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SUPPLEMENT OF SODIUM BICARBONATE, CALCIUM CARBONATE AND RICE STRAW IN LACTATING DAIRY COWS FED PINEAPPLE PEEL AS MAIN ROUGHAGE

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ABSTRACT

The aim of this study was to evaluate the effect of buffering agents on performance of lactating cows fed pineapple peel as main roughage. Four mid-lactation primiparous crossbred Holstein dairy cows averaging 443.5 ± 10.6 kg BW were assigned in a 4 x 4 Latin square design. Each cow was fed one of four experimental diets including: T1) control, pineapple peel (PP) to commercial pellet (CL) ratio of 70:30 without added buffer; T2) PP to CL ratio of 70:30 with 1.2 % sodium bicarbonate ($NaHCO_3$); T3) PP to CL ratio of 52.5: 30 with mixture of 17.5 % rice bran and 1.2 % calcium carbonate ($CaCO_3$); and T4) PP to CL ratio of 50:30 with 20 % rice straw (RS). The results revealed that feed intake, digestion coefficient, digestible nutrient intake were unaffected by supplementation of $NaHCO_3$, $CaCO_3$, and RS in diets ($P > 0.05$). The daily quantities of ME and NEL intake were not altered by treatments ($P > 0.05$), but PDIE and PDIN were increased by supplementing $CaCO_3$ in the diet ($P < 0.05$). Weight gain was higher for cows supplemented with $NaHCO_3$ and RS compared with other groups ($P < 0.05$). Cows receiving supplemental $NaHCO_3$, $CaCO_3$ and RS had the same concentration of volatile fatty acids ($P > 0.05$). Acetate to propionate ratio ranged between 2.18 to 2.96 ($P > 0.05$) with the highest (2.96) in the RS supplement group. The $NaHCO_3$, $CaCO_3$, and RS supplement did not influence blood metabolites, blood electrolytes, milk yield and milk composition ($P > 0.05$). No sign of acidosis was observed. Therefore, it could be concluded that $NaHCO_3$, $CaCO_3$, or RS supplementation had no significant impact on performance of lactating cows fed PP as main roughage. Further research should be conducted to test the influence of such diets on milk production with larger number of animals in longer period of time.

Key words: sodium bicarbonate; calcium carbonate; rice straw; pineapple peel; dairy cow

INTRODUCTION

Local feed resources and agricultural by-products are of prime importance for ruminants raised in the tropics (Wanapat, 2000). Pineapple peel is a cannery by-product of Pineapple (*Ananas comosus*), a tropical fruit which largely grows in Brazil, Thailand, Philippines, China and several other countries (FAO, 2013). Pineapple peel is a potential roughage source for ruminants due to the large amount of effective fiber and some sugars (Datt *et al*., 2008; Paengkoum *et al*., 2013; Nadzirah *et al*., 2013) which can be used by rumen microbes to digest and synthesize for animal energy supply as well as lactose synthesis in mammary gland (Russell, 2002). The nutrients in pineapple peel consists of dry matter (DM), organic matter (OM), crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF) and ash which are 12.6, 88.6, 8.7, 67.7, 50.3 and 11.4, respectively (Paengkoum *et al*., 2013). However, chemical property of pineapple peel is rather low in pH (3.47-3.84) (Nadzirah *et al.*, 2013) which may affect rumen ecology and productive performance if large amount of pineapple peel is being fed to dairy cows. Feeding diets high in nonstructural

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carbohydrates or acid load usually decreases ruminal pH and may cause ruminal acidosis (Owens *et al*., 1998; Rustomo *et al*., 2006). In clinical acidosis, cows will suffer from rumenitis, metabolic acidosis, lameness, hepatic abscessation, pneumonia and death while those in subclinical acidosis will lower feed intake, lower feed digestibility and subsequent lower milk fat content (Lean *et al*., 2000).

