



Effects of Two Levels of Dietary Energy Content on Milk Production and Serum Metabolites in Early Lactation Temperate Crossbred Dairy Cows Fed Guinea Grass (*Megathyrsus maximus*) Based Diets

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ABSTRACT

The current study investigated the association of two levels of metabolizable energy (ME) content on milk production, composition, and blood metabolites of temperate crossbred (Jersey x Friesian) dairy cows fed with Guinea grass (*Panicum maximum*) and concentrates (50:50 on a DM basis) based total mixed ration (TMR). Twelve multiparous temperate crossbred dairy cows were randomly allocated to each dietary treatment (n=6) in a randomized block design. The required energy diet (RE) was predicted to supply the recommended level (100%) of ME, and the high energy diet (HE) was predicted to supply 10% more than the recommended level (110%) of the daily ME requirements of the dairy cows. Dry matter intake, body weight, and milk yield were recorded, and feed and blood samples were obtained for proximate and blood metabolite analysis during the 14 weeks experimental period. No treatment effects were observed ($P>0.05$) on BW, milk fat, SNF, protein, and milk urea nitrogen or on blood metabolites such as serum non esterified fatty acids, beta-hydroxy butyric acid, Albumin, Ca and P. Compared to the cows fed with RE, HE diet fed cows had high ($P<0.05$) dry matter intake at 4, 10, 12, and 14 weeks but dietary treatment had no effect on dry matter intake ($P>0.05$) at 2, 6, and 8 weeks of the experimental period. Until the 4th week of lactation, dietary treatment had no effect ($P>0.05$) on milk yield, but from the 4th to the 14th week, cows fed with HE had a higher ($P<0.05$) milk yield than those fed with RE. Thus, the cumulative milk yield of the cows fed with HE were higher ($P<0.05$) than that of cows fed with RE from the 10th to 14th weeks of lactation. It was concluded that the milk yield of temperate crossbred dairy cows fed diets based on Guinea grass can be enhanced by incorporating high energy content into the diet.

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INTRODUCTION

Temperate crossbred (Jersey x Friesian) dairy cows represent a small proportion of cattle population in Sri Lanka but make a significant contribution to national milk production. These cows have a higher average production per cow (> 10 L/cow/day) compared to tropical crossbred cows (*Bos indicus* × *Bos taurus*) commonly found in the dry zone of the country. The average production of tropical crossbred cows managed semi-intensively or intensively, is about 3-5 L/cow/day (Subasinghe and Abeygunawardena, 2011). Although temperate crossbred cows produce more milk than the national average, their milk production is still below their genetic potential. Poor nutrition, primarily due to the lack of good quality forage and limited availability of concentrate feeds, is the main contributing factor (Ranaweera et al., 2019; Weerasinghe, 2019). When nutrient requirement (especially energy) is not fulfilled through the diet, body fat is mobilized in the form of non esterified fatty acids (NEFA) to fulfill the energy requirement for milk production, subjecting dairy cows into negative energy balance (NEB) which is quite common during early lactation (Adewuyi et al., 2005). When this condition is not corrected through proper feeding, it may lead to low milk production, metabolic diseases and poor reproductive performances (Gillmore et al., 2011).

Increasing the energy density in the diet is known to be the most promising solution to overcome energy demand and NEB, especially during early lactation (Nielsen et al., 2007). Supplementation of energy dense diets to transition and early lactation cows is more advantageous as it would positively react on dietary energy content by partitioning more nutrients towards milk synthesis compared to the later parts (i.e., mid and late) of the lactation (Kokkonen et al., 2000). Most economical way of providing energy rich diets is inclusion of high proportion of good quality forages or silages as the response to concentrate feeding diminishes with increasing basal diet (roughage) quality (Nielsen et al., 2007). As observed by Lawrence et al. (2015),

concentrate feeding strategy was found to have no significant effect on milk yield (MY) in early to mid-lactation dairy cows when good quality grass silage was offered to appetite over a period of 19 to 24 wks. This may be due to the enhanced digestibility of the energy rich basal diet that fulfills the significant proportion of energy need of animals. Therefore, additional nutrients supplied by concentrate feeding may have less effect (Lawrence et al., 2015). It implies that utilization of low quality roughages can be enhanced by proper inclusion of energy rich concentrate feed. This was proved by Ferris et al. (2001) who reported increased milk production as a response to concentrate supplementation when basal diet is low in quality.

