Feeding of whole cottonseed on performance, carcass characteristics and intestinal morphology of Zandi lambs

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The present study was conducted to determine the effect of including different levels of whole cottonseed (WCS) in the finishing diet of lambs on their dry matter intake (DMI), live weight gain, feed conversion ratio (FCR, kg feed/kg gain), carcass characteristics and small intestinal morphology. Twenty Zandi male lambs (29.8 ± 1.6 kg body weight) were assigned to one of four diets in a completely randomized experimental design. The experimental diets contained 0%, 4%, 8% and 16% WCS on a dry matter (DM) basis. The diets were prepared as total mixed rations and fed to the lambs *ad libitum*. The lambs were slaughtered on day 90 and carcass data was collected. The DMI and average daily gain (ADG) were significantly greater for lambs fed the 8% WCS diet compared with the other treatments. The FCR was significantly lower in the diets containing WCS compared to the control, *viz.* 6.11, 5.6, 5.46 and 5.68, for treatments 0, 4, 8 and 16% of WCS, respectively. However, the positive effects of including 8% cottonseed in the diet were significant in hot carcass weight, dressing percentage, liver, fat tail, intestinal fat and spleen compared with the control group. Furthermore, villous morphology and depth of crypt gland were affected by both diet and sampling site. No negative effects were observed in the organs and carcasses of the lambs that received different diets. The results indicate that the inclusion of 8% WCS in lamb diets could have a positive effect on most of the traits, but increasing the WCS inclusion up to 16% in the diet may have negative effects on lamb performance.

Introduction

Whole cottonseed (WCS), a by-product of the cotton industry, has been used as a supplemental feedstuff in ruminant nutrition for over 100 years. It is unprocessed and contains a high level of crude protein (CP) with a high digestible energy content, making it a very useful by-product (Poore & Rogers, 1995). Due to its high content of fat and protein, it can be defined as a concentrate. Furthermore, regarding effectiveness in the rumen, it has properties similar to forage fibre sources (Arieli, 1998). Increasing demands for energy and protein sources by ruminants have increased the importance of WCS as an energy and protein supplying ingredient. Due to the high energy, oil and protein contents, WCS is a popular feedstuff for ruminants and it has been accepted as an alternative to cereal grain in many rations (Kandylis *et al.*, 1998).

However, WCS contains gossypol, a polyphenolic yellow pigment that may have detrimental effects on male reproduction (Randel *et al.*, 1992). Moreover, the high content of fat and toxicity of gossypol had damaging effects on microbial activity in the rumen which is a limitation when including it in the diets of high-yielding dairy cattle (Arieli, 1998).

Considering the low heat increment in the metabolism of WCS, this product seems to be desirable as a dietary supplement to animals kept under high environmental temperatures. In addition, WCS may promote growth and stimulate the functional development of the rumen (Anderson *et al.*, 1982). Although WCS has been used extensively in cattle feeding systems (Poore & Rogers, 1995; Arieli, 1998), it has not been fully investigated as a diet ingredient for small ruminants, especially for meat and dairy sheep. The purpose of this study was to evaluate the effects of different levels of WCS on dry matter intake (DMI), average daily gain (ADG), feed conversion ratio (FCR), carcass characteristics and intestinal morphology of Zandi fattening male lambs.

Materials and Methods

The experiment was conducted at the Research Station of Abureyhan, College of Agriculture University of Tehran, Iran. Twenty Zandi male lambs with an average age of 160 (± 5 days) and weighing 29.8 (± 1.6 kg), were randomly allocated to 20 individual pens (1 × 1.4 m). The experimental animals were assigned to one of the four treatments in a completely randomized design (five lambs per treatment).

