Effects of Dietary Whole Cottonseed Ratio on Fattening Performance, Serum Biochemical Indices, and Apparent Nutrient Digestibility in Holstein Bulls (Postprint)

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Abstract

This study aimed to investigate the effects of dietary whole cottonseed proportion on the fattening performance, serum biochemical indices, and apparent nutrient digestibility in Holstein bulls. Forty-four healthy Holstein bulls with similar body weight [(286±52) kg] were selected and randomly allocated into 4 groups (n=11 per group). Groups , , , and were fed diets containing 0, 5%, 10%, and 15% whole cottonseed, respectively, with essentially equivalent energy and crude protein levels across all groups. The pre-experimental period lasted 7 days, followed by a 90-day experimental period. The results indicated: 1) Group exhibited the highest average daily gain, which was 9.80% greater than that of Group (P<0.05); Group also had the highest dry matter intake and the lowest feed conversion ratio, although differences among groups were not statistically significant (P > 0.05). 2) Serum insulin-like growth factor 1 concentrations in Groups and were 4.79% and 6.76% higher than that in Group , respectively (P<0.05); serum alanine aminotransferase and aspartate aminotransferase activities, as well as free fatty acid and growth hormone concentrations, tended to increase with increasing dietary whole cottonseed proportion, but no significant intergroup differences were observed (P > 0.05). 3) The apparent digestibility of neutral detergent fiber, acid detergent fiber, and calcium all tended to increase with increasing whole cottonseed proportion, though no significant differences were detected among groups.

Full Text

Effects of Dietary Whole Cottonseed Ratio on Fattening Performance, Serum Biochemical Parameters and Nutrient Apparent Digestibility of Holstein Bulls

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Abstract

This experiment was conducted to investigate the effects of dietary whole cottonseed (WCS) ratio on fattening performance, serum biochemical parameters, and nutrient apparent digestibility in Holstein bulls. Forty-four healthy Holstein bulls with similar body weight [(286±52) kg] were randomly allocated into four groups (n=11 per group). Groups I, II, III, and IV were fed diets containing 0%, 5%, 10%, and 15% WCS, respectively, with essentially identical energy and crude protein levels across all groups. The pre-trial period lasted 7 days, followed by a 90-day experimental period. The results showed that: (1) Group IV achieved the highest average daily gain (ADG), which was 9.80% higher than that of Group I ($P<0.05$). Group IV also had the highest dry matter intake (DMI) and lowest feed-to-gain ratio, though these differences were not statistically significant ($P>0.05$). (2) Serum insulin-like growth factor-1 (IGF-1) concentrations in Groups III and IV were 4.79% and 6.76% higher than in Group I, respectively ($P<0.05$). Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities, as well as free fatty acid (FFA) and growth hormone (GH) concentrations, tended to increase with higher WCS ratios, but no significant intergroup differences were observed ($P>0.05$). (3) The apparent digestibility of neutral detergent fiber (NDF), acid detergent fiber (ADF), and calcium tended to improve with increasing WCS proportion, though differences were not significant ($P>0.05$). (4) Compared with Group I, the feed cost per kilogram of weight gain decreased by 0.13, 0.17, and 0.26 RMB in Groups II, III, and IV, respectively. In conclusion, under the conditions of this experiment, a dietary WCS ratio of 15% effectively improved the fattening performance of Holstein bulls.

Keywords: fattening performance; serum biochemical parameters; whole cottonseed; Holstein bulls

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China is a major cotton-producing country, with total cotton output reaching 5.343 million tons in 2016, equivalent to approximately 3.473 million tons of whole cottonseed. Whole cottonseed is a high-energy, high-protein, high-fiber feed ingredient that can supplement protein, energy, and fiber simultaneously. It contains 19.3% fat (with unsaturated fatty acids accounting for 70%) and 23.5%

