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# The Effect of Long-term Grazing Sheep in the Cotton Stubble on the Blood Biochemicals and Free Gossypol Residues

R. Halida\*, Y. Aibibula

College of Grassland and Environmental Science, Xinjiang Agricultural University, Urumqi 830052, China.

**Abstract** The objective of this study was to investigate the effect of long-term grazing sheep in cotton stubble on variation of biochemistry components and free gossypol residual in the blood. The survey found that 15% ewe of grazing group suffered miscarriage or premature birth, compared with the captive group ewes without this phenomenon. Blood test result shows that the free gossypol concentration in serum of grazing group was significantly higher than in indoor captive feeding group ( $P < 0.01$ ). In order to ensure the health of sheep grazing on cotton stubble and improve breeding rate, there is a need to do further reasonable research of grazing and feeding methods.

**Key words** Cotton stubble, Sheep, Blood biochemicals, Free gossypol residual

## 1 Introduction

Cotton is one of the major crops in Xinjiang, China. Cotton is planted on 1.46 million hectares of land in the region, and about a third of the total arable land of Xinjiang. The cotton by-products are widely used as ruminant feed in agricultural areas, especially the cotton stalk is used in grazing sheep in the winter. In a previous study, we treated the harvested cotton stalk by chemical, thermophilic fermentation and inoculation with edible basidiomycete to improve the nutrient and the digestibility (Resalet *et al.* 2012 a, b). Also, by chemical [ $\text{Ca}(\text{OH})_2 + \text{urea}$ ], biological (inoculation with edible basidiomycete) treatment or making pellet feed, its free gossypol detoxification rate reached more than 50% (Halidai *et al.* 2013). Aibibula *et al.* (2012) report that the feed intake from the crushed cotton stalk ranged from 0.50 to 0.70 kg  $\cdot$  d<sup>-1</sup> and the dry matter intake was equivalent to 1.3–1.8% of the sheep's weight. Wei *et al.* (2003) report that the leaves, main stems and bolls of cotton stalks contain main nutrients for grazing ruminant. Liu *et al.* (1991) find that the sheep grazing all day on cotton stubble from December to March in the next year caused miscarriage and mortality's rate of ewes reached to 20% and 10%, respectively. Currently, some researches of Xinjiang cotton stalk as animal feed focus on component analysis and pretreatment methods, but there is not a study report about effects on sheep health and production performance before us. Therefore, the objective of this study was to investigate the effect of long-term grazing sheep in cotton stubble on variation of biochemistry components and free gossypol residual in the blood.

## 2 Materials and methods

In November 2013 to April the next year in Shaya County of Akesu

Prefecture in the northern part of the Tarim basin in Xinjiang, thirty sheep were fed with mixed indoor ration all day and another thirty sheep grazed the whole day on the cotton stubble, with no supplementary feeding in the night. Grazing 3 months later in mid-February 8:00 a.m., the blood samples were taken from the jugular vein using vacutainer tubes containing sodium heparin or no anticoagulant agent for separation of plasma and serum, respectively. Samples were immediately placed on ice, transported to the laboratory within 3 h of collection, and centrifuged at 2000  $\times$  g for 15 min in a refrigerated centrifuge at about 10°C. Plasma samples were analyzed for concentrations of glucose and urea. Serum samples were analyzed for their total protein, albumin, globulin, aspartate aminotransferase (AST) and gamma-glutamyl transferase (GGT), and blood routine indexes were also determined at the Hospital Diagnostic Laboratory using a clinical photo spectrometric analyzer. Serum was frozen at 20°C and later analyzed for free gossypol. Plasma free gossypol was analyzed by HPLC (Column: Reprosil 100–C18(3 mm  $\times$  250 mm, 5  $\mu$ m); Mobile phase: acetonitrile:H<sub>2</sub>O + 0.5% H<sub>3</sub>PO<sub>4</sub> 9:1; The wavelength for UV detection 235 nm).

