

## Nutrient intake, acid base status and weight gain in water buffalo calves fed different dietary levels of sodium bicarbonate

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### Abstract

The impact of different dietary levels of sodium bicarbonate ( $\text{NaHCO}_3$ ) on nutrient intake, acid-base status, nitrogen balance and weight gain was examined in growing male buffalo (*Bubalus bubalis*) calves exposed to hot summer conditions. In a complete randomized block design 60 animals of similar age and weight were divided into five treatment groups of 12 per group. Five isonitrogenous and isoenergetic diets were formulated and randomly allocated to a treatment group. The control diet (0SB) contained no  $\text{NaHCO}_3$ , while diets 4SB, 8SB, 12SB and 16SB contained 0.4, 0.8, 1.2 and 1.6%  $\text{NaHCO}_3$ , respectively. An increase in nutrient intake was recorded with increasing dietary  $\text{NaHCO}_3$  level while the reverse was true for nutrient digestibility. Calves fed the 12SB and 16SB diets had higher nitrogen retentions than those fed the 0SB and 4SB diets. Significant increases in blood pH, serum  $\text{HCO}_3$  and urine pH were recorded with increasing  $\text{NaHCO}_3$  levels, with the highest in calves receiving the 16SB diet. Calves fed the 14SB and 16SB diets gained more weight than those fed the 0SB and 4SB diets. These results indicate that the best nutrient intake, acid-base status, nitrogen retention and weight gain occurred in the calves receiving the diet containing 1.2%  $\text{NaHCO}_3$ .

**Keywords:** *Bubalus bubalis*, buffer, ruminal pH, acidosis, weight gain

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### Introduction

A consistent supply of macro and micro nutrients to growing water buffalo (*Bubalus bubalis*) calves is important to attain high growth rates, and would contribute to a profitable income (Sarwar *et al.*, 2002a; Shahzad *et al.*, 2009a; b). This can be achieved by ensuring ingestion of sufficient nutrients and energy, thus ensuring the efficient utilization of nutrients for weight gain (Mandebvu & Galbraith, 1999). However, feeding diets containing high levels of concentrates reduce ruminal pH (Javaid *et al.*, 2008). Rumen pH represents the balance between the acids generated from the fermentation of feeds and the bicarbonate and phosphate buffers in saliva which neutralize these acids. Increased proportions of readily fermentable nutrients in the diet reduce the proportion of effective fibre in the diet, which is essential for copious saliva production through stimulated chewing activity, and consequently effective rumen buffering. Saliva flow rates in mature ruminants are estimated to be in the range of 108 to 308 litres per day which can supply from 1 134 to 3 234 g sodium bicarbonate ( $\text{NaHCO}_3$ ) per day for the buffering of the rumen content (Erdman, 1988). A reduced effective fibre concentration in the diet reduces salivary activities thus preventing the required supply of salivary  $\text{NaHCO}_3$  to counteract acidity produced by rumen fermentation. This chain of events reduces rumen buffering capacity and favours the accumulation of volatile fatty acids and lactic acid in the rumen that ultimately leads to ruminal acidosis (Owens *et al.*, 1998).

Metabolic or systemic acidosis is aggravated in the hot summer because rapid respiration by calves to dissipate heat reduces carbonic acid concentration in the blood leading to respiratory alkalosis, which, because of excessive loss of  $\text{HCO}_3$  in urine, reduces the buffering capacity in blood. The net effect is a reduction in intake of energy and nutrients, and a reduced growth rate.

Several nutritional therapies have been suggested, with variable success, to maintain the buffering capacity in the rumen and blood of ruminants consuming high concentrate diets. Among these, the addition

of buffers such as sodium bicarbonate ( $\text{NaHCO}_3$ ) does not only prevent the reduction in ruminal pH (Owens *et al.*, 1998) but also improves blood buffering capacity (Sarwar *et al.*, 2007a; Shahzad *et al.*, 2007). The favourable effects of  $\text{NaHCO}_3$  supplementation in intensive ruminant farming systems are well documented in the literature (Belibasakis & Triantosa, 1991; West *et al.*, 1991; Sanchez *et al.*, 1997). However, the direct application of these results, mostly obtained in temperate zones to water buffalo calves reared under hot and humid environmental conditions appears to be inappropriate. Furthermore, water buffalo calves lack sweat glands and, being black in colour, can absorb twice the amount of radiation during the hot summer than white animals. Limited scientific information is available on the effect of including  $\text{NaHCO}_3$  in concentrate diets fed to male buffalo calves in hot summer conditions. Therefore, the present experiment was designed to determine the effect of different levels of  $\text{NaHCO}_3$  on dry matter intake (DMI), crude protein (CP), neutral detergent fibre (NDF) and acid detergent fibre (ADF), their digestibility, nitrogen (N) balance and growth performance of growing male water buffalo calves fed a high concentrate diet.

