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Effects of Grazing Dairy Cows on Bt and Non-Bt Cotton Crop Residues on the relationship between Rumen Total Bacterial Count and the proportion of Oleic acid in milk in Gezira State, Sudan

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ABSTRACT

The objective of this experiment was to the relationship between rumen total bacteria count and milk Oleic (g\100g) acid. 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 stored 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. Fatty acids analyzed determine by GC. Approximately 250 mL rumen fluid sample from each cow was collected on weeks 0 (Before introduction of animals for grazing), 1, 2, 3, 4 and 5 of the grazing period via stomach tubing. They were acidified with either 200 µL of 50% (volume/volume) sulfuric acid or 2 mL of 25% (weight/volume) meta phosphoric acid and stored at −20°C until later analyses of total bacterial count. The results revealed the rumen total bacteria, *Rumunococus flavefaciens*, *Fibrobacter succinogens,* Methanogenes from grazing on Bt CCR were negatively correlated with the milk Oleic acid. While rumen *Rumunococus albus,* from grazing on Bt and Non-CCR were negatively correlated with the milk Oleic acid.

Keywords: Grazing, Dairy Cows, Bt and Non-Bt Cotton Crop Residues, total bacteria rumen fluid, milk Oleic acid, correlation between total bacteria rumen fluid and milk Oleic acid.

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). Due to fear

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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) was 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 (Hashim, 2016; ElObied *et al.,* 2016). Lactating dairy cow digestion is strongly determined by the microbial population in the rumen. In the rumen, microbial fermentation of feedstuffs produces volatile fatty acids (VFAs), which are the main energy supply substances in ruminants. There is a significant relationship between ruminal pH and the profile of VFAs available for absorption (Dijkstra *et al.,* 2012). Hence, the composition and amount of milk fatty acids are determined by the proportions and the total amounts of fermentation end-products in ruminants (Sutton1985). Many studies have examined the effects of microbial protein synthesis and microbial nucleic acid composition in the rumen on protein nutrition (Fujihara and Shem 2011). The composition of the ruminal microbial ecosystem in the forestomach of ruminants is known to be affected by the type and quantity of the ration, feeding intervals, specific additives (e.g., antibiotics), and the host animal itself (Bryant 1968). To identify the relationships between bacterial populations and milk OBCFA concentrations, seven kinds of bacteria species (cellulolytic or amylolytic bacteria) were selected for the current study. Ruminococcus albus, R. flavefaciens, Fibrobacter succinogenes (Shi *et al.,* 1997), and Eubacterium ruminantium (Bryant 1959) are the predominant ruminal cellulolytic bacteria. The genus Butyrivibrio fibrisolvens is a heterogeneous bacterial taxon (Miyagawa 1982). Selenomonas ruminantium (Fernando *et al.,* 2010) and Streptococcus bovis (Klieve *et al.,* 2003) are important for the degradation of starch and lactate, which are abundant in high-grain diets. Therefore in depth research with the objectives to investigate the effect of grazing on CCR on relationship between rumen total bacteria count and milk Oleic (g\100g) acid will be carried out in this study.

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. thuringesis). 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 storeded 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 Oleic acid in milk fat. However determination of rumen bacteria count in the rumen fluid was carried out.

2.4. Determination of Oleic 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 modifiations. 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 isooctane 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® flsks for later chromatographic analysis. The original method includes fast heating under reflx 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 flme 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 flw rates (White Martins) used were 1.4 mL min-1 carrier gas (H2), 30 mL min-1 make-up gas (N2), and 30 and 300 mL min-1 flme gases, H2 and flme 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 (189-19 and O-5626, 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 189-9 (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 transesterifiation, 1.00 mL of internal standard solution (1 mg mL-1) was added to all samples and the solvent was evaporated under N2 flw. The sample fatty acids were quantified after the verifiation of the agreement between the theoretical and experimental response factors. The sample fatty acid concentrations were calculated according to Joseph and Ackman (1992).

2.4.4. Rumen fluid Sampling

Rumen fluid sample from each cow was collected on weeks 0, 1, 2, 3, 4 and 5 of the of 5 weeks grazing period via stmach tubing. After discarding the first 200 mL of fluid to minimize saliva contamination, approximately 250 mL of rumen fluid was collected. Samples was immediately measured for pH using a pH meter (Waterproof pH Testr 30). Samples were filtered through 4 layers of cheesecloth, and 2 aliquots (10 mL) was acidified with either 200 µL of 50% (volume/volume) sulfuric acid or 2 mL of 25% (weight/volume) meta phosphoric acid and stored at −20°C until later analysed of total bacterial count, total protozoa and rumen pH. Total sample in Bt and non-Bt CCR was 200 samples (5weeks × 20 cows×2 Treatment).