crude protein [1]. As an important feed resource for ruminants, the scientific and rational application of WCS in beef cattle production is significant for reducing feeding costs, improving rumen metabolism, and enhancing production efficiency. While WCS has been widely used in dairy production, research in beef cattle remains limited. The presence of gossypol, a harmful compound in WCS, restricts its usage in animal diets. Practical experience indicates that WCS inclusion levels up to 15% of dry matter intake (DMI) in diets for growing and finishing cattle do not impair growth performance. Wang et al. [2] reported that adding 25% WCS to beef cattle diets significantly improved the apparent digestibility of acid detergent fiber (ADF) and ether extract (EE), though other nutrient digestibility values were lower than those observed with 13% WCS inclusion. Conversely, Li et al. [3] found that ADG was significantly higher when WCS comprised 22% of the concentrate compared to low-WCS groups. These inconsistent reports regarding the optimal WCS inclusion level in beef cattle diets warrant further investigation. This study aimed to evaluate the effects of different WCS ratios on fattening performance, serum biochemical parameters, and nutrient apparent digestibility in Holstein bulls under conditions of similar dietary energy and crude protein levels, thereby providing a reference for WCS application in Holstein bull fattening operations.

1.1 Experimental Period and Location

The experiment was conducted from December 2016 to March 2017 at the Baoding Mancheng Hongda Dairy Farm.

1.2 Experimental Animals

Forty-four healthy Holstein bulls with normal body condition and similar initial body weight [(286±52) kg] were selected from the Baoding Mancheng Hongda Dairy Farm in Hebei Province. Following a 7-day pre-trial period, the bulls were fasted and weighed again to ensure no significant differences in average body weight among groups ($P>0.05$). This weight served as the initial body weight for the formal 90-day experimental period.

1.3 Experimental Design and Grouping

A single-factor completely randomized block design was employed. The 44 Holstein bulls were randomly divided into four groups (n=11 per group) and housed in free-stall pens. Groups I, II, III, and IV were fed diets containing 0%, 5%, 10%, and 15% WCS, respectively, with essentially identical energy and crude protein levels across all groups. Diet composition and nutrient levels are presented in Table 1 .

The WCS used in this experiment was purchased from Kashgar, Xinjiang Uygur Autonomous Region, with the following nutrient composition: 23.39% crude protein, 16.4% ether extract, 54.89% neutral detergent fiber (NDF), 39.84%

ADF, 0.26% calcium, and 0.63% phosphorus. Free gossypol content ranged from 0.04% to 0.05%.

1.4 Feeding Management

Total mixed rations (TMR) were fed twice daily at 07:00 and 18:00. Bulls had ad libitum access to water throughout the trial period. The barns were regularly cleaned and disinfected to maintain a dry and hygienic environment.

1.5.1 Fattening Performance Measurements

Body weight was measured on two consecutive days before morning feeding at the beginning and end of the experimental period, and the average value was used to calculate ADG. DMI was determined by measuring feed intake for three consecutive days each week during the trial. Feed-to-gain ratio was calculated based on DMI and ADG.

1.5.2 Serum Collection and Analysis

Five bulls were randomly selected from each group for blood collection. Fasting jugular blood samples (25 mL per bull) were collected into centrifuge tubes, incubated in a 37°C water bath for 0.5 h, and then centrifuged at 3,000 rpm for 15 min to obtain serum for biochemical analysis. All serum samples were stored at -20°C prior to analysis.

Serum ALT and AST activities, as well as glucose (GLU), urea nitrogen (UN), total protein (TP), triglyceride (TG), and cholesterol (CHO) concentrations, were measured using a semi-automatic biochemical analyzer (Microlab-300) according to the manufacturer' s instructions (Zhongsheng Beikong Biological Company). Growth hormone (GH), insulin-like growth factor-1 (IGF-1), and free fatty acid (FFA) concentrations were determined by enzyme-linked immunosorbent assay (ELISA) using a microplate reader (Power Wave XS2).

1.5.3 Fecal Sample Collection

At the end of the experiment, fecal samples were collected from five randomly selected bulls per group for three consecutive days. Each bull provided 600 g of fresh feces daily, which was divided into two portions. One portion was treated with 20 mL of 4.5 mol/L H SO per 100 g fresh feces and stored at -20°C for crude protein determination. The other portion was placed directly in sealed sample bags without pretreatment for analysis of other nutrients.

