Winter Grazing on Cotton Stubble Affects Grazing Behavior and Feed Intake of Ewes

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Research

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Abstract

**Background:** In this study, we investigated the effects of cotton (*Gossypium spp*) stubble grazing during winter on the feed intake, grazing behavior, and blood chemical parameters of Altay ewes in Xinjiang, China. Eighty adult and twenty-five young ewes grazed continuously on cotton stubble from sunrise to sunset for 90 d during the winter, without receiving any supplementary feed. We measured the biomass and components of cotton stubble before, and at 1 and 2 months of grazing; the ewes’ feed intake and grazing behavior at 1 and 2 months; and their blood parameters and immunity indices at the beginning and end of the grazing period.

**Results:** The biomass of cotton stalk was 5486.08 kg/hm² at the beginning and decreased to 4340.90 ± 917.10 kg/hm² and 3599.09 ± 689.56 kg/hm² at 1 and 2 months, respectively. The proportion of cotton leaves and boll shells in each component of cotton stalk decreased significantly (*P* < 0.05). At 1 and 2 months, the walking time of ewes was 93.58 ± 3.08 min and 115.99 ± 2.25 min and the distance covered was 6.8 ±0.92 km and 10.2 ± 2.30 km, respectively; both increased significantly during the late grazing stage (*P* < 0.05). At 1 and 2 months, the feed intake and dry matter digestibility did not differ significantly (*P* > 0.05). The intake of digestible energy and digestible crude protein levels at 1 and 2 months in sheep were 7.91~9.58 MJ/d and 47.30~69.04 g/d, respectively, which were lower than the nutritional requirements of grazing ewes. Compared with those before grazing, the blood platelet count and iron concentration in ewes at the end of grazing decreased significantly (*P* < 0.05), whereas the concentrations of free gossypol and aspartate aminotransferase significantly increased (*P* < 0.05). The most common activity of the ewe’s day was grazing, the grazing time of ewes accounted for over 60 % of the total pasture time.

**Conclusion:** Owing to the limited feed intake and digestibility of cotton stubble in winter, the demand for digestible energy and digestible crude protein in ewes cannot be satisfied by grazing alone.

**Background**

Crop stubble is often used for winter grazing by ruminants in the oasis agricultural area of Xinjiang, China. In some countries, crop stubble is considered an important part of the set of options required to maintain a seasonal supply of feed for the livestock.

However, animal production from crop stubble is limited owing to certain nutrient deficiencies in the available material. The low productivity of animals grazing on crop stubble is also associated with low digestibility [1] and resultant low intake[2]of the available material. Crop stubble has low levels of nitrogen (N) and available carbohydrates, high cell wall content, and poor digestibility[1] , rendering it unsuitable to meet the high nutrient requirements of productive sheep [3].

Cotton (*Gossypium spp*) is one of the major crops cultivated in Xinjiang, China and the annual cotton planting area is more than 2,000,000 ha. In this region, the dwarf close planting cultivation mode is often used for cotton, and the cotton stalks in the cotton stubble are widely used for sheep grazing during the
winter. Most of the farmers in the cotton areas of Xinjiang take the cotton stubble after harvest as the grazing land. From the perspective of feed value, the grazing land should be equipped with conditions such as highly suitable forages for feeding the corresponding livestock and sufficient forage nutrients.

Although the leaves and boll shells of cotton stalks contain some nutrients, the lignification degree of the whole plant is higher than that of other crop residues [4-5]. A previous study found that free gossypol (FG) concentrations in blood, liver, and kidney increased significantly and damaged the liver and kidneys after sheep were fed a diet containing 50% ground cotton stalk[6].

The feeding value of crop stubbles varies widely, and the feed intake of sheep has been reported to range from 4.4 to 9.8 MJ/sheep/day depending on the crop type, grazing intensity, and whether green summer plants have grown in the stubble [2].

Grazing crop residues need to be carefully managed because livestock will quickly consume the highest quality residues, which will affect subsequent grazing behavior. The objective of this study was to investigate the effect of cotton stubble grazing on grazing behavior, feed intake, and digestibility in the body of ewes, to provide scientific basis for the nutritional regulation of ewes in cotton stubble grazing.

