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**Araştırma Makalesi**

# FARKLI ORANLARDA PAMUK TOHUMU İÇEREN RASYONLARIN SÜT İNEKLERİNDE BAZI KAN PARAMETRELERİ ÜZERİNE ETKİLERİ*

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## Effect Of Different Levels Of Whole Cottonseed In Rations On Some Hematological Parameters In Dairy Cattle

**Özet:** Bu çalışmada diyette farklı oranlarda bulunan pamuk tohumunun bazı kan parametreleri [hematokrit, hemoglobin, ortalama eritrosit hemoglobin konsantrasyonu (MCHC), eritrositlerin ozmotik frajilitesi] üzerine etkileri araştırılmıştır. Bu amaçla 4 Holştayn ırkı inek toplam 4 dönemden oluşan 4X4 Latin kare metoduna göre kullanılmıştır. Her dönem 14 gün adaptasyon ve 7 gün veri toplama süresinden oluştu. Bütün kan örnekleri sabah yemlemesinden ve sağımından sonra alındı. İneklerin diyetinde bulunan konsantre yemde pamuk tohumu oranları, sırasıyla %0 (Kontrol; C), % 12.5 (W-I), % 25 (W-II) ve % 37.5 (W-III) pamuk tohumu olacak şekilde ayarlandı. Diyette bulunan pamuk tohumu sırasıyla 0 (C), 1 (W-I), 2 (W-II), 3 (W-III) kg/gün olarak hesaplandı. Yemde bulunan pamuk tohumunun % hematokrit (C=40.75 ± 1.55, W-I=41.00 ± 3.19, W-II=40.25 ± 5.03 ve W-III=43.25 ± 4.96), hemoglobin (C=10.93 ± 0.72, W-I=11.19 ± 1.01, W-II=10.26 ± 0.56, ve W-III=10.89 ± 0.31 g %) veya MCHC (C=267 ± 12, W-I=277 ± 30, W-II=270 ± 40 ve W-III=263 ± 35 g/L) seviyeleri üzerine herhangi bir etkisi gözlenmedi. Uygulamanın eritrositlerin ozmotik fragilitesi üzerine de herhangi bir etkisi bulunmadı. Sonuç olarak, yeme kısa süreli % 37.5'e varan oranlarda pamuk tohumu ilavesinin ineklerin kan parametreleri üzerine herhangi bir olumsuz etkisi gözlenmedi.

**Anahtar kelimeler:** Pamuk tohumu, Hematokrit, Hemoglobin, MCHC, Eritrosit Frajilitesi

**Summary:** The effects of different levels of whole cottonseed (WCS) in the diet on hematological parameters (hematocrit %, hemoglobin, mean corpuscular hemoglobin concentration and osmotic fragility of erythrocytes) were examined in lactating cows. Four Holstein cows were assigned to a 4X4 Latin Square design with four stages. All stages had 14 d of adaptation and 7 d of data collection periods. All blood samples were collected from vena jugularis after morning feeding and milking. The dietary treatments included 0% (control; C), 12.5 % (W-I), 25 % (W-II) and 37.5 % (W-III) WCS of total concentrate in diet. Total WCS in the diet was calculated to be 0 (C), 1 (W-I), 2 (W-II), 3 (W-III) kg/d. No significant effects of WCS concentration in diet fed were observed for mean % hematocrit (C=40.75 ± 1.55, W-I=41.00 ± 3.19, W-II=40.25 ± 5.03 and W-III=43.25 ± 4.96), hemoglobin (C=10.93 ± 0.72, W-I=11.19 ± 1.01, W-II=10.26 ± 0.56, and W-III=10.89 ± 0.31 g %) or MCHC (C=267 ± 12, W-I=277 ± 30, W-II=270 ± 40 and W-III=263 ± 35 g/L). Osmotic fragility of erythrocytes showed no treatment effect. In conclusion, short time feeding of up to 37.5% WCS had no negative effects on some blood parameters of animals.

