma sample

Table 1 Recoveries and coefficient of variation (CV) of gossypol from plasma and rumen fluid samples spiked with gossypol isomer standards

Group	Gossypol isomer concentration (μg/ml)	Intraday (n=3)		Interday (n=3)	
		Recovery (%)	CV (%)	Recovery (%)	CV (%)
Plasma					
(+)-Gossypol	0.20	97.72	9.6	96.84	6.4
	0.75	94.68	5.8	100.37	3.9
	1.50	102.48	6.6	97.82	3.8
(−)-Gossypol	0.20	89.35	9.5	98.75	6.4
	0.75	95.64	6.5	103.54	6.8
	1.50	105.68	7.2	98.52	4.6
Rumen fluid					
(+)-Gossypol	0.20	83.28	10.7	71.56	7.6
	1.00	79.66	1.2	81.02	3.9
	3.00	83.49	4.6	78.27	4.9
(−)-Gossypol	0.20	83.15	5.6	77.56	5.1
	1.00	80.14	2.9	83.49	3.8
	3.00	84.44	6.3	77.19	5.9

CV: percentage of the standard deviation relative to the mean

Table 2 Whole cottonseed intakes, gossypol isomer content in whole cottonseed, and gossypol isomers ingested by the surveyed animals

Farm	Cottonseed intake* (kg/(d·herd))	Gossypol isomer content in cottonseed (mg/kg)			Gossypol isomer intake* (mg/(d·herd))		
		(+)	(−)	(±)	(+)	(−)	(±)
Farm 1	2.00±0.16	4255	2971	7226	8493±688	5930±480	14 423±1168
Farm 2	1.96±0.17	3725	2495	6220	7286±630	4880±422	12 166±1052
Farm 3	1.30±0.12	4283	2919	7203	5576±518	3801±353	9377±872

(+): (+)-gossypol; (−): (−)-gossypol; (±): (±)-gossypol. * Data were presented as mean±SE (n=10)

Table 3 Concentrations of gossypol isomers in the plasma of surveyed dairy cows

Farm	Plasma gossypol concentration (μg/ml)		
	(+)-Gossypol	(−)-Gossypol	(±)-Gossypol
Farm 1	0.48±0.24	0.59±0.30	1.07±0.54
Farm 2	0.45±0.08	0.58±0.11	1.03±0.18
Farm 3	0.31±0.07	0.39±0.09	0.7±0.16

Data were presented as mean±SE (n=10)

The guidelines for the use of plasma gossypol to establish safe levels of cotton by-products in the diets of lactating dairy cattle indicate that plasma concentrations of <5 μg/ml for (±)-gossypol and <3.5 μg/ml for (−)-gossypol are safe for animals (Calhoun, 2011). The results of our study showed that both the (±)- and (−)-gossypol levels in the plasma were below these safe limits, indicating that the intake of gossypol on the three farms would not have any adverse effects on the animals.

3.3 Ratios of gossypol isomers in cottonseed and plasma of dairy cows

The ratios of (+):(−) gossypol isomers in both whole cottonseed and plasma were calculated (Table 4). The ratio in whole cottonseed was between 1.43 and 1.49, which is consistent with the results of Stipanovic et al. (2005) that showed that (+)-gossypol usually predominates slightly in cottonseed. The ratio of the (+):(−) gossypol isomers in plasma was reversed, and was between 0.79 and 0.83, indicating that the (−)-gossypol concentration was higher. The reversal of the (+):(−) gossypol ratio in the plasma observed in this study is consistent with reports in the literature (Noftsger et al., 2000; Mena et al., 2001, 2004; Santos et al., 2002, 2003) which have shown that the concentrations of (−)-gossypol in the plasma were higher than those of (+)-gossypol in dairy cattle fed a diet containing a greater percentage of (+)-gossypol.