The treatments were 0%, 4%, 8% and 16% WCS (based on DM) in the diets (Table 1). All diets were isonitrogenous and isoenergetic and contained 135 g CP/kg DM, thus meeting the requirements for finishing male lambs (NRC, 1985). The experiment lasted 90 days. The lambs were fed twice daily at 08:00 and 16:00. A total mixed ration diet (TMR) was offered to the lambs *ad libitum*, with free access to fresh water and a mineral lick. Orts were collected and weighed once a day before morning feeding, and the level of feeding was adjusted daily to yield orts at about 10% of intake. The animals were given a 15-day adaptation period. During the adaptation phase all the animals were dewormed and sprayed against internal and external parasites. They were also vaccinated against pasteurelosis and anthrax. The lambs were weighed at weekly intervals before morning feeding. The FCR was calculated as daily DM intake divided by daily body weight gain (BWG).

Table 1 Ingredient and chemical composition of the four experimental diets (DM basis)

Item	% Whole cottonseed in diet			
	0	4	8	16
Ingredient (% of DM)				
Whole cottonseed (WCS)	0	4	8	16
Barley grain	60	58	55	51
Cottonseed meal	7	5	4	1
Lucerne hay	15	15	15	15
Barley straw	18	18	18	17
Chemical composition				
ME (MJ/kg DM)	11.1	11.2	11.3	11.5
Crude protein (g/kg)	132	130	136	137
Ether extract (g/kg)	20.1	27.0	33.9	47.8
Neutral detergent fibre (NDF) (g/kg)	353	363	373	387
Acid detergent fibre (ADF) (g/kg)	213	223	234	251
Calcium (g/kg)	4.8	4.9	3.8	4.2
Phosphorus (g/kg)	3.4	3.1	3.3	3.0
Iron (mg/kg)	171	192	184	165

ME - metabolizable energy, calculated according to NRC (1985) from diet component.

Diet samples were analyzed for crude protein (CP) (method 988.05; AOAC, 1990), neutral detergent fibre (NDF) (using heat-stable α-amylase; Van Soest *et al.*, 1991), acid detergent fibre (ADF) (method

973.18; AOAC, 1990), ether extract (method 920.39; AOAC, 1990), calcium (titration method) and phosphorous (UV 2100–VIS Spectrophotometer Shimadzu, Kyoto, Japan). Iron concentrations in the diets were measured using atomic absorption spectrophotometry (Model 5000, Perkin Elmer, USA). At the end of the experiment all lambs were transported to a slaughterhouse, where they were weighed before being slaughtered to evaluate carcass characteristics. The lambs were prepared by trained personnel using standard slaughtering procedures (Abdullah *et al.*, 1998) after being fasted for *ca.* 12 h with free access to fresh water. After slaughtering, the organs were removed according to normal dressing procedures. All edible carcass components (heart, head, liver, fat tail, kidneys and intestinal fat) and non-edible carcass components (skin with wool, testes, lungs and trachea, legs and spleen) were weighed immediately. The rumen and small intestine were cleaned and washed under cold running water before being weighed. Post-slaughter empty body weights were recorded. Hot carcass weight was computed by excluding edible and non-edible offals. The dressing percent was calculated as a proportion of the hot carcass weight after slaughter as well as the empty body weight (BW).

Sections of the small intestines were collected, i.e. the duodenum at 10 cm from the pyloric sphincter, the jejunum at 10 cm distal to the ligament of trite, and the ileum at 10 cm proximal to the ileocaecal junction; and also cleaned from digesta. These samples were placed in a formaldehyde and glutaraldehyde mixing fluid (50 mL 0.2 M, pH = 7.4, sodium phosphate buffer, 10 mL 25% glutaraldehyde, 20 mL 10% formaldehyde polymer and 20 mL double-distilled water), 80% (v/v) alcohol and Carnoy fluid (60 mL 100% ethanol, 30 mL chloroform and 10 mL glacial acetic acid), respectively, and kept at 4 °C until further analysis (Gu & Li., 2004). Cross-sections of the preserved intestinal samples were prepared using standard paraffin-embedding techniques. Samples were sectioned at 8 μm thickness and stained with haematoxylin and eosin (Gu & Li., 2004). Villous height (VH), villous width (VW) and crypt depth (CD) were measured on the stained sections under a microscope at 100 × magnification and an ocular micrometer. A minimum of 20 straight, intact villi in each intestinal section were measured in triplicate. The VH parameter was measured from the tip of the villi to the villous crypt junction. The CD was defined as the depth of the invagination between adjacent villi and the VW was considered at the mid of the villous. The ratio of villous height : crypt depth (VH/CD) was determined as the ratio of VH to CD.