Guinea grass (*Megathyrsus maximus*) was introduced to Sri Lanka in the 1820s and has since become the most abundant basal forage, naturalizing in various ecological zones, ecosystems, and habitats throughout the country (Weerasinghe, 2019). In comparison to high-quality roughages like Sorghum (*Sorghum bicolor*) or medium-quality varieties such as hybrid Napier var. CO3 (*Pennisetum purpureum* × *P. americanum*), Guinea grass is considered a nutritionally low-quality roughage (Weerasinghe, 2019). Consequently, despite its abundance and value, this feed resource is currently underutilized in Sri Lanka. In addition, temperate crossbred cows in the country are supplemented with less amount of concentrate feeds and about 30% of that consist of rice polish and dhal husk, which are comparatively low in ME compared to energy rich ingredients such as maize. As a result, the current feeding level is sufficient to support milk production of around 10.5 L/cow/day (Ranaweera et al., 2020).

There are several methods to enhance the energy density of a dairy cow's diet. For instance, providing 250 g/cow/day of bypass fat (Calcium salts of palm oil) to tropical crossbred dairy cows fed a typical basal diet consisting of Guinea and Napier grasses resulted in 132.3 L/cow more cumulative milk production by 15 weeks of lactation (Ranaweera et al., 2019). However, bypass fat is not produced in Sri Lanka and is relatively

expensive. Nonetheless, other energy-rich concentrate feed raw materials, such as coconut poonac and maize, are locally available. While the effects of different energy contents in low-quality roughage-based diets on temperate crossbred dairy cows have not been investigated under Sri Lankan conditions, studies have been conducted on the effects of energy levels in diets based on good quality roughages on dairy cow production performance (Van Kneusel et al., 2007; Nielsen et al., 2007; Sandro et al., 2014; Lawrence et al., 2015). To our knowledge, scientific information on milk production performance and changes in blood metabolites of dairy cows fed with Guinea grass as the basal diet is scarce. Previous literature has reported the digestibility of urea ammonia-treated Guinea grass hay in sheep (Brown and Adjei, 1995), proximate composition and in-vitro digestibility of Guinea grass (Aganga and Tshwenyane, 2004), and the nutrient digestibility and weight gain of goats fed Guinea grass (Ajayi et al., 2008). Therefore, the present study aimed to test the hypothesis that "increasing dietary energy content would positively affect milk production and blood metabolites of early lactation temperate crossbred cows fed a basal diet of Guinea grass".

MATERIALS AND METHODS

Experimental Site

The study was conducted at Sri Lak Farm, a medium-scale, stall-fed dairy cattle farm located in the Nawalapitiya veterinary division of the Kandy district in Sri Lanka. The farm is situated at 7°04'30.74"N and 80°56'54.70"E, with an elevation of 589 meters above sea level. The feeding experiment took place from May to August, during which the minimum and maximum temperatures recorded were 11.4 °C and 19.3 °C, respectively.

Experimental Design and Feeding

The experimental protocol was approved by the committee on ethics review of research projects involving animals, University of Peradeniya (2018-3-NRC TO 14-10). Twelve

