# Effects of Varying Levels of Whole Cottonseed on Blood, Milk and Rumen Parameters of Dairy Cows

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**ABSTRACT :** Four lactating Holstein cows were used in a 4×4 Latin-square design to determine the effects of various levels of whole cottonseed (WCS) in diets on parameters including milk (yield and fat content), rumen fluid (pH, ammonia and TVFA) and blood (β-carotene, vit. A, vit. E, urea, $NH_3$, Ca, P and Mg levels). Cows consumed 0, 1, 2 or 3 kg WCS per day. No significant differences were observed among the groups on analysed parameters except plasma vitamin E concentration. In addition, when the amount of cottonseed was increased, milk yield and milk fat content also tended to increase but this increase was not statistically significant. In conclusion, feeding of WCS up to 3 kg per day with *ad libitum* maize silage did not cause negative effects on milk yield, milk fat and blood vitamin levels in the short term in dairy cows. ***(Asian-Aust. J. Anim. Sci. 2006. Vol 19, No. 6 : 852-856)***

**Key Words :** Whole Cottonseed, Dairy Cow, Rumen, Blood

## INTRODUCTION

Whole cottonseed (WCS) is used as a source of energy, protein and fiber in rations fed to lactating dairy cows. Most energy-rich feeds are relatively low in fiber. However, WCS contains 21% crude fibre (CF), which assists in maintenance of normal milk fat percentage. It is inadequate in lysine, calcium, and fat soluble vitamins (Puschner, 2003). It also contains 80-96% TDN, 21-23% crude protein (CP), 21% CF and 15-23% ether extract (EE) (Lane, 2001). Because of the high fat content (more than 20%), cattle should not consume more than 0.75% of body weight (Lalman, 1996). Whole cottonseed is often fed at 10 to 15% of the total dietary dry matter (DM) for dairy cows. A primary concern with feeding large amounts of WCS is the possibility of gossypol toxicity in cows (Arieli, 1998). Whole cottonseed and other cotton products contain gossypol, a yellow pigment, polyphenolic binaphthylaldehyde, that is toxic to young ruminants and non-ruminant species (Gray et al., 1993). Gossypol exists in free and bound forms. In the intact whole seed, gossypol is mostly found in the free form. However, when cottonseed is processed, gossypol binds to protein, possibly to the epsilon-amino group of lysine (Mena et al., 2001).

Whole cottonseed is widely produced, and it is very cheap in Turkey but it is not used in animal feeding especially for dairy cows. Therefore, the purpose of this study was to determine the effects of WCS on some blood parameters, milk yield, milk fat and rumen parameters in dairy cows.

## MATERIALS AND METHODS

Four lactating Holstein cows (500 kg average body weight, multiparous) were used in the experiment. The experiment started approximately 2 months after parturition; cows were in their third or fourth parity. Cows were assigned to a 4×4 Latin square experimental design with 4 diets. The concentrates contained 0, 12.5, 25 and 37.5% WCS and were formulated as isocaloric and isonitrogenous (Table 1). All animals were fed 4 kg concentrate and 2 kg wheat straw in the morning feeding and 4 kg concentrate and *ad libitum* maize silage in the evening feeding. Thus, each cow was fed 0, 1, 2, 3 kg/d of WCS for each period. Cows were kept under similar conditions (half-open barn). The animals had free access to water throughout the experiment.

Each experimental period was 21 d (14 d for dietary adjustment followed by 7 d of data collection). Cows were milked twice daily at 07:00 and 16:30 h. Milk samples were collected from each animal on day 20 of each period during am and pm milkings and analysed for milk fat. Milk yield was recorded in each data collection period (7 d) during am and pm milkings.

In the middle of each data collection period (d 18), blood samples were taken from the left jugular vein, using vacutainer (20-gauge), 2.54 cm, 10×100-mm tubes containing lithium heparin (VENOJECT, TERUMO), for determination of plasma parameters (vitamin E, Mg, Ca, $NH_3$ and P). For serum collection, vacutainer brand tubes containing no anticoagulant were used (10×100-mm blood

