Effects of Feeding Graded Levels of Whole Cottonseed on Blood Serum Parameters of Arsi-Bale Growing Male Goats

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ABSTRACT

An experiment was conducted with 24 Arsi-Bale growing male goats of about 15 kg body weight and aged 6–12mth in a randomized complete block design to assess the effect of graded levels of whole cottonseed of *Gosspium hirsutum* spp. (WCS) supplementation of the goats' diets on serum parameters. Goats were allocated at random to one of the four treatments: the control of sole local grass hay (T1), control + 10%WCS (T2), control + 20%WCS (T3) and control + 30% WCS (T4), with all amounts based on of dry matter intake (DMI). Blood samples were collected at 30, 60 and 90 d of the experimental period and analyzed for the serum parameters: albumin, glucose, blood urea nitrogen, creatinine, total serum protein and alkaline phosphatase. The level of blood urea N, glucose and alkaline phosphatase increased significantly ($P < 0.05$) with the increasing level of inclusion of WCS in the diet when compared to the control diet. However, there was no clinical sign of gossypol toxicity observed on any goats during the experimental period. It was concluded that the WCS supplementation at 10% of DMI supports good performance of goats and could be used as an alternative option at any critical period of feed shortage.

Keywords: goats, graded level supplementation, serum parameters, whole cottonseed

INTRODUCTION

Whole cottonseed is the most preferred feed supplement in developed countries; it is a unique feed supplement blend containing relatively high CP (crude protein), fiber, fat (23.5, 44 and 20%,respectively, on a dry matter (DM) basis) and metabolizable energy content (36.782 J) (National Research Council,1984). In Ethiopia, cottonseed is grown in the lowland parts of the country that are free of frost and below an elevation of 1,400 meters above sea level. An estimated potential area of 2.6 million ha and a cultivated area of 1.7 million ha is used for cottonseed production, with annual production of cottonseed being about 84,000 t—mainly under irrigation and some rain fed (Ethiopian Science and Technology Commission, 2006). Cotton is one of the most important industrial crops whose fiber and seed is supplied to textile factories and oil mills, respectively.

Natural cottonseed contains gossypol, a polyphenolic yellow pigment found in cotton plants mainly in the pigment glands of seed, roots

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Received date : 11/04/13 Accepted date : 12/07/13

and leaves (Berardi and Goldblatt, 1980; Willard *et al*., 1995). Gossypol disrupts the cell membrane metabolism and can cause the rupture of red blood cells when cottonseed is fed at higher levels beyond the tolerance level of ruminants or their ability to detoxify gossypol in the rumen (Reiser and Fu,1962). In developing countries like Ethiopia, goats make a very valuable contribution to the livelihood of the poor (Devendra *et al*., 1983).The traditional goat production system is dominantly extensive whereby goats are highly dependent on grazing and browsing on natural grazing land and it is characterized by low production and productivity (Solomon *et al*., 2010). Many factors contribute to the low production and productivity of the indigenous goat population such as poor feeding, feed shortage, breeding, disease and poor management; however, poor nutrition accounts for the largest share of the low production and productivity of the goat population in the country (Tolera *et al*., 2000). On the other hand, there has been a recently growing export market for goats and a demand for goats with a specific body weight (Ethiopian Ministry of Industry and Trade, 2008). Importation of the Boer breed for the promotion of goat production has provided good opportunities for increased interest in indoor goat production to cope with the growing demand of the export market (Ethiopian Sheep and Goat Productivity Improvement Project, 2008). With grazing areas disappearing in small territories and production systems changing from grazing to confinement (Adugna, 2007), it appeared relevant to study indoor goat feeding. Thus, supplementation of indoor goat feeding with locally available natural feed resources such as whole cottonseed to enhance faster growth rates and to facilitate finishing goats at a specific weight in a short time is paramount. Therefore, the objective of this work was to assess the effect of graded level supplementation of whole cottonseed (WCS) on some blood serum parameters of growing Arsi-Bale male goats.

MATERIALS AND METHODS

Description of the study area

The study was conducted in the Adami Tulu Agricultural Research Center, located in the Mid Rift Valley of Ethiopia at a latitude of 7°9'N and a longitude of 38°7'E, 167 km south of Addis Ababa, the capital city of Ethiopia. The Center is at an altitude of 1,650 m above sea level. It has a relative humidity of 60% and an average annual rainfall of 760.9 mm with minimum and maximum average annual temperatures of 12.7 and 27.2 °C, respectively (Abule *et al.*, 1998).

