Effects of Grazing Dairy Cows on Bt and Non-Bt Cotton Crop Residues on the Ratios of each Ruminal Acetate and Propionate to Milk Palmitic Acid and the Correlation of both Ratios to Acetate to Propionate in Gezira State, Sudan

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ABSTRACT
The objective of this experiment was to investigate milk yield and mean molar proportion of Acetic (Mol/100Mol) to Palmitic (g/100g) and mean molar proportion of Propionic (Mol/100mol) to Palmitic (g/100g) acids. Milk from cows grazed on Bt and non-Bt cotton crop residues (CCR). Cows were milked once a day by employing full hand milking, put in individual bottle, weigh, recorded, after weigh each group was collected and store in separate tanks. (Bt and non-Bt) Immediately after reaching the tank, it was cooled labeled around 3°C. Milk sample from each cow was collected at week 0 (Before introduction of animals for grazing), 1, 2, 3, 4 and 5 during the 5 weeks of experimental feeding. The collected milk was frozen and then transferred to the University of Gezira Laboratories for chemical analysis. The results revealed significant differences in milk yield mean molar proportion of Acetate (Mol/100Mol) to Palmitic acid (g/100g) and mean molar proportion of Propionic (Mol/100mol) to Palmitic (g/100g), it was found to be higher in Bt CCR than in non-Bt CCR in all weeks of the grazing period. Also milk yield was significantly (P<0.01) higher in animals grazed on Bt than that non-Bt.

Keywords: milk yield, molar proportion, cows grazed, Cotton, crop residues

1. Introduction
Cotton is the major natural textile fiber crop worldwide. In Sudan, cotton has been grown for centuries. The cotton plant is indigenous and a number of its wild relatives exist in various parts of the country, as well as, it is a way of life, reduced poverty and encouraged the settlement in rural areas. Commercial growing of the crop started in 1867. In Sudan, the big jump in cotton production took place in 1926, which marked the official start of functioning of the Gezira Scheme (Elfadil, 2009). The crop contributed to different economical aspects, which included fiber export, oil production and grazing on its residues after harvesting. Due to decline of grazing land, sheep and goats are let loose in the cotton fields for grazing by the farmers and shepherds after harvesting the cotton (Reiser and Fu 1962) in Sudan cows commonly graze on cotton crop residues. 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 provide insect protection or herbicide tolerance are being used as feed for livestock (James and Clive 2014). Bt cotton has been genetically modified by the insertion of one or more genes from a common soil bacterium, Bacillus thuringiensis (Scott 2007). These genes encode for the production of insecticidal proteins, and thus, genetically transformed plants produce one or more toxins as they grow (Scott 2007). Due to fear among animal owners from grazing on Bt-cotton crop residues an investigation in the form of
questionnaire was conducted in two sites (South Gezira locality and Um-Algura locality) to know the effect of grazing on Bt-cotton crop residues (Bt-CCR) on animal health and milk characteristic (Hashim et al., 2017). Also the yield of cotton crop residues (CCR), the botanical composition and the animal intakes from the potentially available dry matter (PADM) from both types of Bt compared to non-Bt cotton crop residues was obtained by the same authors (Hashim et al., 2017a). In another experiment the chemical composition of both Bt and non-Bt cotton crop residues (CCR) and in vitro digestibility of each type of CCR was also determined (Hashim et al., 2017b). The effect of Bt CCR grazing by dairy cows on milk chemical composition and physiochemical properties (acidity, iodine value, and milk pH) were also investigated (Hashim et al., 2017c).

Since the introduction of Bt–cotton crop in 2012, there was increasing fear between people in the country particularly farmers. However, in Sudan and some other cotton-producing countries, cotton crop residues (CCR) grazed by many thousands of different ruminants. For example in Sudan CCR grazed by more than 200,000 animal units in Gezera scheme and 60000 AU in Rahad scheme. This feed source represent a valuable summer feed that contribute in bridging the summer feed gap. To date very little researches had concern on the effect of CCR grazing on some characteristics of milk are cited by (Hashim, 2016; ElObied et al., 2016). Therefore this study was conducted to investigate the effect of grazing on CCR on Milk yield and mean ratios of Acetate (Mol\100Mol) to Palmitic (g\100g) and of Propionate (Mol/100mol) to Palmitic (g\100g).

