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Research Article

Effect of Protein to Metabolizable Energy Ratio in Pineapple Waste Silage-Based Diets on Performance of Holstein Heifers

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Abstract | This study evaluated the effects of crude protein (CP) to metabolizable energy (ME) ratio on responses of heifers fed pineapple waste silage-based diets. Three Holstein heifers were assigned in a 3x3 Latin square design with three of a 20-days period and allocated to one of three diets: T1, 40.17:1 g/Mcal CP:ME ratio; T2, 49.68:1 g/Mcal CP:ME ratio and T3, 56.92:1 g/Mcal CP:ME ratio. The CP:ME ratios were adjusted by altering the CP content (9.18, 10.54, and 12.17%CP) with ME (2.32, 2.24 and 2.15 Mcal/kgDM), respectively. Diets were formulated to provide 0.50 kg/day of the expected average daily gain and comprised 50% of pineapple waste silage in dry matter (DM) basis. Daily DM intake was not affected by elevated CP:ME ratios (P = 0.914). However, CP intake as expressed in g/kgDM increased linearly when CP:ME ratios increased (P = 0.001). In addition, CP intake was the highest for heifers consuming T3 diet. No treatment effect was detected on intake of ME per kgDM (P = 0.974), net energy for maintenance (NEM) per kgDM (P = 0.741), and net energy for growth (NEG) per kgDM (P = 0.970). As the CP:ME ratio was increased, rumen fermentation parameters, plasma metabolites and thyroid hormones did not significantly altered (P>0.05). These results suggest that feeding of CP:ME ratio at 56.92:1 g/Mcal would be a better practice of dietary CP and ME in diets based on pineapple waste silage to maintain feed efficiency of Holstein heifer at 0.50 kg/day of the expected average daily gain.

Keywords | Protein, Energy, Pineapple, Dairy, Heifer

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INTRODUCTION

Dairy replacement heifers are important as prospective milking herds and reflect their potential in milk production (Mohd Nor et al., 2014). For those young animals, appropriate dietary protein and energy supplies are crucial to promote growth performance, reproduction and subsequent production potentials (Brown et al., 2005; Krpálková et al., 2014). In general, minimal requirement of crude protein (CP) to metabolizable energy (ME) (CP:ME ratio) at 56.7:1 g/Mcal or 11.90 %CP with mean of 2.10 Mcal/kgDM of ME should be an appropriate practice for dairy heifer to gain at average of 0.50 kg/day (NRC, 2001). Gabler and Heinrichs (2003a) reported that dietary CP:ME ratio greater than 48.3:1 g/Mcal was suitable for Holstein heifers weighting between 125 to 234 kg of BW and gaining 0.80 kg/day while Dong et al. (2017) reported that CP:ME ratio of 56.19 g/Mcal with dietary CP at 14.60% showed improvement in feed efficiency, rumen fermentation characteristic and some blood metabolites in Holstein heifers for 0.90 kg/day rate of gain. Meanwhile, the thyroid gland plays important roles particularly in regulatory of energy metabolism which effects on growth and puberty of animals (Nixon et al., 1988; Nikolić et al., 1997; Huszenicza et al., 2002).

Due to long drought and shortage of high quality forages in tropical regions, substitution of feed ingredient with agro-industrial and/or agricultural by-products in mixed diets is usually an alternative practice to maintain CP:ME ratio supplying to dairy herds. One of agro-industrial by-products that have been used extensively in adjacent areas of the fruit processing factories is a residue from pineapple fruit (*Ananas comosus*) such as area of Prachuap Khiri Khan province, lower-central region of Thailand (Saenphoom et al., 2016). In lactating dairy herds, feeding of residues from pineapple fruit has no adverse effects on feed intake, rumen fermentation and blood metabolites (Sruamsiri et al., 2007; Wittayakun et al., 2015a; Wittayakun et al., 2015b). However, information on how dietary CP content and CP:ME ratio in mixed diets based on pineapple waste silage affecting productive performance and metabolic responses of Holstein dairy heifers is not available. This study, therefore, aimed to evaluate effects of CP:ME ratios in dairy heifers fed pineapple waste silage-based mixed diets on feed intake, rumen fermentation, blood metabolite and thyroid hormone response to enhance efficient use of pineapple fruit residue as alternative feeding practice for dairy heifers.