Experimental animals and treatments

A total of 24 growing Arsi-Bale male goats aged 6–12 mth were purchased from local markets. The goats were quarantined for 10 d and vaccinated for goat pox and injected with Ivermectin (Ivormic®) for internal and external parasite control and housed in individual pens in a sheltered, wooden-floored, open-sided barn. They were partitioned into blocks of animals by their body weight, with six goats per block and randomly assigned to four treatments: (T1) local grass hay only (control), (T2) control + 10% whole cottonseed (WCS), (T3) control + 20% WCS and (T4) control + 30% of dry matter intake (DMI) WCS-supplemented diets. All four groups were fed a control diet for 15 d prior to the adaptation period and the actual data collection was started after 15 d of the adaptation period after the experimental animals had been gradually acclimatized to their respective experimental feeds. A mineral mix was added to all diets and all goats had free access to fresh water. The amount of WCS added to each level was expressed as a percentage of the daily total DMI of the goats (10, 20 and 30% of DMI) and was mixed with wheat bran in the proportions indicated in Table 1. The local grass hay used was a mixture of *Pennisetum clandestinum*, *Chloris gayana*, *Andropogon abyssinicus*, *Trifolium* spp. and some legumes such as *Alysicarpus rugosus* and was traditionally produced.

Table 1 Proportions of supplement ingredients in experimental diets.

Ingredient	Levels of inclusion of WCS (%DMI)			
	0	10	20	30
Hay (%)	*ad libitum*	*ad libitum*	*ad libitum*	*ad libitum*
WCS (%)	0	10	20	30
Wheat bran (%)	0	40	30	20
Salt (%)	1	1	1	1
Mineral (%)	1	1	1	1
Total proportion(%)	2	52	52	52

WCS = Whole cottonseed, DMI = Dry matter intake.

The treatment diets were fed twice a day (0800 and 1700 hours) and the refusals were collected every morning before 0800 hours. Individual animal intake was calculated using the daily feed offered less the refusals. The animal body weight was determined every 15 d while the average daily gain was calculated as the difference between the initial and final body weights over the interval of the experimental period (90d). Blood samples were collected at intervals of 30 d in the experimental period and were centrifuged at 3,000 rpm for 30 s and serum was separated from plasma and the separated serum samples were frozen at -20 °C until they were analyzed.

Laboratory analysis

The DM concentration of hay, whole cottonseed, wheat bran and the experimental diets was determined by oven drying at 60 °C. Thereafter, the whole cottonseed was decorticated and samples were ground to pass through a 1 mm screen before analyses for DM by oven drying at 100 °C and for OM by muffle furnace incineration, while nitrogen (N) content was determined by the Kjeldahl method, following the procedure of Association of Official Agricultural Chemists (1995). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were estimated by the method of Van Soest *et al.* (1991). The stored serum samples were obtained and total protein, albumin, glucose, creatinine, urea N, alkaline phosphatase activities were analyzed as outlined by Solaiman *et al*. (2009) using similar kits and suppliers.

Statistical analysis

The data generated were subjected to analysis of variance using the general linear model of SAS (2002) in a randomized complete block design. When the analysis of variance revealed the existence of significant differences among the treatment means, Tukey's test was used to determine if treatment means were significantly different from one another.

Table 2 Chemical composition of experimental diets.

Composition (%)	Hay	10%WCS	20%WCS	30%WCS
DM	93.6	94.3	94.9	94.6
CP	4.61	15.8	16.72	17.12
Ash	9.44	5.60	5.81	6.00
OM	90.56	94.4	94.19	94.0
DOMD	90.58	94.4	94.19	93.98
NDF	87.8	47.9	46.8	44.9
ADF	47.9	17.5	15.6	19

DM = Dry matter, CP = Protein content, OM = Organic matter, DOMD = Digestible organic matter in dry matter, NDF = Neutral detergent fiber, ADF = Acid detergent fiber, WCS = Whole cotton seed.

RESULTS AND DISCUSSION

The chemical composition of the experimental diets is shown in Table 2.

The dry matter and energy composition of the experimental diets increased with increasing levels of WCS in the diet. The crude protein content of the control diet was very low compared to the supplemented diets due to the lower CP content of the local grass hay used as the basal diet.

Different studies (Wang *et al*., 1987; Deora *et al.,* 1997; Nagalakshmi *et al*., 2000; Risco *et al*., 2002; Mena *et al*., 2004; Solaiman *et al*., 2009) have linked cottonseed ingestion and gossypol exposure to serum biochemistry disturbance and the serum chemistry changes allow for the recognition of an impeding toxicological effect due to gossypol ingestion prior to the development of clinical signs.

The findings of the present study showed no significant difference (P >0.05) between treatments in the blood serum albumin levels of the goats fed on the WCS-supplemented diet as shown in Table 3.