Table 4 Ratios of gossypol isomers in whole cottonseed and plasma of surveyed dairy cows

Farm	(+):(−) Gossypol isomers	
	Whole cotton seed	Plasma*
Farm 1	1.43	0.83±0.08
Farm 2	1.49	0.79±0.10
Farm 3	1.47	0.80±0.09

* Data were presented as mean±SE (n=10)

The absorption, distribution, biotransformation, and elimination of gossypol isomers in humans and monogastric animals have been investigated (Chen et al., 1987; Wu, 1989; Gamboa et al., 2001; Jia et al., 2008). The oral bioavailability of (−)-gossypol (17.6%) is slightly higher than that of racemic gossypol (12.2%) in mice, suggesting that (−)-gossypol is more bioavailable than (+)-gossypol (Jia et al., 2008). Similar tissue distributions of (+)- and (−)-gossypol were found in rats (Chen et al., 1987). However, in broiler chickens, the proportions of (−)-gossypol in the plasma (26.7%), kidney (25.6%), muscle (19.1%), and liver (16.0%) were all lower than those of (+)-gossypol (Gamboa et al., 2001). Pharmacokinetic studies have shown that the half-lives of (+)- and (−)-gossypol were 133 and 4.55 h, respectively, in humans (Wu, 1989), and 7.80 and 3.96 h, respectively, in rats (Chen et al., 1987). These results imply that (+)-gossypol is more slowly eliminated than (−)-gossypol. In ruminants, the half-lives of racemic gossypol were 67.0, 67.5, and 40.0 h, respectively, in three dairy cows administered 450 mg/kg (feed) gossypol for 7 d (Lin et al., 1991). The results of our own and other studies showed that the concentrations of (−)-gossypol in the plasma were higher than those of (+)-gossypol in cattle fed a diet containing a greater percentage of (+)-gossypol (Noftsger et al., 2000; Mena et al., 2001, 2004; Santos et al., 2002, 2003). Note that the elimination of (−)-gossypol appeared to take longer than that of racemic gossypol in mice (Jia et al., 2008). Logically, the binding of (−)-gossypol to protein may prevent its clearance from the bloodstream as the unbound free molecule, which may explain the higher plasma concentrations of (−)-gossypol than that of (+)-gossypol. Whether this difference can be attributed to species variation, especially the variation between monogastric animals and ruminants, requires further investigation.

3.4 Gossypol isomers degraded by rumen microbes

Non-linear regression analysis showed that gossypol levels (Y) in the rumen fluid were correlated negatively with fermentation time (X) during the in vitro study (R^2>0.99; Fig. 2). Tukey's tests showed that concentrations of the two isomers in rumen fluid at 6 h in both groups were significantly decreased compared with those at 0 h. Although there was some variability in the concentrations in samples taken at 12, 18, 24, and 48 h, there was no great change in the concentrations with time (P>0.05). These results were similar to those of a previous study which showed that gossypol isomers remained at stable levels at 4, 8, and 12 h during the in vitro fermentation of cottonseed (Schneider et al., 2002). The percentages of gossypol isomers degraded by rumen microbes at different time points during in vitro fermentation are presented in Fig. 3. At 6 h, 67.4% and 85.7% of (−)-gossypol were degraded in the 500 and 1000 μg/g groups, respectively, which increased to 83.6% and 92.5%, respectively, at 12 h. From 12 to 48 h, the degradation rates varied slightly. The degradation dynamics of (+)-gossypol is similar to those of (−)-gossypol. The degradation of gossypol by rumen microbes partly explains the high tolerance of gossypol among ruminants. Our results demonstrated the strong degradation of gossypol by rumen microbes and were consistent with those of a previous study in which 95.2% of the gossypol in cottonseed was degraded after in vitro fermentation for 24 h (Feng and Wang, 2011). Our in vitro study indicated that the degradation of gossypol by rumen microbes occurs very soon during fermentation, possibly within 6 h, and that this degradation is not enantioselective.

4 Conclusions

In this study, we found that the gossypol in plasma showed a slight predominance of the (−)-form, although dairy cows in the three farms surveyed were all fed cottonseed with a higher proportion of (+)-gossypol, indicating the different metabolism kinetics of the two isomers in ruminants. An in vitro study showed that gossypol was degraded rapidly by rumen microbes and this degradation is not enantioselective. Further studies exploring whether the absorption of gossypol isomers in the gastrointestinal tract is enantioselective would be of great value.