2.4.5. Total bacterial count

The rumen content was transferred to thermoses and immediately taken to the Animal Nutrition

Laboratory. In the laboratory, the samples were diluted with an anaerobic solution (ADS, Bryant and Burkey, 1953), homogenized under an atmosphere of CO2, and sequentially diluted (10-2 to 10-12). The analysis was conducted in triplicate, and aliquots of each sample were incubated in test tubes containing half of the selected culture prepared under anaerobic conditions. , and antibiotics were used (700 UI of streptomycin sulfate and 1600 UI of penicillin sodium) to inhibit bacterial growth in the medium. To quantify the bacterial population, successive dilutions were performed (to 10-12), and the microbes were inoculated at 39 °C in a complex medium containing soluble sugars and cellulose as a source of carbon and energy (Dehority *et al.,* 1989). To estimate the Microbial Population Number (MPN) (Alexander, 1982) of bacteria, sequential dilutions of 10-10 to 10-12 were performed. After inoculation, the tubes used to estimate the bacterial content were incubated at 39 °C for up to 7 days. Visual confirmation of growth was performed by determining the turbidity of the tubes (in triplicate), measuring the pH, and recording the growth count and average value per treatment.

2.5. Statistical Analysis

Means and differences between means of rumen metabolite of Bt and non- Bt CCR and that of Oleic acid in milk. Mean of rumen total bacteria count and Oleic (g\100g) of milk 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

A negative strong correlation (r^2 = -0.48) existed between the total bacteria and Oleic acid on rumen fluid and milk from grazing on Bt CCR (Fig 1). On the contrary in this study a positive correlation existed between the rumen total bacteria and milk Oleic acid from grazing on non-Bt CCR (r^2 = 0.09) (Fig 2). However, correlated between rumen pH and the milk Oleic acid was low R-squared values.

The rumen *Rumunococus albus* from grazing on Bt and non-Bt CCR (Fig 3 and 4) were negatively correlated with the milk Oleic acid (r^2 = -0.82) and (r^2 = -0.74) respectively.

A negative strong correlation (r^2 = -0.10) existed between the *Rumunococus flavefaciens* and Oleic acid on rumen fluid and milk from grazing on Bt CCR (Fig 5). On the contrary in this study a positive correlation existed between the rumen *Rumunococus flavefaciens* and milk Oleic acid from grazing on non-Bt CCR (r^2 = 0.08) (Fig 6). However, correlated between rumen *Rumunococus flavefaciens* and the milk Oleic acid was low R-squared values.

A negative strong correlation (r^2 = -0.88) existed between the *Fibrobacter succinogens* and Oleic acid on rumen fluid and milk from grazing on Bt CCR (Fig 7). On the contrary in this study a positive correlation existed between the rumen *Fibrobacter succinogens* and milk Oleic acid from grazing on non-Bt CCR (r^2 = 0.16) (Fig 8). However, correlated between rumen *Fibrobacter succinogens* and the milk Oleic acid was low R-squared values.

A negative strong correlation (r^2 = -0.89) existed between the Methanogenes and Oleic acid on rumen fluid and milk from grazing on Bt CCR (Fig 9). On the contrary in this study a positive correlation existed between the rumen Methanogenes and milk Oleic acid from grazing on non-Bt CCR (r^2 = 0.65) (Fig 10).