This could possibly have been due to the serum albumin concentration not being affected by the diet or the level of WCS was not high enough to affect the serum albumin concentration. This was in agreement with Solaiman *et al*. (2009) who reported that the profile of serum metabolites was not different (P > 0.05) between treatments as shown in Table 4.

Likewise, Mena *et al*. (2004) indicated that the serum concentration of albumin was not influenced by diets. In contrast to the present

Table 3 Blood serum parameters of whole-cottonseed-supplemented goats (mean±SD).

Indicators	Levels of whole cottonseed inclusion (% DMI)				SL
	0	10	20	30	
Albumin (g.dL^{-1})	1.71±1.69	2.60±1.28	2.38±1.87	2.16±1.26	NS
Total serum protein (g.dL^{-1})	3.94±4.23	4.64±1.53	3.24±2.36	4.71±2.02	NS
Blood urea nitrogen (mg.dL^{-1})	8.14±7.36^{b}	20.63±4.31^{a}	16.09±10.66^{a}	21.60±4.46^{a}	*
Glucose (mg.dL^{-1})	17.94±7.46^{b}	26.44±0.78^{a}	24.94±6.65^{ab}	29.89±15.07^{a}	*
Creatinine (mg.dL^{-1})	17.17±40.28	1.35±0.66	0.86± 0.49	1.11± 0.24	NS
Alkaline phosphatase (units.L^{-1})	79.61±71.1^{b}	291.22±03^{a}	275.78±205.1^{a}	222.17±178.05^{a}	*

DMI = Dry matter intake, SL = Significant level, * = Significantly different at (P< 0.05), NS = not significantly different at (P < 0.05).

Means in the same row with different lowercase superscripts are significantly different at (P< 0.05).

Table 4 Blood serum parametersof whole-cottonseed-supplemented goats by intervals of blood samples collection (mean±SD).

Indicator	Interval of blood sample collection(d)			SL
	30	60	90	
Albumin (g.L^{-1})	2.52±1.26	2.39±1.70	1.50±1.50	NS
Total serum protein (g.L^{-1})	4.63±2.09	4.35±2.66	3.09±3.44	NS
UreaNitrogen (mg.dL^{-1})	14.57±8.17^{a}	19.75±9.01^{b}	14.13±8.19^{a}	*
Glucose (mg.dL^{-1})	36.00±16.70^{b}	18.07±11.12^{a}	12.07±17.50^{a}	*
Creatinine (mg.dL^{-1})	0.91±0.38^{a}	1.08±0.64^{a}	17.50±40.13^{b}	*
Alkaline phosphatase (units.L^{-1})	204.13±133.44	243.53±208.48	190.72±136.31	NS

SL = Significance level, * = Significantly different at (P< 0.05), NS = Not significantly different at (P < 0.05).

Means in the same row with different lowercase superscripts are significantly different at (P< 0.05).