2. Materials and Methods

2.1. The experiment

The experiment was conducted in two localities of Gezira state where cotton crop was cultivated. This included, South Gezira locality (Al- Madina Arab) and Um-Algura locality. In Um-Algura locality a herd of 25 animal units (AU) 12.6 animal units of them were milking were grazed on non-Bt CCR. In South locality a herd of 22 animal units (AU) 11.2 animal units of them were milking were grazed on Bt CCR (Bt = (B. thuringensis). The lactating cows in the herd of Um- Algura and South Gezira were 50.4% and 51% respectively. The herd in each of the localities was of mixed breeds (local and Crosses between local and Friesian cows).

2.2. Milking schedule

Cows were hand milked once a day in the morning. Each cow milk was measured and sample was taken from it. The sample from each group for each week was pooled and stored for chemical analysis. Total samples of milk from Bt and non-Bt CCR grazing was 200(5weeks × 20 cows×2 Treatment).

2.3. Chemical analysis

These included determination of palmatic acid in milk fat. However determination of Acetic and propionic acids in the rumen fluid was carried out.

2.4. Determination of palmatic acid

2.4.1. Milk sampling

Milk sample from each cow was collected on weeks 0, 1, 2, 3, 4 and 5 of the grazing period of 5 weeks. During milking 2 liter from each cow was taken in a clean bottle. Pooled milk samples consisting milk of each milking in proportion of milk yield of individual cow. The collected milk was frozen and then transferred to the Laboratories. Total sample in Bt and non-Bt CCR was 30 samples (5weeks × 2 Treatment ×3 Replication).

2.4.2. Fatty acids analysis

Fatty acids analyzed according to Simionato et al., (2010). The total lipids were determined by the Folch et al., (1957) method with chloroform, methanol, and water (2:1:1). The lipids were converted into FAMEs as described by Bannon et al., (1982) with modifications. To a screw-cap tube containing approximately 150 mg lipids was added 5.0 mL 0.25 mol L-1 sodium meth oxide inmethanol-diethyl ether (1:1) and it was vigorously agitated for about 3 min. Next, 3.0 mL of isoctane and 15 mL of saturated sodium chloride were added. The tube was vigorously agitated again and rested until phase
separation. The supernatant was collected in labeled Eppendorf flasks for later chromatographic analysis. The original method includes fast heating under reflux after the addition of the transesterifying agent; however, this was not done to prevent the isomerization of the conjugated dienes of linoleic acid. FAMEs were analyzed by gas chromatography in Varian model CP-3380 equipped with a flame ionization detector and a fused silica capillary column (100 m × 0.25 mm i.d. × 0.39 μm 100% bonded cyanopropyl, Varian, EUA). The gas flow rates (White Martins) used were 1.4 mL min-1 carrier gas (H2), 30 mL min-1 makeup gas (N2), and 30 and 300 mL min-1 flame gases, H2 and flame synthetic air, respectively. The sample injection rate (split) was 1/100. The injector and detector temperatures were 235 °C. The column temperature was programmed to 65 °C for 4 min, followed by a ramp of 16 °C min-1 up to 185 °C, which was kept for 12 min. A second ramp of 20 °C min-1 was run up to 235 °C for 14 min. The total analysis time was 40 min. The peak areas were determined using Software Star (Varian). Injections of 2 μL were performed in triplicate.

2.4.3. Identification of fatty acids

The identification of fatty acids was based on the comparison of retention times of standard methyl esters containing linoleic acid geometric isomers c9t11 and t10c12 (18:9-19:0 and 18:5-26:0, Sigma, USA) and on equivalent chain length (ECL). The ECL of fatty acid esters were determined according to Ackman (1972) based on ECL values determined for standard 18:9 (Sigma, USA). The fatty acids in mg g-1 total lipids were quantified in relation to the internal standard, methyl tricosanoate (23:0) from Sigma. Before transesterification, 1.00 mL of internal standard solution (1 mg mL-1) was added to all samples and the solvent was evaporated under N2 flow. The sample fatty acids were quantified after the verification of the agreement between the theoretical and experimental response factors. The sample fatty acid concentrations were calculated according to Joseph and Ackman (1992).