MATERIALS AND METHODS

Pineapple Waste Silage Preparation

The pineapple waste composing of peel and core parts of Pattawia or Smooth Cayenne varieties was collected from a factory producing concentrated pineapple juice in Lampang province, northern Thailand. Then, it was ensilaged separately in plastic bag sized 0.51 x 0.76 m^2 for 3 weeks and kept as silage until the feeding trial was commenced.

Animal and Experimental Design

Three Holstein heifers averaged 207.38 ± 2.42 kg body weight were assigned in a 3x3 Latin square design with three of a 20-days period. Each 20-days period consisted of 14-days for adaptation to treatment, followed by 6-days of sample collection. The least number of animals to ensure acceptable statistical results was applied in this trial due to facility limitations and availability of limited numbers of heifers of similar body weight and average daily gain. The heifers were randomly allocated to one of three diets: Treatment 1 (T1): 40.17:1 g/Mcal CP:ME ratio, Treatment 2 (T2): 49.68:1 g/Mcal CP:ME ratio and Treatment 3 (T3): 56.92:1 g/Mcal CP:ME ratio. The CP:ME ratios were adjusted by altering the CP content (9.18, 10.54, and 12.17%CP) with ME (2.32, 2.24 and 2.15 Mcal/kgDM of ME), respectively. The basis selection of various CP: ME ratios was based on those previous literature reports (NRC, 2001; Gabler and Heinrichs, 2003a; Gabler and Heinrichs, 2003b; Dong et al., 2017). All diets contained 50% of DM as pineapple waste silage (Table 1). Diets were formulated and expect to contain 11.90% CP and 2.10 Mcal/kgDM of ME for large, non-bred heifers, approximate 200 kg body weight for 0.50 kg/day of average daily gain (ADG) based on recommendation of NRC (2001). Feed ingredients were weighted and mixed thoroughly before distribution as a mixed feed. Heifers were individually housed in a 1.5 x 4.0 m^2 pen with free access to water and mineral blocks. Diets were fed twice at 0600 and 1600 h daily throughout the trial. Animal management and experimental protocol were performed with respect to animal care and welfare according to the guidelines of the Animal for Thai Scientific Purposes Acts, B.E. 2558 (AD, 2015).

Sample and Data Collection

The amounts of offered feed and refusal were recorded daily. In the first 7-days of each adaptation period, feed ingredients were collected and dried at 60C in a hot air oven for 72 h for DM daily feed intake adjustment. All heifers were weighed two times (day 2 and day 20) of each period. Feed samples were collected, dried at 60°C for 72 h; ground and composited to analyze for dry matter (DM) and crude protein (CP) (AOAC, 1990). Neutral detergent fiber (NDF) was measured by the method of Van Soest et al. (1991). Organic matter (OM) was calculated as follows: OM = 100 – Ash %. During the last 3-days of each data collection period, fecal samples were collected individually, dried at 60°C for 72 h, ground and analyzed for DM (AOAC, 1990) and acid insoluble ash (AIA) (Van Keulen and Young, 1977). Dry matter digestibility was calculated as following: DM digestibility, % = 100 – (100 x [Marker feed]/ [Marker feces]) (Schnieder and Flatt, 1975). The value of metabolizable energy (ME) was calculated according to digestible organic matter intake (DOMI) as following: 1 kg DOMI = 3.801Mcal ME (Kearl, 1982). Net energy for maintenance (NEM) and growth (NEG) of diets were calculated according to the equations: NEM (Mcal/kg of DM) = $1.37\ ME - 0.138\ ME^2 + 0.0105\ ME^3 - 1.12$ and NEG (Mcal/kg of DM) = $1.42\ ME - 0.174\ ME^2 + 0.0122\ ME^3 - 1.65$ (Garrett, 1980). Microbial crude protein (MCP) was estimated from the equation: MCP = 0.130 kgDOMI (ARC, 1980). In the last day of each experimental period, rumen fluid was taken using esophageal tube under mild vacuum (Oil rotary vacuum pump, Nakabo, Co. Ltd., Japan) from the reticulum near the reticulo-omasal orifice at 4 h post feeding. Then, it was filtered through four layers of sheet cloth and measured pH immediately with portable pH meter (pHtestr 30®, EUTECH Instruments, Singapore). The 50 ml of rumen fluid was filtered through four layers of sheet cloth, added with 5 ml of 6 N H_2SO_4 to stop fermentation, centrifuged at 3,000 rpm for 10 min and frozen supernatant at –20°C until later analyzed for volatile fatty acids with High Performance Liquid Chromatography (HPLC, Agilent technologies 1100 series, Germany). Total volatile fatty acid was measured by the method described by Briggs et al. (1957). Approximated 10 ml of blood samples were taken from coccygeal vein at 4 h after feeding and subsequent analysis for packed cell volume (PCV) on Automate Cellcounter LH780 (Beckman Coulter Inc.), glucose and blood urea nitrogen (BUN) by enzymatic and kinetic methods (Synchron LXSystem/Lxi725, Beckman Coulter Inc.) whereas free triiodothyronine (FT3) and free thyroxine (FT4) using chemiluminescence immunoassay methods (Access II Analyzer, Beckman Coulter Inc.).

