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## **Effect of Grazing of Dairy Cows on Bt Cotton Crop Residues on Milk and Cheese Chemical Composition and Sensory Properties**

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### **Abstract**

This experiment was conducted in two localities in Gezira state, Sudan. The objective of the experiment was to investigate; the effect of grazing cattle on genetically modified cotton crop residues (designated as Bt-CCR) on milk and cheese chemical composition and sensory characteristics. The results revealed that, there was significant differences ( $P < 0.01$ ) between milk produced from grazing on Bt and on non- Bt CCR. With exception of fat, all other milk components were significantly ( $P < 0.01$ ) higher in milk produced from grazing on Bt CCR. Also the same trend was found in cheese produced from Bt milk. In sensory evaluation of milk, consistency, flavor, taste and overall assessment were significantly better in non Bt milk. While there was no significant difference in sensory characteristics between the cheese produced from Bt and non-Bt milk. It was concluded that, grazing on Bt CCR significantly affect milk and cheese chemical composition but only sensory characteristics in milk. However, more investigation to elucidate these reasons is needed.

**Keywords:** Bt- cotton crop residues; grazing; milk,cheese.

### **1. Introduction**

Sudan is one of the African countries that grow cotton as a cash crop.

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Cotton production started in the country before the establishment of Sinnar dam in 1925. The crop contributed to different economical aspects, which included fiber export, oil production and grazing on its residues after harvest.

Cotton crop residues (CCR) amount to a range of 5 – 7 t/ha [1]. Also [2] estimated a total of 1.8 million tons from 368000 ha (4.9 t/ha) in Sudan. CCR supports about 100.000 cows and 200.000 of each sheep and goats for 4 – 5 weeks. Period of CCR grazing witnesses increased milk production due to increased animal influx from out side of Gezira scheme. Surplus milk resulted from increased production, usually used for white cheese production.

Due to introduction of Bt – cotton (genetically modified cotton) since 2012, grazing on its residues and the animal products from feeding on it, began to be a matter of heated debate.

However currently most genetically enhanced plants in market place provide insect protection or herbicide tolerance, are being used as feed for livestock [3].

Generally reports on genetically modified crops as animal feed included for example, Bt – corn silage [4, 5, 6], Soybean [7], cotton seeds [8] were cited. Many authors agreed on that, transgenic crops did not affect milk production and composition [4, 8, 5]. While some authors reported some effect on milk composition, for instance [6] reported higher contents of milk fat, lactose and proteins and [5] found an increase in the contents of milk protein, lactose and SNF. However [7] recorded a dramatic reduction on average protein content in the colostrum and reduced fat, when goats were fed on GM soybean.

Reference [9] reviewed the relationships between ruminant management and sensory characteristics of cheese. The authors concluded that ripened cheese characteristics depend on a lot of technological factors and when these factors are not controlled enough, it is difficult to reveal and interpret the effects of upstream factors (genetic, physiological or dietary). Among the various milk production conditions which may influence cheese characteristics, the floristic composition of forage used by animal. Reference [10] compared the different characteristics of Beaufort cheese made when herd successively grazed on different parts of the same highland pasture, while [11] studied. The influence of the composition of the Alpine highland pasture on the chemical, rheological and sensory properties of Abundance cheese. In both experiments [10, 11], sensory characteristics varied according to the sward botanical composition. Difference concern texture and flavor (Abundance cheese) or only flavor (Beaufort cheese). Generally, in the available literature, effects of grazing on cotton crop residues either Bt or non – Bt CCR, on milk and cheese production and composition or sensory evaluation of these products is lacking. Therefore, this research was designed with the following objectives. To investigate the effect of Bt CCR grazing by dairy cows on milk and cheese chemical composition and sensory properties.

## **2. Materials and Methods**

### **Location of the experiment:**

The experiment was conducted in two localities of Gezira state where cotton crop was cultivated. South Gezira

locality lies south Greater Wad Medani locality. Greater part of this locality found in Gezira irrigated scheme, where cotton crop is a main cash crop. From this locality cattle herd composed of 66 animal units (AU) in Al-Madina Arab was selected (selections based on animal owner cooperation). This herd grazed on genetically modified cotton crop residues which designated in this study as Bt CCR ( Bt =(*B.thuringensis*). Another herd of 46 AU also in South locality was selected from Ganib Al-Asad area where animals grazed on non-Bt CCR. The second locality was East Gezira locality. From this locality a herd of 62 AU in Tambol area was selected for Bt CCR grazing and a herd of 52 AU in Um Algura area for non-Bt CCR grazing. The lactating cows in Al-Madina Arab and Um- Algura was 40%. While it was 52% and 46% in Ganib Al- Asad and Tambol respectively. Each herd was of mix breeds (local and Crosses between local and Friesian cows).