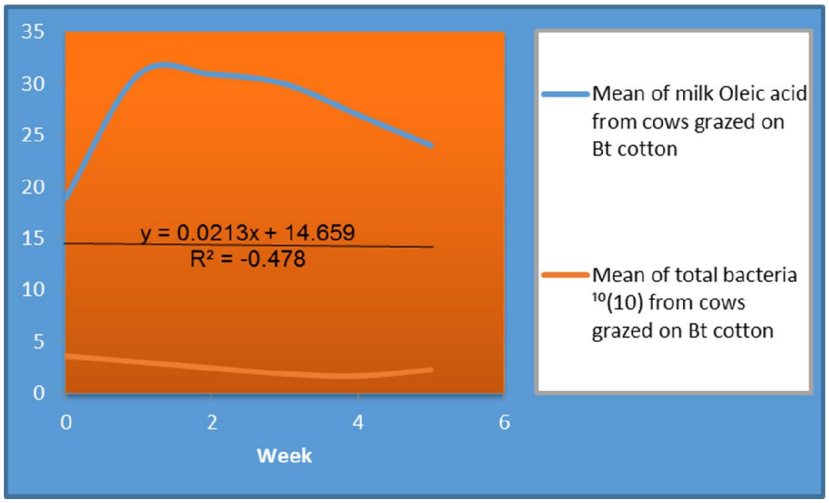

Fig. 1: Correlation between rumen total bacteria and milk Oleic acid from grazing on Bt CCR.

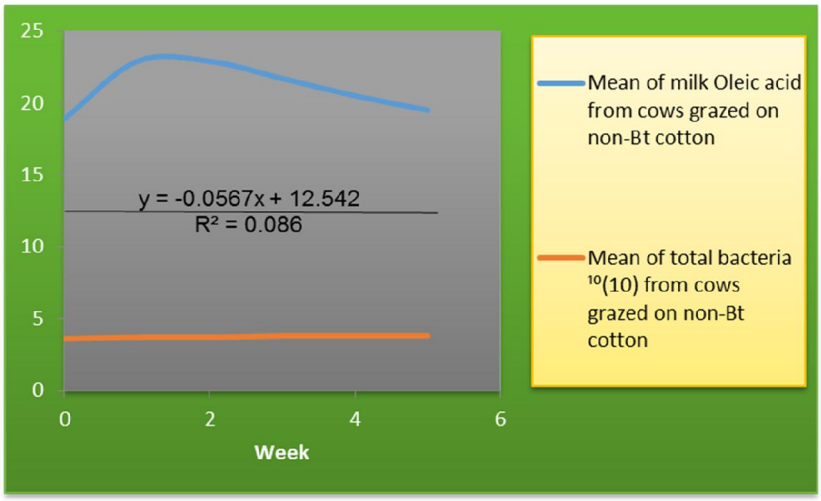

Fig. 2: Correlation between rumen total bacteria and milk Oleic acid from grazing on non-Bt CCR.

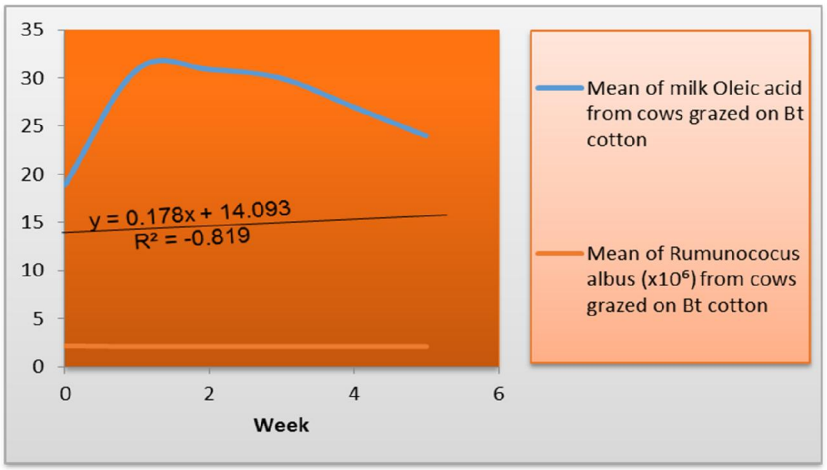

Fig. 3: Correlation between rumen *Rumunococus albus* and milk Oleic acid from grazing on Bt CCR.