The differences between experimental treatments were tested by the analysis of variance using the General Linear Model (GLM) procedures of SAS (2005) for a completely randomized design. The body weight was initially considered as a covariate factor in the statistical model. After analysis, the date of the initial body weight was removed due to it being insignificant. Statistical significance was considered at $P \leq 0.05$. The differences between treatments were tested using Duncan's multiple range tests.

Results and Discussion

The data on initial body weight, final live weight, live weight gain, ADG, DMI and FCR of the lambs is presented in Table 2.

The DMI was significantly higher ($P < 0.05$) in lambs fed the 8% WCS diet compared with the other three treatments. These results differ from those of Luginbuhl *et al.* (2000) who reported that, using 24% of cottonseed in the diet of Boer goats decreased DMI compared with a control group. However, the increase in DMI, observed in the present study, is consistent with the results of Karalazos *et al.* (1992) who included 0, 17.5, 35.5 and 53.0% WCS in a diet for rams. Coppock *et al.* (1987) stated that the DMI of dairy cattle did not change when up to 25% WCS was included in their diet. However, in some studies executed under hot environmental conditions (Coppock *et al.*, 1985; Lanham *et al.*, 1992), a decline in DMI did occur when WCS was fed. This drop in intake could be explained by the higher fibre content of diets high in WCS. In an experiment conducted in hot weather conditions where the fibre contents of the treatments were balanced, the supplementation of 20% WCS did not reduce DMI (Belibasakis & Tsirgogianni, 1995).

The increase in DMI due to the feeding of a higher percentage of WCS could be attributed to the regulatory effect of dietary energy intake. When the lambs were fed finishing rations, particle size of forage was small enough to avoid the regulation of feed intake by physical fill (Fimbers *et al.*, 2002). The factors which can influence the DMI are divided into physiological, environmental and dietary factors. Of them, body composition, sex, physiological status and body size are categorized as physiological factors, and digestibility, dietary CP, amino acid situation, the imbalance of other ingredients in the diet, restriction of water, flavour and taste of feed and composition of diets are regarded as dietary factors. In the present study

the concentration of fat, fibre, energy and perhaps CP degradability in the ration might have influenced the DMI of fattening lambs consuming WCS containing diets.

There were no significant differences between treatments in initial body weight (averaging 29.8 ± 1.6 kg), final live weight and live weight gain of the experimental lambs (Table 2). However, different levels of inclusion of WCS resulted in different ADGs, with the highest ADG being in the 8% WCS treatment (Table 2). A gradual increase was recorded in the daily gain of the control group due to the 8% WCS treatment, and then a decrease in gain between the 8% and 16% WCS treatments.

The lower ADG in treatment 16% WCS could be related to both a higher gossypol content of the diet and a lower DMI compared with the other treatments. Kandylis *et al*. (1998) reported increased ADGs at levels of 10, 20 and 30% WCS in the diets compared to a control diet. Bird *et al*. (1987) indicated that the addition of WCS to oaten chaff diets fed to sheep resulted in a lower growth rate. Luginbuhl *et al*. (2000) reported that the rate and efficiency of growth decreased at the higher levels of WCS inclusion in diets (up to 24%). These studies suggest that the growth rate may be negatively affected by high dietary levels of WCS. The decline in the growth rate may be due to the depression in feed intake which was mentioned in previous studies, using high levels of WCS (Luginbuhl *et al*., 2000).