2.5. Determination of acetic and propionic acids in the rumen fluid:

The rumen liquid (n = 30) was sampled by a stomach tube and filtered through an ordinary sieve in the standard way. Approximately 10 mL of the sample was preserved with 2-3 drops of toluene to prevent fermentation. The samples preserved in this way were immediately analyzed or stored at -20 °C temperature pending analysis. For the gas chromatography (GC) analysis, the samples were prepared in the following way: 200 μl of metaphosphoric acid (25%) and formic acid (3:1) mixture was added to 1 mL of rumen liquid (Cottyn and Boucque 1968). After 30 min of centrifugation, the clear supernatant was × 10 diluted in water and injected in the chromatograph.

2.6. Chemicals used

Metaphosphoric acid, ε-aminocaproic acid (EACA), hydroxyethylcellulose (HEC), caproic acid – SigmaAldrich, CR, HCl – refined by isothermic distillation, formic acid (Lachema Brno, CR). Standard solutions of appropriate concentration were prepared from the individual substances of analytical purity (Sigma-Aldrich, CR).

2.6. GC Analysis

The analyses were conducted on a 6820 GC System gas chromatograph, Agilent Technologies. A FFAP capillary column was used, 30 m × 250 μm × 0.25 μm (Quadrex Corporation). Carrier gas – nitrogen, flow 1.0 mL/min, detector – FID, temperature programme used: 60–200 °C (20 °C/min, 10 min), injector – 250 °C, detector – 300 °C. The injector was equipped with a glass liner of glass wool to separate particles of dirt from the sample. The samples were dosed by a HT 300A automatic dosing device at an injection size of 1 μl using the split method and a 30:1 splitting ratio. The analysis time is approximately 15 min.

2.7. Calculations

Ratio of acetic to propionic acids in the rumen fluid. Ratio of acetic acid in the rumen fluid to palmitic acid in milk fat and ratio of propionic acid in the rumen to palmitic acid in milk fat Estimation of weekly milk yield.
2.8. Statistical Analysis

Means and differences between means of rumen metabolite of Bt and non-Bt CCR and that of palmitic acid in milk. Mean molar proportion of rumen Acetate (Mol/100Mol) to Palmitic (g/100g) of milk, mean molar proportion of rumen Propionic (Mol/100mol) to Palmitic (g/100g) and Acetate to Propionic ratio were calculated and. Statistical design used, was complete randomized design (CRD). Statistical analysis was performed using SPSS. To compare the means t-test was used.

3. Results

Milk yield produced from grazing on Bt and non-Bt CCR in week-0 (Table 1) was not significantly different. While milk yield in weeks of the grazing period were highly significantly differed (P<0.01). It was found to be higher in Bt CCR than in non-Bt CCR. Whereas the variation between the weeks in both Bt-cotton and Non-Bt-Cotton was highly significant (P<0.01). It is clear that in both groups the milk yield increased weekly when compared to week zero.

Table 1: Means of milk yield (Ib/week) from cows grazed on Bt-cotton and Non-Bt cotton

| Week | Non-Bt cotton | Bt-cotton | SE     | Level of sig. |
|------|--------------|-----------|--------|---------------|
| 0    | 120.4033     | 121.2000  | 0.77460| 0.332         |
| 1    | 140.0000     | 158.0000  | 2.34521| 0.000         |
| 2    | 138.0000     | 156.8000  | 1.85472| 0.000         |
| 3    | 132.0000     | 155.0000  | 2.04939| 0.000         |
| 4    | 124.6000     | 146.4000  | 2.21811| 0.000         |
| 5    | 121.4000     | 135.0000  | 1.36382| 0.000         |
| SE   | 3.49777      | 5.98799   | 0.000  |               |
| SD   | 8.49777      | 14.66752  | 0.000  |               |

The results in (Table 2) the Acetate to Palmitic ratio compared, was found to be significantly differed (P<0.01) between Bt and non-Bt - CCR in all weeks of the grazing period, it was found to be higher in Bt CCR than in non-Bt CCR. While in week-0 was not significantly (P>0.05) differed. Also the variation between the weeks in both Bt-cotton and non-Bt CCR followed the same trend of the previously mentioned parameter. It is clear that in Bt-CCR the molar proportion of Acetate to Palmitic acid increased weekly when compared to week zero in each of the two groups. While in non-Bt-CCR the molar proportion of Acetate to Palmitic acid decreased in all weeks of the grazing period when compared to week zero in each of the groups.