**Material And Methods**

The study was conducted in the Dunkuotan Township (N 40°46′ ~ 42°35′, E 82°35′ ~ 84°17′), Kuqa City, Aksu prefecture, Xinjiang, China. The climate is warm, temperate, continental, dry with little precipitation. The temperatures reach 33 °C in summer and fall to -4 °C in winter, with large differences in annual and daily temperatures. The annual average temperature in this area is 11.4 °C, average precipitation is 65 mm/year, and the altitude varies from 930 to 1225 m.

Animals, diets and experimental design

The experiment was conducted from November to 2017 February. Ten Xinjiang Altay sheep (51.60 ± 9.90 kg) with similar body conditions were selected and, in November, we began to follow the flock of the sheep (105) for a 90 d grazing experiment. The total grazing land was 10.6 hm², and grazing took place from 1000 to 1900 h (Beijing time). The grazing method is fixed grazing, where in the sheep were herded into the grazing experimental area daily and allowed to feed freely, to rest in the barn at night, provided drinking water once at noon, and given no supplementary feeding after grazing. All sheep were treated with insect repellent before grazing, weighed on an empty stomach, and numbered (ear tag) before the study. The experiment can be divided into three stages – (a) before grazing on cotton stubble – when blood was collected and each experimental sheep was weighed before grazing, (b) early grazing - after one month, and (c) late grazing - after two months, when the aboveground biomass of cotton stubble was determined. During each grazing period, we selected 10 sheep to install dung bags for the determination of feed intake and digestibility after 1 and 2 months of grazing.

Sampling and laboratory analysis
Feed samples

We collected cotton stalk samples from the grazing area and separated cotton leaves, boll shells, slender stalks, and main stalks to calculate their proportions. The samples of each component were oven-dried at 60 °C for 48 h and passed through a 1-mm screen prior to chemical analyses.

Fecal samples

Fecal samples were collected from each ewe. The feces samples were mixed and weighed and a small amount of formaldehyde was added for preservation. Samples of feces were dried in a forced-air oven at 60 °C and ground in order to go through a 1-mm screen and stored for subsequent analyses.

Blood samples

Blood samples were drawn from ten ewes by puncture of the vein into vacutainer tubes at the beginning and end of the grazing period (at 0600 h). No anticoagulants were used in order to facilitate the separation of serum. Blood samples were centrifuged at 3000 × g for 20 min to harvest serum and were subsequently stored at -30 °C until required for analyses of blood composition and immunity indices.

Measurement and analysis

Aboveground biomass

Before, and after 1 and 2 months of grazing, five sample plots were selected in the cotton stubble grazing area and three quadrants were set in each plot to measure the aboveground biomass. The plant height and density of the cotton stalk were measured before grazing.

Chemical analyses

The plant samples were analyzed chemically for dry matter (DM), crude protein (CP), and ether extract (EE) content according to AOAC [7] procedures, and for neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) content we followed the method of Van Soest [8-9]. The FG content was determined using the phloroglucinol colorimetric method [10]. The chromium concentrations were measured using colorimetry [11]. We carried out acid insoluble ash (AIA) analysis using the modified 3 N HCl method by Keulen and Young[12].

Animal behavior

Ewes were fitted with GPS collars (Model 3300SL GPS unit with x-y motion sensors; Lotek Wireless, Newmarket, Ontario, Canada) and an Ice Tag activity monitor (Ice Robotics Ltd, Midlothian, UK) on the rear left leg. The GPS collars were used to estimate horizontal distance traveled and duration for which the head was in a ‘down’ position. The ‘head down’ determination from GPS collars arises from a motion/position sensor. The collars were scheduled to acquire a GPS fix every 5 min. Fixes were downloaded and post-differentially corrected using proprietary software (N4, Lotek Wireless). Corrected
fixes were then imported into Arc Map 9.3 (ESRI, Redlands, CA, USA). The x and y coordinates, in meters, were calculated for each fix. Only fixes within the boundary and buffer shape files were exported. Distance between consecutive fixes was calculated using Euclidean geometry. The area within the pasture did not markedly vary in elevation; therefore, vertical distance traveled was not computed. Time standing from Ice Tags encompasses both grazing/eating and non-grazing/eating periods, and ‘active’ is walking at a relatively fast pace presumably without grazing/eating.