#### **Milk collection:**

Milk was collected at week 3 of the grazing period of 4-5 weeks. During milking 1litre from each cow was taken in a clean bottle. Each herd milk was pooled for each of the 4 areas. The collected milk was frozen and then transferred to the University of Gezira Laboratories for sensory evaluation and chemical analysis for both milk and cheese made from it.

The pooled sample was divided into two parts; one part was boiled and cooled for sensory evaluation of milk. While the other part was used for cheese making.

#### **Cheese Making:**

Cheese making was conducted at the Department of animal Science , Faculty of Agricultural Science, Gezira University . The soft white cheese was made by the traditional method according to [12]. Where coagulation was done by rennet tablets [Chris Hansen's Laboratory, Copenhagen Denmark] that purchased from local market. After the cheese was processed it was cut into small cubes (about 50 g) for sensory evaluation.

#### **Physicochemical analysis of milk:**

Milk samples were analyzed for physicochemical composition such as pH, acidity, fat, protein, casein, moisture and lactose according to [13]. All the samples were determined in triplicates

#### **Chemical analysis of cheese:**

The moisture content in Bt and non Bt cheese was determined by the Method No. 926.08 of [13]. Where samples were kept in oven at  $103 \pm 5^{\circ}\text{C}$  till the constant weight of dried cheeses is obtained. While fat content was determined by Gerber method as described by [14]. Total protein content was measured by Kjeldahl's method No. 20A: 1986 of [15]. However, ash content was determined by igniting the cheese sample according to method No. 935.42 of [13]. (All the sample were determined in triplicates).

#### **Determination of cheese pH:**

Twenty grams from each Bt and non Bt cheese was blended with 12 ml water to prepare the cheese slurry and pH was measured. The samples were measured in triplicates by a pH meter (Inolab WTW Series 720) after calibrating it with fresh pH standard buffers of 7.0 and 4.0 [16].

#### **Analysis of whey:**

Moisture and ash contents were determined according to [17] and the crude protein content was measured according to Kjeldahl method as described by [18]. While the fat content was determined by Gerber method as described by [14].

#### **Sensory evaluation:**

For sensory evaluation of milk, 30 university students who already have experience with milk and cheese were volunteered to perform the test.

Each student was given a cup of 100 ml from each milk sample. The students filled a form containing the following properties:

Color, consistency, flavor, taste and overall assessment. The score allocated as follows:

4 = excellent

3 = very good

2 = good

1 = fair

0 = not acceptable

#### **For cheese, the following characteristics were evaluated:**

Color, texture, flavor, taste and overall assessment. The same students were asked to perform cheese sensory evaluation.

The same scoring pattern of milk was also applied for cheese.

#### **Statistical analysis:**

Means and differences between means of chemical composition of Bt and Non- Bt milk, cheese and whey were performed using SPSS.

Chi-square test was employed for sensory evaluation and the t-test was used for difference between means

### 3. Results and Discussion

**Table 1:** Percent chemical composition of milk produced from grazing on Non – Bt and Bt cotton crop residues:

Component	Mean		SE	P – value
	Non – Bt	Bt		
Protein	3.50	4.50	0.120	0.001
Casein	2.40	3.00	0.159	0.023
Lactose	3.60	4.20	0.047	0.000
Fat	4.50	4.00	0.47	0.000
Ash	0.51	0.37	0.018	0.001
Total solids	12.11	13.07	0.191	0.005
Acidity	0.19	0.34	0.021	0.002
Protein to fat	0.78	1.125	0.033	0.002
Casein to total protein*	0.686	0.667	-	-