Sodium bicarbonate ($NaHCO_3$) is one of dietary buffer commonly used to prevent ruminal pH reduction and enhance ruminal fermentation in low roughage diet (Le Ruyet and Tucker, 1992; Russell and Chow, 1993). NRC (1989) suggested that $NaHCO_3$ should be added 1.2 – 1.6 % in concentrate mixture to control ruminal pH when diets were high in nonstructural carbohydrates or acids. Calcium carbonate ($CaCO_3$) or limestone is locally available buffer. However, $CaCO_3$ has little or no buffering effect when the rumen pH is 6.0 or above because of its low solubility in ruminal fluid at pH above 5.5 (Clark *et al*., 1989). Rice straw (RS) is a local agricultural by product which is abundant in effective fibre which promote chewing activity and saliva secretion. Saliva contains $NaHCO_3$ which acts as a buffer to control ruminal pH in ruminants (Russell and Chow, 1993). The available data involved in PP feeding to lactating dairy cows concerning the incident of rumen acidosis is limited. Our study was conducted to evaluate whether feeding PP supplemented with $NaHCO_3$, $CaCO_3$ and RS would reduce risk of subclinical acidosis as measured by feed intake and digestible nutrient intake variation, ruminal fermentation, blood metabolites, blood electrolytes, milk production and milk composition.

MATERIAL AND METHODS

Animals, Experimental Design and Diet

Four primiparous, midlactation (84 ± 18 d in milk) crossbred Holstein cows (n = 4) initially averaging 443.5 ± 10.6 kg body weight (BW) were assigned with four successive periods in 4 x 4 Latin square design. Each 21-d experimental period consisted of 14-d for animal adaptation to the diet and 7-d for sample and data collection. Treatments consisted of: T1) control diet, pineapple peel (PP) to commercial pellet (CL) (Charoen Pokphand PCL, Thailand) ratio of 70:30 on dry matter basis without added buffer, T2) PP to CL ratio of 70:30 with 1.2 % $NaHCO_3$, T3) PP to CL ratio of 52.5: 30 with mixture of 17.5 % rice bran (RB) and 1.2 % $CaCO_3$, and T4) PP to CL ratio of 50:30 with 20 % rice straw (RS) (Table 1). Feed for each cow was balanced depending on its body weight, milk yield and milk fat following the recommended nutrient requirement as stated by NRC (2001). All diets were formulated to support nutrient need for maintenance and lactation of cows approximately 63.49 ± 1.95 $MJ.d^{-1}$ of NEL and 1.17 ± 0.05 $kg.d^{-1}$ of dietary CP concentration (Table 1). Each feed ingredient was weighed individually before distribution as a mixed feed.

The PP using in this experiment was collected from a cannery factory in Kanchanaburi province in the west of Thailand, stored approximately 30 kg each in a sealed double layer polyethylene plastic bag without any preservative agents. In T1 and T2, PP was used as the roughage and energy sources, whereas in T3 and T4 a reduction in PP was replaced with RB and RS, respectively. RB was added in T3 to increase palatability and it was a locally available feed. However, RB was high in fat content. RS was added in T4 to stimulate chewing activity to increase saliva secretion. Each lactating dairy cow was housed individually in a 3.0 x 6.0 m^2 pen, in which drinking water and mineral blocks were available throughout. Cows were fed twice daily at 07:00 h and 17:00 h at 110 % of expected intake throughout the experiment. Cows were moved to milking parlour and milked twice daily at 06:00 and 15:00 h. Animal management and experimental protocol was performed with respect to animal care and welfare.

Measurement, Sample Collection and Analyses

Feed offered and refused were recorded daily in all last 7-d of each data collection period. In the first 7-d of each adaptation period, PP and CL were collected and dried in a 60 °C hot air oven for 72 h for DM concentration determination in order to correct daily feed intake. All cows were weighed three times (d1, d14 and d21) during each period to calculate and predict feed intake. Regular feed samples from individual cows were collected during the last 7-d of each period. Then, feed samples were dried at 60 °C for 72 h; ground and composited and analyzed for chemical composition including DM, CP, EE, ash, Ca and P by the method of AOAC (1984). Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were measured by the method of Goering and Van Soest (1970). Acid insoluble ash (AIA) as a natural marker in feed was measured by the method of Van Keulen and Young (1977).