## Materials and Methods

The experiment was conducted on five commercial feedlot farms in the district, Toba Tek Singh, in Pakistan. The average day-time temperature recorded during the study period remained at  $40.4 \pm 4.55$  °C. The study lasted for 12 weeks. The first three weeks were an adaptation period and the fourth week served as the first collection period, while every last week of the remaining two months served as collection periods, (i.e. three collection periods of one week each). Sixty growing male water buffalo calves, 10 - 12 months of age, were allocated to five treatments according to a complete randomized block design. Five isoenergetic and isonitrogenous total mixed diets were formulated in which different levels of  $\text{NaHCO}_3$  were included (Table 1). The control diet (0SB) was formulated without  $\text{NaHCO}_3$ , while the other four diets, 4SB, 8SB, 12SB and 16SB, contained 0.4, 0.8, 1.2 and 1.6%  $\text{NaHCO}_3$ , respectively. The calves were kept in individual pens. Feed was mixed daily and offered twice a day in the morning and evening at a 10% refusal level with daily recording of feed intake. The animals had free access to drinking water throughout the study.

Initial body weights of the animals were recorded prior to the start of the experiment. Thereafter body weights were recorded every 15 days. Urine collections were made fortnightly, using special leather buckets tied to the belly of the calves and fitted with plastic pipes ending in large cylindrical containers. The urine was acidified with 50%  $\text{H}_2\text{SO}_4$ , and 20% of it was sampled and preserved at -20 °C (Shahzad *et al.*, 2008a). The preserved urine samples were pooled per animal after thawing and 10% was used for analysis at the end of each collection period. Similarly, faecal grab samples were collected twice daily during every collection period and digestibility determined using an internal marker, acid insoluble ash. Feed, orts and faecal samples were mixed thoroughly to obtain composite samples of each animal at the end of a collection period. These samples were dried at 55 °C, bulked and analyzed for dry matter (DM), CP, ash, sodium (Na), potassium (K), chlorine (Cl), calcium (Ca), phosphorus (P), magnesium (Mg) and sulphur (S) (AOAC, 2003), and NDF (Van Soest *et al.*, 1991) and ADF (Goering & Van Soest, 1970).

During each collection period blood samples were collected twice at 6 and 12 h post feeding. Twelve mL blood were collected from the jugular vein into two different heparinized vacuum tubes. One of the tubes was placed immediately on crushed ice to determine blood pH within two hours (Tucker *et al.*, 1991). Serum from the other tube was separated and used to analyze Na, K, Cl, Ca, P, Mg and S concentrations via atomic absorption spectrophotometry (AOAC, 2003). Serum  $\text{HCO}_3$  concentration was determined by the method of Harold (1976). Analysis of variance (ANOVA) was used to analyze the data (SAS, 1988). Differences between treatment means were compared using the Duncan's New Multiple Range test at a 5% probability level (Steel & Torrie, 1984).

## Results

Nutrient intake increased ( $P < 0.05$ ) with increasing levels of dietary  $\text{NaHCO}_3$  (Table 2). Minimum (6.23 kg/d) and maximum (7.45 kg/d) DM intakes were recorded in calves fed the 0SB and 16SB diets, respectively. The CP consumption was highest (1176 g/d) for calves fed the 16SB diet and lowest (984 g/d) in calves fed the 0SB diet. Likewise, the NDF intake was the highest (2638 g/d) in the 16SB treatment and lowest (2206 g/d) for calves fed the 0SB diet. A similar trend was recorded for ADF (Table 2). Dry matter digestibility decreased linearly with increasing levels of  $\text{NaHCO}_3$  in the diet (Table 2). The lowest and