findings, Calhoun *et al*. (1990) reported a decrease in the serum albumin levels as the level of gossypol acetic acid increased in the diet and similarly, Risco *et al*. (1992) reported that free gossypol depressed serum albumin. Furthermore, in the present investigation no statistically significant ($P > 0.05$) differences between treatments in the level of the serum total protein were observed with increasing levels of inclusion of WCS in the diets of goats compared to goats consuming the control diet as shown in Table 3. The lack of a significant change in the serum total protein observed with increasing levels of inclusion of WCS could possibly have been due to the low level of gossypol in the diet which was not sufficient to have a boosting or depressing effect on the level of the serum total protein. Likewise, Mena *et al*. (2004) reported that the serum total protein was not influenced by diet. In contrast, to the present findings, Solaiman *et al*. (2009) reported that the blood serum protein increased linearly ($P < 0.02$) as the dietary EasiFlo cottonseed (ECS) level increased in the diets of goats receiving WCS-supplemented diets compared to goats consuming the control diet as shown in Table 4. On the other hand, Calhoun *et al*. (1990) indicated that the total protein level decreased as the levels of gossypol acetic acid increased in the diets of lambs receiving WCS-supplemented diets compared to goats fed a control diet and this was consistent with Risco *et al*. (1992) who reported that free gossypol depresses the serum total protein concentration. Furthermore, the present findings indicated that the blood serum urea N level increased significantly ($P < 0.05$) with an increasing level of WCS in the diets of goats as compared to the control group as shown in Table 3. An increase in the blood serum urea nitrogen could possibly have been due to the inefficient utilization of the urea nitrogen produced by the rumen microflora due to the negative effect of gossypol on the rumen microflora activity or numbers. In line with the present findings, Luginbuhl *et al*. (2000) reported that serum urea N increased with a quadratic trend ($P < 0.05$) with increasing levels of WCS in the diet of goats compared to goats consuming a control diet and suggested that even though the serum urea N value increased as more WCS was consumed, they were within the range reported by Calhoun *et al*. (1990) and Keery *et al*. (1991) in lambs fed diets of varying proportions of WCS, as shown in Table 4. They suggested that this may have been due to less efficient capture of ruminal ammonia as a result of a decrease in ruminally degradable carbohydrates such as starch or due to the reduced microbial growth resulting from the toxic effects of polyunsaturated fatty acids on the ruminal flora. Likewise, Harrison *et al*. (1995) reported increased levels of blood urea N with an increasing level of whole cottonseed in the diet for dairy cows and this finding was also in agreement with Mena *et al*. (2004) who reported increased blood urea N from a high total and free gossypol diet. Further, Salehpour *et al*. (2010) reported that blood urea increases with increasing levels of 0, 7, 14 and 21% WCS in the diet of supplemented animals. However in contrast to the present findings, Dayani *et al*. (2010) reported lower blood urea N in lambs fed 20% WCS. Moreover, the results of this study showed that the level of blood serum glucose increased significantly ($P < 0.05$) with increasing levels of inclusion of WCS in the diets of goats compared to goats fed a control diet as shown in Table 3. In line with the present findings, Solaiman *et al*. (2009) reported that serum glucose tended to be higher in goats consuming 15.7% ECS according to a quadratic ($P < 0.06$) increase. This could probably have been due to the increase in fermentable carbohydrates with increasing levels of supplements that improved the nutritional status and energy source of the animals because the level of glucose is an indicator of the nutritional status of the animal (Dayani *et al*., 2010).

In contrast to the present results, Calhoun *et al.* (1990) indicated that the blood serum glucose level decreased as the levels of gossypol acetic acid increased in the diets of lambs. On the other hand, the finding of the present study showed decreased levels of creatinine with increasing levels of WCS in the supplemented diets of goats compared to goats fed the control diet as shown in Table 3. This might have been due to the low effect of the gossypol content of WCS or the relatively high tolerance of the goats to the negative effects of gossypol that can lead to creatiniemia and increased creatinin levels. In contrast to the present findings, Morgan *et al*. (1988) reported that serum creatinine kinase is usually high in diseases of the heart, skeletal muscle and brain because there are large quantities of the enzyme in these organs and even a mild injury can release it into the circulation system. Likewise, Warren *et al.* (1988) observed a significant ($P < 0.01$) increase in the serum creatinine level and creatinine kinase activity in wethers after 127 d of 50% whole cottonseed feeding and indicated that this was attributed to muscle damage because of gossypol. In line with this report, Solaiman *et al.* (2009) reported on goats and Mena *et al.* (2004) reported on dairy cows that creatinine kinase was higher with increased levels of WCS in the diets. On the other hand, Barraza *et al*. (1991) found no variation in the creatinine levels of Holstein calves fed on 50% WCS in their diet.

Furthermore, the present investigation showed a significantly ($P < 0.05$) increased level of alkaline phosphatase with an increasing level of WCS in the diets of goats consuming WCS-supplemented diets compared to goats fed on the control diet as shown in Table 3.

This could possibly have been due to hepatic disfunction attributed to the toxic effect of gossypol. Likewise, Solaiman *et al*. (2009) reported that alkaline phosphatase was higher in goats fed on a diet containing 15.7% ECS for 140 d and they also indicated that alkaline phosphatase is of interest because of its location in the plasma membrane. Moreover, Murray *et al.* (1993) indicated that a high alkaline phosphatase concentration might be a direct response to liver and bone injury as a result of gossypol toxicity. On the other hand, Colin-Negrete *et al*. (1996) reported that there was no change in the alkaline phosphatase concentration in growing Holstein cows that had consumed 30% WCS for 144 d but observed a linear decrease ($P < 0.05$) in the alkaline phosphatase levels after 430 d. This result was in agreement with Mena *et al*. (2004) who reported that the level of alkaline phosphatase did not differ among the treatments as shown in Table 4.