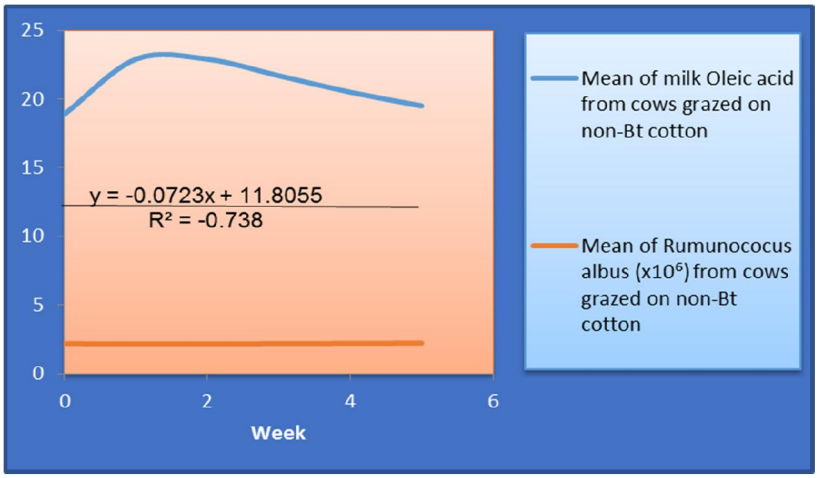

Fig. 4: Correlation between rumen *Rumunococus albus* and milk Oleic acid from grazing on non-Bt CCR.

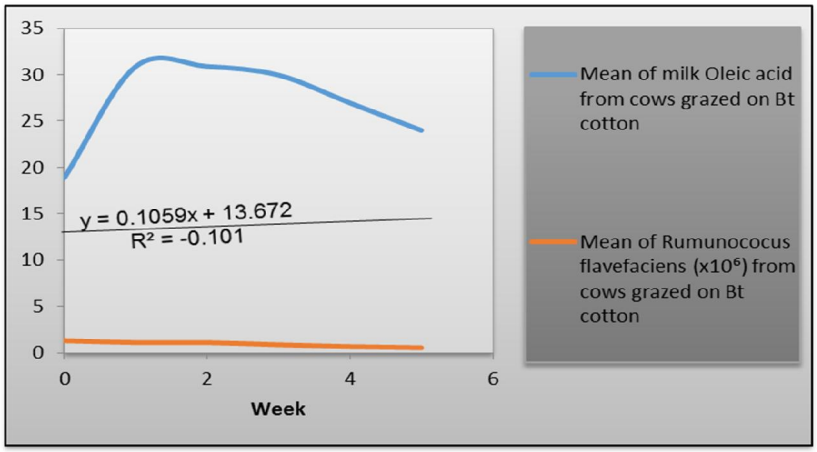

Fig. 5: Correlation between rumen *Rumunococus flavefaciens* and milk Oleic acid from grazing on Bt CCR.

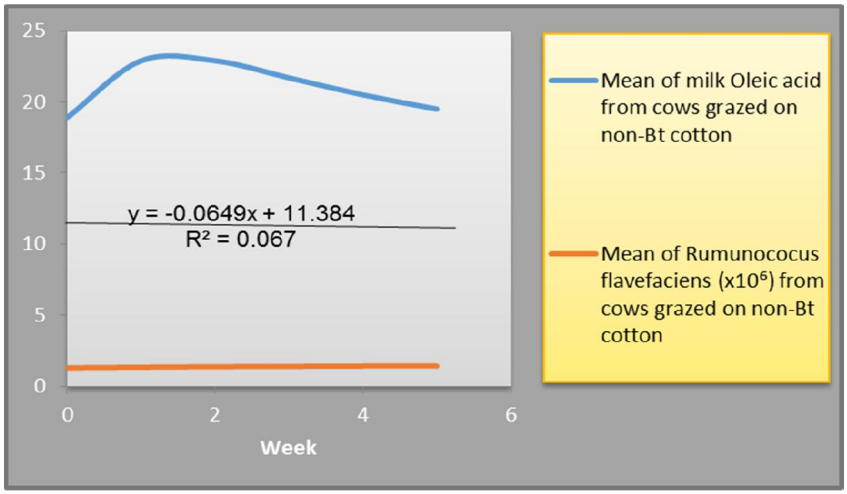

Fig. 6: Correlation between rumen *Rumunococus flavefaciens* and milk Oleic acid from grazing on non-Bt CCR.

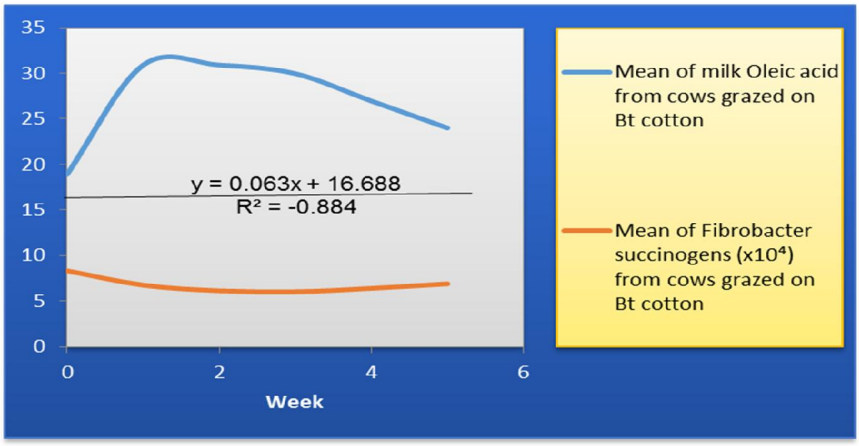

Fig. 7: Correlation between rumen *Fibrobacter succinogens* and milk Oleic acid from grazing on Bt CCR.