During the last 5-d of each data collection period, fecal grab samples were collected twice daily at 12 h intervals, pooled on an equal wet-weight basis for each cows, dried at 60 °C for 72 h, ground and analyzed for DM and CP by the method of AOAC (1984), NDF and ADF by the method of Goering and Van Soest (1970) and AIA by the method of Van Keulen and Young (1977). Digestibility coefficients of nutrients were calculated using equations given by Schneider and Flatt (1975): DM digestibility, % = 100 – [100 × (AIA % in feed) ÷ (AIA % in feces)]; Nutrient digestibility, % = 100 – [(100 × AIA % in feed ÷ AIA % in feces) x (nutrient % in feces ÷ nutrient % in feed)]. Organic matter (OM) or the loss

Table 1: Ingredients, chemical composition of diets and daily nutrient requirement of dairy cows

Items					Complete feed Mixtures			
					T1	T2	T3	T4
Ingredients, kg.100 kg DM^{-1}								
PP					70.00	69.16	51.87	50
CL					30.00	29.64	29.64	30
RB					-	-	17.29	-
RS					-	-	-	20
$NaHCO_3$					-	1.2	-	-
$CaCO_3$					-	-	1.2	-
Total					100	100	100	100
Chemical composition, g.kg DM^{-1}								
	PP	CL	RB	RS	T1	T2	T3	T4
DM	340.96	958.50	960.10	949.60	929.45	918.29	925.74	935.97
OM	908.50	885.50	901.20	849.30	901.60	890.78	889.51	889.76
CP	73.80	187.80	142.00	44.30	108.00	106.70	118.49	102.10
EE	23.30	52.40	220.60	16.50	32.03	31.64	65.75	30.67
NDF	584.80	358.20	315.80	763.10	516.82	510.61	464.10	552.48
ADF	278.00	165.50	78.20	469.70	244.25	241.31	206.77	288.59
Ash	91.50	114.50	98.80	150.70	98.40	97.21	98.48	110.24
Ca	8.20	16.90	3.60	4.10	10.81	10.68	9.93	9.99
P	2.00	8.50	19.90	0.80	3.95	3.90	6.99	3.71
pH	3.55	-	-	-	-	-	-	-
Daily nutrient requirement					T1	T2	T3	T4
NEL, $MJ.d^{-1}$					61.88	62.84	62.92	66.35
NEL, MJ.kg DM^{-1}					5.81	5.94	5.85	6.06
CP, $kg.d^{-1}$					1.13	1.16	1.16	1.26
NDF, $kg.d^{-1}$					2.98	2.96	3.02	3.07
ADF, $kg.d^{-1}$					2.23	2.22	2.26	2.30
Ca, $g.d^{-1}$					47.26	48.22	48.3	52.10
P, $g.d^{-1}$					30.80	31.39	31.44	33.80

PP = pineapple peel, CL = commercial pellet, RB = rice bran, RS = rice straw, T1 = PP to CL ratio of 70:30, T2 = PP to CL ratio of 70:30 with 1.2 % $NaHCO_3$ supplement, T3 = PP to CL ratio of 52.5: 30 with supplement of 17.5 % RB and 1.2 % $CaCO_3$ mixture, T4 = PP to CL ratio of 50:30 with 20 % RS.

in DM weight after incubation at 550 ℃ for 15 h was calculated as follows: OM = 100 – Ash %.