STATISTICAL ANALYSIS

All data were analyzed for Analysis of Variance (ANOVA) using general linear procedure. Significance was set at P-value <0.05. Treatment means were compared by Duncan's new multiple range test (SPSS, 2006). The statistical model used was described by: $Y_{ij(k)} = \mu + {}_{i} + {}_{j} + {}_{(k)} + {}_{ij}$ where $Y_{ij(k)}$ = dependent variable, μ = overall mean, ${}_{i}$ = effect of time period (i=1,2,3), ${}_{j\,=}$ effect of animal (j = 1,2,3), ${}_{(k)}$ = effect of treatment, and ${}_{ij}$ = random error (Petersen, 1985). Trend response was analyzed using orthogonal polynomial contrasts and significance was declared when P-value <0.05 (SPSS, 2006).

RESULTS

CHARACTERISTICS OF EXPERIMENTAL DIETS

Ingredients, nutrient composition and digestion coefficient of experimental diets are presented in Table 1. Diets contained similar amounts of DM, OM, and ME, but differed in CP and CP:ME ratios. Mean concentrations of ME were similar for T1, T2, and T3 at 2.32, 2.24, and 2.15 Mcal/kgDM, respectively. Meanwhile, the T3 diet had higher CP concentration than T1 (12.17 vs. 9.18%) and T2 (12.17 vs. 10.54%). The CP:ME contents of T1, T2, and T3 were 40.17, 49.68, and 56.92 g/Mcal, respectively. Diets were formulated to provide 11.90 % CP and 10.70 Mcal/d or 2.09 Mcal/kgDM for large breed non-bred heifers (NRC, 2001). Diets had similar concentrations of NDF, but T3 contained slightly more ADF (+8.89%) and ash (+8.48%) than both T1 and T2.

FEED INTAKE AND NUTRIENT INTAKE

Feed intake, nutrient intake and body weight are presented in Table 2. Increasing CP:ME ratio of the diets had no significant effect on feed consumption of DM as kgDM/day (P = 0.914), g/kg metabolic body weight (P = 0.854), and percentage of body weight (BW) (P = 0.827) as the CP content and the CP:ME ratio increased. The nutrient intake in form of organic matter was unaffected by increasing of CP to ME ratio (P = 0.895). However, daily CP intake tended to increase by 25% with elevated dietary CP:ME ratio (P = 0.140). Meanwhile, daily CP intake as expressed in g/kgDM increased linearly with increasing of dietary CP:ME ratio (P = 0.001). The elevated CP:ME in ratio in diets linearly increased the CP:ME ratio in feed consumption with mean intake of 43.13, 49.81 and 56.87 g/Mcal in T1, T2 and T3, respectively (P = 0.004). Daily energy intake as expressed in ME, NEM and NEG did not differ among treatments, showing overall mean intakes of 12.73 (ME) (P = 0.925), 13.27 (NEM) (P = 0.786), and 4.32 (NEG) (P = 0.921) Mcal per day, respectively. Dietary CP:ME ratio had no effect on intake of NDF (P = 0.596), ADF (P = 0.308) and ash (P = 0.488). Average body weights were similar among groups of the heifers, ranging from 205.66 to 210.16 kg (P = 0.373).

RUMEN FERMENTATION, BLOOD METABOLITES AND THYROID HORMONE RESPONSES

Rumen fermentation, blood metabolites and thyroid hormone responses are presented in Table 3. The rumen pH across treatments ranged from 6.97 to 7.30. The average rumen pH was not significantly affected by dietary CP:ME ratio (P = 0.145). Increasing of CP:ME ratios from 40.17:1 to 56.92:1 g/Mcal did not alter concentrations of rumen fermentation parameters including acetate (P = 0.972), propionate (P = 0.565), butyrate (P = 0.882), acetate to propionate ratio (P = 0.748), and total volatile fatty acids (P = 0.183). In this study, the acetate to propionate ratio ranged from 2.58 to 2.80 (P = 0.885). Packed cell volume (PCV) (P = 0.119), glucose (P = 0.575) and blood urea nitrogen (BUN) (P = 0.295) of heifers were not affected by manipulation of CP:ME ratios. In this study, altering dietary CP:ME ratios did not have significant effect on the serum concentration of thyroxine (FT4) (P = 0.598) and free triiodothyronine (FT3) (P = 0.578). However, plasma concentrations of FT4 and FT3 tended to decrease slightly from 1.20 to 0.80 ng/dl and 7.17 to 3.72 pg/ml with increasing of CP:ME ratios.

