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Changes Occurring in Some Biochemical Markers of Fat Supplemented Dairy Cows during Transition Period under Tropical Conditions

Ulfina Galmessa[1], S. P. Kimothi[2], Seid Mohammed[3] and A. K. Dang[3]
[1]*Department of Animal science, College of Agriculture and Veterinary Sciences, Ambo University, P. O. Box 19, Ambo, Ethiopia. E-mail Address:ulfinaag@gmail.com*
[2]*Principal Scientist & Assistant Director General (ADG) ICAR-DARE New Delhi India*
[3]*National Dairy Research Institute (NDRI), Karnal, Haryana-India*

Abstract

The transition period is the most critical period where cow's metabolism shifts from the demands of pregnancy to those of lactation. This results in negative energy balance especially in early lactation. To meet this demand both long chain (a-linolenic acid) or short chain (butyric acid) fatty acid were supplemented to Karan Fries crossbred (Tharpakar x Holstein Frisian) dairy cows starting one month before parturition to one month after parturition. Various biochemical markers like NEFA (non-esterified fatty acids), BHBA (Betahydroxy butyrate), BUN (Blood Urea Nitrogen), TRG (triglycerides) and creatinine were estimated in plasma samples of both control and supplemented cows. Results revealed significant improvement in most biochemical markers of fat supplemented cows compared to the control cows. Plasma glucose increased significantly (P<0.05) and plasma total protein increased non-significantly, whereas plasma NEFA, BHBA, BUN, TRG and creatinine decreased in cows supplemented with a-linolenic acid compared to cows supplemented with either butyric acid or cows kept under control diet, indicating the cows in the former group were in positive balance compared to the other groups of cows. There was a marked improvement in reproductive performance like open days, service per conception and conception rate in cows supplemented with a-linolenic acid. Hence, supplementation of fat (high density energy) especially polyunsaturated fat acid (PUFA) like a-linolenic acid is safely recommended during transition period under tropical conditions as it has been proved to improve reproductive performances in addition to enhanced energy status in early lactation.

Keywords: Biochemical markers, transition period, fat supplementation, reproductive performance

Introduction

Transition cow biology and management has become a focal point for research during the last two decades. Dairy farmers as well as scientist world over are looking for significant breaking through which could ensure successful transition cow management. This transition period is defined as 3 week pre-calving to 3 week post-calving (Grummer, 1995) and is the most critical 6 weeks of the production cycle as it imparts the level of success realized during the subsequent lactation. It is regarded as one of the most challenging periods of the production cycle, which entails profound physiologic and metabolic changes that often disrupt the homeostatic mechanisms of the cow. This is because at this stage the liver adapts from a minimal glucose demand to an overwhelming demand for glucose (Defrain *et al.,* 2005).

Transition period is characterized by a substantial decline in feed intake around parturition, lipid mobilization leading to elevated plasma NEFA and hepatic triglycerides (TRG) content and protein mobilization. Albeit an increased nutrient requirement for foetal development and lactogenesis in late pregnancy, the feed intake in ruminants is drastically reduced. The onset of lactation in the dairy cow is thus characterized by a dramatic increase in the nutrient demands for milk synthesis that coincides with a prepartum decline in dry matter intake (DMI); this lead to negative energy Balance (NEB) in early lactation (Castaneda-Gumierrez *et al.*, 2005). This down-regulation of the appetite is reportedly caused by an increased concentration of sex hormones, an incipient mobilization of lipid from body deposits and reduced rumen capacity as a result of the growing foetus (Ingvartsen *et al.*, 1999; Ingvartsen and Andersen, 2000).

Hence, due to the fact that rumen capacity also gets reduced in this stage, it is unlikely that the requirement of nutrients can be met through conventional feed supplementation for energy. It is therefore imperative to provide high energy density diet that could be achieved by fat supplementation. Even though both saturated and unsaturated fatty acids are being used as energy sources for ruminant livestock, the extra benefit of feeding unsaturated fatty acids (USFA) over that of saturated fatty acids (SFA) is becoming the current topic of research globally. In the present study, α-lenoleniec acid (USFA) and butyric acid (SFA) were supplemented to transition cow's diet to determine changes occurring in the profile of biochemical markers and effect on the cows' subsequent production and reproduction performance.