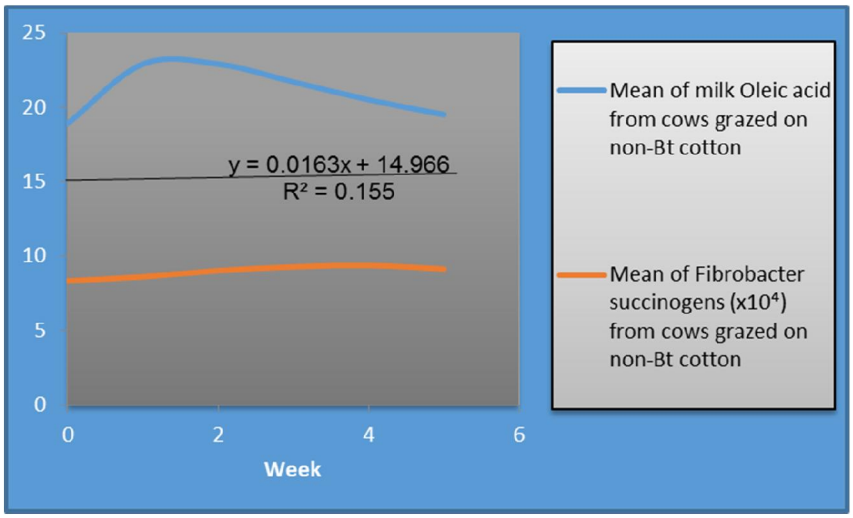

Fig. 8: Correlation between rumen *Fibrobacter succinogens* and milk Oleic acid from grazing on non-Bt CCR.

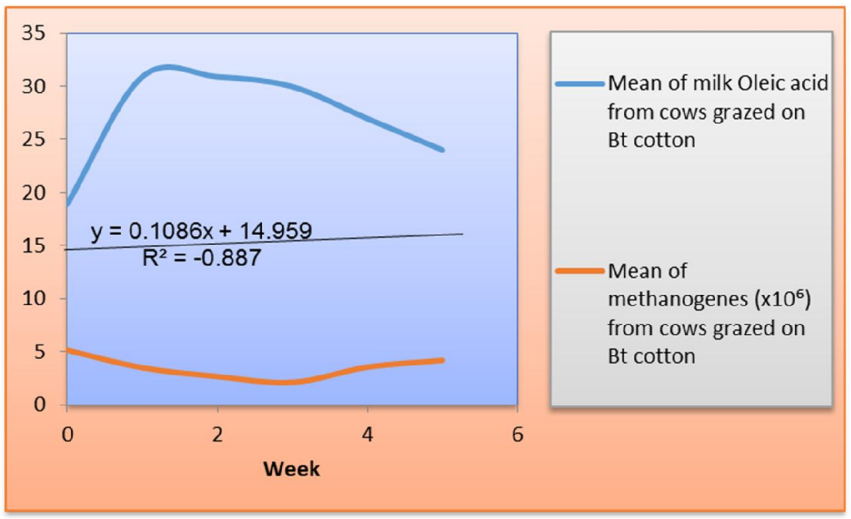

Fig. 9: Correlation between rumen Methanogenes and milk Oleic acid from grazing on Bt CCR.