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**Table 1.** Composition of the concentrates used in this experiment

| Items | Treatments | | | |
|---|---|---|---|---|
| | Control | WCS 1 | WCS 2 | WCS 3 |
| Ingredients (%) | | | | |
| Barley | 5.5 | 20 | 21 | 25 |
| Wheat | 55 | 36 | 30 | 23 |
| Whole cottonseed (WCS)[1] | 0 | 12.5 | 25 | 37.5 |
| Cottonseed meal | 9.5 | 8 | 5.3 | 2 |
| Soybean meal | 10.5 | 10 | 8.2 | 7 |
| Wheat bran | 1 | 1 | 2 | 1 |
| Sunflower meal | 15 | 9 | 5 | 1 |
| Dicalcium phosphate | 0.1 | 0.1 | 0.1 | 0.1 |
| Limestone | 2.5 | 2.5 | 2.5 | 2.5 |
| NaCl | 0.5 | 0.5 | 0.5 | 0.5 |
| Premix[2] | 0.4 | 0.4 | 0.4 | 0.4 |
| Chemical composition | | | | |
| Dry matter (% ) | 89.64 | 88.98 | 89.96 | 89.95 |
| Ash (% DM ) | 7.44 | 7.28 | 7.40 | 7.15 |
| Crude protein (% DM) | 20.33 | 20.78 | 20.33 | 20.69 |
| Ether extract (% DM) | 4.29 | 5.11 | 6.89 | 8.28 |
| Acide detergent fiber (% DM) | 11.95 | 14.30 | 15.34 | 16.56 |
| Neutral detergent fiber (% DM) | 19.61 | 21.11 | 23.91 | 24.91 |
| ME (Mcal/kg DM) | 2.92 | 2.91 | 2.92 | 2.93 |
| Gossypol[3] (g/kg) | 0 | 0.418 | 0.836 | 1.254 |

[1] Contains free gossypol 3,346 ppm, CP 23.1%, EE, 11.4%, ADF, 28.6%.
[2] per kg premix, Vitamin A, 15,000,000 IU; Vitamin $D_3$, 3,000,000 IU; Vitamin E, 30,000 mg.
Mn, 50,000 mg; Zn, 50,000 mg; Fe, 50,000 mg; Cu, 10,000 mg; I, 800 mg; Co, 150 mg; Se, 150 mg.
[3] The amounts originated from WCS.

**Table 2.** Milk yield and composition

| Item | Treatments | | | | Prob. |
|---|---|---|---|---|---|
| | Control | WCS 1 | WCS 2 | WCS 3 | |
| Milk yield (L/d) | 18.30±0.77 | 19.77±0.73 | 20.09±0.81 | 20.45±0.91 | NS |
| Milk fat (%) | 3.98±0.36 | 3.99±0.36 | 4.06±0.15 | 4.13±0.24 | NS |

NS: Non significant. Mean±SE.

**Table 3.** Effects of feeding level of whole cottonseed on the parameters of rumen and blood in dairy cow

| Items | Treatments | | | | Prob. |
|---|---|---|---|---|---|
| | Control | WCS 1 | WCS 2 | WCS 3 | |
| Rumen pH | 5.98±0.29 | 6.21±0.19 | 6.13±0.21 | 6.22±0.17 | NS |
| Rumen TVFA[1] (mmol/L) | 125.8±8.9 | 115.9±8.5 | 121.3±6.4 | 117.6±6.0 | NS |
| Rumen ammonia (mmol/L) | 9.07±2.82 | 8.2±1.02 | 8.4±1.13 | 8.66±1.96 | NS |
| Serum β-carotene (μmol/L) | 0.69±0.17 | 0.91±0.32 | 0.83±0.08 | 1.04±0.38 | NS |
| Serum vitamin A (μmol/L) | 1.02±0.09 | 1.30±0.22 | 1.14±0.08 | 1.33±0.18 | NS |
| Serum urea (mmol/L) | 14.21±1.7 | 14.71±1.28 | 12.80±1.01 | 12.69±1.60 | NS |
| Plasma vitamin E (μmol/L) | 107.9±7.6[a] | 113.9±3.5[a] | 133.2±5.3[b] | 125.6±5.8[ab] | * |
| Plasma ammonia (μmol/L) | 27.8±4.9 | 30.0±5.0 | 21.5±3.7 | 23.3±4.3 | NS |
| Plasma calcium (mmol/L) | 2.36±0.10 | 2.37±0.11 | 2.52±0.09 | 2.48±0.06 | NS |
| Plasma magnesium (mmol/L) | 1.05±0.05 | 0.97±0.02 | 1.06±0.05 | 1.07±0.04 | NS |
| Plasma phosphorus (mmol/L) | 1.80±0.31 | 1.81±0.17 | 1.69±0.23 | 1.57±0.10 | NS |

[1] Total volatile fatty acid. Mean±SE. NS: Non significant; * $p<0.05$.
[a, b] Different letters within same row mean significant differences between treatments.

collection tubes; Becton-Dickinson). Serum samples were used for analyses of serum parameters (β-carotene, vitamin A, urea).