multiparous temperate crossbred dairy cows (n=6) weighing 402±3.8 kg and producing an average milk yield of 10.2 L/cow/day in the first week post-partum were randomly assigned to two treatments using a complete randomized design. The allocation of animals to the treatments was based on their milk yield, milk fat yield, body weight, and body condition score, measured over 7 consecutive days prior to allocation (Russell et al., 1969). The cows were milked twice a day at 0700 and 1600 h in a standard milking parlor. It was assumed that the cows would reach peak milk production by the eighth week of lactation (Vinay-Vadillo et al., 2014). Two total mixed rations (TMR) were formulated using Guinea grass (*Megathyrsus maximus*) and concentrate in a 50:50 dry matter basis ratio (Table 1) to meet the nutrient requirements according to the National Research Council (NRC, 2001). The required energy diet (RE) was formulated to supply 100% of the daily metabolizable energy (ME) requirement, while the high energy diet (HE) was formulated to supply 110% of the daily ME requirement. The different energy levels of the two diets were primarily adjusted by manipulating the maize content in the two concentrate feeds. The cows of both treatments were fed *ad-libitum* twice a day (At 0730 and 1530) and had free access to clean fresh drinking water. The cows were housed in a well-ventilated shed with a concrete floor.

Sampling

Body weight and fresh matter intake were measured, and feed samples (500 g) were collected weekly throughout the experiment. The collected feed samples were stored at -20 °C and composited before analysis. Morning and evening milk records were collected weekly. Milk samples from each milking were collected, composited by volume, and stored at 4 °C in 50 mL sterile specimen containers containing 0.1 mL of formalin (40%) until analysis of milk composition. Blood samples (3 mL) were collected weekly from the jugular vein using sterile syringes without anticoagulant. The samples were chilled and allowed to clot. Within 5-6 hours of collection,

Table 1. Ingredients and proximate composition of the experimental diets (RE and HE)

Item	Diet	
	RE	HE
Ingredient, g/kg/DM		
Grass (<i>Megathyrsus maximus</i>)	500	500
Rice polish	250	65
Dhal husk	145	60
Coconut poonac	45	120
Maize	45	240
Urea	5	5
Mineral mixture [†]	10	10
Proximate composition		
DM, g/kg	379	382
CP, g/kg/DM	142	138
ADF, g/kg/DM	257	203
NDF, g/kg/DM	502	431
EE, g/kg/DM	60.7	58.4
Ash, g/kg/DM	83.6	77.3
ME, MJ/kg/DM [‡]	8.48	9.52

[†] Composition (g/100 g): Ca 18.1, P 8.6, Mg 0.7, Na 10.0, Zn 0.6, Cu 0.15, Co 0.1, K 0.1, Se 0.1. [‡] Calculated using proximate composition values according to NRC 2001.

the samples were centrifuged (Beckman Avanti, Beckman, Buckinghamshire, UK) at 4000 g for 15 minutes. Serum was collected into microcentrifuge tubes and stored at -20 °C for analysis of serum metabolites.

Chemical analysis

Feed samples were analyzed for dry matter (AOAC 2005 934.01), ash (AOAC 1995a, b 942.05), crude protein (AOAC 1995 984.13), and crude fat (AOAC 2000 920.39). Feed samples were analyzed for NDF by the method described by Van Soest et al. (1991) and for ADF by the method described by Goering and Van Soest (1970). Milk composition (Fat, SNF and protein) was analyzed using a previously calibrated ultrasonic milk analyzer (Lactoscan MCC, Milkotronic Ltd., Bulgaria). The milk urea nitrogen (MUN) content of milk samples were estimated using the method described by Malik and Sirohi (1998). Thawed serum samples to room temperature were analyzed using commercial biochemical test kits (Randox Laboratories Ltd., UK) for NEFA: (Cat. No. FA 115), BHBA: (Cat. No. RB 1008), albumin (Cat. No. AB 362), calcium (Cat. No. CA 2390), and phosphorous (Cat. No. PH 1016) using serum auto analyzer (3000 Evolution®, Biochemical System International, Arezzo, Italy).