**Key words:** Whole cotton seed, Hematocrit, Hemoglobin, MCHC, Erytrocyte Fragility

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## Introduction

Whole cottonseed (WCS) and cottonseed meal (CSM) are an excellent source of protein, fat, fiber and energy in cattle diets (**3, 20**) and feeding WCS to milking cows can stimulate higher milk fat test and help maintain milk persistency (**11, 17, 21, 25**). However, cottonseed contains gossypol, a natural toxic compound that protects the cotton plant from insect damage (**18, 26**). Ruminants with a well developed rumen microbial population are able to detoxify gossypol by converting free to bound gossypol within the rumen, thereby impeding its absorption into the blood (**21**). Thus, gossypol toxicity usually is not a common problem in ruminants (**11, 15**). However, reports of gossypol toxicity in mature cattle demonstrate that the capacity of the rumen to detoxify gossypol may be exceeded (**11**) or bound gossypol may be released during the digestive process and have a physiological effect on the animal (**25**).

The gossypol in cottonseed is mostly free gossypol and is toxic to livestock (**25**). Risco et al. (**20**) reported that 400 ppm of free gossypol approaches toxicity and 800 ppm results in death was not accurate for ruminants with active pregastric fermentation. They did not demonstrate those effects and, in other studies, feeding diets with up to 1000 ppm of free gossypol for 170 days did not cause death or toxicity (**21**). Moreover, Mena et al. (**13**) fed diets up to 1800 ppm of gossypol with no death or clear signs of toxicity, except for increases in plasma gossypol and erytrocyte fragility.

Gossypol toxicity can alter some blood parameters. For example, Nikokyris et al. (**16**) reported elevations of lactate dehydrogenase and the increase of plasma urea as signs of gossypol toxicity in growing lambs. Alterations in normal erythrocyte structure, erythrocyte fragility, and function at 30 % WCS of the diet appear to be the major physiological effects of gossypol in lactating dairy cows (**11**).

Dietary gossypol from cottonseed products has caused hematological changes in ruminants may be concidered dangerous if fed for long periods (**4**). Free gossypol binds to Fe reflecting apparent loss of Fe for normal biosynthesis of hemoglobin (**2**) and may interact directly with erythrocyte membrane (**23**) causing anaemia and increased erythrocyte fragility (**15**). Therefore, the objective of this experiment was to evaluate the effects of feeding a diet containing 0 % WCS, 12.5 % WCS, 25 % WCS and 37.5 % WCS in dairy cattle on some blood parameters such as hematocrit %, hemoglobin, MCHC and osmotic fragility.

## Materials and Methods

Experimental Design: Four multiparous Holstein cows (5 to 6 years old) with similar milk production, body weight and lactation periods were used in the experiment. The experimental protocol was approved by the Institutional Animal Care and Use Committee by Akdeniz University. Parity of the cows ranged between 3 and 4 with body weights between 474 and 527 kg. Cows were assigned to a 4 x 4 Latin square design with four periods. Each experimental period lasted 21 d including 14 d of dietary adjustment and 7 d of data collection periods.

Feeding Program: Cows were fed four different concentrates containing 0% (C), 12.5% (W-I), 25% (W-II), or 37.5% (W-III) WCS during experimental periods. The diets were formulated as isocaloric and isonitrogenous (Table 1). Animals were fed 8 kg of the formulated ration (4 kg for a.m. feeding and 4 kg for p.m. feeding), 2 kg straw (a.m. feeding) and *ad libitum* corn silage (p.m. feeding). The amounts of WCS in concentrates were formulated to result in daily intakes of 1, 2, 3 kg of WCS. The gossypol content of cotton seed was measured as 3346 ppm (**1**).

Collection of Blood Samples: All blood samples were collected using 20 gauge Vacutainer needles after the morning feeding in the middle of each data collection period (d 18). Blood samples were directly collected from the jugular vein into 10 ml (10X100 mm) Vacutainer blood collection tubes with EDTA (Becton-Dickinson, Fairlawn, NJ). They were placed on ice after collection and analyzed within an hour.

Determination of Blood Parameters: Percent hematocrit and hemoglobin concentrations were estimated by microhematocrit and cyanmethemoglobin methods, respectively (**14**). Mean corpuscular hemoglobin concentrations (MCHC) were calculated from the values of hematocrit % and Hb (**10**). The osmotic fragility of erythrocytes was determined as described by Jain (**10**) using a 1% NaCl solution in phosphate buffer at room temperature (pH 7.4). Hemolysis in distilled water (0%) was set as 100 % and the percentage of hemolysis in each NaCl concentration was evaluated. Erythrocyte fragility index defined as the salt concentration at which 50 % hemolysis of erythrocytes occurred (**6**).