Feed intake and digestibility

The intake of digestible dry matter (DM), CP, and energy was determined for ten ewes. At each grazing period, the ewes were dosed with a 20 g chromic oxide (Cr2O3) mixed pellet (containing 10 % Cr2O3, 80 % concentrate feed, and 10 % molasses) at 9:00 to 21:00 h. Fecal bags were used, and the fecal samples were continuously collected twice daily, morning and evening, after the 10 d pre-test period.

Acid insoluble ash was analyzed in stubble and fecal grab samples, an estimation of the digestibility coefficient was made assuming total recovery of AIA in feces. Digestibility values were obtained as follows:

\[
\text{AIA dry matter digestibility} = 100 - \left[ 100 \times \left( \frac{\% \text{ marker in diet}}{\% \text{ marker in feces}} \right) \right]
\]

Blood chemicals and immunity indices

Concentrations of glucose (Glu), thyroglobulin (TG), urea, low density lipoprotein (LDL), and high-density lipoprotein (HDL) in serum samples were determined using an Automatic Clinical Chemistry Analyzer (Sysmex XN-1000B3 automatic blood analyzer, Germany). The concentrations of IgG, IgA, and IgM in the blood samples were determined using single radial immunodiffusion (using a Hitachi 7170 automatic analyzer, Tokyo, Japan). The assays were conducted using the assay kits (Naijiang Jiancheng Institute of Bioengineering, Nanjiang, China).

Statistical analysis

Data for aboveground biomass, feed intake, grazing behavior, and digestibility were reported as least square means ± SEM. They were analyzed as a Latin square with a factorial treatment arrangement using SPSS v. 17.0 (IBM Corp., Armonk, NY, USA). Overall differences in treatment means were considered significant when p < 0.05.

Result

Biomass and composition of cotton stubble
Aboveground biomass and component composition of the cotton stalks during the grazing period are shown in Table 1. During the grazing period, the plant height of the cotton stalk on the cotton stubble did not change significantly. Before grazing, the above ground biomass and herbage allowance of the cotton stubble were 5486.08 kg/hm² and 9.23 kg/d, respectively. After grazing on cotton stubble for 2 months, the aboveground biomass and herbage allowance decreased to 3599.09 kg/hm² and 6.06 kg/d, respectively. Changes in the proportions of the composition of the cotton stalks were observed during the grazing period, with the highest decrease in the proportion of the cotton leaves, followed by that of the boll shells. The reduction in each part of the plant occurred in the following order: Leaves > Boll shells > Slender stalks > Main stalks. The proportion of slender stalks and main stalks gradually increased with the extension of grazing time respectively.

During the later stage of grazing, the CP content of aboveground biomass decreased slightly and the NDF, ADF, and ADL content increased, however, these changes between the grazing periods were not significant (P > 0.05). There was no significant difference in the mineral content of calcium (Ca), phosphorus (P), magnesium (Mg), potassium (K), sodium (Na), and zinc (Zn) in the cotton stalk between the beginning and end of grazing. However, iron (Fe) content at the end of grazing was 146.13 ± 2.30 mg/kg, which was a significant decrease from that at the beginning (P < 0.05). The concentration of FG in cotton stalk increased with the extension of the grazing period, and showed a significant difference between grazing times.

Grazing behavior
The daily time spent feeding, walking, standing, and ruminating by the ewes at each grazing stage is shown in Table 3. The most common activity of the ewe's day was grazing and grazing time increased with the extension of grazing time, but no significant difference between each grazing period (P > 0.05). In the early and later period of stubble grazing, the grazing time of ewes accounted for 64.33 and 68.60 % of the total grazing time, respectively. They spent significantly (P < 0.05) more time walking in the second stage (115.99 ± 2.25) than in first (93.58 ± 3.08). Standing time reached 11.50 min/d after 2 months of grazing, and accounted for 2.13 % of the total grazing time, which was significantly lower than that after 1 month (P < 0.05). Rumination time accounted for about 9 % of the total grazing time, but did not differ significantly during the whole grazing period (P > 0.05). The grazing behavior of the ewes during the entire grazing period followed the following order: feeding > walking > standing > ruminating. The total distance covered by ewes in 1 d increased with the increase in grazing time, and was significantly higher at 2 months after grazing than that at 1 month after grazing (P < 0.05).