Data analysis

The data were analyzed using PROC GLM by SAS 9.1 statistical software (SAS 9.2, SAS Institute Inc., 2003). The initial milk yield was considered as a covariate. The results were presented as treatment means with standard error (SE), and differences were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

Dry matter intake and body weight

Dairy cows fed with HE diet had higher ($P < 0.05$) dry matter intake (DMI) at 4, 10, 12 and 14 weeks but the dietary treatment had no effect on DMI ($P > 0.05$) at 2, 6 and 8 weeks of the experimental period (Figure. 1). A gradual increase in DMI was observed as lactation progressed in both treatment groups. As a result, DMI at weeks 2 and 14 of the experiment accounted for 3.3%, 3.6% of BW for RE diet fed animals and 3.5%, 3.7% of BW for HE diet fed animals, respectively. Dietary treatment had no effect ($P > 0.05$) on BW of the dairy cows, although HE diet fed cows showed reducing trend of BW until 8th week of lactation and gained slightly thereafter (Figure 2).

The basal forage, Guinea grass, included in the total mixed ration (TMR) used to feed

cows in the current study, had lower nutritive quality, especially in terms of metabolizable energy (ME), compared to commonly used roughage varieties for dairy cattle in Sri Lanka, such as sorghum (*Sorghum bicolor*) or hybrid Napier varieties (Weerasinghe, 2019). For example, Guinea grass at the blooming stage contains ME of 6.25 MJ/kg/DM (Thelaksan et al., 2018) compared to the ME contents of 9.2 and 8.3 MJ/kg/DM for fodder sorghum and hybrid Napier var. CO3 (*Pennisetum purpureum* x *P. americanum*), respectively (Jothirathna et al., 2020). Therefore, it can be reasonably assumed that the effects of the diet on production performance of temperate crossbred dairy cows in the current study was enhanced by the different energy contents offered through the concentrate feed, particularly, different amounts of starch in two experimental diets.

Studies have demonstrated that early lactating cows fed high-energy density diets *ad-libitum* exhibit higher DMI and MY compared to cows fed low or normal energy density diets (McNamara et al., 2003). Further, when the diet changed from normal (fed only to meet the energy requirement) to high energy density for early lactation dairy cows, the DMI increased and the vice versa (Nielson et al., 2007). Compared to fiber, the rate of degradation and organic matter digestibility of starch (such as maize) is high in the rumen, thus positively affects DMI (Hatew et al., 2015). The chemical nature of starch also plays a role in digestibility and DMI. A slowly fermentable maize starch-based diet, similar to the one used in the current study, has been shown to increase DMI in dairy cows compared to a ration containing rapidly fermentable barley starch (McCarthy et al., 1989). Additionally, besides the starch content in the diet, several other chemical components act as determining factors for DMI, such as neutral detergent fiber (NDF); (Minson, 1990). The DMI and NDF contents of dairy cow rations are generally inversely related when NDF content of diets exceeds 25% (Allen, 2000). For example, in a dairy cow feed with dietary NDF concentrations ranging from 22.5% to 45.8%, DMI (% of BW and kg DM/d per animal) decreased ($r^2 = 0.595$ and 0.672 , respectively) with increasing NDF content

(Arelovich et al., 2008). Therefore, average NDF content of the HE diet was lower compared to the RE diet in the current study (502 and 431 g/kg/DM for the RE and HE diets, respectively), it could have contributed to the comparatively high DMI of the HE diet-fed cows.

Early lactation is characterized by a gradual decrease of BW mainly due to NEB. However, there was no difference in the BW of the temperate crossbred dairy cows fed with diets containing two levels of energy during 14 weeks experimental period where the average BW of RE diet fed cows observed to be lower than that of HE diet fed dairy cows (BW at 2 and 14 weeks were 425, 424 kg and 440, 436 kg for RE and HE diets fed animals respectively). When the energy balance between RE and HE diet-fed animals was calculated as the difference between the metabolizable energy (ME) requirement and the ME supply through the feed (NRC, 2001), the energy balance at 2 and 14 weeks was -24.0, -19.0, and 0.0, -8.0 MJ/day for RE and HE diet-fed animals, respectively. This could be the reason for the comparatively lower BW of RE diet-fed animals, as they may have lost more weight to support milk production compared to their counterparts who received more energy through their diet, reducing the requirement to utilize body fat to meet energy requirements.