After collection, blood samples (with anticoagulant) were centrifuged for 10 minutes at 2,500 rpm (Hettich Zentrifugen Universal 30F model, Germany) for preparation of plasma. The serum in each blood was prepared by centrifugation.

The blood serum urea levels were measured using a commercial kit (SPINREACT, Bertheleot enzymatic cholorometric test, Espana). The blood serum β-carotene and vitamin A (Suzuki and Katoh, 1990), and blood plasma vitamin E (Baker and Frank, 1968) were measured using a spectrophotometer (Shimadzu UV-1601, Japan). Blood plasma Ca, P, Mg and $NH_3$ were determined using an auto blood chemistry analyser (VETTEST 8008, IDEXX Laboratories, inc Westbrook ME 04092 USA).

Rumen fluid was collected carefully via a stomach tube approximately 2 h post morning feeding on the same day that blood was collected. Milk fat contents were determined using the Gerber Method (Marshall, 1992). The nutrient composition (Dry matter, Ash, Crude protein and ether extract) of the experimental diets were determined according to the AOAC (1984), and free gossypol was analyzed according to the AOCS (1985). ADF and NDF were determined by the methods of Georing and Van Soest (1970). Rumen total volatile fatty acid (TVFA) and NH3-N were analyzed according to Markham (1942). Rumen pH was determined using a pH meter (Metrohm 744, Metrohm limited CH-9101 Herisau, Switzerland).

Milk fat, rumen and blood parameters were statistically analysed by the Kruskal-Wallis test and milk yield was analysed by one-way analysis of variance. Differences between group means were determined using Duncan's multiple range test using the SPSS statistical package (version 10.0).

## RESULTS AND DISCUSSION

The composition of experimental diets are shown in Table 1. Whole cottonseed used in the present study contained 3,346 ppm free gossypol, 23.1% CP, 11.4% EE and 28.6% ADF. The ether extract (EE) levels in the concentrate diet were 4.29%, 5.11%, 6.89%, and 8.28 in control, WCS 1, WCS 2 and WCS 3 groups, respectively.

There were no significant differences between the groups in terms of either milk yield or milk fat levels (Table 2).

The rumen TVFA, rumen ammonia, rumen pH, serum β-carotene, serum vitamin A, plasma vitamin E, urea, Mg, Ca, $NH_3$ and P values are shown in Table 3. Plasma vitamin E level was significantly higher in the WCS2 group than in the control group ($p<0.05$).

Although the milk yield and milk fat concentration levels increased linearly with an increased level of WCS supplementation, this effect was not statistically significant. In the WCS3 group the milk yield and milk fat levels were 11.75% and 3.77% higher respectively than in the control group. Similar results were found in other studies. For example, Tomlinson (1981) reported increased milk fat levels with an addition of cottonseed to the ration. When 0, 1.8, 3.6 and 5.4 kg of cottonseed were added to the daily ration, the milk fat levels were 4.04, 4.53, 4.66, 4.71%, respectively. In another study, increased levels of both milk yield and percent milk fat but decreased percent milk protein were reported from the addition of WCS to the rations of lactating cows (Coppock and Wilks, 1991). In this context, Campbell (2002) indicated that increased milk fat content depended on the amount of cottonseed in the ration, and such an increase in milk fat could be due to the combination of the fat and the cellulose in cottonseed which can stimulate the milk fat production. On the other hand, unlike our current findings, decrease in milk fat level by dietary cottonseed was also reported (Mena et al., 2002). It has been shown that the fatty acids in cottonseed are largely unsaturated, and can reduce fiber digestion and milk fat yield levels if their levels are too high (Hoffman, 2002). Furthermore, decreased milk yield was also reported when WCS diets were replaced with soybean meal in dairy cows (Barraza et al., 1991).