In this study the FCR of the lambs receiving 8% WCS in their diets was the best, i.e. the lowest, of all treatments ($P < 0.05$). In all treatments where WCS was included in the diets the FCR was lower than in the control diet. Similarly, Kandylis *et al*. (1998) found a reduction in FCR when animals were fed WCS at ration levels of 10, 20 and 30%. Kuhlmann *et al*. (1991) reported that live weight gains increased and FCR decreased in heifers receiving 10 - 30% WCS in their diets.

Table 2 Performance of lambs fed four diets containing different levels of whole cottonseed for 90 days

Traits	% Whole cottonseed in diet				SEM
	0	4	8	16	
Initial body weight (kg)	29.9	29.9	30.0	29.8	1.6
Final live weight (kg)	51.0	52.6	54.5	51.8	2.1
Live weight gain	21.1	22.7	24.5	22.0	1.3
Average daily gain (g/day)	234[c]	252[b]	272[a]	244[bc]	8.13
Dry matter intake(DMI, kg/day)	1.43[b]	1.41[b]	1.48[a]	1.38[c]	0.012
FCR (kg DMI/kg gain)	6.11[a]	5.59[b]	5.44[c]	5.65[b]	0.05

[abc] Means with different superscripts in the same row are significantly different ($P < 0.05$).
SEM = standard error of mean; FCR – feed conversion ratio.

The effects of the different levels of WCS on hot carcass weight, dressing percentage and the weight of various internal and external organs of the lambs are presented in Table 3.

The inclusion of 8% WCS in the diets increased the hot carcass weight and dressing percentage compared with the other treatments ($P < 0.05$). This is consistent with the results obtained by Kandylis *et al*. (1998). Diets and nutrient contents may also affect carcass characteristics and meat quality. Kandylis *et al*. (1998) indicated that growing lambs fed WCS had a higher live weight, increased hot carcass weight and dressing percentage in comparison with a control diet. The low dressing percentage (slaughter-weight basis) in the control group could be due to a high gut fill which would reduce the dressing percentage. The dressing percentage of the Zandi breed, based on body weight at slaughter, has been recorded as 51.6 (Kyanzad, 2001; Kashan *et al*., 2005). The average slaughter weight and hot carcass weight of lambs fed the diet containing 8% WCS were heavier than in the other treatments, similar to final live weight.

In the present study no significant differences were recorded for most of the carcass performance data, and the weights of the internal and external organs (Table 3). The weights of the empty small intestines, empty rumen, lungs and trachea, heart, head, kidney, skin with wool, testes and legs were not affected by

dietary WCS levels. However, the weights of intestinal fat, fat tail, liver and spleen were significantly influenced by the level of dietary WCS; probably because of a higher dietary fat content.

Butler-Hogg & Johnsson (1986) recorded 260 g intestinal fat for lambs slaughtered at 32 kg live weight. However, in the present study we obtained values ranging from 489 to 597 g that is highly related to the greater weight of the lambs. It has also been reported that with increasing amounts of WCS in the diet, the site of fat depots tends to change (Huerta-Leidenz *et al*., 1991). Fat-tailed sheep breeds that are found in the most parts of Asia, are probably more tolerant to protracted feed shortages, compared to European breeds. Some researchers showed that the changes in weight of some organs, especially the small intestine, large intestine, heart, lung, liver and kidney, are related to metabolic activity which is affected by breed, diet, husbandry system and slaughtering age (Pearson & Dutson, 1991). In order to yield a high carcass percentage, the lamb must have a light pelt, the well finished and heavily muscled, and free from paunchiness. Due to the higher value of the offal of sheep compared with other species, especially the high value of the pelt, a high dressing percentage in sheep is not as important economically as in cattle or pigs. Wool yield is not usually an important item in slaughter and the dressing return, and dressing percentage lowered by a heavier wool yield may actually mean a greater total return. For this reason, the fleece characteristics should be an important production component in the production of mutton or lamb of high quality (Enseminger, 2002).