Milk yield and composition

Until the 4th week of lactation, dietary treatment had no effect ($P>0.05$) on milk yield (3.5% fat corrected) of the cows (Table 2). However, from 4th week to the end of the experimental period (14th week), dairy cows fed with the HE diet exhibited a higher ($P<0.05$) milk yield than those fed with the RE diet. Consequently, the cumulative milk yield from the 10th week to the 14th week was higher ($P<0.05$) in animals fed with the HE diet compared to animals fed with the RE diet.

Early lactation is characterized by high-energy demand, especially to meet the increasing milk production during this period (Nielsen et al., 2007). This could be the reason for dairy cows fed HE diet showing



Figure 1. Dry matter intake (mean±SE) of temperate crossbred dairy cows ($n = 6$) fed with RE or HE diets.

Note: Significant difference ($P < 0.05$) at 4, 10, 12 and 14 weeks only.

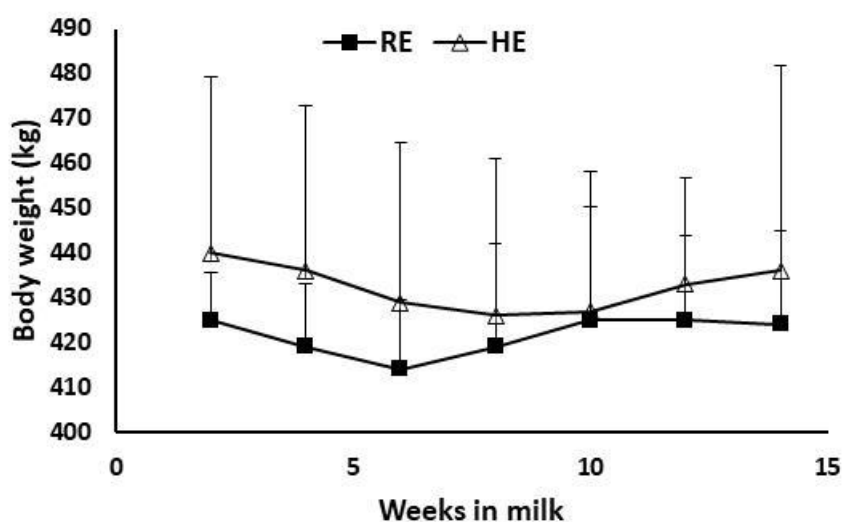


Figure 2. Body weight (mean±SE) of temperate cross bred dairy cows ($n = 6$) fed with RE or HE diets.

Table 2. Mean milk yield of temperate crossbred dairy cows ($n=6$) fed RE or HE diets[†]

Weeks in milk	Milk yield (L/cow/day)		Cumulative milk yield (L/cow) [‡]	
	RE	HE	RE	HE
2	17.2 ± 1.41 ^a	18.5 ± 0.70 ^a	179.0 ± 24.2 ^a	194.0 ± 21.2 ^a
4	18.6 ± 1.46 ^a	19.2 ± 1.15 ^a	446.3 ± 39.8 ^a	460.3 ± 29.5 ^a
6	18.3 ± 1.42 ^a	20.0 ± 0.91 ^b	686.8 ± 77.0 ^a	738.8 ± 39.8 ^a
8	18.1 ± 1.46 ^a	20.2 ± 0.90 ^b	942.3 ± 76.7 ^a	1022.3 ± 51.0 ^a
10	18.0 ± 1.31 ^a	20.1 ± 0.84 ^b	1175.0 ± 101.2 ^a	1305.0 ± 61.9 ^b
12	17.5 ± 1.14 ^a	20.2 ± 0.72 ^b	1442.0 ± 109.6 ^a	1609.0 ± 56.8 ^b
14	17.4 ± 0.96 ^a	20.0 ± 0.76 ^b	1686.2 ± 122.8 ^a	1873.2 ± 80.3 ^b

[†] Means (±SE) in a row with superscripts without a common letter differ at $P < 0.05$. [‡] Fat corrected (3.5%) cumulative milk yield.