**Table 1** Ingredients and chemical composition of experimental diets for growing male water buffalo calves

	Diets <sup>1</sup>				
	0SB	4SB	8SB	12SB	16SB
Ingredients (g/kg)					
Wheat straw	300	300	300	300	300
Maize grain cracked	150	150	150	150	150
Rice polishing	100	100	100	100	100
Sunflower meal	80	80	80	80	80
Maize oil cake	100	100	100	100	100
Maize gluten 30%	180	180	180	180	180
Molasses	62	58	54	50	46
Salt	5	5	5	5	5
Urea	10	10	10	10	10
Dicalcium phosphate	13	13	13	13	13
NaHCO <sub>3</sub>	0	4	8	12	16
Chemical composition (g/kg)					
ME (MJ/kg)	9.7	9.7	9.7	9.7	9.7
Crude protein	158	158	158	158	158
Neutral detergent fibre	354	354	354	354	354
Acid detergent fibre	212	212	212	212	212
NFC	199	199	199	199	199
Calcium	7	7	7	7	7
Phosphorus	4	4	4	4	4
Sodium	3	4	5	6	8
Potassium	4	4	4	5	5
Magnesium	2	2	2	2	2
Chlorine	2	2	2	2	2
Sulphur	2	2	2	2	2
DCAD, mEq/kg	30.3	80.1	120.9	190.8	260.4

<sup>1</sup> Diet 0SB contained no sodium bicarbonate while the 4SB, 8SB, 12SB and 16SB diets contained 0.4, 0.8, 1.2 and 1.6% sodium bicarbonate, respectively.

ME – metabolizable energy (calculated); NFC - Non-fermentable carbohydrate.

DCAD - Dietary cation-anion difference {(Na + K) – (Cl + S)}.

highest DM digestibility ( $P < 0.05$ ) were recorded for calves fed the 0SB and 16SB diets, respectively. Crude protein digestibility followed a similar trend. The NDF and ADF digestibilities also decreased with increasing NaHCO<sub>3</sub> levels.

Nitrogen intake increased with increasing NaHCO<sub>3</sub> level (Table 2). Consequently, N intake was the highest (188.1 g/d) in the calves on the 16SB diet and lowest (157.5 g/d) on the 0SB diet. Despite that, calves fed the 16SB diet retained more ( $P < 0.05$ ) N than those on the other diets, resulting in the highest N balance in calves on the 16SB diets and the lowest in calves on the control (0SB) diet (Table 3).

Blood pH was the lowest (7.26,  $P < 0.05$ ) in calves fed the 0SB diet and highest (7.57,  $P < 0.05$ ) in those fed the 16SB diet (Table 4). A consistent increase ( $P < 0.05$ ) in blood HCO<sub>3</sub> concentration was recorded with increasing NaHCO<sub>3</sub> level in the diet (Table 4). The highest (28.3 mmol/L) and lowest (24.1 mmol/L) serum HCO<sub>3</sub> concentrations ( $P < 0.05$ ) were recorded in calves fed the 16SB and 0SB diets, respectively. A linear increase in urine pH ( $P < 0.05$ ) was observed with increasing levels of NaHCO<sub>3</sub> in the

**Table 2** Effect of increasing levels of sodium bicarbonate on nutrient intake and their digestibility in growing male water buffalo calves

	Diets <sup>1</sup>					SEM
	0SB	4SB	8SB	12SB	16SB	
Nutrient intake (g/d)						
Dry matter (kg/d)	6.23 <sup>c</sup>	6.38 <sup>bc</sup>	6.75 <sup>b</sup>	7.16 <sup>ab</sup>	7.45 <sup>a</sup>	0.39
Crude protein	984 <sup>c</sup>	1008 <sup>bc</sup>	1065 <sup>b</sup>	1130 <sup>ab</sup>	1176 <sup>a</sup>	98.75
Neutral-detergent fibre	2206 <sup>c</sup>	2259 <sup>bc</sup>	2390 <sup>b</sup>	2535 <sup>ab</sup>	2638 <sup>a</sup>	220.5
Acid-detergent fibre	1323 <sup>c</sup>	1355 <sup>c</sup>	1433 <sup>b</sup>	1520 <sup>ab</sup>	1582 <sup>a</sup>	145.4
Nutrient digestibility (expressed as coefficients)						
Dry matter	0.712 <sup>a</sup>	0.690 <sup>ab</sup>	0.682 <sup>b</sup>	0.681 <sup>bc</sup>	0.681 <sup>c</sup>	0.0008
Crude protein	0.731 <sup>a</sup>	0.720 <sup>ab</sup>	0.711 <sup>b</sup>	0.711 <sup>bc</sup>	0.709 <sup>c</sup>	0.0004
Neutral-detergent fibre	0.654 <sup>a</sup>	0.642 <sup>ab</sup>	0.642 <sup>b</sup>	0.641 <sup>bc</sup>	0.634 <sup>c</sup>	0.0090
Acid-detergent fibre	0.633 <sup>a</sup>	0.623 <sup>ab</sup>	0.622 <sup>b</sup>	0.620 <sup>bc</sup>	0.618 <sup>c</sup>	0.0072