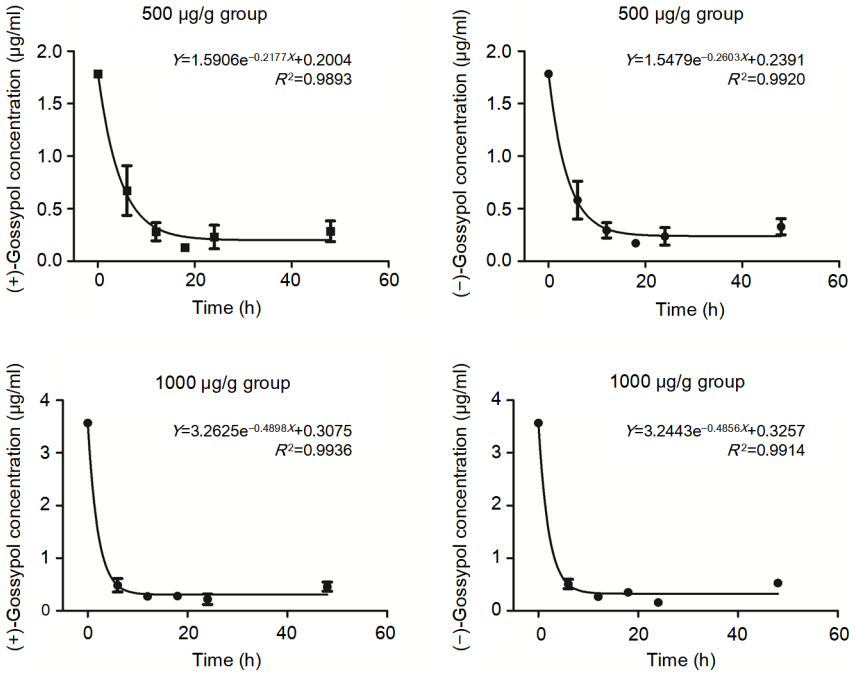

Fig. 2 Non-linear regression analysis of time (*X*) vs. gossypol levels (*Y*) in rumen fluid during in vitro fermentation (*n*=5, mean±SE)

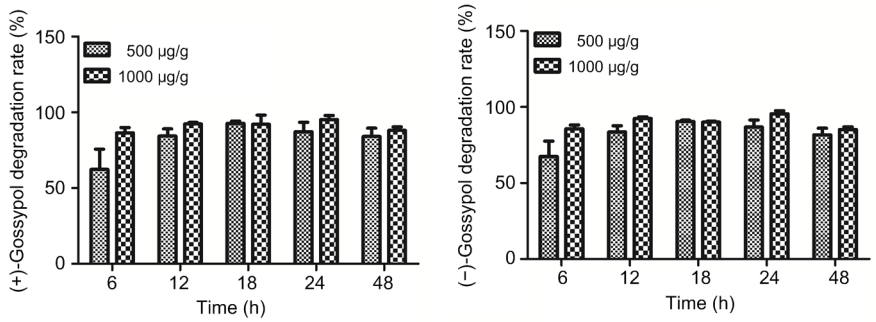

Fig. 3 Percentage of gossypol isomers degraded by rumen microbes at different time points during in vitro fermentation (*n*=5, mean±SE)

Compliance with ethics guidelines

Chao-hua TANG, Jia LIU, Qing-yu ZHAO, and Jun-min ZHANG declare that they have no conflict of interest.

All institutional and national guidelines for the care and use of laboratory animals were followed.

References

Abou-Donia MB, 1976. Physiological effects and metabolism of gossypol. In: Gunther FA, Gunther JD (Eds.), Residue Reviews, Vol. 61. Springer, New York, p.125-160. https://doi.org/10.1007/978-1-4613-9401-3_5

Alexander J, Andersson HC, Bernhoft A, et al., 2008. Gossypol as undesirable substance in animal feed: scientific opinion of the panel on contaminants in the food chain. *EFSA J*, 6(9):1-55.

AOCS (American Oil Chemists Society), 2009. Recommended Practice Ba 8a-99: Determination of (+)-, (−)-, and Total Gossypol in Cottonseed, Cottonseed Meats, and Meal by HPLC. https://www.aocs.org/attain-lab-services/methods/methods/method-detail?productId=118850 [Accessed on June 1, 2017].