Values for metabolizable energy (ME) were calculated by prediction from digestible organic matter intake (DOMI) as follows: 1 kg DOMI = 3.8 Mcal ME.kg DM^{-1} 4.184 (Kearl, 1982). Net energy of lactation (NEL) was estimated at actual intake when feed EE content was above 3 % by the equation: NEL (MJ.kg DM^{-1}) = 0.703 × ME – 0.19 + ([(0.097 × ME + 0.19) /97] × [EE–3]) × 4.184 (NRC, 2001). Values for feed protein truly digestible in the small intestine (PDIA); protein truly digestible in the small intestine where N is limiting microbial protein synthesis (PDIN); and protein truly digestible in the small intestine where energy is limiting microbial protein synthesis (PDIE) were calculated using the equations given by Jarrige (1989): PDIN (g.kg M^{-1}) = PDIA + [0.64 × CP(g.kg DM^{-1}) × (deg – 0.1)] where PDIA = CP(g.kg DM^{-1}) × 1.11(1 – deg) × dsi; PDIE = PDIA + DIME where PDIME (g.kg DM^{-1}) = 0.093 × [FOM – EE (g.kg DM^{-1})].

FOM is the fermentable organic matter content (g.kg DM^{-1}) (Jarrige, 1989; Beatriz Tobias *et. al.*, 2006). The deg value is theoretical degradability of feeds *in sacco* and dsi value is the true digestibility of undegraded dietary protein in the small intestine (Jarrige, 1989). Both deg and dsi were obtained from published data (Jarrige, 1989; Susmel *et. al.*, 1989; Pozdíšek *et. al*., 2003; Beatriz Tobias, *et. al.*, 2006).

Ruminal samples were taken by suction pump at 4 h post feeding and measured pH immediately by portable pH meter (pH Tester 30®, EUTECH Instruments, Singapore). The 50 ml of rumen fluid were filtered through four layers of cheesecloth, added with 5 mL of 6*N* H_2SO_4 to stop fermentation, centrifuged at 3,000 rpm for 10 minutes and kept supernatant frozen at –20 °C until later analyses for volatile fatty acid using an analytical High Performance Liquid Chromatography (HPLC, Agilent technologies 1100 series, Germany). 10 mL of blood samples were taken from coccygeal vein and subsequent analysis for glucose, urea nitrogen and electrolytes using enzymatic and kinetic methods (Synchron LXSystem/Lxi725, Beckman Coulter Inc.). Milk samples of each cows were collected during milking in the morning and afternoon at ratio of 60 to 40 for 4 consecutive days, composited and analyzed for fat, protein, lactose, solid not fat and total solid by Combi Foss 6000 (Foss Electric, Hillerød, Denmark).

Statistical Analysis

Data were analyzed using the general linear model procedure wherein treatment means were compared by Duncan's new multiple range test and significance was declared when P-value < 0.05 (SPSS, 2006). The statistical model used was $Y_{ij(k)} = \mu + \rho_i + \gamma_j + \tau_{(k)} + \varepsilon_{ij}$ where $Y_{ij(k)}$ = dependent variable, μ = overall mean, ρ_i = effect of period (i=1,2,3,4), γ_j = effect of animal (j = 1,2,3,4), $\tau_{(k)}$ = effect of treatment, and ε_{ij} = random error (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

The chemical composition of ingredients and complete feed mixtures are presented in Table 1. The average DM content of PP was 340.96 g.kg DM^{-1} or 34.09 % DM. Previous studies (Datt *et al*., 2008); Suksathit *et al.*, 2011) reported that DM content of pineapple waste is 138.50-168.70 $g.kg^{-1}$. The higher DM content of PP in this experiment is due to the moisture loss before packing into double layer polyethylene plastic bags. Proper conservation such as storage in sealed plastic bags could prevent mold growth and help to control both nutritive value and palatability of PP throughout the experiment. The CL used in this study contained CP, NDF and ADF of 187.80, 358.20 and 165.50 g.kg DM^{-1}, respectively.