1.5.4 Nutrient Apparent Digestibility Determination

Fecal samples were analyzed for ether extract, calcium, and phosphorus using conventional laboratory methods as described in "Feed Analysis and Feed Quality Detection Technology" (2nd edition) edited by Zhang Liying. NDF and ADF concentrations were determined using an automatic fiber analyzer (ANKOM

A2000i), and crude protein content was measured using a semi-automatic Kjeldahl nitrogen analyzer (FOSS 8400). The acid-insoluble ash (AIA) method was used as an internal marker for digestibility determination according to GB/T 23742–2009.

Apparent digestibility of a nutrient (%) = [1 - (b × c)/(a × d)] × 100, where: a = nutrient content in diet; b = nutrient content in feces; c = AIA content in diet; d = AIA content in feces.

1.6 Statistical Analysis

Experimental data were analyzed using the ANOVA procedure in SPSS 19.0 software. Duncan' s multiple range test was used for intergroup comparisons when significant differences were detected. Results are expressed as "mean ± standard deviation."

2.1 Fattening Performance

As shown in Table 2 , Group IV achieved the highest ADG at 1.68 kg, which was significantly higher than Group I ($P<0.05$). DMI tended to increase with higher WCS inclusion, though differences were not significant ($P>0.05$). Group IV had the lowest feed-to-gain ratio, representing a 5.67% reduction compared to Group I ($P>0.05$).

2.2 Serum Biochemical Parameters

Table 3 shows that serum IGF-1 concentrations in Groups III and IV were significantly higher than in Group I ($P<0.05$), increasing by 4.79% and 6.76%, respectively. No significant differences were observed among groups for other serum biochemical parameters ($P>0.05$), although ALT and AST activities and FFA and GH concentrations tended to increase with higher WCS ratios.

2.3 Nutrient Apparent Digestibility

Table 4 indicates that dietary WCS ratio had no significant effect on nutrient apparent digestibility ($P>0.05$). However, the apparent digestibility of NDF, ADF, and calcium tended to improve with increasing WCS proportion.

2.4 Feed Cost Analysis

Based on feed ingredient prices during the experimental period, daily feed cost per bull was calculated (Table 5). Although WCS inclusion increased total feed cost, the cost per kilogram of weight gain decreased with higher WCS ratios. Compared with Group I, feed cost per kilogram of gain was reduced by 0.13, 0.17, and 0.26 RMB in Groups II, III, and IV, respectively.

3.1 Effects of Dietary Whole Cottonseed Ratio on Fattening Performance

Dry matter intake is a critical parameter in animal nutrition. Insufficient DMI limits growth performance, while excessive intake increases feed costs and causes unnecessary waste. Zhao et al. [4] reported that replacing part of the concentrate with WCS in TMR increased DMI by 6.35% and 4.19% in Holstein cows and brown cattle, respectively, consistent with our findings. This may be attributed to the slow release of fat from WCS in the rumen and the formation of smaller digesta particles due to the cotton lint, which reduces mixing with other forages, shortens rumen retention time, accelerates gastric emptying, and increases DMI, thereby enhancing daily nutrient intake in Holstein bulls [5].

Reports on the effects of dietary WCS on ADG have been inconsistent. Wang et al. [2] found that WCS supplementation increased propionate concentration in rumen fluid, which enters the gluconeogenic pathway for glucose synthesis and promotes body fat deposition. As a high-energy nutrient, fat is an efficient energy source. Increased fat intake with higher WCS proportions can maximally meet the growth requirements of Holstein bulls, allowing full expression of growth potential, which aligns with our results. However, Li et al. [3] reported that ADG in low and medium WCS groups was lower than the control, while the high WCS group outperformed the control, partially contradicting our findings. These discrepancies may be related to differences in diet composition, nutrient levels, concentrate-to-forage ratios, or supplementation methods. Whether inclusion levels exceeding 15% WCS could yield better fattening results requires further investigation.

3.2 Effects of Dietary Whole Cottonseed Ratio on Serum Biochemical Parameters

Serum biochemical parameters are important indicators reflecting animal nutritional metabolism and physiological health, closely related to dietary nutrient levels and growth performance [6].

ALT and AST play crucial roles in amino acid metabolism and the interconversion of protein, fat, and carbohydrates. Their activities reflect protein synthesis and degradation status [7-8], indirectly indicating growth rate and performance. In this study, although no significant differences were observed in serum ALT and AST activities among groups, a tendency toward increased activity with higher WCS ratios suggests that WCS can promote amino acid metabolism and the conversion of protein, fat, and carbohydrates, thereby enhancing beef cattle growth [9].