Bullock SL, Hewitt DG, Stanko RL, et al., 2010. Plasma gossypol dynamics in white-tailed deer: implications for whole cottonseed as a supplemental feed. *Small Rumin Res*, 93(2-3):165-170. https://doi.org/10.1016/j.smallrumres.2010.05.018

Calhoun MC, 2011. Cottonseed Meal and Whole Cottonseed: Optimizing Their Use in Dairy Cattle Rations. Texas Agricultural Experiment Station, Texas A&M University System, San Angelo, Texas. http://cottonseed.org/publications/Cottonseed%20Meal%20and%20Whole%20Cottonseed.pdf [Accessed on June 1, 2017].

Cass QB, Bassi AL, Matlin SA, 1999. First direct resolution of gossypol enantiomers on a chiral high-performance liquid

chromatography phase. *Chirality*, 11(1):46-49. https://doi.org/10.1002/(SICI)1520-636X(1999)11:1<46::AID-CHIR8>3.0.CO;2-I

Cass QB, Oliveira RV, de Pietro AV, 2004. Determination of gossypol enantiomer ratio in cotton plants by chiral higher-performance liquid chromatography. *J Agric Food Chem*, 52(19):5822-5827. https://doi.org/10.1021/jf049626p

Chen QQ, Chen H, Lei HP, 1987. Comparative study on the metabolism of optical gossypol in rats. *J Ethnopharmacol*, 20(1):31-37. https://doi.org/10.1016/0378-8741(87)90116-4

Feng Y, Wang Y, 2011. The nutrition value and metabolism of cottonseed in ruminants. *Feed Res*, 4:13-16 (in Chinese).

Gamboa DA, Calhoun MC, Kuhlmann SW, et al., 2001. Tissue distribution of gossypol enantiomers in broilers fed various cottonseed meals. *Poult Sci*, 80(7):920-925. https://doi.org/10.1093/ps/80.7.920

He XW, 2013. Quantitative Analysis Method of Gossypol Enantiomers and Research of Their Metabolic in Rats. MS Thesis, Xinjiang Medical University, Xinjiang Uygur Autonomous Region, China.

Hron R, Kim H, Calhoun M, et al., 1999. Determination of (+)-, (−)-, and total gossypol in cottonseed by high-performance liquid chromatography. *J Am Oil Chem Soc*, 76(11):1351-1355. https://doi.org/10.1007/s11746-999-0149-5

Jia L, Coward LC, Kerstner-Wood CD, et al., 2008. Comparison of pharmacokinetic and metabolic profiling among gossypol, apogossypol and apogossypol hexaacetate. *Cancer Chemother Pharmacol*, 61(1):63-73. https://doi.org/10.1007/s00280-007-0446-3

Lin YC, Nuber DC, Gu Y, et al., 1991. Gossypol pharmacokinetics in mid-lactation Brown Swiss dairy cows. *Vet Res Commun*, 15(5):379-385. https://doi.org/10.1007/BF00366995

Lordelo MM, Davis AJ, Calhoun MC, et al., 2005. Relative toxicity of gossypol enantiomers in broilers. *Poult Sci*, 84(9):1376-1382. https://doi.org/10.1093/ps/84.9.1376

Mena H, Santos JEP, Huber JT, et al., 2001. The effects of feeding varying amounts of gossypol from whole cottonseed and cottonseed meal in lactating dairy cows. *J Dairy Sci*, 84(10):2231-2239. https://doi.org/10.3168/jds.S0022-0302(01)74670-X

Mena H, Santos JEP, Huber JT, et al., 2004. The effects of varying gossypol intake from whole cottonseed and cottonseed meal on lactation and blood parameters in lactating dairy cows. *J Dairy Sci*, 87(8):2506-2518. https://doi.org/10.3168/jds.S0022-0302(04)73375-5

Menke KH, Steingass H, 1988. Estimation of the energetic feed value obtained from chemical analysis and in vitro gas production using rumen fluid. *Anim Res Dev*, 28(1):7-55.