Materials and Methods

Description of the study area

The study was conducted on Karan Fries (KF) crossbred (Holstein Friesian X Tharparkar) cows maintained at Livestock Research Centre of National Dairy Research Institute, Karnal, situated in the Trans-Gangeticplain region of India. The NDRI, Karnal is located on $29^0 43'$ N latitude and 76^0 $58'$ E longitudes at an altitude of 245 meters above the mean sea level. There are four major seasons in the year viz. winter (December to March), summer (April to June), rainy (July to September) and autumn (October and November). The minimum ambient temperature falls to near freezing point in winter and maximum goes approximately up to 45^oC in May/June months of summer. The average annual rainfall is 700 mm, most of which is received from July to September.

Experimental animals and their management

A total number of 36 pregnant crossbred cows (30 days prior to expected date of calving) were initially recruited for the present study. All the selected cows were of 2-3 parity, apparently healthy and vaccinated against common diseases (Brucellosis, Foot and Mouth Diseases, Hemorrhagic Septicemia and Black Quarter) as per the standard management practices of the farm. Cows were maintained in loose housing system under individual feeding pen during experimental period. The selected cows were randomly allotted on the basis of their parity and weight to one control and two treatments of 12 cows each under Completely Randomized Design (CRD). Control cows (Treatment 1) were fed the routine ration of the Institute for pregnant and lactating cows. Under treatment 2, cows were supplemented with 750 g/d/head α-linolenic acid (crushed flaxseed), and cows under treatment 3 were supplemented with 250 g/d/head butyric acid over and above the routine feeding of the Institute**.** All cows were fed on isoenergetic diet formulated to meet or exceed the predicted requirements of National Research Council (NRC, 2001). The concentrate was fed at the rate of 2.0 kg per animal for body maintenance. Milking cows were given additional concentrate at the rate of 1.0 kg for every 2.5 kg milk production, above 5.0 kg milk yield. Water was available *ad lib* to them throughout the day.

Blood sampling protocol

Blood samples were collected from 6 cows in each group starting from 21 days before expected parturition through 21 days postpartum at (day −21, day −14, day −7, day −2, day + 2, day + 7, day +14 and day +21) through jugular venipuncture using 9 mL blood collection tubes with EDTA as anticoagulant (Vacuette®, Greiner Bio-one Gmbh, Austria). Immediately after collection, blood samples were centrifuged at 4°C (3000 rpm for 20 min), plasma was separated, and stored in cryovials at −20°C till assay.

Analysis of blood metabolites

Glucose was estimated in plasma samples by using B-Bridge International, Inc. Glucose colorimetric Detection Kit (Catalog# K3039-1); which is designed to quantitatively measure glucose in a variety of samples. Standard curve was generated for the assay and all samples were read off the standard curve. Total protein concentration (gm/dl) was estimated by modified Biuret method (Span diagnostic Ltd. Surat, India).

The B-Bridge Urea Nitrogen (also known as BUN) Colorimetric Detection Kit (catalog # k3024-1) quantitatively measure urea nitrogen in serum, plasma, urine, saliva, and tissue culture medium without phenol red. A urea nitrogen standard curve for the assay was generated and all samples were read off the standard curve. Triglycerides were estimated by Triglycerides Colorimetric Assay Kit -1" of EnzyChroTM, Hayward,CA 94545, USA.

Plasma NEFA was estimated by bovine NEFA kit, Qayee-Bio for Life Science, USA. The kit uses a double antibody sandwich enzyme-linked immunosorbent one-step process assay (ELISA) to assay level of NEFA in samples. The standard curve was generated by plotting the average OD obtained for each of the standard concentrations on the vertical (Y) axis versus the corresponding concentration the horizontal (X) axis. Plasma BHBA was estimated by "β-Hydroxy butyrate (Ketone Body) ELISA Kit" of Qayee-Bio for Life Science, USA. The kit uses a double antibody sandwich enzyme-linked immunosorbent one-step process assay (ELISA) to assay level of BHBA in samples. Creatinine was estimated by QuantiChrom™ Creatinine Assay Kit (Item No. DICT-500). Bioassay systems creatinine kit was designed to measure creatinine directly in biological samples without any pre-treatment. The improved Jaffe method utilized picrate that forms a red coloured complex with creatinine and the intensity of the colour, measured at 510 nm was directly proportional to creatinine concentration in the sample.