Glucose is the transport form of sugar in the body, providing energy for most tissue activities, and must be maintained at adequate levels to support organ and tissue function. The absence of significant differences in serum glucose concentrations among groups indicates that WCS supplementation does not disrupt blood glucose homeostasis in Holstein bulls [5].

Urea nitrogen is the end product of protein catabolism and an indicator of protein synthesis efficiency from blood amino acids [10]. When amino acid balance is favorable and nitrogen utilization is high, blood urea nitrogen levels are low, demonstrating a negative correlation with nitrogen utilization efficiency [11-13]. In this study, serum urea nitrogen concentrations in Groups II, III, and IV were lower than in Group I, suggesting that WCS supplementation accelerated protein synthesis or reduced protein degradation, improved protein utilization efficiency, and promoted growth in finishing cattle.

Total protein represents both dietary protein nutritional level and animal protein digestion and absorption capacity. Enhanced protein synthesis increases blood total protein concentrations [14-15]. Colin-Negrete et al. [16] reported that serum total protein increased over the experimental period but not significantly, consistent with our results, indicating that WCS promotes protein biosynthesis.

Serum triglyceride and cholesterol concentrations are two important indicators of lipid metabolism. Research shows that unsaturated fatty acids can reduce blood triglyceride and cholesterol levels. Jump et al. [17] suggested that blood cholesterol is not related to dietary cholesterol intake but is positively correlated with saturated fatty acid intake and negatively correlated with unsaturated fatty acid intake. Zhong et al. [5] confirmed that feeding WCS reduced serum cholesterol in cattle, consistent with our findings. This may be attributed to the high polyunsaturated fatty acid content in WCS, which prevents serum cholesterol elevation from high-cholesterol diets [18]. Additionally, fat and cholesterol micelles wrapped in cotton lint are excreted with feces, blocking intestinal absorption and interrupting enterohepatic circulation of bile salts, thereby reducing cholesterol levels.

Blood FFA concentrations are related to lipid, glucose, and endocrine metabolism. The highest serum FFA concentration in Group IV may result from the natural protection of unsaturated fatty acids by whole cottonseed, reducing ruminal biohydrogenation and increasing FFA entry into the bloodstream.

Growth hormone is a primary regulator of animal growth, promoting cell division and proliferation in bone, cartilage, and other tissues, while stimulating fat mobilization and protein synthesis to enhance weight gain [19]. Vestergaard et al. [20] found that growth rate and ADG in Holstein bulls were positively correlated with blood GH concentration, and Sarkar et al. [21] reported a positive correlation between serum GH and ADG in yaks, both confirmed by our study.

Insulin-like growth factor-1 acts as a mediator of GH, with cooperative effects that promote protein synthesis and maintain nitrogen balance. The significantly higher serum IGF-1 concentration in Group IV compared to Groups I and II indicates that increasing WCS proportion enhances protein synthesis, thereby improving growth performance in finishing cattle.

3.3 Effects of Dietary Whole Cottonseed Ratio on Nutrient Apparent Digestibility

Our results showed that increasing WCS proportion had no significant effect on nutrient apparent digestibility. Crude protein apparent digestibility tended to increase initially and then decrease with higher WCS levels, consistent with findings by Wang et al. [22], possibly because cottonseed protein is encapsulated by the hull, making it less digestible.

The apparent digestibility of NDF and ADF tended to improve with increasing dietary WCS proportion, consistent with Bernard et al. [23]. Palmquist et al. [24] reported that dietary WCS supplementation significantly improved ADF apparent digestibility in dairy cows, a trend consistent with our results. However, Wang et al. [2] reported that cottonseed meal reduced ADF apparent digestibility in beef cattle, possibly because fat in cottonseed inhibits microbial growth, thereby affecting fiber digestibility.

4 Conclusion

1. Feeding WCS-supplemented diets improved ADG and serum IGF-1 concentration in Holstein bulls, while DMI and apparent digestibility of NDF, ADF, and calcium also tended to increase.
2. Under the conditions of this experiment, a dietary WCS ratio of 15% effectively improved the fattening performance of Holstein bulls.

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Note: Figure translations are in progress. See original paper for figures.