comparatively higher MY than the RE diet fed counterparts. This observation is consistent with the study conducted by Sandro et al. (2014), which demonstrated that increasing the dietary energy content through the inclusion of different levels of concentrate feed led to an increase in MY. Similarly, when the dietary energy content was increased primarily by raising the maize content of the concentrate feed from 16.8% to 31.3% (on a dry matter basis), the cows produced 2.75 kg more milk per day than their counterparts (Hernandez-Urdaneta et al., 1975). As mentioned earlier, animals from both treatment groups experienced NEB during the 14-week study period in the current study, although the NEB was more pronounced in the RE diet-fed animals. This suggests that even though the diets were formulated based on the nutrient requirements of the cows according to their previous milk production records, the two diets were still marginally deficient in energy, preventing the animals from recovering from NEB. This may also explain why there was no difference in body weight (BW) between the two treatments and why BW did not increase towards the end of the 14-week experimental period. However, under ideal feeding conditions that fulfill energy requirements, dairy cows typically experience weight loss until 7-8 weeks of lactation and then start

gaining weight (Kellems and Church, 2010). In addition, it was also interesting to observe that the dairy cows used in the current study were under NEB during the experiment, although no difference in BW was observed, DMI was not greatly increased with the advancement of the lactation (Fig. 1) and milk yield was slightly increased towards the end of the experiment. According to Chamberlain and Wilkinson (1996), this could be attributed to the efficient conversion of nutrients into milk by reducing the energy required for maintenance as milk production increases.

The contents of milk fat, SNF, protein and MUN were not affected ($P>0.05$) by the dietary treatment (Figure 3). However, compared to the cows fed the HE diet, those fed the RE diet tended to have higher milk fat from the 6th week of lactation until the end of the experiment. This could be due to well described fact that milk fat depression is generally observed when lactating animals are fed highly fermentable carbohydrates (Griinari & Bauman, 2003). Oldham and Emmans, (1988) described the basis of this phenomenon as the reduced production of acetate and butyrate leading to low milk fat owing to reduced *de novo* fatty acid synthesis in the mammary gland.

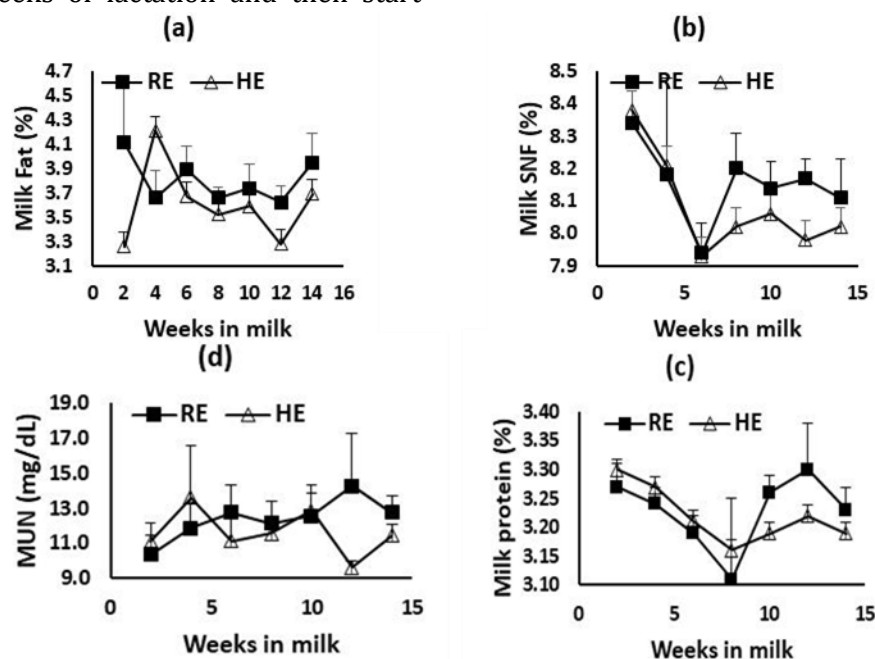


Figure 3. Milk composition; (a: milk fat, b: milk SNF, c: milk protein, d: milk MUN) of temperate crossbred dairy cows ($n = 6$), fed with diets containing RE or HE.