<sup>a,b,c</sup> Means within the same row having different superscripts differ significantly ( $P < 0.05$ ).

<sup>1</sup>Diet 0SB contained no sodium bicarbonate while the 4SB, 8SB, 12SB and 16SB diets contained 0.4, 0.8, 1.2 and 1.6% sodium bicarbonate, respectively.

**Table 3** Effect of increasing levels of sodium bicarbonate on nitrogen balance in growing male water buffalo calves

Nitrogen metabolism	Diets <sup>1</sup>					SEM
	0SB	4SB	8SB	12SB	16SB	
Intake	157.5 <sup>c</sup>	161.3 <sup>bc</sup>	170.4 <sup>b</sup>	180.8 <sup>ab</sup>	188.1 <sup>a</sup>	21.41
Faecal excretion	41.2 <sup>c</sup>	44.1 <sup>bc</sup>	46.9 <sup>b</sup>	51.6 <sup>ab</sup>	52.2 <sup>a</sup>	3.95
% of intake	26.2	27.4	27.5	28.5	27.8	0.37
Apparent absorption	116.3 <sup>a</sup>	177.2 <sup>c</sup>	123.5 <sup>b</sup>	129.2 <sup>ab</sup>	135.9 <sup>a</sup>	11.52
% of intake	73.8	72.7	72.5	71.5	72.2	1.78
Urinary excretion	10.22 <sup>c</sup>	11.4 <sup>bc</sup>	11.4 <sup>b</sup>	11.8 <sup>ab</sup>	11.9 <sup>a</sup>	1.97
% of intake	6.49	7.06	6.71	6.50	6.32	0.45
Apparent retention	106.1 <sup>c</sup>	105.8 <sup>bc</sup>	112.1 <sup>b</sup>	117.5 <sup>ab</sup>	124.0 <sup>a</sup>	4.11
% of intake	67.34	65.6	65.8	65.0	65.9	1.01
Balance	51.4 <sup>c</sup>	55.5 <sup>bc</sup>	58.3 <sup>b</sup>	63.3 <sup>ab</sup>	64.1 <sup>a</sup>	12.31

<sup>a,b,c</sup> Means within the same row having different superscripts differ significantly ( $P < 0.05$ ).

<sup>1</sup>Diet 0SB contained no sodium bicarbonate while the 4SB, 8SB, 12SB and 16SB diets contained 0.4, 0.8, 1.2 and 1.6% sodium bicarbonate, respectively.

diet (Table 4). The lowest (7.33) and highest (8.02) urine pH readings ( $P < 0.05$ ) were recorded in calves fed the 0SB and 16SB treatment diets, respectively. The urine pH's of calves fed the 8SB and 12SB diets were 7.60 and 7.81, respectively.

Serum Na concentration increased ( $P < 0.05$ ) with increasing levels of  $\text{NaHCO}_3$  in the diet while K, Cl, S and Mg concentrations remained unaltered (Table 5). Serum Ca increased with decreasing  $\text{NaHCO}_3$  levels in the diet. In the case of serum Mg, a slight decrease was noticed with increasing  $\text{NaHCO}_3$  levels. Serum DCAD increased with an increased level of  $\text{NaHCO}_3$  in the diet.

An increasing weight gain ( $P < 0.05$ ) of the buffalo calves was observed with increasing  $\text{NaHCO}_3$  levels in the diet (Table 6), though FCR between treatments did not differ significantly. Calves fed the 0SB

showed the lowest (625 g/d) weight gains and those fed the 16SB diet, the highest (735 g/d) ( $P < 0.05$ ). Calves fed the 8SB and 12SB diets gained 678 and 708 g/day, respectively.