DISCUSSION

The DM concentration of diets was largely influenced by moisture concentration and proportion of pineapple waste silage in the diet. DM contents of all diets ranged from 64.72 to 64.96 % which were at a desirable level for ordinary mixed feed without water adding. If the diet is too high in DM content, water adding is flavored to drop DM content to reduce feed sorting behavior in dairy cattle (Leonardi et al., 2005). The CP:ME ratio in T3 was higher than those T1 and T2 due to the addition of urea and commercial concentrate. In T1 and T2, the CP contents had 22.85 and 12.90 % in deficit of requirement compared with T3 (NRC, 2001). Whitlock et al. (2002) reported that the diet with 15.43% deficit of CP requirement had no major effects on growth and mammary development of heifers while Schiavon et al. (2013) observed the similar outcome in the diet with 26.16% deficit of CP requirement. These may benefit to reduce feed cost and the concern about the environment impact of dairy farming (Sheppard et al., 2011). Concentrations of NDF and ADF were above recommended by NRC (2001) for dairy heifers with 0.50 kg/day of the expected average daily gain.

Table 1: Ingredients, nutrient composition and digestion coefficient of experimental diets

Items	**Experimental diets**		
	T1	**T2**	**T3**
Ingredients, kg/ as fed basis			
Pineapple waste silage	50	50	50
Cassava chip	10	10	-
Urea	-	0.5	0.5
Pangola grass hay	20	20	20
Commercial Concentrate	20	19.5	29.5
Total	100	100	100
Nutrient composition, %DM			
DM	64.72	64.73	64.96
OM	92.76	92.80	92.15
ME, Mcal/kgDM	2.32	2.24	2.15
CP	9.18	10.54	12.17
CP:ME ratio, g/Mcal	40.17	49.68	56.92
NDF	52.62	52.45	53.57
ADF	33.54	33.35	36.42
Ash	7.63	7.58	8.25
Digestion coefficient , %DM			
DM	59.95	59.31	59.21
OM	66.88	62.60	61.85

T1: 40.17:1 g/Mcal CP:ME ratio; T2: 49.68:1 g/Mcal CP:ME ratio; T3: 56.92:1 g/Mcal CP:ME ratio
DM: dry matter; OM: organic matter; ME: metabolizable energy; CP: crude protein; NDF: neutral detergent fiber; ADF: acid detergent fiber

Table 2: Feed intake, nutrient intake and body weight of heifers

Items	**Experimental diets**			**SEM**	**P-value**		
	T1	**T2**	**T3**		**Treatment**	**Linear**	**Quadratic**
DM intake							
kg/d	5.78	5.76	5.87	0.34	0.914	0.893	0.910
g/kgBW$^{0.75}$	106.02	108.71	105.97	6.55	0.854	0.973	0.646
% BW	2.80	2.86	2.78	0.17	0.827	0.935	0.485
OM intake	5.29	5.41	5.38	0.31	0.895	0.891	0.914
Nutrient intake							
CP, kg/d	0.54	0.64	0.72	0.06	0.140	0.073	0.933
CP, g/kgDM	94.13^{a}	108.36^{b}	122.56^{c}	2.21	0.008	0.001	0.242
CP:ME, g/Mcal	43.13^{a}	49.81^{ab}	56.87^{b}	2.57	0.045	0.004	0.632
ME, Mcal/d	13.44	12.88	12.65	0.28	0.925	0.950	0.840
ME, Mcal/kgDM	2.32	2.24	2.15	0.09	0.974	0.903	0.981
NEM, Mcal/d	14.02	13.81	13.27	1.29	0.786	0.770	0.918
NEM, Mcal/kgDM	2.42	2.30	2.28	0.22	0.741	0.648	0.871
NEG, Mcal/d	4.29	4.44	4.24	0.60	0.921	0.973	0.799
NEG, Mcal/kgDM	0.74	0.74	0.73	0.07	0.970	0.895	0.972
NDF, kg/d	2.99	3.07	3.13	0.14	0.596	0.690	0.966
ADF, kg/d	1.94	1.99	2.16	0.13	0.308	0.443	0.760
Ash, kg/d	0.44	0.46	0.49	0.04	0.488	0.246	0.576
Body weight, kg	206.33	205.66	210.16	3.24	0.373	0.866	0.871