Table 2: Acetate (Mol/100Mol) to Palmitic (g/100g) acid ratio

| Week | Non-Bt cotton | Bt-cotton | SE     | Level of sig. |
|------|--------------|-----------|--------|---------------|
| 0    | 3.01         | 3.00      | 0.16292| 0.423         |
| 1    | 2.75         | 3.37      | 0.11846| 0.000         |
| 2    | 2.68         | 3.30      | 0.15846| 0.000         |
| 3    | 2.68         | 3.20      | 0.11652| 0.000         |
| 4    | 2.65         | 3.13      | 0.14267| 0.000         |
| 5    | 2.69         | 2.98      | 0.19757| 0.000         |
| SE   | 0.97978      | 0.58603   | 0.000  |               |
| SD   | 2.39997      | 1.43547   | 0.000  |               |

Investigation of Propionate to Palmitic acid ratio in all weeks of the grazing period in both Bt and non-Bt CCR groups (Table 3) was highly significantly (P<0.01) different. It was found to be higher in Bt CCR than in non-Bt CCR. However, Propionate to Palmitic acid ratio in week-0 in each group was found to be not significantly different. Also the variation between the weeks in both Bt-CCR and non-Bt-CCR was highly significant (P<0.01). On the contrary to that in non-Bt the ratio of acetate to palmitic acid in non-Bt, in both Bt-CCR and non-Bt-CCR the molar proportion of Propionate to Palmitic acid ratio increased weekly when compared to week zero in each of the two groups.
The milk yield of week 0 and that of other weeks of Bt and non Bt-CCR in this study was higher than that of (69.51, 77.19 and 88.41 lb/week) reported by Hayaz Uddin et al. (2013) for milk yield of crossbred dairy cattle fed on ration having different level of cotton seed cake (0, 20, 35 % respectively). While the milk yield of week 0 of Bt and non Bt-CCR in this study and other weeks was lower than that of (210.26 and 203.88 lb/week) for cows fed on non-Bt and Bt cotton seed respectively reported by Ranjan et al. (2010) and lower than that of (539.00, 523.60 and 540.54 lb/week) for milk yield of cows fed diets containing whole cottonseed (WCS) with increasing concentrations of free fatty acid (FFA) in the oil (0, 24.1 and 22.3% respectively) reported by Cooke et al., (2007). While the milk yield of all weeks of Bt and non Bt-CCR in this study was similar than that of reported by Singhal et al., (2006a) for cows fed on non-Bt and Bt cotton seed. Based on the results of a long-term study (over 25 months) with dairy cows, Steinke et al., (2010) reported that feeding with GM Bt (MON810) maize did not adversely affect milk yield. This result was on line with the work of Hashim et al. (2017) who found a higher milk yield in cows grazed on Bt and Non Bt cotton residues of dairy cows. Also this results of this research disagree with the finding of ( Singhal et al., 2001; Singhal et al., 2006; Calsamiglia et al., 2007 ; Singhal et al., 2008, Flomer et al., 2002 ) who reported no significance difference in milk yield produce from cows fed on transgenic and non-transgenic cotton, corn and soybean. Ranjan et al. (2010) reported that effect of feeding transgenic cottonseed (Bt-cry1Ac gene) on nutrient utilization, production performance and blood biochemical status in lactating dairy cows, milk yield did not vary significantly between the groups. While in this study cows grazed on Bt cotton crop resides produced more milk than that grazed on non-Bt CCR.

The Acetate to Palmitic ratio of week 0 (3.00) and that of other weeks of Bt and non Bt-CCR in this study (Table 2) was higher than that of (2.63) and reported by Liu et al., (2019) for dairy cows at different milk stages and higher than that of (2.61, 2.60 and 2.51 respectively) for milk yield and composition for pasture grazing dairy cows supplemented with (3.5, 7.0 and 10.5 kg/d respectively) of

### Table 3: Propionate (Mol/100mol) to Palmitic g/100g acid ratio

| Week | Non-Bt cotton | Bt-cotton | SE | Level of sig. |
|------|---------------|-----------|----|--------------|
| 0    | 0.76          | 0.76      | 0.16292 | 0.423        |
| 1    | 0.92          | 1.23      | 0.11846 | 0.000        |
| 2    | 0.94          | 1.33      | 0.15846 | 0.000        |
| 3    | 0.90          | 1.16      | 0.11652 | 0.000        |
| 4    | 0.88          | 1.13      | 0.14267 | 0.000        |
| 5    | 0.86          | 1.10      | 0.19757 | 0.000        |
| SE   | 0.97978       | 0.58603   |    |              |
| SD   | 2.39997       | 1.43547   |    |              |
| Level of sig. | 0.000       | 0.000     |    |              |