Feed intake and digestibility
The Dry Mater Intake (DMI) and nutrient digestibility of ingested DM of ewes is shown in Table 4. The DMI of ewes during late grazing stages showed a slight increase but no significant difference (P > 0.05) from that in the early grazing stages, and the DM intake of body weight (BW) ranged from 3.93 in the first stage to 3.87 the second. Fecal excretion was not affected by grazing time, and was 0.670 ± 0.12 kg d⁻¹ and 0.724 ± 0.34 kg d⁻¹ during the early and late stages of grazing, respectively, with no significant difference during the total grazing period (P > 0.05). The digestibility of dry mater (DMD) of ewes in total
grazing period was 32.94 and 32.07 %, respectively. The digestibility of CP after grazing for 2 months was significantly higher than that after 1 month (P < 0.05). The digestible crude protein (DCP) content of ewes at early and late grazing stages was 47.30 and 69.04 g/kg, the digestible energy (DE) was 7.91 and 9.58 MJ/kg, respectively, and showed no significant difference with each grazing period (P > 0.05).

Blood metabolites and immune responses
There was a significant difference between the number of white cells and the number of platelets in the blood before and after grazing in cotton stubble (Table 5). Liver function index AST and gamma-glutamyl transferase (GGT) content after grazing was significantly higher than that before grazing (P < 0.05). Analysis of blood samples obtained at the end of the grazing period revealed a significant decrease in the Fe content (P < 0.05). Blood FG content was significantly higher than that before grazing.

At the end of grazing, the levels of blood immunoglobulin A (IgA), immunoglobulin M (IgM), and immunoglobulin G (IgG) were 2.39, 3.61, and 10.19 mg/mL, respectively (Table 6). Although these levels were higher than those obtained before grazing, the difference was not significant (P < 0.05).

Discussion
Xinjiang is one of the most important pastoral areas in China. Sheep account for more than 80 % of the total number of herbivorous livestock here. Although Xinjiang has a large area of natural grassland, overgrazing and global climate change have led to the degradation of most natural grasslands, and herders have to consider changing their traditional winter-feeding patterns. Large cotton plantations provide cotton products such as cotton stalk, cotton seed hulls, and cottonseed meal that serve as feed resources for animal husbandry in Xinjiang.

In northern Xinjiang, cotton is generally planted in the farming-pastoral junction area, whereas, in southern Xinjiang, it is planted in the oasis farming area; the cotton stubble leftover after harvesting is an important feeding resource for small ruminants in the both areas. In addition, cotton plantations in this area adopt the dwarf and high-density planting method, which results in a plant height of no more than 60-70 cm and is suitable for small ruminant grazing. Wei et al[4] and Xu [13] reported that before grazing the CP content of cotton leaves reached 17 %, but the ADL content was 2-3 times higher in parts other than the grain straws.

Forage availability seemed to affect grazing behavior. Our results show that the proportion of cotton leaf and boll shells in cotton stubble before grazing was more than 50 % of that at the end, and the amount of the edible herbage reduced from 9.23 kg sheep-1 d-1 at the beginning of the grazing period to 6.06 kg sheep-1 d-1 at the end. During grazing, the proportion of cotton leaves decreased followed by that of boll shells, indicating that cotton leaves had good palatability. Grazing and walking time of grazing sheep accounted for 64.3 and 17.3 % of the total pasture time, respectively, and showed a significant increase during the late grazing period. The difference in the quantity and quality of the plants available in the pasture produced significant changes in the grazing behavior of ewes and showed a significant increase
in grazing time and reduced ruminating time with the decrease in pasture availability [14]. There was no significant difference in the DMI and DMD in ewes between early and late grazing stages (P > 0.05).