Note: Percentage values are means ($\pm$ SE) of 6 cows.

The current findings of no significant effects of dietary energy content on milk solid-not-fat (SNF), protein, and milk urea nitrogen (MUN) are consistent with Nielson (2007), who demonstrated that changing dietary energy content does not affect these parameters. Similarly, studies have shown that increasing dietary energy either by linearly increasing the amount of concentrate in the diet (Blum et al., 1985; Ferris et al., 1999; Ferris et al., 2003) or by directly infusing glucose through the duodenum (Hurtaud et al., 2000) does not result in any changes in milk protein content.

Serum metabolic profile

The effects of dietary energy content on the serum metabolic profiles of the cows fed with RE or HE diets during 14 weeks experimental period is presented in Figure 4. The different energy content in the diet (RE or HE) had no effect ($P>0.05$) on the serum metabolic profiles. Examination of the temporal pattern of the serum NEFA during the experiment revealed that NEFA content gradually declined with the advancement of the lactation for both RE and HE diets.

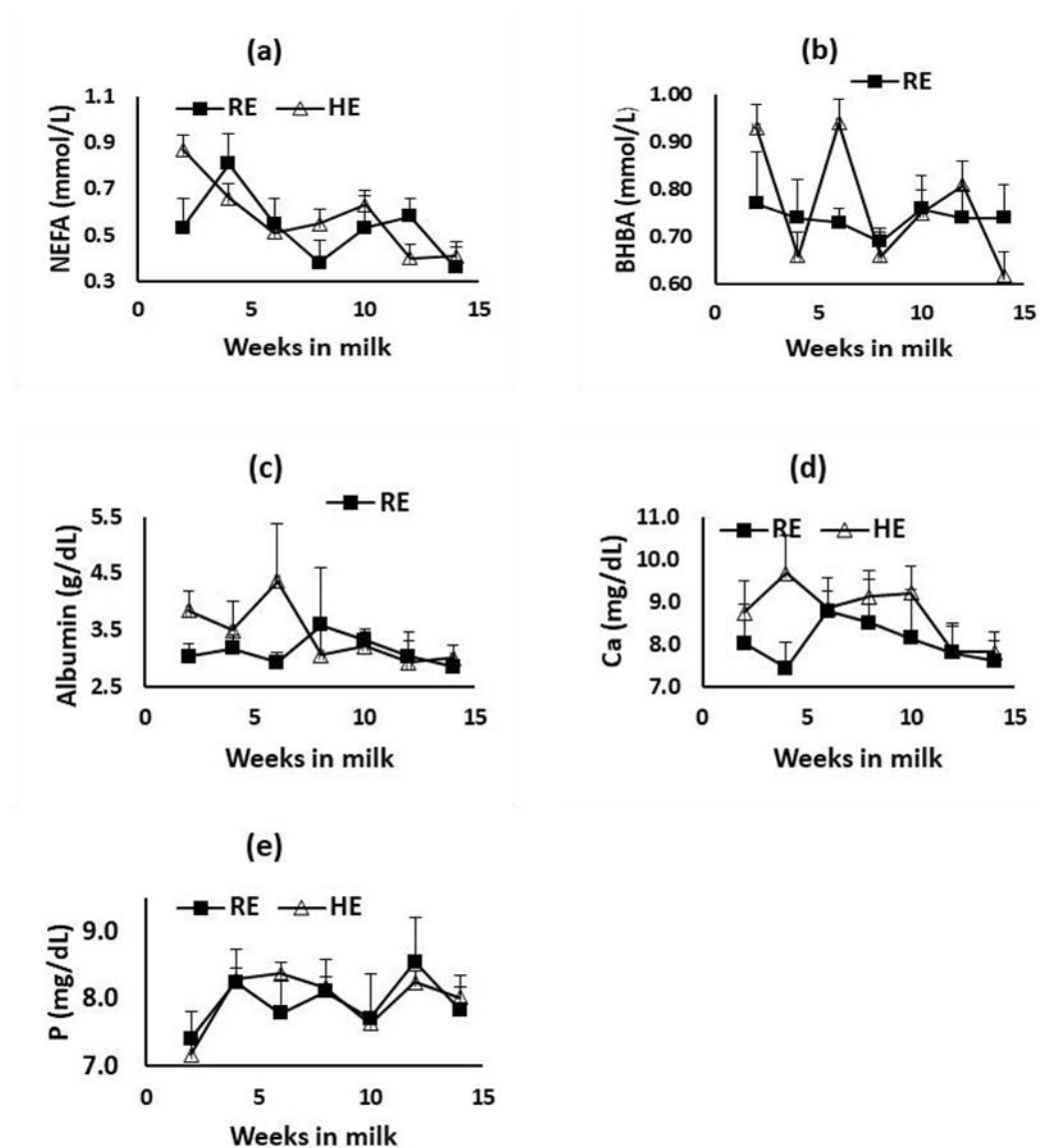


Figure 4. Serum metabolic profiles (a: serum NEFA, b: serum BHBA, c: serum albumin, d: serum Ca, e: serum P) of temperate crossbred dairy cows ($n = 6$) fed with RE or HE diets.

Note: Percentage values are means ($\pm$ SE) of 6 cows.

Reference ranges for serum non-esterified fatty acids (NEFA), beta-hydroxybutyrate (BHBA), albumin, calcium (Ca), and phosphorus (P) contents in healthy, temperate crossbred transition and early lactation dairy cows are as follows: 0.01–0.52 mmol/L for NEFA, 0.30–1.50 mmol/L for BHBA, 2.80–3.90 g/dL for albumin, 7.00–11.00 mg/L for Ca, and 4.30–8.00 mg/L for P (Puls, 1989; Kahn & Line, 2011; Lager and Jordan, 2011; Penn State Extension, 2017). Normally, serum NEFA content increases usually up to 3 weeks postpartum (Chapinal et al., 2012) due to suppression of the *de novo* synthesis or uptake and then esterification of fatty acids, promotion