Total feed intake in all lactating cows was not affected by $NaHCO_3$, $CaCO_3$ or RS supplementation (P > 0.05) (Table 2). However, PP intake was significantly increased by $NaHCO_3$ supplementation and averaged 13.87 kg $DM.d^{-1}$ or 3.12 % BW/d (P<0.05) (Table 2). The increasing response in feed intake by $NaHCO_3$ supplement has been demonstrated in other trials (Rogers *et al*., 1985; Vicini *et al*., 1988). In contrast, a number of trials reported the lack of response to $NaHCO_3$ supplementation on feed intake (Erdman *et al*., 1982; Wittayakun *et al*., 2006 a,b; Doepel and Hayirli, 2011). Supplementation with $NaHCO_3$ may have had an effect on osmolality and pH in the rumen. Addition of $CaCO_3$ and RS in other groups did not affect PP intake (P > 0.05).

The digestion coefficient and digestible nutrient including OM, CP, NDF and ADF were not affected by addition of $NaHCO_3$, $CaCO_3$ or RS (P > 0.05) (Table 2). The physical form of PP containing high fiber content is also an important factor which may alter digestibility. Suksathit *et al.* (2011) reported that the use of pineapple waste as sole roughage source had a positive effect on digestibility when compared with hay. ME and NEL intake were not affected by treatments (P > 0.05). The ME intake averaged 11.16 ± 0.72 ranging from 10.81-11.54 MJ.kg DM^{-1}. The NEL intake averaged 7.06 ± 0.51 MJ.kg DM^{-1} or 117.77 ± 17.45 $MJ.d^{-1}$ which was higher than the nutrient requirement recommended by NRC (2001). The $CaCO_3$ supplement increased supply of PDIN and PDIE (P < 0.05). In this study, PDIN was always lower than PDIE (Table 2).

Influence of treatments on body weight, rumen pH, VFA concentration, blood metabolites, milk yield, and milk composition of dairy cows are shown in Table 3. Initial and final body weight of the cows were similar in all cows (P > 0.05). However, average daily weight change of those cows supplemented with $NaHCO_3$ and RS tended to increase more than those fed only PP or PP with $CaCO_3$ (P < 0.05). Those cows fed PP with $CaCO_3$ had significantly decreased body weights (P < 0.05). Changes in body weight may indicate efficiency of productive improvement. However, the influence of treatments on body weight may need longer time to verify.

The average ruminal pH across treatments was 6.78 ± 0.34. The average rumen pH was not significantly affected by $NaHCO_3$, $CaCO_3$ and RS supplementation (P > 0.05) (Table 3). However, $CaCO_3$ supplementation tended to increase ruminal pH when compared with other treatments (Table 3). Normally, $CaCO_3$ has

Table 2: Influence of treatments on intake, digestion coefficient, digestible nutrient intake and nutritive values

Items	T1	T2	T3	T4	SE	*P-value*
Total Feed Intake						
kg DM	15.29	17.55	17.79	15.79	1.27	0.076
% BW	3.43	3.95	3.95	3.50	0.27	0.068
PP Intake						
kg DM	11.61[a]	13.87[b]	10.85[a]	11.32[a]	1.14	0.037
% BW	2.61[a]	3.12[b]	2.40[a]	2.51[a]	0.24	0.026
Digestion coefficient, %						
DM	72.30	74.37	77.79	76.36	5.66	0.580
OM	75.36	76.95	80.46	79.18	5.05	0.532
CP	57.32	58.88	69.70	65.69	10.48	0.378
NDF	70.60	72.57	74.58	75.25	5.54	0.653
ADF	68.37	70.64	70.65	71.89	5.49	0.836
Digestible nutrient intake, kgDM/d						
DM	12.54	13.08	13.91	12.05	1.93	0.602
OM	10.43	12.23	12.98	11.16	1.63	0.229
CP	0.89	1.01	1.36	1.02	0.23	0.114
NDF	5.74	6.86	6.53	6.42	1.01	0.514
ADF	2.63	3.16	2.76	2.96	0.48	0.475
Nutritive value						
ME[1], MJ.kg DM^{-1}	10.81	11.05	11.54	11.23	0.72	0.580
NEL[2], MJ.kg DM^{-1}	6.80	6.97	7.36	7.09	0.51	0.530
NEL, MJ.d^{-1}	104.44	122.78	131.68	112.16	17.45	0.235
PDIN[3], g.kg DM^{-1}	76.95[a]	73.68[a]	83.66[b]	74.95[a]	2.23	0.003
PDIE[4], g.kg DM^{-1}	81.06[a]	78.09[a]	91.07[b]	79.50[a]	2.24	0.001