**Table 4** Effect of increasing levels of sodium bicarbonate on blood pH, urine pH and serum bicarbonate concentration in growing male water buffalo calves

Variables	Diets <sup>1</sup>					SEM
	0SB	4SB	8SB	12SB	16SB	
Blood pH	7.26 <sup>c</sup>	7.32 <sup>bc</sup>	7.38 <sup>b</sup>	7.43 <sup>ab</sup>	7.47 <sup>a</sup>	0.06
Urine pH	7.33 <sup>c</sup>	7.56 <sup>bc</sup>	7.67 <sup>b</sup>	7.81 <sup>ab</sup>	8.02 <sup>a</sup>	0.28
Serum HCO <sub>3</sub> (mmol/L)	24.1 <sup>c</sup>	25.1 <sup>bc</sup>	25.5 <sup>b</sup>	27.0 <sup>ab</sup>	28.3 <sup>a</sup>	1.01

Means within the same row having different superscripts differ significantly ( $P < 0.05$ ).

<sup>1</sup>Diet 0SB contained no sodium bicarbonate while the 4SB, 8SB, 12SB and 16SB diets contained 0.4, 0.8, 1.2 and 1.6% of sodium bicarbonate, respectively.

**Table 5** Effect of increasing levels of sodium bicarbonate on serum minerals in growing male water buffalo calves

Minerals	Diets <sup>1</sup>					SEM
	0SB	4SB	8SB	12SB	16SB	
Na, mEq/L	131.1 <sup>c</sup>	132.2 <sup>c</sup>	135.2 <sup>b</sup>	138.4 <sup>ab</sup>	141.3 <sup>a</sup>	1.89
K, mEq/L	4.71	4.91	5.10	5.32	5.45	0.18
Cl, mEq/L	91.8	90.1	89.9	89.7	89.4	0.08
S, mEq/L	1.45 <sup>c</sup>	1.44 <sup>c</sup>	1.45 <sup>b</sup>	1.45 <sup>ab</sup>	1.43 <sup>a</sup>	0.07
Serum (Na+K)-(Cl+S), mEq/L	42.6	45.6	49.0	52.6	55.9	3.12
Ca, mg/dL	9.39 <sup>a</sup>	9.32 <sup>a</sup>	9.26 <sup>b</sup>	9.16 <sup>bc</sup>	9.07 <sup>c</sup>	0.01
Mg, mg/dL	2.41	2.38	2.36	2.32	2.27	0.02
P, mg/dl	6.98	7.01	7.12	7.12	7.13	0.02

Means within the same row having different superscripts differ significantly ( $P < 0.05$ ).

<sup>1</sup>Diet 0SB contained no sodium bicarbonate while the 4SB, 8SB, 12SB and 16SB diets contained 0.4, 0.8, 1.2 and 1.6% sodium bicarbonate, respectively.

## Discussion

During hot climatic conditions, rapidly growing buffalo calves usually experience a severe problem of heat dissipation, generally referred to as heat stress. It disrupts homeostasis by evoking thermo-regulatory responses to maintain heat balance that lead to decreased feed consumption and growth (Sarwar *et al.*, 2007b; Shahzad *et al.*, 2007). Feeding high levels of NaHCO<sub>3</sub> can influence the acid-base status of animals that plays a key role in body physiology (Sarwar *et al.*, 2007a; b). Certain changes in blood chemistry can occur when high dietary NaHCO<sub>3</sub> concentrations are fed; for example, if dietary concentration of NaHCO<sub>3</sub> increases, it may lead to a decrease in blood H<sup>+</sup> and an increase in blood HCO<sub>3</sub><sup>-</sup>, blood pH and urine pH (Spanghero, 2004). Alteration in blood pH has been associated with many vital physiological functions of the body such as insulin secretion and its effectiveness may be affected (Robertson, 1987) as well as that of the growth hormone (Challa *et al.*, 1993), dry matter intake and growth in growing animals (Jackson & Hemken, 1994).

**Table 6** Effect of increasing levels of sodium bicarbonate on growth performance of growing male water buffalo calves

Variables	Diets <sup>1</sup>					SEM
	0SB	4SB	8SB	12SB	16SB	
Weight gain, g/d	625 <sup>c</sup>	645 <sup>bc</sup>	678 <sup>b</sup>	708 <sup>ab</sup>	738 <sup>a</sup>	18.91
FCR <sup>2</sup>	9.98	9.89	9.96	10.11	10.14	0.58

Means within the same row having different superscripts differ significantly ( $P < 0.05$ ).