Statistical analysis

All data were subjected to two-way ANOVA with interaction using General Linear Model (GLM) procedure of SAS Institute, Inc., (SAS, 2002). Least squares means of all concentrations were calculated and presented as mean ± SEM. Significance was declared at $p \leq 0.05$ and a trend at $0.05 < p \leq 0.1$, unless otherwise stated. When a significant F-test was detected, pair wise multiple comparisons were performed to discriminate among the means using Tukey Honestly significant difference (HSD) test as post hoc test.

Results and Discussions

Plasma Concentration of Glucose

The least squares means and changes in plasma concentration of glucose in cows fed on diets supplemented with α-linoleneic acid (crushed flaxseed) or butyric acid during transition period is presented in Table 1 and Figure 1 respectively.

The result revealed significantly ($p<0.05$) higher plasma glucose concentration in cows supplemented with α-linolenic acid compared with cows under control diet. It was also noticed that the overall mean concentration of cows supplemented with α-linolenic acid and butyric acid were statistically ($P>0.05$) similar, indicating that both saturated and unsaturated fatty acid play comparable role in energy balance of dairy cattle. Working with Murrah buffaloes, Nazir *et al*. (2013) reported a non-significant difference in plasma concentrations of glucose between flaxseed supplemented and un-supplemented groups. The plasma concentration of glucose was observed to have increased from -21 day to -14 in all group of cows and drastically decline thereafter till one week after parturition, where it slightly tend to increase (Figure 1). Two-way ANOVA depicted also changes due to transition period was statistically significant ($p<0.05$). The plasma concentration of glucose decreased and that of BHBA increased in control cows as compared to either cows supplemented with α-linolenic acid or butyric acid cows. Even though, Huhtanen *et al*. (1993) observed decreased blood concentration of glucose and linearly increased BHBA when the amount of butyric acid was raised from 0 to 600 g/d, the present study failed to substantiate as the animals were under isoenergic diets. However, there were variations in the trends of change in different feeding groups. Cows fed on diets supplemented with α-linolenic acid (flaxseed) showed more or less constant glucose concentration up to day 21 after calving; whereas, control cows and butyric acid group showed a decline in glucose concentration after calving owing to the increased glucose demand for milk synthesis. The blood glucose level increases in response to the increased demand for the initiation of milk synthesis. Bertics *et al*. (1992) reported that glucose supply calculated from digestive energy intake exceeded estimated demand before parturition; it may be associated with increased glycogen synthesis in liver and muscle. After calving, demand for glucose increases significantly and cows adapt to this requirement by gluconeogenesis from propionates, amino acids, lactate and glycerol (Drackley *et al*., 2000).

Table 1. LSM ± SE and overall mean for Biochemical markers in KF cows fed diet supplemented with α-linolenic acid or butyric acid DTP

Blood Metabolites	Treatment			Overall Mean
	α-linolenic acid	**Butyric acid**	**Control**	
Glucose, mg/dl	59.95 ^{a} ± 0.599	58.36 ^{a} ± 0.873	56.84 ± 1.433^{b}	58.02± 0.657
Total Protein, mg/dl	66.48 ± 1.010	64.99 ± 2.679	60.90 ± 2.965	64.115 ± 2.53
BUN, mg/dl	39.99 ±1.610 ^{b}	41.06 ± 2.512^{ab}	47.47 ^{a} ± 1.538	42.84 ± 1.131
TRG, mg/dl	24.11^{c}± 0.534	29.38^{b} ± 0.829	33.34^{a} ± 0.803	28.94 ± 1.084
NEFA, mmol/L	0.245 ^{b} ± 0.061	0.416 ^{a} ± 0.077	0.531 ^{a} ± 0.115	0.396 ± 0.096
BHBA, mmol/L	0.564 ^{b} ± 0.023	0.756 ± 0.041^{a}	0.775 ± 0.059^{a}	0.697 ± 0.038
Creatinine, mmol/L	1.078 ± 0.1416	1.330 ± 0.1120	1.160 ± 0.1006	1.189 ± 0.120

*means bearing different superscripts in a row are significant at P<0.05; LSM-least squares means, SE-standard error, KF-Karan fries, DTP-during transition period

There was extremely high glucose demand during early lactation. In this regard, Aiello *et al*. (1984) found the t rates of gluconeogenesis from propionate in liver slices to be three fold greater at 30 day of lactation as compared to 90 and 180 day of lactation. Hammond (1983) reported that the sharp increase of plasma glucose after parturition might be due to a rise in plasma glucocorticoids. More intense glucoconeogenesis also may be due to the increase in protein concentration in cows, whose protein requirements had already been met (Rastini *et al*., 2006; Westwood *et al*., 2008).