Statistical Analyses: Data were analyzed by Kruskal-Wallis test (blood parameters). Differences between means were tested by Duncan's test by SAS (**22**).

**Table 1:** Composition of the concentrate mixtures fed to animals (% DM).

**Tablo 1:** Hayvanlara verilen konsantre yemlerin kompozisyonu (% kuru madde)

| | Treatments[1] | | | |
|---|---|---|---|---|
| Ingredients | C | W-I | W-II | W-III |
| Barley | 5.5 | 20 | 21 | 25 |
| Wheat | 55 | 36 | 30 | 23 |
| 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 |
| Whole cottonseed | 0 | 12.5 | 25 | 37.5 |
| Dicalcium phosphate (DCP) | 0.1 | 0.1 | 0.1 | 0.1 |
| Limestone | 2.5 | 2.5 | 2.5 | 2.5 |
| Salt (NaCl) | 0.5 | 0.5 | 0.5 | 0.5 |
| Vitamin Premix* | 0.2 | 0.2 | 0.2 | 0.2 |
| Mineral Premix** | 0.2 | 0.2 | 0.2 | 0.2 |
| CP %*** | 18.93 | 18.94 | 18.93 | 18.96 |
| ME, Mcal/kg*** | 2.92 | 2.91 | 2.92 | 2.93 |

* per kg vitamin premix; Vitamin A 15,000,000 IU, Vitamin D3 3,000,000 IU, Vitamin E 30,000 mg.

** per kg of mineral premix; Mn=50,000 mg, Zn=50,000 mg, Fe=50,000 mg, Cu 10,000 mg, I=800 mg, Co=150mg, ve Se=150mg.

*** calculated values.

[1] C=Control (0% WCS), W-I=12.5% WCS, W-II=25%WCS and W-III=37.5% WCS.

**Table 2:** Average blood values of cows fed concentrates containing 0, 12.5, 25, 37.5 % whole cotton seeds.

**Tablo 2:** Konsantre yemlerinde %0, 12.5, 25, 37.5 pamuk tohumu ihtiva eden yemlerle beslenen ineklerin ortalama kan değerleri.

| | Treatment groups[1] | | | | |
|---|---|---|---|---|---|
| | C | W-I | W-II | W-III | |
| Hematocrit% | 40.75± 1.55 | 41.0 ±3.19 | 40.25 ±5.03 | 43.25 ±4.96 | NS[2] |
| Hemoglobin (g %) | 10.93 ±0.72 | 11.19 ±1.01 | 10.26 ±0.56 | 10.89 ±0.31 | NS[2] |
| MCHC (g/L) [3] | 267 ±12 | 277 ±30 | 270 ±40 | 263 ±35 | NS[2] |
| OF (%) [4] | 0.55-0.35 | 0.55-0.35 | 0.55-0.35 | 0.55-0.35 | NS[2] |

[1] C=Control (0% WCS), W-I=12.5% WCS, W-II=25%WCS and W-III=37.5% WCS.

[2] NS=Not Significant (P<0.1),

[3] MCHC=Mean Corpuscular Hemoglobin Concentration.

[4] OF=Osmotic fragility; % Values of NaCl where hemolysis began and completed.

## Results

The mean values for hematocrit %, hemoglobin, MCHC, erythrocyte fragility are in Table 2. No effects of WCS treatment were observed for hematocrit %, hemoglobin, MCHC or osmotic fragility. In addition, no specific trends were observed for different diets with WCS tested in the experiment.

The lowest value for hematocrit % and hemoglobin were observed in animals fed 25 % WCS contained diet. The values for MCHC were numerically different in all groups and tended to decrease with the increasing concentration of WCS in diet. The lowest values for MCHC of blood were observed in animals fed with 37.5 % WCS. In addition, no differences were observed in osmotic fragility of blood samples among the treatments. The osmotic fragility of both samples started to increase at 0.65 % NaCl concentrations and riched to a plateau around 0.45 % NaCl concentrations. The NaCl concentration at which 50 % of hemolysis of erytrocytes seen was at around 0.55 % NaCl for all groups (Figure 1).