Morphology results of the duodenum, jejunum and ileum are presented in Table 4. The level of WCS in the ration had significant effects on villi height, villous width and villous/crypt ratio in the duodenum (P <0.05). The greatest values for villi height and villous/crypt ratio were observed in the 8% dietary WCS

Table 3 Effect of whole cottonseed on carcass measurements of lambs slaughtered after 90 days

Item	% Whole cottonseed in diet				SEM
	0	4	8	16	
Slaughter live weight (kg)	51.0	52.6	54.5	51.8	2.1
Hot carcass weight (kg)	25.8[b]	26.9[ab]	29.6[a]	26.4[b]	0.63
Dressing percentage, hot carcass	50.6[b]	51.2[b]	53.2[a]	50.9[b]	0.38
Edible carcass (g)					
Heart	227	245	252	237	6.2
Head	2058	2203	2203	2115	38.8
Liver	827[b]	830[b]	897[b]	1051[a]	28.7
Kidney	231	317	297	299	16.4
Empty rumen	1220	1371	1282	1343	37.9
Empty small intestine	559	551	592	532	13.4
Fat tail	3582[b]	3710[ab]	3734[ab]	4511[a]	165.8
Testes	436	488	457	411	18.1
Intestinal fat	489[b]	512[b]	583[a]	597[a]	21.8
Non-edible carcass offal (g)					
Skin with wool	5990	6680	6900	6350	162.4
Lungs and trachea	551	641	593	588	20.3
Legs	926	931	1025	956	19.2
Spleen	97[b]	121[a]	127[a]	130[a]	4.6

[abc] Means with different superscripts in the same row are significantly different (P <0.05).
SEM = Standard error of means.

among treatments. However, the villous width was larger in the control treatment compared with the other treatments. In the jejunum, different levels of WCS in the ration had significant effects on villi height and the

villous height : crypt depth ratio. Similar to the parameters observed in the duodenum, the villi height and VH/CD were the highest in the 8% WCS treatment in the present study. As it has shown in Table 4, all morphological parameters in the ileum were affected by diet (P <0.05).

Table 4 Effect of feeding whole cottonseed on morphology of different segments in the small intestines

Site	% Whole cottonseed in diet				SEM
	0	4	8	16	
Duodenum					
Villous height (μm)	290[b]	303[ab]	346[a]	344[a]	9.7
Villous width (μm)	300[a]	297[ab]	297[ab]	2633[b]	6.2
Crypt depth (μm)	273	240	263	243	7.2
VH/CD	1.06[b]	1.27[ab]	1.42[a]	1.31[ab]	0.05
Jejunum					
Villous height (μm)	237[c]	280[b]	327[a]	303[ab]	11.3
Villous width (μm)	257	280	283	253	7.4
Crypt depth (μm)	210	247	250	247	7.4
VH/CD	1.12[b]	1.13[ab]	1.32[a]	1.23[ab]	0.03
Ileum					
Villous height (μm)	243[c]	283[b]	327[a]	290[b]	10.2
Villous width (μm)	253[b]	273[ab]	300[a]	280[ab]	7.2
Crypt depth (μm)	217[b]	237[ab]	257[a]	237[ab]	5.3
VH/CD	1.12[b]	1.19[ab]	1.27[a]	1.22[ab]	0.02

[abc] Means with different superscripts in the same row are significantly different (P <0.05).
SEM = standard error of mean.
VH/CD = villous height : crypt depth.

The villi of the small intestine changes with both age and weaning time, from a long and fine finger-like shape to a short and thick tongue-like shape (Kenworthy, 1976; Cera *et al.*, 1988). A high sensitivity to antigens in the diet may be responsible for morphological changes such as villous atrophy and crypt hypertrophy (Kenworthy, 1976). Diets containing high CP levels, especially those with high levels of plant protein that may contain strong antigens, may greatly affect villous morphology (Li *et al.*, 1991). In this experiment the villous height was significantly affected by different levels of WCS. Villous height : crypt depth ratio in the duodenum, villous height and VH/ CD in the jejunum and villous height, villous width, crypt depth and VH/CD in the ileum were also affected.