The transit fall in glucose levels in the first week of lactation (Figure 1) could be the result of high demands for lactose synthesis and of insufficient gluconeogenesis. This is mainly because energy losses could not be fully compensated by energy intakes. immediately before calving and at calving there was high utilization of large proportion of glucose by foetus and mammary gland which could be the reason for the fall of glucose concentration at parturition.

It was also noticed that the increase in NEFA concentration increases the liver triglycerides accumulation in control group of cows and in those cows supplemented with butyric acid as compared to those cows supplemented with α-linolenic acid (flaxseed); which further prevents conversion of propionate to glucose (Piepenbrink and Overton, 2003). The overall better performance of cows supplemented with cows supplemented with α-linolenic acid may be attributed to the defaunation property of flaxseed as it is known to be antimethanogenic bacteria, where retardation of these methane producing bacteria leads to reduced intrinsic methane production and thereby reduced energy losses. Piepenbrink and Overton, (2003) had reported an increase accumulation of triglycerides in the liver of control group of cows and in those cows supplemented with butyric acid due to increase in NEFA concentration which further prevents the conversion of propionate to glucose

Plasma Concentration of Total Protein

The overall mean of total protein for cows supplemented with α-linolenic acid (flaxseed) and butyric acid is presented in Table 1. Results of two way ANOVA revealed that there was no significant ($p>0.05$) difference either due to treatment groups or transition period in the concentration of total protein. This finding was at par with Nazir *et al*. (2013) who did not observe significant difference in plasma concentrations of total protein between flaxseed supplemented and un-supplemented Murrah buffaloes.

Functionally, plasma proteins are involved in nutrition, maintenance of osmotic pressure, and development of resistance to infection. Even in adverse situations except diseased condition, the body tries to maintain a consistency of plasma proteins. This may be the reason why no any adverse impact of treatment was observed on the concentration of protein in the present study. The concentration of the plasma protein at any given time is a function of several factors such as age, water balance, status of health, breed, stage of lactation and nutritional status of the dairy cow (Jain, 1986). It was observed in this study that the concentrations of plasma protein tend to increase after parturition until day 14 and seems to decline thereof in all groups of cows.

This drop coincides with the movement of blood protein into colostrum (Rowland and Manston, 1983).

Higher total plasma protein concentration in flaxseed supplemented cows as compared to the other two groups of cows could be explained in terms of the defounation properties of flaxseed. Because of this property flaxseed has been known to reduce the rumen ammonia-N concentration which increase the duodenal flow of microbial-nitrogen (Abreu *et al*. 2004; Hess *et al*., 2004), via their toxicity to rumen ciliate protozoa (Headon *et al*.,1991) and formed complexes of protein (Sen *et al*., 1998a), thereby enhancing protection of protein from degradation in the rumen and increasing the availability of protein post-ruminally (Wallace *et al*., 1994), which enhance production and productivity. This could partly be responsible for better reproductive performance recorded in cows supplemented with α-linolenic acid compared to other groups of cows (Figure not shown).

Blood Urea Nitrogen (BUN) Concentration

Blood urea nitrogen (BUN) is the major end product of N metabolism in ruminants and its high concentration is indicative of an inefficient utilization of dietary nitrogen. In the present study the concentration of BUN generally increased just before parturition and reached the highest peak value at calving and then tends to decrease for all groups even though increased trends was observed after 14 days postpartum in the case of butyric supplemented and control cows (Figure 3). Results indicated that Blood Urea Nitrogen concentration was maintained significantly ($P<0.01$) at lower level in cows supplemented with α-linolenic acid compared to the other groups of cows. Contrary, Nazir *et al*. (2013) did not observe significant difference in plasma concentrations of BUN between flaxseed supplemented and un-supplemented Murrah buffaloes. The present findings clearly indicated positive energy-protein balance in the flaxseed supplemented group of cows as BUN concentration in the blood plasma was used as indicator of energy-protein balance (Dhali *et al*., 2006; Campanile *et al*., 1998). The BUN concentration is typically increased in cows deficient in energy denoting the imbalance between protein and energy levels in the diet as in the case of control cows and cows fed on diets supplemented with butyric acid. Likewise, increased protein intake prepartum has been reported to cause elevated prepartum plasma urea nitrogen concentration (Campanile *et al*., 1998; Doepel *et al*., 2002) while high protein diet fed to prepartum cows has been observed to cause increased blood urea and reduced fertility.