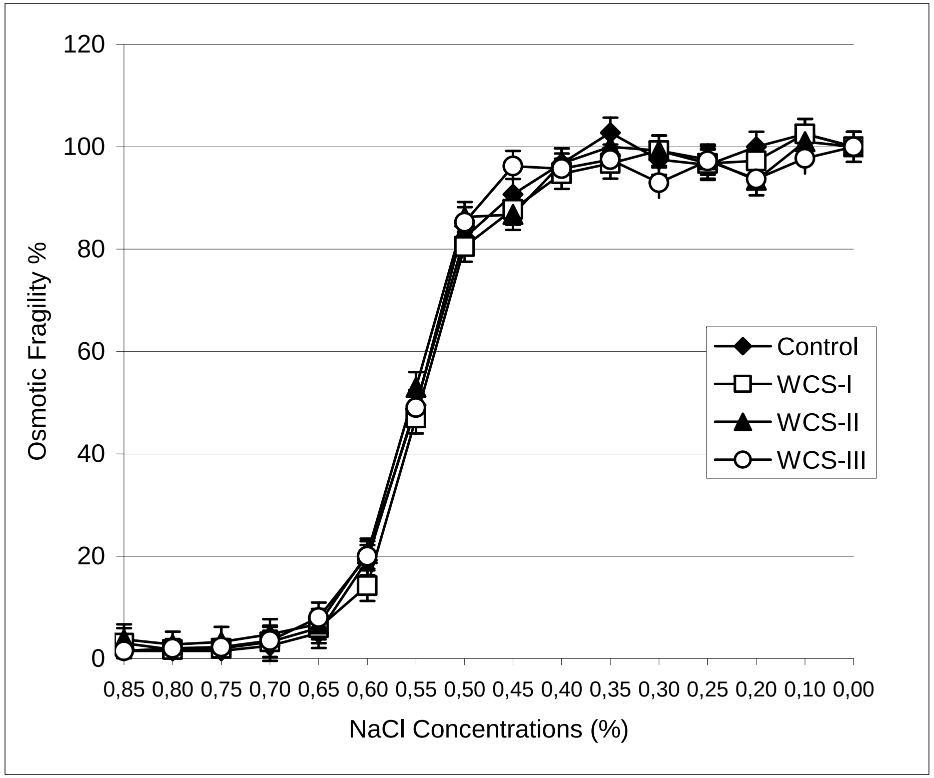


**Figure 1:** Percent osmotic fragility of erythrocytes for treatment groups in different NaCl concentrations. C=Control (0% WCS), W-I=12.5% WCS, W-II=25%WCS and W-III=37.5% WCS

**Şekil 1:** Farklı NaCl konsantrasyonlarında tedavi guruplarının eritrositlerinin ozmotik frajiliteleri. C=Kontrol (%0 pamuk tohumu), W-I=%12.5 pamuk tohumu, W-II=%25 pamuk tohumu and W-III=%37.5 pamuk tohumu

## Discussion

An important finding of this study was that short time feeding of diets containing different amounts of WCS did not affect the blood parameters examined. In addition, values for MCHC, hematocrit %, and hemoglobin were within the accepted ranges for cattle (**10, 24**).

Erythrocyte osmotic fragility measures ability of erythrocytes to resist osmotic stress when they are incubated with decreasing NaCl concentrations. In the current study, the amount of WCS in the diet did not affect the osmotic fragility of erythrocyte and erythrocyte fragility index for the blood samples tested was at the 0.55 % NaCl concentration for both treated and control animals. Percent hemolysis of cattle erythrocytes in NaCl solutions were previously reported to be between 0.44 % and 0.59 % (**8, 10**). Some researchers (**4, 11, 13, 19, 20, 25, 26**) reported an increase in erythrocyte fragility with increasing levels of WCS or cotton byproducts in the diet. However, consistent with our findings, no change in erythrocyte fragility was reported when lactating diary cows were fed whole gin-run cottonseed for one lactation period (**8**), lactating dairy cows were fed diets with increasing levels of cracked pima cottonseed (**18**) or lactating dairy cows were fed up to 30 % WCS (**5**). The frequently different levels of gossypol used in different studies or detoxification of gossypol by rumen microorganisms could explain part of the different responses in erythrocyte fragility in previous studies. Thus, the relatively lower level of gossypol or effective detoxification of gossypol in WCS in the current study might be the reasons for no change in erythrocyte fragility.