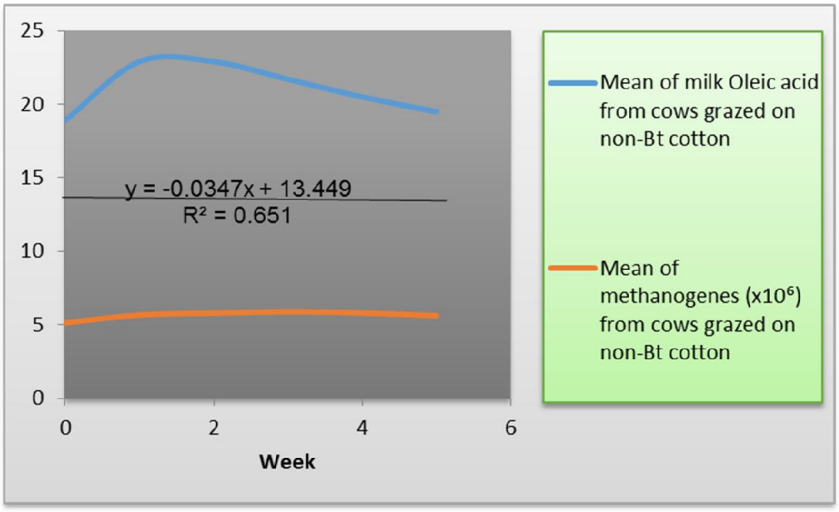

Fig. 10: Correlation between rumen Methanogenes and milk Oleic acid from grazing on non-Bt CCR.

4. Discussion

A negative correlation between the rumen total bacteria and milk Oleic acid from grazing on Bt CCR (Fig 1). This result agreed with reported by Stergiadis *et al.,* (2021) who stated that, negatively correlated between rumen total bacteria and milk Oleic acid. Also it is agreed with Petri *et al.,* (2013) who found a positive correlation between rumen bacteria and milk Olic acid in dairy cows. This result is disagreed with Chen *et al.,* (2012) who found significant correlation between rumen bacteria and milk Oleic acid in dairy cows. On the contrary in this study a positive correlation existed between the rumen total bacteria and milk Oleic acid from grazing on non-Bt CCR (Fig 2). This may be explained on the basis of increasing number of rumen bacteria has a positive effect on milk fat content as reported by Davidson *et al.,* (2008). This result agreed with reported by Buitenhuis *et al.,* (2019) who impact of the rumen microbiome on milk fatty acid composition of Holstein cattle. The variation of rumen total bacteria between weeks in the same group was highly significant ($P<0.01$) in both Bt and Non-Bt-CCR. It is clear that in Bt CCR the number of total bacteria count decreased weekly when compared to week zero. Li *et al.,* (2017) reported decreased for rumen total bacteria in lambs when compared to control. While in Non- Bt-CCR total bacteria count increased weekly when compared to week zero. Nagpal *et al.,* (2010) reported increased for rumen total bacteria in buffalo when compared to control. The variation of milk Oleic acid between the weeks in both Bt-CCR and non-Bt-CCR was highly significant ($P<0.01$). It is clear that in Bt-CCR the molar proportion of milk Oleic acid increased weekly when compared to week zero(Before introduction of animals for grazing). Moralesa *et al.,* (2015) reported increased of milk Oleic acid for grazing system when compared to control.

The rumen *Rumunococus albus* from grazing on Bt and non-Bt CCR (Fig 3 and 4) were negatively correlated with the milk Oleic acid. This result agreed with reported by Yang (2007) who found no significant correlation between *Rumunococus albus* and milk Oleic acid in dairy cows. Also it is agreed with Smith (1994) who found a negative correlation between rumen *Rumunococus albus* and milk Oleic acid in dairy cows. Liu *et al.,* (2019) found that milk C11:0 and C13:0 were negatively correlated with the rumen of Ruminococcus albus. In this studies though the relationship between C11:0 and C13:0 was not investigated but the same terned was found between C18:1 and *Rumunococus albus* ratio. Also it is agreed with Vlaeminck *et al.,* (2006) found a negative correlation between rumen *Rumunococus albus* and odd- and branched-chain fatty acids in milk in dairy cows. The variations of *Rumunococus albus* between the weeks in both Non-Bt-CCR and Bt-CCR were highly significant ($P<0.01$). It is clear that in Bt CCR *Rumunococus albus* decreased weekly when compared to week zero. Lijun Wang *et al.,* (2020) reported decreased for ruminal fermentation characteristics of cows fed two different diets high forage and high concentrate when compared to control. While in Non- Bt-CCR it was increased weekly when compared to week zero. Kim *et al.,* (2018) reported increased for bacterial communities in Holstein cattle fed a high-grain diet HAY Period and CON Period when compared to control.