Calculated casein to total protein\*

As presented by table (1), there were significant ( $P < 0.01$ ) differences between the milk produced from grazing on Non – Bt CCR and Bt – CCR in all components. When milk composition in this study was compared to that reported by [19] for milk produced by conventional system in Gezira University dairy farm, it is clear that, the moisture contents of Non – Bt CCR was similar, while in Bt – CCR was slightly lower. Other components were different for fat (higher) in Non – Bt CCR milk, while protein was higher in both milks of this study. It is worth mentioning that, protein in Bt – CCR milk was extremely higher even than in milk from Non – Bt grazing. In addition, lactose in both milk in this experiment was lower, than of that reported by [19]. Generally in Non – Bt - CCR milk, the moisture and protein were about the range of 87.3 – 87.8% and 3.13 – 3.4% moisture and protein respectively reported by [20, 21, 22, 23] While only fat in Bt – was about the range of 3.53- 3.9% reported by the previous authors. The protein to fat ratio (table 1) was extremely higher in Bt – milk compared to Non – Bt milk. However the ratio in Non – Bt was about the lower limit of the range (0.8 – 0.9) reported by [24] while that of Bt was higher than the upper limit of the range, reported by [20, 21, 22, 23]. These results agreed well with [6] who reported significant increase in milk fat, protein and urea in experiment included Bt – corn (Bt – MON 810) in the first lactation. In the second lactation the authors found that cows fed CON (Non – genetically modified diet) has a significantly lower milk, lactose.

While [5] found a significant increase in milk protein lactose and SNF when cows fed the 2 GM corn. On the other hand, many authors reported no significant effect on milk composition when lactating cows were fed on Bt – crops. For example [25] on feeding Bt – protein (Cry 3 Bb1), [26] who studied the effect of glyphosate tolerant corn vs near isogenic hybrids and [27].

Generally the results of this experiment is unique due to that grazing on Bt – cotton crop residues was not discussed at least in the available literature.

**Table 2:** Percent chemical composition and pH of cheese made from milk produced from grazing on Bt and Non - Bt cotton crop residues:

Component	Mean		SE	P – value
	Non – Bt	Bt		
DM	43.5	45.3	0.163	0.000
Protein	15.1	18.8	0.663	0.007
Fat	17.2	13.3	0.145	0.001
Ash	7.5	9.0	0.183	0.001
pH	5.2	6.7	0.546	0.136

Chemical composition of cheese made from milk produced from grazing on Bt – CCR and Non – Bt – CCR (table 2) showed significant ( $P < 0.001$ ) increase in DM, protein and ash in cheese made from milk produced from Bt – CCR grazing compared to Non – Bt CCR grazing. In cheese made from milk produced from Non – Bt CCR grazing, only fat was significantly ( $P < 0.01$ ) higher. It is clear that chemical composition of cheese followed the same trend of milk. The moisture content of cheese made from milk produced from Non – Bt CCR grazing was higher than from that made from milk produced from Bt – CCR grazing. This trend may simulate the result reported by [28] who found that, cheese produced from organic milk had slightly (7%) higher moisture content. The DM of both cheese of this study were lower than 48 and 53% in rennet and lemon set cheese reported by [19] who used milk produced by conventional feeding. Also lower than  $56.31 \pm 9.2\%$  reported by [29] for cheese made from cow milk without addition of cassava.

Fat content of cheese in this study was lower than that of  $20.25 \pm 1.84\%$  reported by [29]. However only in Non Bt cheese was about to that reported by [30]. While the protein content of Non – Bt cheese was higher than that reported by [29]. Generally it was reported by [31] that, cheese with higher fat were higher in moisture in mozzarella cheese. This finding is also valid to Non – Bt cheese in this study where fat content was significantly higher than in Bt cheese. Ash content in both cheese of this experiment was also higher than that reported by [29]. The protein to fat ratio (table 1) was higher in Bt – cheese. This may explain the increased DM of that cheese produced from Bt – milk. Protein to fat ratio in Non – Bt cheese was about the lower limit of the range (0.8 – 0.9) reported by [24, 32]. While in Bt – cheese the ratio of protein to fat was higher than the upper limit of the range reported by the authors. Generally [33] reported yield of cheese obtained from milk with ratio of protein to fat of 0.7 – 0.85 was significantly higher than that obtained from milk with ratio of protein to fat of 0.88 – 1.00 or 1.01 – 1.15.

However, [34] found that, increase ratio of protein to fat in milk raise the protein, calcium and phosphorus content in cheese and has extensive impact on the moisture content. When the ratio of casein to whey protein was examined (table 1), it is clear that, this ratio in both cheese of this study was far below to the normal range of 4.96 – 5.49% mentioned by [35, 36].