The concentrations of ruminal pH, TVFA and ammonia were not significantly different among treatments. The numerically lower concentrations of rumen ammonia in WCS 1, WCS 2 and WCS 3 may reflect the slower degradable nitrogen in these groups compared with that of control group. The slightly lower concentration of TVFA in treatment groups WCS 2 and WCS 3 could be due to the high fat content of these diets. Lalman (1996) indicated that the forage digestibility could decrease when the fat content exceeds 6% of the diets. Harvatina et al. (2002) reported that ruminal pH, acetate and propionate levels were decreased when cottonseed was increased in the ration.

In the present study, plasma vitamin E concentration was higher in the WCS treatments than in the control ($p<0.05$). Although there were no significant differences in the concentrations of serum β-carotene and vitamin A, these tended to be higher in cows fed the WCS diets. In contrast, Lane and Stuart (1990) showed that there was a negative relationship between free gossypol intake and serum retinol and tocopherol.

The concentration of blood urea and ammonia were not influenced by supplementation of WCS in this study; Barraza et al. (1991), however, reported that plasma urea-N (PUN) was decreased by WCS supplementation. They concluded that this might be an indicator of gossypol intoxication due to damage of liver cells and, thus, diminished capability of the liver to synthesise urea. In addition, Nikokyris et al. (1999) reported that PUN was associated with differences in dietary free gossypol intake. This was obviously not the case in the present study.

No statistical differences were observed in plasma Ca, P or Mg levels between groups, a finding which did not agree with Colin-Negrete et al. (1996) or Larson and Olson (2002). They reported that cottonseed meal was low in Ca and high in P, and inorganic P in blood increased linearly ($p<0.05$) as WCS increased. Moreover, Hoffman (2002)

**Table 4.** The phenotypic correlation coefficients among some blood and rumen parameters (n = 16)

| | β-carotene | Vitamin A | Vitamin E | Urea | Magnesium | Ammonia | Calcium | Phosphorus | Rumen TVFA | Rumen Ammonia |
|---|---|---|---|---|---|---|---|---|---|---|
| Vitamin A | 0.621* | | | | | | | | | |
| Vitamin E | 0.052NS | 0.124NS | | | | | | | | |
| Urea | -0.693** | -0.145NS | -0.066NS | | | | | | | |
| Magnesium | 0.237NS | 0.374 NS | 0.160NS | 0.118NS | | | | | | |
| Ammonia | -0.374NS | -0.613* | -0.202NS | -0.257NS | -0.547* | | | | | |
| Calcium | -0.,060NS | 0.107 NS | 0.257NS | -0.099NS | 0.005NS | -0.341NS | | | | |
| Phosphorus | -0.309NS | -0.167NS | 0.033NS | 0.658** | 0.265NS | 0.316NS | -0.372NS | | | |
| Rumen TVFA | -0.501* | -0.123NS | -0.182NS | 0.584* | 0.361NS | -0.162NS | 0.199NS | 0.067NS | | |
| Rum ammonia | -0.598* | -0.106 NS | -0.70NS | 0.577* | 0.215NS | -0.170NS | 0.280NS | 0.015NS | 0.600* | |
| Rumen pH | 0.550* | 0.117 NS | -0.009NS | -0.819** | -0.338 | 0.057NS | -0.002NS | -0.499* | -0.786** | -0.556* |

* Correlation is significant at the 0.05 level.
** Correlation is significant at the 0.01 level.

indicated that the fat fraction of WCS can reduce Ca and Mg absorption. The phenotypic correlation coefficients among blood and rumen parameters were also analyzed (Table 4). Results indicated that carotene was highly correlated with serum urea ($p<0.01$), rumen TVFA, ammonia and rumen pH ($p<0.05$). Magnesium and vitamin A were highly correlated with ammonia ($p<0.05$). These results should be investigated in future experiments.

In conclusion, feeding WCS up to 3 kg per day with *ad libitum* maize silage did not cause a negative effect on milk yield, milk fat and blood vitamin levels in the short term in dairy cows.

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## REFERENCES

AOAC. 1984. Official Methods of Analysis of the Association of Analytical Chemists. 14th ed., The William BYRD Press, Inc., Richmond, Virginia.

AOCS. 1985. Determination of free gossypol. Official method Ba 7-58. Official and Tentative Methods of Analysis. 3rd ed. American Oil Chemists Society, Chicago, IL.

Arieli, A. 1998. Whole cottonseed in dairy cattle feeding: A review. Anim. Feed Sci. Technol. 72:97-110.