<sup>1</sup> Diet 0SB contained no sodium bicarbonate while the 4SB, 8SB, 12SB and 16SB diets contained 0.4, 0.8, 1.2 and 1.6% of sodium bicarbonate, respectively.

<sup>2</sup> Feed conversion ratio (kg feed/kg gain).

Increased DMI in male water buffalo calves fed the higher NaHCO<sub>3</sub> levels in diets could be attributed to a higher rumen pH (Tucker *et al.*, 1991), blood HCO<sub>3</sub><sup>-</sup> and acid-base balance (Sanchez *et al.*, 1994; Shahzad *et al.*, 2008a; b), suggesting an increased rumen buffering capacity in addition to an increased rumen fluid dilution rate (Rogers *et al.*, 1979). Rogers *et al.* (1982) reported an increased DMI in dairy cows when a high level of NaHCO<sub>3</sub> was supplemented. They further stated that, in addition to a buffering effect, NaHCO<sub>3</sub> also increased ruminal osmotic pressure and liquid dilution rate. In the rumen NaHCO<sub>3</sub> is disassociated into Na<sup>+</sup> and HCO<sub>3</sub><sup>-</sup> with non-buffering and buffering effects, respectively (Schneider *et al.*, 1986). Erdman (1988) hypothesized that rumen buffering reduces the extent of acidity produced by volatile fatty acid and lactic acid production in the rumen and, therefore, improves the systemic acid-base status. It is also proposed that in the absence of strong buffering effects, propionate decreases feed intake by ruminants through stimulating oxidative metabolism in the liver (Allen, 2000). The decrease in digestibility of nutrients with increasing dietary NaHCO<sub>3</sub> levels could be attributed to higher rates of passage of digesta through the digestive tract, leading to reduced rumen retention of nutrients and hence reduced digestibility. A positive correlation between nutrient retention time and digestibility has been documented (Sarwar *et al.*, 1996). However, inconsistent responses have been reported on the effects of NaHCO<sub>3</sub> supplementation on nutrient digestibility. A plausible explanation, suggested by Cetinkaya & Unal (1992), was that differences could be due to differences in feeding regimens, particularly concentrate to forage ratio and level of intake, and types and levels of added mineral buffers.

The higher N intake and retention were due to increased DMI of the calves fed the 16SB diet. The higher level of NaHCO<sub>3</sub> might have prevented systemic acidosis induced by rapid degradation of concentrates and a concomitant drop in ruminal pH. This more desirable pH for rumen microbes might have increased the post-ruminal supply of amino acids by accelerating rumen microbial multiplication and eventual enhanced N retention.

A consistent increase in blood pH with increasing dietary NaHCO<sub>3</sub> could be attributed to the gradual increase in dietary Na content. Sodium is absorbed from the posterior segment of the intestine and the excess in chloride is exchanged for hydrogen ions to sustain electrical neutrality of the body. This activity increases blood pH and serum bicarbonate concentration by reducing the H<sup>+</sup> concentration in the blood pool. Similar to the results here, Jackson *et al.* (2001) reported increased serum bicarbonate levels in dairy calves fed 1.75% NaHCO<sub>3</sub>. Waterman *et al.* (1991) also reported increased blood H<sup>+</sup> with reduced Na. Furthermore, the phosphate and ammonia buffer system contributes to hydrogen ion excretion. Hydrogen ions combine with phosphate or ammonia after entering the renal tubules and a bicarbonate ion is formed that enters the extracellular fluids to further buffer acids in the extracellular fluids.