The intake of DE and DCP in sheep grazing on cotton stubble was 7.91-9.58 and 47.30-69.04 g/d, which could not meet the daily requirements of 18.4-21.8 MJ/d and 90-130 g/d, respectively, recommended for 50 kg grazing ewes in the NRC[15] feeding standard. In this study, although feed intake did not differ during the entire grazing period, the nutrition digestibility showed several changes, e.g., increased CP and total DE, owing to the changes in the composition of the cotton stubble, such as the significantly decreased proportion of highly nutritious cotton leaves during the second stage of grazing. The increased intake of FG may also affect the digestion and absorption of proteins, fats, and other nutrients in the sheep [16].

Some studies have found that FG toxicity is associated with alterations in blood parameters[17]. Gossypol may combine with iron, inhibit the synthesis of respiratory enzymes, and generate excessive reactive oxygen species (ROS) in the mitochondria [10]. In the present study, the serum AST and GGT content increased, whereas the Fe content decreased in response to CS ingestion. Elevated AST and GGT activities were correlated with liver metabolism impairment.

Conclusions

Above ground biomass in cotton stubble reaches 5486.08 ± 766.34 kg/ha after fixed grazing, the proportion of cotton leaf and boll shells on the cotton stalk decreases, and the proportion of edible parts account for more than 50% of the plant. The grazing and walking time of ewes accounted for more than 80% of the pasture time, and the walking time increased significantly in the late grazing period covering a grazing distance of more than 10 km. In the early and late grazing periods, the DM digestibility was 32.94 and 32.07%, DE was 7.91 and 9.58 MJ/kg, and DCP intake was 47.30 and 69.04 g/kg, respectively, which do not meet the nutrient requirements of ewes according to the NRC feeding standards.

Cotton stubble widely used in winter grazing in Xinjiang. With the increase of grazing time and traveling distance, the intake of digestible nutrients is insufficient, and the excessive intake of free gossypol will affect the health of ewes. It is suggested to ensure healthy breeding by limiting grazing time and supplementary feeding.

Abbreviations

CP: Crude protein; NDF: Natural detergent fiber; ADF: Acid detergent fiber; ADL: Acid detergent lignin; Ca: Calcium; P: Phosphorus; Mg: Magnesium; K: Potassium; Na: Sodium; Fe: Iron; Zn: Zinc, Cr2O3: Chromic Oxide, Dry Mater Intake (DMI) Note: DMD: Dry Mater Intake; DCP: Digestible Crude Protein; DE:Digestible Energy,AST; aspartate aminotransferase; GGT: gamma-glutamyl transferase; Fe: Iron; NEFA:non estesterified fatty acid, IgA: immunoglobulin A, IgM: immunoglobulin B, IgG: immunoglobulin G
Declarations

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Authors’ contributions

H Rehemujiang: Conceptualization, methodology, software data curation, writing- Original draft preparation, A Yimamu: Supervision, validation, reviewing and editing the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article [and its supplementary information files].

Ethics approval and consent to participate

The study received approval from the institutional review board of Xinjiang agricultural University

Consent for publication

I would like to declare on behalf of my co-authors that the work described was original research that has not been published previously, and not under consideration for publication elsewhere, in whole or in part. All the authors listed have approved the manuscript that is enclosed.

Competing interests

No conflict of interest exits in the submission of this manuscript, and manuscript is approved by all authors for publication.

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### Tables

Table 1. Aboveground biomass and components of cotton stubble

|                          | Before Grazing | Early grazing stage | Late grazing stages | \(P\) value |
|--------------------------|----------------|---------------------|---------------------|-------------|
| Plant height (cm)        | 66.51 ± 6.70   | 61.77 ± 6.30        | 62.92 ± 6.20        | 0.07        |
| Stalk biomass (kg DM/hm\(^2\)) | 5486.08 ± 766.34\(^{a}\) | 4340.90 ± 917.1\(^{b}\) | 3599.09 ± 689.56\(^{c}\) | 0.01        |
| Herbage allowance (kg. sheep\(^{-1}\). d\(^{-1}\)) | 9.23 ± 1.23\(^{a}\) | 7.30 ± 1.54\(^{b}\) | 6.06 ± 1.16\(^{c}\) | 0.02        |