T1 = PP to CL ratio of 70:30, T2 = PP to CL ratio of 70:30 with 1.2 % $NaHCO_3$ supplement, T3 = PP to CL ratio of 52.5: 30 with supplement of 17.5 % RB and 1.2 % $CaCO_3$ mixture, T4 = PP to CL ratio of 50:30 with 20 % RS.
Within rows, means followed by different letters are significantly different at $P < 0.05$.
[1]1kg DOMI = 3.8 McalME.kg DM^{-1} × 4.184 (Kearl, 1982).
[2]NEL (Mcal.kg^{-1}) = 0.703 × ME – 0.19 + ([(0.097 × ME + 0.19)/97] × [EE – 3]) × 4.184 (NRC, 2001).
[3]PDIN = protein truly digested in the small intestine with nitrogen-limiting microbial protein synthesis in the rumen (Jarrige, 1989).
[4]PDIE = protein truly digested in the small intestine with energy-limiting microbial protein synthesis in the rumen (Jarrige, 1989).

no buffering effect when rumen pH is greater than 6.0 due to its low solubility (Rogers *et al*., 1985). Physical form of PP had thick and long particle size which contained 584.80 g.kg^{-1} NDF and 278.00 g.kg^{-1} ADF (Table 1). It may stimulate chewing activity and saliva secretion which may affect fluid dilution rate and pH control in the rumen. The $NaHCO_3$ in saliva is also an extra buffering agent involved in ruminal pH control which can act effectively when rumen pH is above 5.7 (Russell, 2002).

The supplementation of $NaHCO_3$, $CaCO_3$ and RS did not significantly affect concentration of volatile fatty acids in ruminal fluid including acetic, propionic, and butyric acids ($P > 0.05$) (Table 3). However, acetic acid concentration in $NaHCO_3$ and $CaCO_3$ groups tended to be lower than those without RS supplementation. Furthermore, there was a tendency for the lowest acetic acid concentration in ruminal fluid of cows fed PP supplemented with $CaCO_3$ (Table 3). This may reflect low buffering ability of $CaCO_3$ because rumen pH is greater than 6.0 (Rogers *et al*., 1985). The ratio of acetic acid (A) to propionic acid (P) was in the range of 2.18 to 2.96 which was not significantly different ($P > 0.05$) (Table 3). The ratio of acetic acid to propionic acid reflects the pattern of ruminal fermentation and ratio of roughage to concentrate in total feed.

Table 3: Influence of treatments on body weight, rumen pH, VFA, blood metabolites and milk of dairy cows