In the current study, blood hemoglobin and hematocrit were not different among WCS treatments and were within normal values reported for cows (**10**). Similarly, Valesquez-Pereira et al. (**25, 26**) did not observe any significant changes in hematocrit % or hemoglobin when cows fed cotton seed meal (CSM) with 0.13 % free gossypol/kg of BW/d to bulls or 400 mg of free gossypol/kg of diet DM to calves. Risco et al. (20) also reported similar results when cows fed 0 % and 15 % WCS to cows upto 120 d postpartum. Although Lindsey at al. (**11**) reported lower hemoglobin concentration and hematocrit percentage in animals receiving gossypol in solvent extracted CSM, Nikokyris et al. (**15**) found no significant differences in hemoglobin, hematocrit and MCHC in lambs fed diets with varying amounts of WCS diets. Hemoglobin and hematocrit concentrations are directly affected by the rate of intravascular hemolysis. In the present study, osmotic fragility of erythrocytes was not affected by treatment. Lack of alteration of membrane integrity due to WCS feeding may explain the normal hemoglobin and hematocrit values. Thus, no or minimal effect of gossypol on erythrocyte function was observed in current study.

The differences among studies could be explained by the different diets fed, difference in the age of the animals or the duration of feeding period. For example, the development of toxicity might depend on the free gossypol content of the food or their processing methods. Researchers concluded that processing of cottonseed apperas to alter nutrient digestibility and the ability of gossypol to produce a toxic response. In the intact whole cottonseed, gossypol is mostly found as free gossypol (**21**). It was noted that methods of processing, except for roasting, may reduce concentrations of both free



gossypol in cotton products and plasma gossypol, and increase the proportion of the positive isomers of gossypol (**3**). Increased plasma gossypol concentrations were observed when WCS was grained or cracked (**12**). Lindsey et al. (**11**) found that cows fed screw pressed CSM had consistently less Hb than those fed soybean meal.

The age of the ruminants used in different trials can also alter the results. The researchers suggest that younger animals are more susceptable to gossypol toxicity (**9, 15, 21**). Even though 10-16 weeks old young calves were fed high concentration of cotton seed meal resulted in death with lesions compatible with gossypol toxicity (**9**), Nikokyris et al. (**15**) reported the absence of gossypol toxicity in growing lamb.

Another possible reason for contradictory results might be the shorter duration of our feeding period. Free gossypol is detoxified by the formation of gossypol-protein complex and only high levels of free gossypol intake for a long time result in toxicity in ruminants (**15**). Erythrocyte membrane fragility was increased when 20 g of free gossypol was fed lactating dairy cows and heifers for 33 week (**7**). In addition, intoxication is possible in mature ruminants consuming CSM containing free gossypol but dose- dependent and dependent on the situation of animals (**15**). Lindsey et al. (**11**) suggested that gossypol intoxication may be more possible when the animals are subjected to the different stress-situations. Age can be an important factor whether the animal become a ruminant and can influence resistance to gossypol toxicity. Colin-Negrete et al. (**4**) reported that one heifer fed a diet containing 30% WCS died on d 396 of feeding possibly from gossypol toxicosis. Moreover, diet containing 100 to 220 mg/kg free gossypol resulted toxicity and deaths in calves between 7 to 15 weeks of age (**27**). In the current study, parity of the cows was ranged from 3 to 4. Thus, well-grown rumen microbial population of these adult ruminants could detoxify gossypol within the rumen, thereby was able to could hinder its absorption into the blood.

In conclusion, no adverse effects on hematologic parameters were observed in lactating dairy cows fed concentrates varying in the content of WCS. Dietary gossypol concentrations in the three WCS diets did not have any harmful effect on blood parameters. Thus, supplementing WCS for a short time to cows had no toxic effects on the hematology of animals.

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