As the dietary WCS level increased, all morphological parameters of the small intestines were affected significantly. In the entire length of the small intestines of the experimental lambs, villous heights and villous width as well as crypt depth were higher in lambs that were fed WCS compared to the control group. The lambs that were fed the control diet in this study had the lowest VH/CD which probably relates to the lower digestive capacity of the small intestines in these animals. Villous height and villous width as well as villous height : crypt depth ratios were longer in the duodenum. These results are in agreement with that of Sharifi *et al.* (2007) who studied the small intestine morphology in chickens. Studying intestinal morphology traits when the level of CP in piglet diets was increased, Gu & Li (2004) showed that the villous height in the duodenum was longer than in other segments, in agreement with the present results. We assumed that gossypol in WCS may affect the villous morphology in the small intestines. In another study on broilers, Gabriel *et al.* (2007) reported that villous height, crypt depth and villous height : crypt depth ratios were affected by wholewheat grain and the highest villous height, crypt depth and villous height : crypt depth ratios were observed in the duodenum and the lowest in the jejunum. A similar result in piglets was reported

by Hedemann *et al.* (2007). Changes in the development of the enterocytes and in the structure of villi determine the digestive and absorptive capacity of the small intestines. Hampson (1986) reported that by measuring the villous height and studying villous shape one can estimate the number of entrocyte in the villous. In other words, if the villi are longer and flatter, the entrocyte surface will be longer and consequently, absorption ability will be higher. Villous height, crypt depth and the ratio of VH/CD reflect intestinal health (Wang *et al.*, 2008). Sharifi *et al.* (2007) reported that a reduction in villous height caused by soluble non-starch polysaccharide can reduce nutrients' digestibility. An increase in VH/CD in the intestinal structure is related to better digestion and absorption of nutrients due to the provision of the required nutrients (Gabriel *et al.*, 2008). Wang *et al.* (2008) reported that greater villous height, villous width, crypt depth and villous height : crypt ratios were observed in the duodenum of goats receiving WCS. The positive effect of WCS on villous height and villous height: crypt depth ratio can improve the nutrient uptake from the intestines which could result in improved growth performance in comparison with control diets. The greater duodenal villous height : crypt depth ratio in lambs fed WCS may reflect the higher level of energy absorbed from the duodenum, which could be the result of the volatile fatty acids formed through the degradation of WCS. The increased villous height : crypt depth ratio showed an intestinal structure more conducive to digestion, with improved absorptive and hydrolysis potential, than the required nutrients for intestinal maintenance. Thus, with the inclusion of WCS in the diet, the intestinal structure of the duodenum was more favourable for the animal and may contribute to the improved FCR.

Moreover, pervious studies on the interaction of iron supplementation with cottonseed feeding indicated that iron could ameliorate the negative effects of gossypol in the ruminant (Barraza *et al.*, 1991; Santos *et al.*, 2005; McCaughey *et al.*, 2005). Barraza *et al.* (1991) suggested that iron could bind with gossypol and reduce gossypol availability in the digestive tract. Santos *et al.* (2005) also reported that iron supplementation in the form of iron sulphate decreased plasma gossypol in Holstein steers. However, in our study the relatively low concentration of iron could probably not affect the gossypol absorption in the small intestines and the gossypol concentration in the plasma of fattening lambs.

Conclusion

The inclusion of different levels of WCS in the finishing diets of lambs was investigated in the present study. The results indicated that DMI, FCR, carcass weight, fat tail weight, liver and spleen weights were affected positively by the inclusion of WCS in the diets. Furthermore, WCS feeding affected the morphology of villous and crypt in the small intestines. The results suggested that an inclusion of 8% WCS in the diet was the most effective in improving most of the traits, and that a 16% inclusion of WCS affected some of the performance traits negatively.

Acknowledgements

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