T1: 40.17:1 g/Mcal CP:ME ratio; T2: 49.68:1 g/Mcal CP:ME ratio; T3: 56.92:1 g/Mcal CP:ME ratio
^{abc} Within rows: means followed by different superscripts are significantly different ($P<0.05$)
DM: dry matter; BW: body weight; OM: organic matter; CP: crude protein; ME: metabolizable energy; NEM: net energy for maintenance; NEG: net energy for growth; NDF: neutral detergent fiber; ADF: acid detergent fiber

Table 3: Rumen pH, rumen fermentation, blood metabolites and thyroid hormone responses.

Items	Experimental diets			SEM	P-value		
	T1	T2	T3		Treatment	Linear	Quadratic
Rumen pH	7.30	7.21	6.97	0.12	0.145	0.243	0.662
VFA, mmol/100 mol							
Acetate (C2)	65.16	65.45	66.86	6.78	0.972	0.539	0.632
Propionate (C3)	24.87	25.31	23.83	1.81	0.565	0.811	0.761
Butyrate (C4)	0.88	1.05	0.97	0.41	0.882	0.732	0.666
C2 to C3 ratio	2.62	2.58	2.80	0.25	0.748	0.885	0.888
Total VFA, mmol/l	70.45	84.42	70.77	6.54	0.183	0.638	0.651
Microbial CP, kg/d	0.43	0.44	0.43	0.03	0.925	0.950	0.840
Microbial CP, g/kgDM	74.78	74.53	74.17	3.21	0.974	0.902	0.981
Blood metabolites							
PCV, %	27.66	29.66	30.33	0.88	0.119	0.332	0.847
Glucose, mg/dl	76.33	67.66	70.33	8.95	0.575	0.285	0.328
BUN, mg/dl	5.00	5.66	8.00	1.76	0.295	0.155	0.515
Thyroid hormones							
Free T4, ng/dl	1.20	1.05	0.80	0.42	0.598	0.208	0.750
Free T3, pg/ml	7.17	6.01	3.72	3.56	0.578	0.182	0.679

T1 = 40.17:1 g/Mcal CP:ME ratio, T2 = 49.68:1 g/Mcal CP:ME ratio, and T3 = 56.92:1 g/Mcal CP:ME ratio.
VFA: volatile fatty acids; CP: crude protein; DM: dry matter; PCV: packed cell volume; BUN: blood urea nitrogen; T4: thyroxine; T3: triiodothyronine

The findings in the present study demonstrate that increasing dietary CP:ME ratio had no effect on DM intake in terms of kg/d, $g/kgBW^{0.75}$, and %BW ($P>0.05$) which were in agreement with those previous reports of Schiavon et al. (2013), Dong et al. (2017), and Zhang et al. (2017) who reported that daily DM intake of dairy heifers remained constant with increasing of CP or CP:ME ratio in mixed diets, but not with Gabler and Heinrichs (2003a) who reported that feeding diets of 48.3:1, 59.1:1, 67.5:1, and 76.5:1 g/Mcal CP:ME ratios had a quadratic effect on DM intake of heifers between 125 and 234 kg of BW and gaining 0.80 kg/d. All heifers had DM feed intake in excess of the values of requirement recommended by NRC (2001) who suggested that an intake of 2.55%BW or 5.10 kgDM/day is expected for heifer at 200 kg of BW and gaining 0.50kg/day. Van Soest (1994) pointed out that several factors could attribute to limit feed intake in ruminants such as caloric density in diets, acetate and propionate concentration in rumen, adequate fiber and the energy demand of the animal. The finding in higher CP intake in response to elevated dietary CP:ME ratio is supported by previous studies (Gabler and Heinrichs, 2003a; Dong et al., 2017; Piñeiro-Vázqueza et al., 2017; Zhang et al., 2017). The greater CP intake, which provides ammonia nitrogen available to rumen microbes, may have led to greater efficiency of microbial protein synthesis in rumen to supply microbial protein as amino acid sources for absorption in small intestine to contribute amino acid pools and promote the efficient growth of animals (Hackmann and Firkins, 2015). If animal consumed diets contained below 13-15% CP, the quality of CP digesta flow from rumen output generally exceeds above the quality of input due to microbial activity via microbial protein synthesis (Owens and Zinn, 1993). In addition, the inclusion of pineapple waste silage about half of feed ingredients may have attributed to ME density due to the abundant of effective fiber and some sugars in it (Datt et al., 2008; Nadzirah et al., 2013). These could yield structural carbohydrate (cellulose, hemicellulose) and non-structural carbohydrate (sugars, starches) as the availability of carbon skeleton sources for rumen microbes to incorporate to the microbial protein. The microbial protein synthesis could be maximized, if the availability of carbon skeleton and ammonia nitrogen is synchronized (Ørskov, 1992). In this study, the increase in CP:ME ratios did not alter average daily gain of heifers due to the short-term Latin square design. However, Zhang et al. (2017) reported that heifers fed diets with CP:ME ratios of 48.17 and 54.43 g/Mcal had higher average daily gain compared with those fed CP:ME ratio at 41.29 g/Mcal (P = 0.04).