Investigation of acetate to propionate ratio in weeks 1, 2, 3, 4 and 5 in Bt and non-Bt CCR groups (Table 4) was highly significant (P<0.01). In all weeks of the grazing period, was found to be higher in non-Bt CCR than in Bt CCR. However, acetate to propionate ratio in week- 0 in each group was found to be not significantly different. Whereas the variation between the weeks in both Bt-CCR and Non-Bt CCR was highly significant (P<0.01). It is clear that in both groups the molar proportion of acetate to propionate ratio decreased weekly when compared to week zero in each of the groups.

### Table 4: Weekly ratio of Acetate to propionate from cows grazed on Bt-cotton and Non-Bt cotton:

| Week | Non-Bt cotton | Bt-cotton | SE | Level of sig. |
|------|---------------|-----------|----|--------------|
| 0    | 3.9400        | 3.9000    | 0.00816 | 0.221        |
| 1    | 3.0000        | 2.7000    | 0.00816 | 0.000        |
| 2    | 2.8500        | 2.4900    | 0.00820 | 0.000        |
| 3    | 2.9800        | 2.7000    | 0.00822 | 0.000        |
| 4    | 3.0200        | 2.7600    | 0.00816 | 0.000        |
| 5    | 3.1100        | 2.8000    | 0.00823 | 0.000        |
| SE   | 0.16166       | 0.20634   |    |              |
| SD   | 0.39598       | 0.50543   |    |              |
| Level of sig. | 0.000       | 0.000     |    |              |

4. Discussion
The milk yield of week 0 and that of other weeks of Bt and non Bt-CCR in this study was higher than that of (69.51, 77.19 and 88.41 lb/week) reported by Hayaz Uddin et al. (2013) for milk yield of crossbred dairy cattle fed on ration having different level of cotton seed cake (0, 20, 35 % respectively). While the milk yield of week 0 of Bt and non Bt-CCR in this study and other weeks was lower than that of (210.26 and 203.88 lb/week) for cows fed on non-Bt and Bt cotton seed respectively reported by Ranjan et al. (2010) and lower than that of (539.00, 523.60 and 540.54 lb/week) for milk yield of cows fed diets containing whole cottonseed (WCS) with increasing concentrations of free fatty acid (FFA) in the oil (0, 24.1 and 22.3% respectively) reported by Cooke et al., (2007). While the milk yield of all weeks of Bt and non Bt-CCR in this study was similar than that of reported by Singhal et al., (2006a) for cows fed on non-Bt and Bt cotton seed. Based on the results of a long-term study (over 25 months) with dairy cows, Steinke et al., (2010) reported that feeding with GM Bt (MON810) maize did not adversely affect milk yield. This result was on line with the work of Hashim et al. (2017) who found a higher milk yield in cows grazed on Bt and Non Bt cotton residues of dairy cows. Also this results of this research disagree with the finding of ( Singhal et al., 2001; Singhal et al., 2006; Calsamiglia et al., 2007 ; Singhal et al., 2008, Flomer et al., 2002 ) who reported no significance difference in milk yield produce from cows fed on transgenic and non-transgenic cotton, corn and soybean. Ranjan et al. (2010) reported that effect of feeding transgenic cottonseed (Bt-cry1Ac gene) on nutrient utilization, production performance and blood biochemical status in lactating dairy cows, milk yield did not vary significantly between the groups. While in this study cows grazed on Bt cotton crop resides produced more milk than that grazed on non-Bt CCR.

The Acetate to Palmitic ratio of week 0 (3.00) and that of other weeks of Bt and non Bt-CCR in this study (Table 2) was higher than that of (2.63) and reported by Liu et al., (2019) for dairy cows at different milk stages and higher than that of (2.61, 2.60 and 2.51 respectively) for milk yield and composition for pasture grazing dairy cows supplemented with (3.5, 7.0 and 10.5 kg/d respectively) of
concentrate reported by Salado et al., (2017). However, it was higher than that of (1.70, 1.80, 1.79 and 1.71) due to effects of ruminal VFA infusions (propionate 900 g/d, propionate 600 g/d plus 250 g/d of butyrate, propionate300 g/d plus 500 g/d of butyrate and butyrate750 g/d respectively) on milk fatty acid composition in dairy cows reported by Miettinen and Huhtanen (1996). Also in goats as reported by Requena et al., (2020) it was lower (2.11) than that reported in this study for cows. When compared to that of (1.50 and 1.99) for rumen volatile fatty acids (VFA) and fatty acid composition of milk fat Propionate to Palmitic ratio from cows fed hay and haylage rations respectively reported by Schingoethe et al. (1975).