The significantly ($p<0.001$) higher blood urea nitrogen in control group of cows as compared to both flaxseed or butyric acid supplemented groups of cows might also be due to higher reproductive disorders like RFM recorded for control cows. In this regard, Civelek *et al*. (2011) observed higher (13.89 mg/dL) BUN concentration in RFM Holstein cows as opposed to non RFM cows (11.18 mg/dL). Suvani (2015) also reported significantly higher BUN concentration in RFM affected than normal KF cows during day 30 (36.9 ± 1.2 vs. 31.7 ± 1.2 mg/dL), day 14 (41.4 ± 1.2 vs. 37.8 ± 1.6 mg/dL) and day 7(34.9 ± 1.4 vs. 39.7 ± 1.3 mg/dL) prior to calving. The other possible reason was that protein intake might have been decreased along with the overall decline in DMI during transition period, which in turn resulted in less production of ammonia in the liver. This alteration of liver during transition period when the animals were under stress was also substantiated by lowered glucose and higher creatinine, NEFA and BHBA in control cows as compared to cows fed on diets supplemented with α-linolenic acid.

Elevated plasma urea levels were suggestive of causing fertility problems in dairy animals and wastage and inefficiency of dietary protein (Habib *et al*., 2007). It is apparent from the present study that cows in control groups took longer day to post partum heat and AI; moreover significantly less number of cows became pregnant during the study period (Figure 4) which is corroborated by the findings of Fergusson *et al.* (1991) who reported that the increased blood urea levels adversely affect fertility. So, the nutritional manipulation to mitigate nutrient losses and improve the efficiency of utilization of diet in dairy animals would require technical interventions.

Plasma Concentration of Triglycerides

The overall mean and changes in plasma concentrations of triglycerides in KF cows fed diets supplemented with α-linolenic acid (flaxseed) or butyric acid is also shown in Table 1 and Figure 4, respectively. It was observed that treatment had significant effect in TRG concentration where cows received α-linolenic acid (flaxseed) supplemented diet had significantly ($P<0.001$) lower (24.11 ± 0.534 mg/dl) plasma concentration of triglycerides as compared with the other groups. Moreover, the overall mean plasma concentrations of triglycerides in cows fed on butyric acid supplemented diet was significantly ($p<0.001$) lower (29.38 ± 0.829 mg/dl) when compared to that of control cows (33.34 ± 0.803 mg/dl). Contrary to the present findings, Nazir *et al*. (2013) did not observe significant difference in plasma concentrations of TRG between flaxseed supplemented and un-supplemented murrah buffaloes.

In the present study it was apparent that the concentration of TRG steadily increased until calving and tends to declined slowly thereafter which actually follows the trends of NEFA concentration. This result was corroborated by Gummer (1993) who reported four to five fold increase of liver TRG 17 days prior to calving and day 1 after calving due to increased NEFA and thereby increased fatty acid uptake by liver.