Noftsger SM, Hopkins BA, Diaz DE, et al., 2000. Effect of whole and expanded-expelled cottonseed on milk yield and blood gossypol. *J Dairy Sci*, 83(11):2539-2547. https://doi.org/10.3168/jds.S0022-0302(00)75146-0

Reiser R, Fu HC, 1962. The mechanism of gossypol detoxification by ruminant animals. *J Nutr*, 76(2):215-218. https://doi.org/10.1093/jn/76.2.215

Santos JEP, Villaseñor M, de Peters EJ, et al., 2002. Type of cottonseed and level of gossypol in diets of lactating dairy cows: effects on lactation performance and plasma gossypol. *J Dairy Sci*, 85(6):1491-1501. https://doi.org/10.3168/jds.S0022-0302(02)74218-5

Santos JEP, Villaseñor M, Robinson PH, et al., 2003. Type of cottonseed and level of gossypol in diets of lactating dairy cows: plasma gossypol, health, and reproductive performance. *J Dairy Sci*, 86(3):892-905. https://doi.org/10.3168/jds.S0022-0302(03)73672-8

Santos JEP, Mena H, Huber JT, et al., 2005. Effects of source of gossypol and supplemental iron on plasma gossypol in Holstein steers. *J Dairy Sci*, 88(10):3563-3574. https://doi.org/10.3168/jds.S0022-0302(05)73041-1

Schneider IC, Ames ML, Rasmussen MA, et al., 2002. Fermentation of cottonseed and other feedstuffs in cattle rumen fluid. *J Agric Food Chem*, 50(8):2267-2273. https://doi.org/10.1021/jf010783n

Stipanovic RD, Puckhaber LS, Bell AA, et al., 2005. Occurrence of (+)- and (−)-gossypol in wild species of cotton and in *Gossypium hirsutum* var. *marie-galante* (Watt) Hutchinson. *J Agric Food Chem*, 53(16):6266-6271. https://doi.org/10.1021/jf050702d

Wang JM, Tao L, Wu XL, et al., 1992. Differential binding of (+) and (−) gossypol to plasma protein and their entry into rat testis. *J Reprod Fertil*, 95(1):277-282. https://doi.org/10.1530/jrf.0.0950277

Wang S, Tang L,Wang Y, 2012. RP-HPLC chiral separation of gossypol acetic acid. *J Guangdong Pharm Univ*, 28(4):406-409 (in Chinese).

Wang X, Howell CP, Chen F, et al., 2009. Gossypol—a polyphenolic compound from cotton plant. *Adv Food Nutr Res*, 58:215-263. https://doi.org/10.1016/S1043-4526(09)58006-0

Wu D, 1989. An overview of the clinical pharmacology and therapeutic potential of gossypol as a male contraceptive agent and in gynaecological disease. *Drugs*, 38(3):333-341. https://doi.org/10.2165/00003495-198938030-00001

List of electronic supplementary materials

Table S1 Regular diets fed on the respective farms, as well as the average daily feed intake and the respective average daily milk yield (mean±SE, n=10)

中文概要

题　目：棉酚旋光异构体在牛体内代谢动力学研究：从日

粮向血浆中转移及瘤胃微生物的降解作用

目　的：比较研究棉酚旋光异构体从日粮向血浆中转移以及瘤胃微生物的降解作用。

创新点：从饲粮向血浆中吸收代谢以及瘤胃微生物降解角度揭示了棉酚旋光异构体在牛体内代谢差异，为棉酚毒理学评价、棉籽类产品在畜牧生产中合理应用提供科学依据。

方　法：利用高效液相色谱法（HPLC）测定全棉籽、血浆及瘤胃发酵液中棉酚旋光异构体含量。

结　论：三个调研牛场奶牛日粮右旋、左旋棉酚摄入量分别在 5.6~8.5 和 3.8~5.9 g/(d·herd) 之间。血浆棉酚含量与日粮棉酚摄入量呈正相关，右旋、左旋棉酚浓度分别在 0.31~0.48 和 0.39~0.59 μg/ml 之间。奶牛摄入棉酚中右旋棉酚比率（58.8%~59.8%）高于左旋棉酚，血浆中左旋棉酚比率（54.6%~55.9%）高于右旋棉酚。体外静态培养试验发现：500 和 1000 μg/g 组左旋棉酚的降解率在 6 小时后分别为 67.4%和 85.7%，12 小时后降解率分别升高到 83.6%和 92.5%；同时，棉酚旋光异构体之间降解规律相似。结果表明：相比于右旋棉酚，左旋棉酚在奶牛体内吸收率更高，瘤胃微生物对棉酚旋光异构体的降解没有选择性。

关键词：棉酚旋光异构体；代谢动力学；牛