## 3 Results and discussions

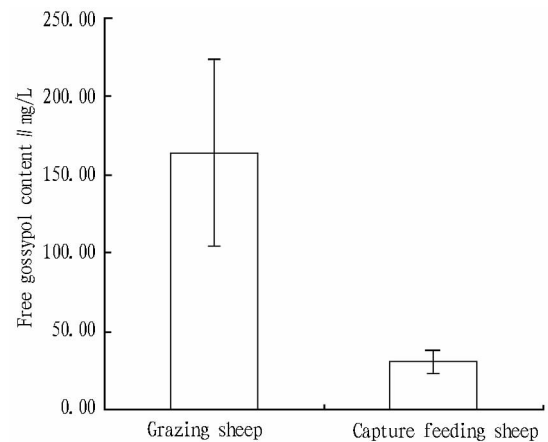
The survey found that 15% ewe of grazing group suffered miscarriage or premature birth, compared with the captive group ewes without this phenomenon. Blood test result shows that (Fig. 1), the free gossypol concentration in serum of grazing group was significantly higher than in indoor captive feeding group ( $P < 0.01$ ). Mena *et al.* (2004) observed that concentrations of plasma gossypol and its isomers were directly proportional to free gossypol intake. We observed that the free gossypol content of cotton stalks from stubble ranged from 150 mg/kg to 175 mg/kg when compared with the diet for capture feeding group which only contained 66.23 mg/kg of free gossypol. Serum free gossypol concentration higher for all day grazing sheep group might be related to more gossypol intake from cotton plant residues. White and red blood cells were

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\* Corresponding author. E-mail: halak08@163.com

not influenced by feeding methods (Table 1), but blood platelet and the hemoglobin concentration were decreased in grazing sheep ( $P < 0.05$ ). Serum concentrations of total proteins, albumin in grazing sheep were slightly higher than those of indoor sheep, but the triglyceride and total cholesterol concentration were decreased in grazing sheep. Serum AST and GGT significantly decreased in grazing sheep. These enzymes occurred in most cells, but were usually increased in serum when hepatocytes or muscle cells had suffered cellular damage (Turk and Casteel, 1997). Plasma glucose and urea concentrations of indoor sheep were higher than grazing sheep's ( $P < 0.05$ ), this effect might be related to more rumen degradable starches and nitrogen from concentration in mixed ration. Calcium and phosphorus concentration in serum of grazing sheep was higher than that of indoor feeding groups ( $P < 0.01$ ), but other minerals were the same between two groups of sheep.



**Fig. 1** Blood free gossypol concentration of sheep grazing on cotton stubble or capture feeding captive feeding

**Table 1** Blood routine and chemical parameters of sheep for grazing cotton stubble or indoor feeding TMR diet

Items	Grazing sheep N = 30	Capture feeding sheep N = 30	P-value
White Blood Cell// $10^9/L$	11.61 ± 0.48a	11.03 ± 0.02a	$P > 0.05$
Red Blood Cell// $10^{12}/L$	8.14 ± 0.06b	7.44 ± 0.48a	$P < 0.05$
Hemoglobin concentration//g/L	99.07 ± 0.71b	144.23 ± 0.64a	$P < 0.05$
Blood platelet// $10^9/L$	277.36 ± 0.23b	414.25 ± 0.01a	$P < 0.05$
Total protein//g/L	76.73 ± 0.22	62.93 ± 0.31	$P < 0.05$
Albumin //g/L	28.30 ± 0.13	20.50 ± 0.35	$P < 0.05$
Triglyceride//mmol/L	0.63 ± 0.03	0.33 ± 0.06	$P < 0.05$
Cholesterol//g/L	0.52 ± 0.07	0.29 ± 0.02	$P < 0.01$
High-density lipoprotein//mmol/L	0.81 ± 0.17	0.90 ± 0.13	$P < 0.05$
AST//U/L	168.23 ± 0.07	124.64 ± 0.16	$P < 0.01$
GGT//U/L	47.54 ± 0.69	37.00 ± 0.35	$P < 0.05$
GLU//mmol/L	2.99 ± 0.44	3.72 ± 0.21	$P < 0.05$
BUN//mmol/L	3.35 ± 0.33	7.13 ± 0.75	$P < 0.01$
Mg//mmol/L	2.55 ± 0.03	2.62 ± 0.01	$P > 0.01$
Ca//mmol/L	2.16 ± 0.04	2.80 ± 0.02	$P < 0.01$
P //mmol/L	2.24 ± 0.01	3.14 ± 0.03	$P < 0.01$
Fe//mmol/L	41.50 ± 0.35	39.65 ± 0.42	$P < 0.01$
Na//mmol/L	144.25 ± 0.58	148.27 ± 0.31	$P < 0.01$
Cl//mmol/L	97.37 ± 0.13	97.53 ± 0.19	$P > 0.05$
K//mmol/L	4.95 ± 0.14	4.38 ± 0.16	$P < 0.05$

## 4 Conclusions

These results suggest that the sheep all day and long grazing on cotton stubble makes blood free gossypol concentration doubling, and causes significant changes of conventional and biochemical indexes of blood in grazing sheep. Blood platelet, hemoglobin, AST, GGT, glucose, calcium and phosphorus concentrations decreased further effect on the health and performance of sheep. In order to ensure the health of sheep grazing on cotton stubble and improve breeding rate, there is a need to do further reasonable research of grazing and feeding methods.

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