Propionate to Palmitic acid ratio in all weeks of the grazing period in both Bt and non- Bt CCR groups (Table 3) was highly significantly (P<0.01) different. The Propionate to Palmitic acid ratio in all weeks of the grazing period in both Bt and non- Bt CCR groups in this study was lower than that of (1.34) reported by Liu et al., (2019) for dairy cows at different milk stages and lower than that of (1.17) for rumen volatile fatty acids (VFA) and fatty acid composition of milk fat for cows fed on corn silage ration respectively reported by Schingoethe et al. (1975). While the Propionate to Palmitic acid ratio in all weeks of the grazing period in both Bt and non- Bt CCR groups in this study was higher than that of (0.61, 0.74 and 0.82 respectively) reported by Salado et al., (2017) for milk yield and composition for pasture grazing dairy cows supplemented with (3.5, 7.0 and 10.5 kg) of concentrate and higher than that of (0.73, 0.71, 0.62 and 0.47) that resulted from ruminal VFA infusions (propionate 900 g/d, propionate 600 g/d plus 250 g/d of butyrate, propionate300 g/d plus 500 g/d of butyrate and butyrate750 g/d respectively) reported by Miettinen and Huhtanen (1996) and higher than that of (0.52) for Propionate to Palmitic ratio from dairy goats reported by Requena et al., (2020).

Acetate to propionate ratio in weeks 1, 2, 3, 4 and 5 in Bt and non- Bt CCR groups (Table 4.8) was highly significant (P<0.01). The Acetate to propionate ratio in all weeks of the grazing period in both Bt and non- Bt CCR groups in this study was lower than that of (4.67 Mol\100 Mol) reported by Lijun Wang et al. (2020) for ruminal fermentation characteristics of cows fed high forage and lower than that of (4.68 and 4.43 Mol\100 Mol) for rumen acetate to propionate ratio from dairy cows grazing a cool-season pasture (CSP) and pearl millet (PM) respectively reported by Bainbridge et al. (2018). While the Acetate to propionate ratio in all weeks of the grazing period in both Bt and non- Bt CCR groups in this study were higher than that of (2.49, 2.44 and 2.46 Mol\100 Mol) reported by Cooke et al. (2007) for acetate to propionate ratio of cows fed diets containing whole cottonseed (WCS) with increasing concentrations of free fatty acid (FFA) in the oil (2.41 and 2.23% respectively) and higher than that of (2.35 and 1.94 Mol\100 Mol) from cows fed whole whey-treated corn silage and treated corn silage rations respectively reported by Schingoethe et al. (1975). While the Acetate to propionate ratio in all weeks of the grazing period in non- Bt CCR groups in this study was higher than that of (2.78 Mol\100 Mol) for rumen fermentation in heifers limit-fed a distillers dried grains with solubles (DDG) concentrate mix with ad libitum grass hay reported by Manthey and Anderson (2018).