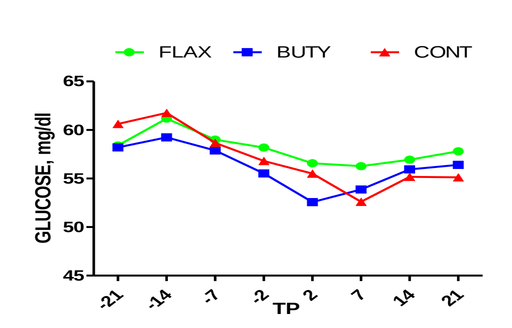

Fig 1. Changes in Plasma Glucose Concentration

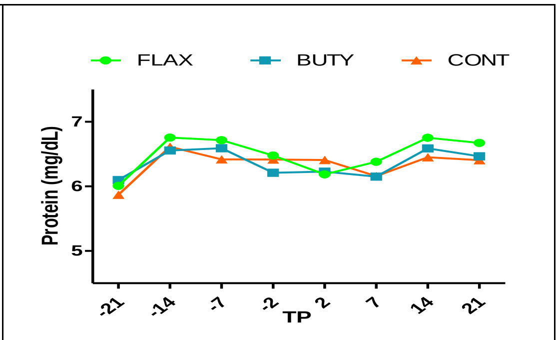

Fig 2. Changes in Total protein Concentration

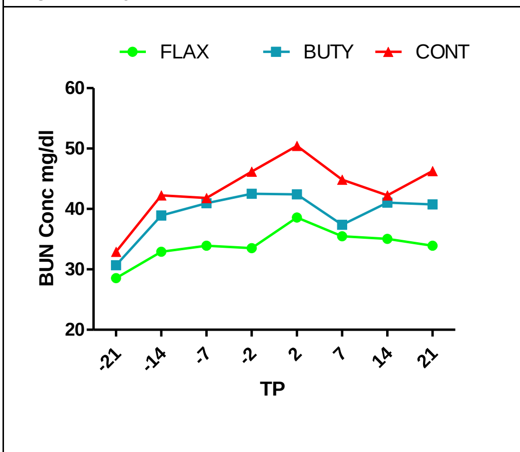

Fig 3. Changes in plasma concentration of BUN

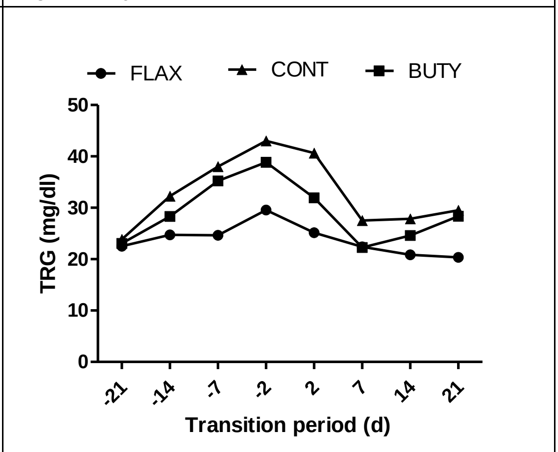

Fig 4. Changes in Plasma concentration of TRG

Plasma concentration of non-esterfied fatty acid (NEFA)

The least squares means and changes in concentration of NEFA in KF cows under different feeding regimes during transition period has been depicted in Table 1 and Figure 5. Perusal of the table revealed that treatments had significant (P<0.01) effect on the concentration of NEFA in KF cows during transition period. It was observed that α-linolenic acid supplemented cows had significantly (p<0.001) lower concentration of NEFA than either butyric acid supplemented or control cows (Table 1). Generally, there was an overall increase in blood NEFA (mmol/L) levels starting day 7 before parturition to day 2 postpartum and a continuous decline thereof in all the treatment groups. It is accepted fact that measurements of NEFA are most useful during the close-up period and in fresh cows for determining energy status of the animal. Normal values for cows with positive energy balance are in range less than 200 micromolar (µM). The overall mean values for control cows and butyric acid supplemented cows were higher than that reported by Drackley *et al.* (2000) who observed slow increase in NEFA from 200 to 300 µM (0.2 to 0.3 mM) during the last week before calving. It is apparent from Figure 5 that the concentration of NEFA in the present study increased sharply at 2 to 3 days before calving and reached peak at 0.3856± 0.036 mmol/L, 0.603 ± 0.093 mmol/L and 0.924 ± 0.193 mmol/L at 2^{nd} days after calving for all groups of cows. The present values were at the minimum range of NEFA concentration reported by Baird *et al.* (1982). They observed a sharp increase in values at 2 to 3 days before calving and generally peak at 800 to 1200 µM on the day of calving due to hormonal changes and the stress of calving.

It was noted that cows fed on control diets showed a negative energy balance when the concentration of NEFA become greater than 0.700 mmol/L at 7 days, because values greater than about 700 µM beyond 7 days after calving indicate severe negative energy balance or health problems and suggest that transition and fresh cow management should be examined (Drackley, 2000). He also suggested that plasma concentration of NEFA should be below 300 µM by 3 weeks after calving. In the present study we observed higher mean concentration of NEFA at 21 days after calving for both butyric acid supplemented and control cows, indicating the cows in these group were in NEBAL. Contrary to the present findings, ruminal infusion of different levels of butyrate (0, 200, 400, 600g/d) in dairy cows was reported not to show clear trend for plasma concentration of NEFA (Huhtanen *et al.*, 1993). The increased NEFA concentration in present study may be related to increased body fat mobilization, which might partly be due to lower dry matter intake (DMI) and increased energy requirement for milk production as suggested by Roberts *et al.* (1981) and Petit *et al.* (2002).