Baker, H. and O. Frank. 1968. Vit. E Clinical Vitaminology. Methods and Interpretation, 169-177.

Barraza, M. L., C. E. Coppock, K. N. Brooks and D. L. Wilks. 1991. Iron sulfate and feed pelleting to detoxify free gossypol in cottonseed diets for dairy cattle. J. Dairy Sci. 74:3457-3467.

Campbell, J. 2002. Whole cottonseed for dairy rations. Accessed Jan. 27, 2002, http://www.burkmann.com/basics/whlcotsd.htm.

Colin-Negrete, J., H. E. Kiesling, T. T. Ross and J. F. Simitt. 1996. Effect of whole cottonseed on serum constituents, fragility of erythrocyte cells, and reproduction of growing Holstein heifers. J. Dairy Sci. 79:2016-2023.

Coppock, C. E. and D. L. Wilks. 1991. Feeding whole cottonseed and cottonseed meal to dairy and beef cattle. Proc. Alternative Feeds for Dairy and Beef Cattle, Natl. Invit. Symp., St. Louis, MO (Ed. E. R. Jordan). p. 43-48. Columbia, MO: Coop. Ext. Univ. of Missouri. Feed Industry Red Book (1994). (J. H. Goihl and R. R. McEllhiney). Edin Prairie, MN: Comm. Marketing, Inc.

Georing, H. K. and P. J. Van Soest. 1970. Forage Fiber Analysis Agric. Handbook No: 379. (Agricultural Research Service) U.S. Dep. Agric. Washington. DC. 1970.

Gray, M. L., L. W. Greene and G. L. Wiliams. 1993. Effects of dietary gossypol consumption on metabolic homeostasis and reproductive endocrine function in beef heifers and cows. J. Anim. Sci. 71:3052-3059.

Harvatina, D. L., J. E. Winkler, J. L. Firkins and M. L. Eastridge. 2002. Whole linted cottonseed as a forage substitute fed with ground or steam-flaked corn: digestibility and performance. J. Dairy Sci. 85(8):1976-1987.

Hoffman, C. P. 2002. Whole cottonseed. Accessed Jan. 27, 2002. from http://www.cf.uvex.edu/ces/pubs/pdf/A3519.PDF. Univ. Wisconsin-Madison.

Lalman, D. L. 1996. Alternative feeds for beef cows and stockers. pp. 1-8. Agricultural publication G2076-New January 15, University of Missouri. Published by University Extension.

Lane, A. G. and R. L. Stuart. 1990. Gossypol intake may affect vitamin status of cattle. Feedstuffs, July 9.

Lane, C. D. 2001. Feeding cottonseed and cotton by-product to beef cattle. Accessed 02. 10. 2001, from http://www.utextension.utk.edu/ansci/feeding-cottonseed. Univ. Tennessee Knoxville.

Larson, R. L. and K. C. Olson. 2002. Cottonseed feed for cattle. University of Missouri Extension. Cattlemen’s Corner. Timely Tips Innovative Beef Producers. Volume 5, No. 2 June/July 2002.

Markham, R. 1942. A steam distillation apparatus suitable for micro-kjeldahl analysis. Biochem. J. 36:790.

Marshall, R. T. 1992. Standard method for the examination of dairy products, 14 th ed. APHA, Washington, DC.

Mena, H., J. E. P. Santos, J. T. Huber, J. M. Simas, M. Tarazon and M. C. Calhoun. 2002. The effects of feeding varying amounts of gossypol from whole cottonseed and cottonseed meal in lactating dairy cows. J. Dairy Sci. 84:2231-2239.

Nikokyris, P. N., K. Kandylis and K. Deligiannis. 1999. Effect of varying levels of dietary free gossypol in whole cotton seed on physiological responses of growing-fattening lambs. J. Sci. Food Agric. 79:1969-1981.

Puschner, B. 2003. Feeding cottonseed to dairy cattle. Accessed July, 15, 2003, http://cahfs.ucdavis.edu/diseaseinfo/cattle.pdf.

Suzuki, J. P. and N. A. Katoh. 1990. A simple and cheap method for measuring serum vitamin A in cattle using only spectrophotometer. Jpn. J. Vet. Sci. 52:1291-1293.

Tomlinson, J. E. 1981. Cottonseed on milk production and composition. J. Dairy Sci. 64(Suppl 1.):141.