The present study demonstrate that increasing dietary CP:ME ratios had no effect on rumen fermentation, blood metabolites and thyroid hormone responses of replacement dairy heifers. According to physical form of pineapple waste silage with quite thick and long in particle size and comprises as main ingredients in mixed diets, these could be beneficial to stimulate chewing activity and saliva secretion which may influence on fluid dilution rate and pH control in the rumen (Wittayakun et al., 2016). This finding in this study was in agreement with Gabler and Heinrich (2003b) who found that rumen pH, volatile fatty acids, and the acetate-to-propionate ratio were not affected with increasing CP:ME ratios of 45.0, 63.3, 69.4 and 77.3 g/Mcal. Meanwhile, Zhang et al. (2017) also found the similar results for heifers fed diets containing CP:ME ratios of 41.29, 48.17, and 54.43 g/Mcal. In contrast, the results regarding to rumen fermentation were not supported by the study of Dong et al. (2017) who found that heifers fed dietary CP:ME ratios of 44.47, 49.68, 56.20 g/Mcal had significantly effect on concentration of acetate, propionate, butyrate and total volatile fatty acids (P<0.05). In this study, the acetate to propionate ratio ranged from 2.58 to 2.80 (P = 0.885). This would imply that these mixed diets were quite rich in non-structural carbohydrate. Russell (1998) reported that the ratio of acetate to propionate was approximately 4.1 in roughage based diet, but dropped to 2.2 in concentrate based diet. No difference for the estimated microbial CP synthesis in rumen was observed ($P \geq 0.925$). The PCV is represented the volume percentage of red blood cells, its ability to transport oxygen and animal health. In this study, PCV was found in normal range (24 to 46 %) (Blood and Studdert, 1995). Plasma levels of glucose were within normal range of 62 ± 8 mg/dl suggested by Kappel et al. (1984). However, the heifers receiving the T1 diet was slightly higher in blood glucose than those fed T2 (+12.81 %) and T3 (+ 8.53%) diets which might relate to high ME content in the T1 compared to T2 and T3 diets. Thyroxine (FT4) and free triiodothyronine (FT3) are iodinated derivatives of the amino acid tyrosine (Taylor and Ritchie, 2007) which are involved in many metabolic aspects of certain nutritional and regulation as well as ovarian functions related to puberty of animals (Huszenicza et al., 2002). Rowntree et al. (2004) reported that concentrations of FT4 and FT3 in neonatal Holstein heifers decreased associate with low energy intake. The concentration of FT4 in this study was higher compared to the report by Lawrence et al. (2016), who reported values for FT4 from 0.37 to 0.44 ng/dl, but lower than those previous studies by Moriel et al. (2011) and Cappellozza et al. (2012). Plasma FT3 concentration may vary from 2.98 to 6.23 pg/ml due to several factors such as seasonal change and stages of lactation (Nixon et al., 1988).

In conclusion, increasing the dietary CP:ME ratio linearly increased dietary CP intake for dairy Holstein heifers weighing 200 kg and gaining 0.50 kg/day. Nevertheless, none of CP:ME ratios had a significant impact on rumen fermentation, blood metabolites and thyroid hormone responses. Therefore, these data suggested that the CP:ME ratio in diets slightly improved feed efficiency of growing Holstein heifers, particularly for those fed high CP:ME ratio diets based on pineapple waste silage.

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CONFLICT OF INTEREST

There is no conflict of interest.

AUTHORS CONTRIBUTION

All authors contributed equally.

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