An increase in concentration of plasma non-esterified fatty acids (NEFA) results from the mobilization of lipids, which increase gradually in the prepartum transition period but rapidly in the last 3 days of gestation (Grummer, 1995), as it was seen in the present study. Thus plasma non-esterified fatty acids (NEFA) reflect body fat mobilization in response to NEBAL or stress conditions. Measuring NEFA concentrations can be used as a tool with other factors to help diagnose problems during the transition period. During times of energy deficit, animals break down triglycerides (fat) stored in adipose tissue. The resultant non-esterified fatty acids (NEFA) enter the blood stream to be transported to organs and tissues throughout the body. The concentration of NEFA measured in blood has been shown to reflect fat mobilized from body fat reserves. The elevated NEFA levels in the present study may indicate that dietary energy intake was insufficient for the cows needs for milk production or foetal growth and that body fat is being broken down to supply the energy deficit.

Plasma Concentration of Beta-hydroxyl butyrate (BHBA)

The overall mean and least squares mean of BHBA in KF cows supplemented α-linolenic acid or butyric acid during transition period has been depicted in Table 1 and Figure 6. Two way ANOVA revealed that treatments had significant ($P<0.01$) effect on BHBA concentration, where, cows fed on diets supplemented with α-linolenic acid had significantly ($p<0.05$) lower (0.564 ± 0.023) BHBA concentration than cows supplemented with butyric acid (0.756 ± 0.041) or control group(0.775 ± 0.059). It was also noted that the α-linolenic group had significantly higher concentration of glucose indicating animals were in positive energy balance and vice versa for other groups. The trend analysis showed an increase of BHBA up to 7 days following parturition in both control cows and butyric acid supplemented cows but in α-linolenic acid supplemented group there seems only a slight increase of BHBA up to calving and constantly decline thereafter (Table 1). This may probably be due to the decrease in DMI and the high demand for nutrients which lead to NEBAL. This is corroborated by Duffiled, (2000) who reported that immediately before calving there was a decrease in dry matter intake which leads to NEBAL. To bridge the gap, the high producing dairy cows adjusts its metabolism to fulfilled nutrient requirements. This is done by extensive mobilization of nutrients particularly fat from adipose tissues, amino acids, minerals and vitamins. Despite the tight adjustments to cope up with the changes in metabolism caused by milk production, 45 to 60% of dairy cows at different levels of milk production, breeds and management systems develop metabolic and infectious diseases in the first months of lactation (Ribeiro *et al.*, 2011; Santos *et al.*, 2010).

Figure 6 presents the changes in BHBA concentration across the transition period. The BHBA follows the opposite pattern to glucose; where cows supplemented with butyric acid could perform between α-linolenic acid group and control cows. But during postpartum of the transition period BHBA became increased in butyric acid and in control groups regardless of high glucose concentration in cows fed on butyric acid supplemented diet. This may indicate that the form or source of energy supply is of paramount importance during stress where animals reduce feed intake like in early lactation of the transition period. This finding clearly indicated that cows under α-linolenic acid (flaxseed) supplementation outsmarted the other groups in all circumstances.

Study revealed that prior to calving; BHBA concentrations generally do not exceed 575-750 μmol/L, unless the animal is in NEBAL or consuming ketogenic silage (Van Saun, 2004).

Since in the present study cows from butyric acid supplemented group and cows under control diet had shown their peak concentration of BHBA at more than 1 mmol/L it was likely that these groups were in NEBAL. Moreover it was reported that cows with BHBA concentrations above 1.0 or 1.4 mmol/L are 3.2 and 4.3 times at greater risk for postpartum disease (Van Saun, 2004; Ulfina *et al*., 2015).

Increased BHBA in the present study also goes along with increase in NEFA concentration. In this regard it was reported that NEFA provide the substrate for BHBA synthesis and increased BHBA concentration reveals incomplete oxidation of NEFA in the tricarboxylic acid cycle during negative energy balance (Grummer, 1993).