Component (% of DM mass)

| Component               | Before Grazing | Early grazing stage | Late grazing stages | \(P\) value |
|-------------------------|----------------|---------------------|---------------------|-------------|
| Leaf                    | 11.36 ± 2.59\(^{a}\) | 10.65 ± 3.49\(^{b}\) | 5.47 ± 1.37\(^{c}\) | 0.01        |
| Boll shells             | 40.11 ± 4.54\(^{a}\) | 38.79 ± 2.65\(^{b}\) | 36.83 ± 2.97\(^{c}\) | 0.04        |
| Slender stalks          | 17.49 ± 6.16\(^{a}\) | 16.10 ± 3.08\(^{b}\) | 18.94 ± 3.24\(^{c}\) | 0.01        |
| Main stalks             | 31.04 ± 3.75\(^{a}\) | 34.46 ± 3.59\(^{b}\) | 38.73 ± 3.95\(^{c}\) | 0.02        |

Note: In the same row, values with different small letter superscripts denote significant difference (\(P < 0.05\)), and same letter superscripts denote no significant difference (\(P > 0.05\)).

Table 2. Chemical composition and free gossypol (FG) content of whole cotton stalk
| Item       | Before Grazing | Early grazing stage | Late grazing stages | P value |
|------------|----------------|---------------------|---------------------|---------|
| CP g/kg    | 60.1 ± 5.7    | 59.2 ± 7.6          | 56.0 ± 2.8          | 0.18    |
| NDF g/kg   | 601.0 ± 4.3   | 612.0 ± 15.5        | 632.0 ± 15.8        | 0.03    |
| ADF g/kg   | 488.0 ± 14.1  | 497.2 ± 8.8         | 511.3 ± 9.4         | 0.13    |
| ADL g/kg   | 180.2 ± 1.7   | 183.5 ± 5.0         | 188.5 ± 56.6        | 0.31    |
| Ca g/kg    | 6.89 ± 0.03   | 652 ± 0.03          | 6.61 ± 0.02         | 0.13    |
| P g/kg     | 1.44 ± 0.01   | 1.43 ± 0.02         | 1.39 ± 0.02         | 0.10    |
| Mg g/kg    | 18.41 ± 0.04  | 17.35 ± 0.02        | 15.96 ± 0.04        | 0.01    |
| K g/kg     | 2.44 ± 0.02   | 2.40 ± 0.02         | 2.15 ± 0.01         | 0.02    |
| Na g/kg    | 1.45 ± 0.02   | 1.44 ± 0.01         | 1.43 ± 0.02         | 0.33    |
| Fe mg/g    | 188.29 ± 13.72| 177.07 ± 2.91       | 146.13 ± 2.30       | 0.01    |
| Zn mg/g    | 11.88 ± 0.40  | 12.78 ± 0.31        | 11.99 ± 0.37        | 0.21    |
| FG mg/kg   | 215.74 ± 2.20 | 210.65 ± 1.77       | 181.74 ± 1.12       | 0.01    |

Note: CP: Crude protein; NDF: Natural detergent fiber; ADF: Acid detergent fiber; ADL: Acid detergent lignin; Ca: Calcium; P: Phosphorus; Mg: Magnesium; K: Potassium; Na: Sodium; Fe: Iron; Zn: Zinc

Table 3. Grazing behavior of ewes on cotton stubble field
| Item                        | Early grazing stage | Late grazing stages | P value |
|-----------------------------|---------------------|---------------------|---------|
| Grazing behavior (min/d)    | 540.00 ± 5.31       | 569.00 ± 16.48      | 0.02    |
| Feeding                     | 347.38 ± 2.42       | 390.32 ± 13.42      | 0.12    |
| Walking                     | 93.58 ± 3.08        | 115.99 ± 2.25       | 0.04    |
| Standing                    | 46.17 ± 4.45        | 11.50 ± 2.70        | 0.01    |
| Ruminating                  | 52.87 ± 10.37       | 51.19 ± 27.39       | 0.24    |
| Proportion (%)              |                     |                     |         |
| Feeding                     | 64.33 ± 0.72        | 68.60 ± 1.15        | 0.01    |
| Walking                     | 17.33 ± 0.92        | 20.38 ± 0.58        | 0.01    |
| Standing                    | 8.55 ± 1.33         | 2.02 ± 0.69         | 0.03    |
| Ruminating                  | 9.79 ± 3.11         | 8.99 ± 7.03         | 0.29    |
| Distance traveled (km/d)    | 6.80 ± 0.92         | 10.20 ± 2.30        | 0.02    |