Items	T1	T2	T3	T4	SE	*P-value*
Initial BW, kg	443.00	441.00	449.00	442.00	9.13	0.629
Final BW, kg	446.00	449.00	445.00	456.00	12.10	0.606
BW change, kg.d^{-1}	0.28^{a}	1.28^{b}	-0.26^{a}	1.69^{b}	0.79	0.042
Rumen pH and VFA concentration						
Rumen pH	6.68	6.70	7.12	6.62	0.34	0.225
Acetic, *mmol.l*$^{-1}$	90.64	81.91	76.09	96.05	8.89	0.906
Propionic, *mmol.l*$^{-1}$	35.69	37.60	30.80	32.41	4.36	0.251
Butyric, *mmol.l*$^{-1}$	3.52	2.61	2.72	3.25	2.37	0.868
A:P ratio	2.53	2.18	2.47	2.96	0.57	0.255
Blood metabolites and electrolytes						
Glucose, *mg.dl*$^{-1}$	67.50	65.25	69.50	64.75	3.78	0.348
BUN, *mg.dl*$^{-1}$	7.75	7.25	7.75	8.75	2.64	0.875
Sodium, *meq.l*$^{-1}$	141.25	141.25	139.75	141.75	1.32	0.263
Chloride, *meq.l*$^{-1}$	101.00	96.5	100.25	103.75	5.71	0.421
Bicarbonate, *meq.l*$^{-1}$	23.50	23.75	22.50	23.50	1.75	0.757
Calcium, *mg.dl*$^{-1}$	9.45	9.55	9.40	9.90	0.46	0.473
Potassium, *meq.l*$^{-1}$	4.35	4.22	4.70	4.70	0.24	0.071
Milk yield and composition						
Milk yield, kg	9.18	9.49	9.53	10.00	1.47	0.887
4 % FCM , kg	8.79	9.16	9.16	10.08	1.32	0.591
Fat, %	3.70	3.80	3.73	4.05	0.46	0.711
Protein, %	2.70	2.91	2.95	2.97	0.79	0.622
Lactose, %	4.73	5.14	5.25	5.06	0.44	0.443
Solid not fat, %	8.13	8.75	8.90	8.72	0.65	0.436

T1 = PP to CL ratio of 70:30, T2 = PP to CL ratio of 70:30 with 1.2 % $NaHCO_3$ supplement, T3 =, PP to CL ratio of 52.5: 30 with supplement of 17.5 % RB and 1.2 % $CaCO_3$ mixture, T4 = PP to CL ratio of 50:30 with 20 % RS.
Within rows, means followed by different letters are significantly different at $P < 0.05$.

Russell (1998) reported that the ratio of acetic acid to propionic acid in cows fed 100 % hay was 4.1 while in cows fed 90 % concentrate was 2.2.

Blood metabolites including glucose and blood urea nitrogen (BUN) were unaffected by the treatments ($P > 0.05$) (Table 3). Glucose ranged from 64.75-69.50 mg.dl^{-1} while BUN ranged from 7.25-8.75 mg.dl^{-1}. Kronfeld *et al*. (1982) reported nutritional status of dairy cows indicated by analysis of blood and suggested that normal glucose and BUN should range between 43-69 and 2-22 mg.dl^{-1}, respectively. Electrolytes showed no significant differences among treatments ($P > 0.05$) (Table 3). Serum sodium concentration was unchanged by treatments. In addition, there was no difference in blood chloride, bicarbonate, calcium and potassium among treatments ($P > 0.05$). The normal ranges of serum sodium, chloride, bicarbonate, calcium and potassium are 0.70-157 meq.l^{-1}, 93-152 meq.l^{-1}, 11-26 meq.l^{-1}, 8.5-14.7 mg.dl^{-1} and 1.7-4.5 meq.l^{-1}, respectively (Kronfeld *et al*., 1982).

The supplementation of $NaHCO_3$, $CaCO_3$ and RS did not influence milk yield of dairy cows ($P > 0.05$) (Table 3). However, there was an increasing trend in daily milk production in those cows supplemented with $NaHCO_3$, $CaCO_3$ and RS. Cows fed PP with $NaHCO_3$, $CaCO_3$ and RS produced slightly more milk than cows fed only PP (3.4, 3.8, and 8.9 % or 0.31, 0.35, and 0.82 kg.d^{-1}, respectively). The composition of milk including fat, protein, lactose, solid not fat and total solid was not affected by added $NaHCO_3$, $CaCO_3$ and RS ($P > 0.05$). These data are in agreement with previous reports (Erdman *et al.*, 1982; Rogers *et al*., 1985).

CONCLUSION

Supplementation of $NaHCO_3$, $CaCO_3$ as dietary buffers or RS as saliva secretion stimulant had no significant impact or major physiological changes on performance of lactating dairy cows. No sign of acidosis was observed by added $NaHCO_3$, $CaCO_3$ or RS. However, this experiment was quite limited in experimental animals and time for data collection. Further research should be conducted to test the influence of such diets on milk production with larger number of animals in longer period of time.

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