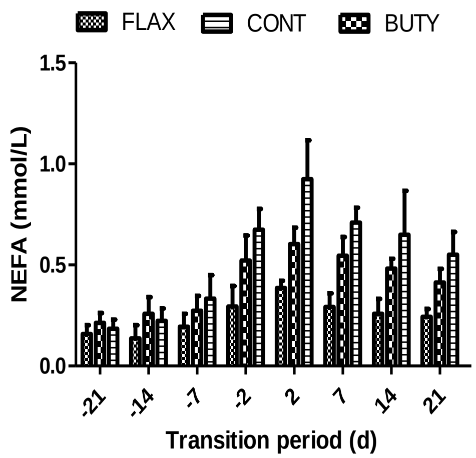

Fig 5. Changes in concentration of NEFA

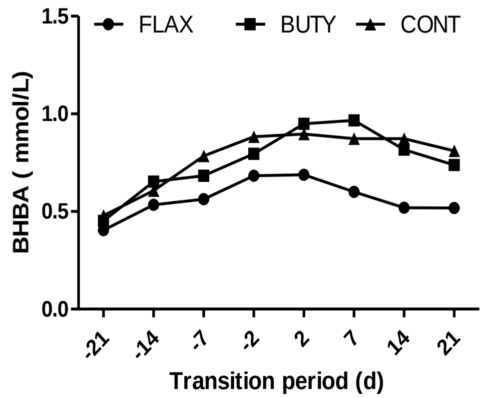

Fig 6. Changes in concentration of BHBA

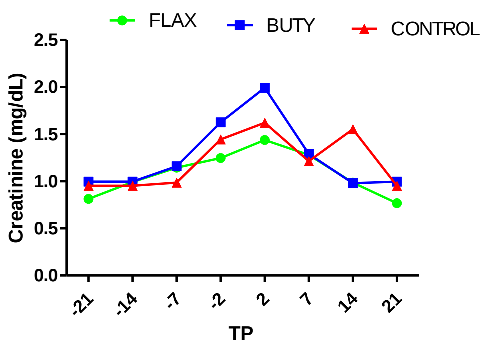

Fig 7. Changes in concentration of Creatinine

Plasma concentration of creatinine

The least squares means and overall mean plasma concentration of creatinine in KF cows supplemented with α-linolenic acid or butyric acid during transition period has been shown in Table 1 and Figure 7. The mean values for cows supplemented with α-linolenic acid, butyric acid and control were, 1.078 ± 0.1416, 1.330 ± 0.1120 and 1.160 ± 0.1006, respectively. It has been observed that cows fed on diets supplemented with α-linolenic acid during transition period had a lower mean concentration of creatinine than the other groups; whereas higher mean concentration of creatinine was recorded for cows fed on diets supplemented with butyric acid. However the mean differences between groups were non-significant. Higher level of creatinine concentration in the blood plasma has been used as biological indictor for stress or as stress marker indicating certain disease condition. Generally the concentration of creatinine was higher at postpartum period when compared to the prepartum period of the transition cows . In agreement to the present study, Nazir *et al*. (2013) did not observe significant difference in plasma concentrations of creatinine between flaxseed supplemented and un-supplemented murrah buffaloes. Contrary to our findings, Mapiye *et al.* (2010) observed the highest concentration of creatinine in pregnant non-lactating cows and lowest in pregnant lactating cows.

Conflict of Interest: None

Conclusion

The present study clearly indicates the improvement of energy balance through supplementation of α-linolenic acid as compared to cows fed either on butyric acid supplemented or on control diet. Higher glucose concentration and total protein, and lowered BUN, TRG, NEFA, BHBA and creatinine were observed at all levels of measurement in cows fed on diets supplemented with α-linolenic acid as opposed to the other groups of cows. Besides the performance of cows that received omega-3 FA source flaxseed outsmarted the other groups in positive energy balance and subsequent reproductive performance. Under tropical conditions, where pregnant cows are already facing double source of stress i.e. stress due to pregnancy as well as stress due to hot environmental conditions, they tend to consume less quantities of feed and their performance decreases accordingly. The supplementation of PUFA such as α-linolenic acid which has multifaceted benefit to dairy cows as it improves the status of energy balance, reproductive performance and health status as observed in the present study can be recommended for dairy farmers rearing crossbred cows under tropical conditions.

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