The changes in urine pH noticed in calves fed the 14SB and 16SB diets reflect changes in blood pH, though the kidneys play a vital role in minimizing this change by increasing the urine pH through the excretion of more HCO<sub>3</sub><sup>-</sup> and the conserving of H<sup>+</sup> (Roche *et al.*, 2003; Sarwar *et al.*, 2007b; Shahzad *et al.*, 2008a; b). A marked increase in urine pH had also been reported with increased dietary Na intake (Waterman *et al.*, 1991). The findings of the present study concur with those of other workers (Jackson *et al.*, 1992; Mosel *et al.*, 1993; Jackson & Hemken, 1994; Pehrson *et al.*, 1999) who reported increased urine pH with increased dietary Na, and those of Jackson *et al.* (2001) who noticed increased urine pH (8.09) in dairy

calves fed a high Na level in the diet compared to those (pH 6.80) fed a low Na level. The increased urinary pH in calves fed the 16SB diet might be attributed to decreased  $H^+$  and increased  $HCO_3^-$  concentrations (Fredeen *et al.*, 1988). Another possible reason for this could be that urine excretion of serum  $HCO_3^-$  carries with it  $Na^+$  or other cations for renal rectification of alkalosis (Guyton & Hall, 2000). Increased urine pH was shown to be an indicator of blood pH and  $HCO_3^-$  as it reliably reflected that the acid load of lactating cows decreased dramatically when  $NaHCO_3$  concentration, particularly the sodium content, increased in the diet (Hu & Murphy, 2004). Moreover, male water buffalo calves fed the 16SB diet tended to have a high blood pH due to more  $HCO_3^-$  production and  $H^+$  excretion (Tucker *et al.*, 1992).

The higher serum  $Na^+$  content was primarily due to the higher dietary concentration of this mineral which significantly altered the serum cation–anion balance. A slight metabolic acidosis in cows induced by low sodium dietary content has been reported to enhance Ca absorption from the alimentary tract (Lomba *et al.*, 1978) or increase Ca mobilization from bones (Joyce *et al.*, 1997). This is also supported by Block (1994) who attributed increased serum Ca to increased Ca supply, derived directly from Ca mobilization from bones and indirectly through increased absorption from the intestine due to increased synthesis of 1,25-dihydroxycholecalciferol (hormonally active form of vitamin  $D_3$ ) in response to metabolic acidosis. This is also supported by Gaynor *et al.* (1987) who observed higher plasma hydroxyproline, an index of bone resorption, in cows experiencing metabolic acidosis. Increased plasma Ca in sheep with a low blood pH has been reported by Espino *et al.* (2003). A slight decreasing trend in serum S with increased DCAD level might be due to dietary S concentration. Moreover, S balance is regulated renally and not intestinally, thus increased intakes will increase blood serum S (Krijgsheld *et al.*, 1979). These findings are consistent with those of Delaquis & Block (1995).

The increased weight gain of the male water buffalo calves fed the 16SB diet was due to an increased DMI. The plausible explanation of the higher weight gain because of higher feed consumption by the buffalo calves fed diets with gradual increasing dietary SB levels could be attributed to its favourable effects on ruminal pH, rumen microbial activity, fermentation and passage rate from the rumen, resulting in higher nutrient consumption. It is also stated that metabolic activities in growing animals take place at a rapid rate, leading to higher production of  $CO_2$  in the cell. When production of  $CO_2$  increases when it is exhaled, the enhanced  $CO_2$  production makes the cellular environment acidic (Robert *et al.*, 2004) as  $CO_2$  acts as an acid (carbonic acid) after combining with water (Guyton & Hall, 2000). This slight acidic situation restricts the cell and its organelles to work optimally and consequently reduced cellular activities, resulting in poor growth rate. The activity of this phenomenon might have operated in calves fed OSB diet. The alkalogenic nature of the high SB diet might have allowed a cell to work to its optimal potential by sustaining the cellular environment slightly alkaline through counteracting the cellular acidity produced by  $CO_2$ . Similar findings were reported by Jackson *et al.* (1992) who recorded a quadratic increase in the average daily gain in growing calves when bicarbonate salts of Na or K were supplemented in diets. It is suggested that the lower growth rate in the calves fed the OSB diet was due to low DMI because of metabolic acidosis induced by low dietary Na. Moreover, when the acid balance of the diet is towards acidosis, apart from the homeostatic welfare, most metabolic pathways cannot work under optimal conditions and are more involved in homeostatic regulation than growth.

The findings of the present study suggest that inclusion of 1.2%  $NaHCO_3$  in the concentrate diets of growing male water buffalo calves increased their feed intake, N balance and weight gain. However, further studies aimed at examining the influence of altering  $NaHCO_3$  levels on rumen ecology and digestion kinetics are warranted in order to get a better understanding of  $NaHCO_3$  supplementation in buffaloes.

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