of lipolysis and reduction of the intracellular re-esterification of fatty acids released by lipolysis (Bell et al., 1995). However, in the current study, serum NEFA content remained higher than the reference values during weeks 4, 6, and 12 for RE diet-fed cows and weeks 6, 8, and 10 for HE diet-fed cows, gradually decreasing as lactation advanced (Figure 4a). Typically, serum NEFA content and body weight (BW) of early lactating dairy cows are inversely related, with serum NEFA content declining while BW starts to increase around 7-8 weeks postpartum (Adewuyi et al., 2005). Similar to the relationships observed for energy balance, MY, and BW, the energy content of the diets used in the present study may not have been sufficient to meet the energy demands of the cows (even with the HE diet), resulting in the continued elevation of NEFA levels even at 14 weeks of lactation. Consequently, the animals were unable to allocate energy for weight gain, leading to no BW gain. This may be a common occurrence among the most temperate crossbred dairy cows in the country. Our recent study also revealed that the serum NEFA content of tropical crossbred cows at post-partum transition stage exceeded the upper critical limit of the reference range proposed for the determination of NEB due to poor nutritional status of the animals (Ranaweera et al., 2020). However, the serum BHBA content was not affected by the dietary energy content in the current study, which is consistent with previous findings that feeding a glycogenic diet (Ruppert et al., 2003) or providing high or normal energy contents to early lactating dairy cows does not

significantly impact BHBA levels (Nielsen et al., 2007).

CONCLUSIONS

The results of the present study demonstrate that low quality grass, such as Guinea grass, can be efficiently utilized to obtain relatively higher milk production from temperate crossbred dairy cows by providing additional energy in their diet through concentrate feed during the early lactating.

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