A positive highly strong correlation (r² = 0.95) existed between the Acetate to Propionate ratio and Acetate to Palmitic acid ratio on rumen fluid and milk from grazing on non-Bt CCR (Fig 1). This may be explained on the basis of increasing the acetate supply has a positive effect on milk yield and milk fat content as reported by Dijkstra (1994). Vlaeminck et al., (2006) found that milk iso-C14:0 and iso-C15:0 contents are positively correlated with ruminal proportions of acetate. In this studies though the relationship between iso C19:0 was not investigated but the same terned was found between C16:0 and acetate to propionate ratio. It was also found a positive relationship between the concentrations of iso-C17:0 in milk and the molar proportions of ruminal acetate, but this relationship was not significant. Moreover, milk iso-C14:0 and iso-C15:0 contents are negatively related to ruminal propionate proportions according to Vlaeminck et al., (2006). On the contrary in this study a negative correlation existed between the molar proportions of Acetate to Propionate ratio and Acetate to Palmitic acid ratio from grazing on Bt CCR (r² = -0.60) (Fig 2). The C16:0 depends on many different factors such as breed, season, lactation stage, lactation number, age of dairy cows, geographical location, and, as most important factor, the diet, which is responsible for 95% of the variance in cow milk fat (Ellis et al., (2006) Jensen (2002), Kelsey et al., (2003) and Pietrzak et al., (2009)), here in this, it seems that feed plays an important role in milk fatty acid composition. This result may be explained on the basis of that, reported by (Rennò et al., (2013) and Liu et al., (2008)) who stated that, diet of dairy cows, which affects the microbiological processes in rumen, and the changes in the bio hydrogenation processes, are the key to modifying the fatty acid composition of milk fat. The variation of acetate between the weeks in both
Bt and non Bt cotton crop residues was highly significant (P<0.01). It is clear that in both Bt and non Bt cotton crop residues the molar proportion of acetate decreased weekly when compared to week zero (Before introduction of animals for grazing). Cooke et al. (2007) reported decreased proportion of acetate for fed whole cottonseed (WCS) on rumen VFA concentrations when compared to control. The variation of Acetate to Palmitic acid between the weeks in both Bt-cotton and non-Bt CCR followed the same trend of the previously mentioned parameter. It is clear that in Bt-CCR the molar proportion of Acetate to Palmitic acid increased weekly when compared to week zero. While in non-Bt-CCR the molar proportion of Acetate to Palmitic acid decreased in all weeks of the grazing period when compared to week zero. Requena et al., (2020) reported decreased Acetate to Palmitic on rumen for a Meta-Analytic approach to predict methane emissions from Dairy Goats using milk fatty acid profile when compared to control. Also in goats the ratio was lower than that reported in this study for cows. The Acetate to Propionate ratio from grazing on Bt and non-Bt CCR (Fig 3 and 4) were negatively correlated with the ratio of Propionate to Palmitic acid (r² = −0.97) and (r² = −0.96) respectively. This result agreed with reported by Emmanule and Kennelly (1984) who stated that, Propionic acid decreased proportions of C16:0. The variation of Propionate between the weeks in each group was highly significant (P<0.01). It was clear that, in both groups the molar proportion of propionate increased weekly. Cooke et al. (2007) reported increased proportion of propionate for fed whole cottonseed (WCS) on rumen when compared to control. Vlaeminck et al., (2006) found that milk iso-C14:0 and iso-C15:0 contents are negatively correlated with ruminal proportions of Propionate. However, in this studies though the relationship between iso-C14:0 and iso-C15:0 was not investigated but the same result was found between C16:0 and acetate to propionate ratio.

Fig 1: Correlation between Acetate to Propionate ratio and Acetate to Palmitic acid ratio from grazing on non-Bt CCR.
Fig 2: Correlation between Acetate to Propionate ratio and Acetate to Palmitic acid ratio from grazing on Bt CCR.

Fig 3: Correlation between Acetate to Propionate ratio and Propionate to Palmitic acid ratio from grazing on non-Bt CCR.

Fig 4: Correlation between Acetate to Propionate ratio and Propionate to Palmitic acid ratio from grazing on Bt CCR.
5. Conclusions

This research evaluated the effects of grazing dairy cows on Bt and non-Bt Cotton Crop Residues on the ratios of each ruminal Acetate and Propionate to milk Palmitic acid and the correlation of both ratios to Acetate to Propionate in Gezira State, Sudan. Based on the study findings it could be concluded that: The milk yield in weeks of the grazing period were highly significantly differed (P<0.01) and had the same trend of Acetate to Palmitic acid ratio and Propionate to Palmitic acid ratio; the milk yield was higher in Bt CCR compared to non-Bt CCR. Also Bt CCR had higher Acetate to Propionate ratio when compared to non-Bt CCR. A positive highly strong correlation existed between the Acetate to Propionate ratio and Acetate to Palmitic acid ratio from grazing on non-Bt CCR. While a negative correlation existed between the molar proportions of Acetate to Propionate ratio and Acetate to Palmitic acid ratio from grazing on Bt CCR. The Acetate to Propionate ratio from grazing on Bt and non-Bt CCR were negatively correlated with the ratio of Propionate to Palmitic acid.

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