In addition, the present findings indicated that there was a significant ($P < 0.05$) difference between the blood urea N, glucose and creatinine levels collected at different periods from goats who had consumed the WCS-supplemented diets compared to goats fed the control diet. A significantly ($P < 0.05$) higher level of glucose was observed at day 30 and later was lower at day 60. In contrast to these present findings, Kaneko (1989) reported higher glucose at day 144 and day 430. Further, the findings of the present study showed that blood urea N was significantly ($P < 0.05$) lower at day 30 and higher at day 60. In contrast to this finding, Kaneko (1989) reported lower blood urea N at day 144 and day 430. The level of creatinine was significantly ($P < 0.05$) lower at day 30 and higher at day 90. However, there was no significant ($P > 0.05$) difference between the levels of albumin, total protein and alkaline phosphatase collected at intervals from goats who had consumed the WCS-supplemented diet. In contrast, Colin-Negrete *et al.* (1996) reported that the profiles of the serum chemistry were not different ($P > 0.05$) on days 0, 144 and 430 for blood serum protein contents such as albumin, globulin, total protein and the ratio of albumin:globulin except for the ratio of albumin:globulin on day 0 which showed a slight quadratic trend for inclusion rates of 0, 15 and 30% WCS, respectively, in diets. Further, they reported a linear decrease in the level of alkaline phosphatase (292.8, 238.2 and 210.8 ± 28.6, respectively) for 0, 15 and 30% WCS inclusion in the diets. Moreover, they reported that the clinical profile of the serum suggested the possible impairment of liver and kidney function on days

144 and 430 and indicated that one heifer death on day 396 from the group fed on the 30% inclusion level of WCS was due to gossypol. Furthermore, Colin-Negrete *et al*. (1996) indicated that on day 144, the levels of glucose, creatinine and uric acid were not different and the total bilirubin (0.19, 0.22 and 0.32±0.0 mg.dL^{-1}) increased linearly ($P < 0.05$) at inclusion levels of 0, 15 and 30%, respectively, of WCS in the diet of WCS-supplemented heifers compared to heifers who had consumed a control diet. According to Kaneko (1989), the data recorded by Colin-Negrete *et al*. (1996) were within the normal ranges for cows, except for the blood urea N which was lower and the glucose which was slightly higher. On the other hand, Murray *et al*. (1993) reported that an albumin concentration greater than 3.6 g.dL^{-1} was a sign of chronic hepatic injury. However, Kaneko (1989) reported serum proteins levels similar to this. Furthermore, Murray *et al.* (1993) indicated that high alkaline phosphatase levels might be a direct response to liver and bone injury as a result of gossypol toxicity.

Measured serum metabolites (albumin, total protein, glucose, urea N, creatinine and alkaline phosphatase activity) were affected by the level of inclusion of WCS. However, the increases or decreases in the serum biochemistry indicators (except for creatinine that decreased below the normal range) were within the standard range for reported goats as shown in Table 4. Further, in the present study, no clinical sign of gossypol toxicity was observed in any goats. This suggested that Arsi-Bale goats were less adversely affected by the negative effect of gossypol.

On the other hand, gossypol has a depressive effect on protein digestibility due to its inhibitory effect on digestive enzymes (Abou-Donia, 1989). In addition, it inhibits glucose 6 phosphate dehydrogenase and causes a decrease in nicotinamide adenine dinucleotide (NADH) production which is necessary to reduce glutathione (an important component of the cell antioxidant system)if animals are fed beyond their threshold level (Solaiman *et al*., 2009).This could suggest the possible impairment of liver and kidney function possibly contributed to by gossypol toxicity. According to Risco *et al.* (2002), ruminants aged from 3 to 8 wk have the ability to detoxify dietary-free gossypol up to safe dietary levels of 200 mg.kg^{-1}, whereas mature ruminants (older than 24 wk) can tolerate up to 600 mg.kg^{-1} in their diet without any adverse effect.

CONCLUSION

From the results of this study, it is possible to conclude that an increase in the level of whole cottonseed in the diet of growing male goats caused a significant change in the serum biochemistry parameters of goats consuming diets supplemented with whole cottonseed. The serum biochemistry indicators (blood urea N, glucose and alkaline phosphatase) increased significantly with increasing levels of WCS in the diets of goats supplemented with WCS. However, the levels of blood albumin, total protein and creatinine showed no significant difference between treatments. Moreover, the blood urea N, glucose and creatinine levels showed significant differences between the interval of blood samples collection (30, 60 and 90 d) while the albumin, total protein and alkaline phosphate levels showed no significant difference between the intervals of blood sample collection. The significant difference indicated in the serum biochemistry with intervals of blood samples collection in the levels of urea N, glucose and creatinine possibly could be an indication of the accumulation effect of gossypol in the whole cottonseed. However, no clinical symptoms were observed and this suggested that Arsi-Bale goats were less adversely affected by high level of WCS in their diets than other goat breeds.

ACKNOWLEDGMENTS

The authors would like to thank the Rural Capacity Building Project for financial support for this work.

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