**Table 3:** Percent chemical composition of whey from cheese made from milk produced from Non – Bt and Bt cotton crop residues:

Component	Mean		SE	P – value
	Non – Bt	Bt		
DM	7.3	8.7	0.170	0.000
Protein	1.0	1.7	0.105	0.003
Fat	0.3	0.9	0.047	0.000
Lactose	5.4	5.1	0.201	0.311
Ash	0.00	1.0	0.083	0.007

Table (3) of the results, showed significant ( $P < 0.01$ ) differences in DM, protein, fat and ash between the whey produced from milk produced from Non – Bt and Bt CCR grazing. Except for lactose all the components of whey were significantly higher in Bt than Non – Bt whey. The calculated ratio of whey casein to whey protein (table 4) was higher in Bt – whey which indicated that part of the casein retained in whey. While in Non – Bt whey, it seems that all the casein was retained in the cheese. However, increased milk acidity as reported by [37, 19] reduced the ratio of whey casein to whey protein. On the contrary, in this study the ratio of whey casein to whey protein increased in Bt – whey though the acidity of Bt – milk was higher than in Non – Bt milk.

**Table 4:** Calculated ratio of whey casein to total whey protein.

Protein component	Non – Bt whey	Bt – whey
% Total protein in milk	3.5	4.5
% Casein in milk	2.4	3.0
% Milk whey protein	1.1	1.5
% Whey protein after cheese making	1.0	1.7
% Whey casein	0.00	0.2
% Whey casein to whey protein	0.00	0.13

**Table 5:** Sensory characteristics of milk produced from grazing on Bt and Non – Bt cotton crop residues.

Sensory characteristics	Mean		SE	P – value
	Non – Bt	Bt		
Colour	3.57	3.17	0.270	0.144
Consistency	3.37	2.47	0.289	0.003
Flavor	3.27	1.70	0.329	0.000
Taste	3.37	2.00	0.297	0.000
Overall assessment	3.22	2.38	0.160	0.000

When the sensory characteristics of milk produced from grazing on Bt – CCR and Non – Bt CCR were compared, it is obvious that, milk produced from grazing on Non – Bt CCR scored significantly ( $P < 0.01$ ) high score in overall assessment than the milk produced from grazing on Bt – CCR. Also for individual characteristics, milk produced from Non – Bt CCR grazing had significantly ( $P < 0.05$ ) excellent consistency, flavor and taste and overall acceptability than the milk from grazing on Bt – CCR. Other characteristics (color and flavor) were not significantly different. It is clear that different types of the grazed cotton crop residues, had impact on the sensory properties of milk. This may be due to the floristic composition of the grazed CCR. This observation may coincided with [10, 11] who found that, the sensory characteristics of cheese varied according to the swards composition.

**Table 6:** Sensory characteristics of cheese made from milk produced by Bt and Non – Bt CCR grazing.

Sensory characteristics	Mean		SE	P – value
	Non – Bt	Bt		
Colour	3.60	3.27	0.237	0.166
Oder	3.27	3.33	0.236	0.779
Texture	3.30	3.13	0.247	0.892
Taste	3.23	3.13	0.251	0.692
Overall assessment	2.74	2.77	0.577	0.874

It is obvious from table (6), there was no difference in sensory characteristics of cheese produced from milk produced from grazing on Bt and Non – Bt cotton crop residues. However, there was a significant difference between the milk produced by grazing on Bt and Non – Bt CCR. The reason for this discrepancy is unclear but it seems that cheese processing may improve the cheese made from milk produced from grazing on Bt CCR.

**4. Conclusion**

It could be concluded that grazing on Bt – cotton crop residues affect, milk composition and its sensory characteristics. While cheese made from such milk was not differing from that made from milk produced from grazing on Non – Bt cotton crop residues in sensory properties, but significantly differed in chemical analysis. Whenever the safety of Bt – cotton crop residues as animal feed is proven, utilization of the milk produced from it for cheese making may be one of the promising marketing channels. Finally, more efforts for investigation of Bt – cotton crop residences as animal feed are urgently needed. However, this grazable substance support thousands of animal units in Africa and Asia. Also the products of animals grazed on Bt- CCR need to be checked for Bt-toxin.

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