Table 4. Feed intake and digestibility of grazing ewes on cotton stubble
| Item                                      | Early grazing stage | Late grazing stages | P value |
|-------------------------------------------|---------------------|---------------------|---------|
| Feed intake and digestibility             |                     |                     |         |
| Daily feed intake (kg/d)                  | 2.03±0.29           | 2.36±0.14           | 0.29    |
| % of body weight                          | 3.93±0.03           | 3.87±0.04           | 0.10    |
| Fecal output (kg/d)                       | 0.670±0.12          | 0.724±0.34          | 0.34    |
| DMD (%)                                   | 32.94±2.88          | 32.07±1.34          | 0.79    |
| Nitrogen (N) intake and digestibility     |                     |                     |         |
| Intake (g/d)                              | 113.68±1.01         | 132.16±3.32         | 0.01    |
| Fecal (g/d)                               | 74.71±2.10          | 70.44±1.02          | 0.02    |
| Apparent N digestibility (%)              | 38.77±3.41          | 49.50±9.07          | 0.01    |
| Digestible Crude Protein (g/d)            | 47.30±0.48          | 69.04±5.65          | 0.04    |
| Total energy intake and digestibility     |                     |                     |         |
| intake (MJ)                               | 31.56±7.21          | 46.62±8.52          | 0.15    |
| Fecal (MJ)                                | 15.49±6.12          | 24.02±3.52          | 0.30    |
| Apparent digestibility (MJ)               | 50.98±4.92          | 47.58±2.58          | 0.27    |
| Digestible Energy(MJ/d)                   | 7.91±0.43           | 9.58±0.26           | 0.03    |

Note: DMD: Dry Mater Intake; DCP: Digestible Crude Protein; DE:Digestible Energy

Table 5. Blood composition and free gossypol (FG) content of grazing ewes in cotton stubble
| Item                     | Before Grazing | After grazing | P value |
|--------------------------|----------------|--------------|---------|
| White cell count $10^9/L$ | 15.44 ± 5.65   | 10.64 ± 2.13 | 0.02    |
| Red cell count $10^{12}/L$ | 8.06 ± 0.93    | 4.95 ± 3.46  | 0.04    |
| Platelet count $10^9/L$  | 605 ± 118.66   | 318.80 ± 57.03 | 0.01 |
| AST(U/L)                 | 81.18 ± 17.97  | 180.20 ± 2.25 | 0.04 |
| GGT(U/L)                 | 30.90 ± 1.33   | 57.72 ± 12.34 | 0.01 |
| Fe (mmol/L)              | 25.31 ± 15.39  | 19.89 ± 0.89  | 0.01 |
| NEFA (mg/ML)             | 0.21 ± 0.07    | 0.32 ± 0.10   | 0.02 |
| FG (mg/L)                | 56.00 ± 11.96  | 131.21 ± 24.55 | 0.03 |

Note: AST; aspartate aminotransferase; GGT: gamma-glutamyl transferase; Fe: Iron; NEFA: nonesterified fatty acid

**Table 6.** Blood immune index of ewes grazing in cotton stubble (mg/ml)

| Item | Before grazing | After grazing | P value |
|------|----------------|--------------|---------|
| IgA  | 2.09 ± 0.23    | 2.39 ± 0.16  | 0.06    |
| IgM  | 3.54 ± 0.40    | 3.61 ± 0.24  | 0.09    |
| IgG  | 10.13 ± 2.13   | 10.19 ± 0.97 | 0.11    |

IgA: immunoglobulin A, IgM: immunoglobulin B, IgG: immunoglobulin G