A negative strong correlation existed between the *Rumunococus flavefaciens* and Oleic acid on rumen fluid and milk from grazing on Bt CCR (Fig 5). This result agreed with reported by Yang (2007) who found no significant correlation between *Rumunococus flavefaciens* and milk Oleic acid in dairy cows. Also it is agreed with Smith (1994) who found a negative correlation between rumen *Rumunococus flavefaciens* and milk Oleic acid in dairy cows. Also it is agreed with Liu *et al.,* (2019) found that milk and odd- and branched-chain fatty acids in milk in dairy cows were negatively correlated with the rumen of *Rumunococus flavefaciens*. Also it is agreed with Vlaeminck *et al.,* (2006) found a negative correlation between rumen *Rumunococus flavefaciens* and odd- and branched-chain fatty acids in milk in dairy cows. On the contrary in this study a positive correlation existed between the rumen *Rumunococus flavefaciens* and milk Oleic acid from grazing on non-Bt CCR (Fig 6). Whereas the variations of *Rumunococus flavefaciens* between the weeks in both Non-Bt-CCR and Bt-CCR were highly significant ($P<0.01$). It is clear that in Bt CCR *Rumunococus flavefaciens* decreased weekly when compared to week zero. Lijun Wang *et al.,* (2020) reported decreased for ruminal fermentation characteristics of cows fed two different diets high forage and high concentrate when compared to control. While in Non- Bt-CCR it was increased weekly when compared to week zero. Kim *et al.,* (2018) reported increased for bacterial communities in Holstein cattle fed a high-grain diet HAY Period and CON Period when compared to control.

A negative strong correlation existed between the *Fibrobacter succinogens* and Oleic acid on rumen fluid and milk from grazing on Bt CCR (Fig 7). This result agreed with reported by Croom *et al.,* (1981) who found no significant correlation between *Fibrobacter succinogens* and milk Oleic acid in dairy cows. Also it is agreed with Vlaeminck *et al.,* (2006) found a negative correlation between rumen *Fibrobacter succinogens* and milk Oleic acid in dairy cows. Liu *et al.,* (2019) found that milk C15:0 and C17:0 were negatively correlated with the rumen of *Fibrobacter succinogens*. In this studies though the relationship between C15:0 and C17:0 was not investigated but the same terned was found between C18:1 and *Fibrobacter succinogens*. On the contrary in this study a positive correlation existed between the rumen *Fibrobacter succinogens* and milk Oleic acid from grazing on non-Bt CCR (Fig 8). The variations of *Fibrobacter succinogens* between the weeks in both Non-Bt-CCR and Bt-CCR were highly significant ($P<0.01$). It is clear that in Bt CCR *Fibrobacter succinogens* decreased weekly when compared to week zero. Wang *et al.,* (2020) reported decreased for ruminal fermentation characteristics of cows fed two different diets high forage and high concentrate when compared to control. While in Non- Bt-CCR it was increased weekly when compared to week zero. Kim *et al.,* (2018) reported increased for bacterial communities in Holstein cattle fed a high-grain diet HAY Period and CON Period when compared to control. A negative strong correlation existed between the Methanogenes and Oleic acid on rumen fluid and milk from grazing on Bt CCR (Fig 9). This result agreed with reported by Stergiadis *et al.,* (2021) who stated that, negatively correlated between rumen Methanogenes and milk Oleic acid. On the contrary in this study a positive correlation existed between the rumen Methanogenes and milk Oleic acid from grazing on non-Bt CCR (Fig 10). The variations of Methanogenes between the weeks in both Non-Bt-CCR and Bt-CCR were highly significant ($P<0.01$). It is clear that in Bt CCR Methanogenes decreased weekly when compared to week zero. While in Non- Bt-CCR it was increased weekly when compared to week zero. Kim *et al.,* (2018) reported increased for bacterial communities in Holstein cattle fed a high-grain diet HAY Period and CON Period when compared to control.

5. Conclusions

This research evaluated the effects of grazing dairy cows on Bt and Non-Bt cotton crop residues on the relationship between rumen total bacterial count and the proportion of Oleic acid in milk in Gezira State, Sudan. Based on the study findings it could be concluded that: A negative correlation existed between the rumen total bacterial count from grazing on Bt CCR and milk Oleic acid and had the same trend *Rumunococus flave faciens*, *Fibrobacter succinogens* , total Methanogenes. Also a positive correlation existed between the rumen total bacterial count from grazing on Non-Bt CCR and milk Oleic acid. The rumen *Rumunococus albus* from grazing on Bt and Non-Bt CCR were negatively correlated with the milk Oleic.

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