

EFFECT OF LYSINE AND SODIUM BUTYRATE SUPPLEMENTATION ON METABOLIC PROFILE AND GROWTH PERFORMANCE OF BUFFALO CALVES

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ABSTRACT

The present study was designed to support the calf growth during winter season with supplementation of crude protein, lysine (the essential amino acid) and sodium butyrate (acidifier) in the calf starter. 15 apparently healthy and infection free buffalo calves above 2 weeks of age were randomly divided into three groups: Group 1: calves were fed calf starter (20% CP), Group 2: calves were fed calf starter (20% CP) + lysine 100 g/100 kg calf starter, Group 3: calves were fed calf starter (20% CP) + lysine 100 g/100 kg calf starter + sodium butyrate -0.3%. Lysine and sodium butyrate were supplemented for 2 months. Group 3 calves (supplemented with lysine and sodium butyrate) exhibited significantly higher body weight value, withers height, length and heart girth as compared to Group 1 calves (Control). The data revealed no significant ($P < 0.05$) effect of sodium butyrate and lysine on Hb, PCV, Liver function tests (Bilirubin, AST, ALT, ALKP, GGT) and Kidney function tests (BUN, Creatinine), total protein, glucose, sodium, chloride, potassium, plasma osmolality and total immunoglobulins.

Keywords: *Bubalus bubalis*, buffaloes, growth, lysine, metabolic profile, sodium butyrate

INTRODUCTION

Poor growth rate of calves leads to delayed maturity than healthy ones thus increasing the age for first calving and decreasing the profit. Calves born during winter are prone to diseases and/or mortality. Moreover, newly born animals with insufficient fat (needed for heat production for protection from severe cold stress) burn body fat for heat generation which culminates into depressed growth, compromised immune system and even death.

Pearlin *et al.* (2020) have highlighted the role of different acidifiers in livestock nutrition with their potent applications that helps in improvement of nutrient digestibility, utilization of minerals, meat quality, enhances immunity, shows antimicrobial effects in countering pathogenic bacteria, boosts performance and increase production, and thus safeguarding health of livestock animals and poultry. Volatile fatty acids

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are required for rapid development of the rumen papillae (Suarez, 2007). Fast rumen development in young calves facilitates important changes in metabolites that may have synergistic effects on growth. Productivity of dairy animals gets compromised with adversity in thermo-neutral zone. Beneficial effects of sodium butyrate on maturation of gastrointestinal functions were seen in milk-fed calves (Guilloteau *et al.*, 2009). As per Kong *et al.* (2021), lysine supplementation may promote the synthesis of body tissue proteins. The incorporation of bypass nutrients (lysine and fat) in the ration of growing jaffrabadi heifers improved live weight gain (Gajera *et al.*, 2013).

Ingestion of solid feed is necessary to stimulate rumen development in the young calf. Concentrate diets are generally provided because dry matter intake and volatile fatty acids production are higher than with diets based only on roughage. Calf starter is a crucial link to proper ruminal development and successful weaning as it has been shown that a four-week-old calf fed starter has a more developed rumen than a twelve-week-old calf that did not receive starter. Early and aggressive feeding of calf starter as per the requirement of developing young calf is the key to good and early rumen development.

The present study was designed to support the calf growth during winter season, with supplementation of crude protein, lysine (the essential amino acid) and sodium butyrate (acidifier) in the calf starter.

MATERIALS AND METHODS

Experimental design

The study was conducted at the Dairy farm of Guru Angad Dev Veterinary and Animal

Sciences University, Ludhiana during the period from December 2021 to February 2022. 15 apparently healthy and infection free buffalo calves above 2 weeks of age were randomly grouped as mentioned in Table 1.

Approval of IAEC/ CPCSE was obtained vide memo no. IAEC/2022/83-100 dated 24.03.2022 - (GADVASU/2022/IAEC/64/07). The experimental procedures using buffalo calves have been conducted as per IAEC guidelines.

Growth parameters

Body weight of individual calves was monitored at weekly intervals using a digital electronic weighing machine in the morning before feeding the animals. The change in the body weight, if any, was considered as body weight gain or growth. Body measurements: Body length (distance between the points of shoulder and point of pin bone), heart girth (circumference of the chest measured directly behind the front leg), withers height (distance from base of the front feet to the withers) were recorded at weekly interval.

Meteorological observations

Environmental temperature (°C) and relative humidity (%) were recorded in the calf shed daily in morning between 7:30 a.m. to 8:00 a.m. and in evening between 3:30 p.m. to 4:00 p.m. at weekly intervals using thermo-hygrometer. The temperature humidity index (THI) was calculated using the following formula given by Tucker *et al.* (2008).

$$THI = (0.81 \times Ta) + (RH \div 100) \times (Ta - 14.4) + 46.6$$

where,

Ta = Average ambient temperature in °C

RH = Average relative humidity

THI during the study period

The THI value ranged from 58.60 to 68.36. The mean THI was 63.82 (Table 2) during the study period.

Physiological parameters

Rectal temperature (°F) of the selected calves was measured with a digital rectal thermometer with a metallic probe. Respiration rate (breaths/min) was recorded by the 'flank method' by standing at the back of animals. Rise and fall of the abdominal flank were observed.

Blood sampling

Blood samples were collected on 0, 30th and 60th day by jugular venipuncture from all buffalo calves. Sodium fluoride vials were used to collect blood for glucose estimation and heparinized vials were used to collect blood for analysis of other biochemical parameters. The blood samples were kept in an ice bucket and transported quickly to the laboratory for further processing.

Processing of samples

Vials containing blood were centrifuged at 2500 to 3000 rpm for 30 minutes. The plasma was separated out carefully and stored in small aliquots at -20°C for analysis of biochemical parameters.

Hemoglobin Hb was estimated by Sahli's hemoglobinometer method. Estimation of Packed cell volume % (PCV) was done with microhematocrit method (Strumia *et al.*, 1954).

Biochemical parameters

Plasma AST (Aspartate aminotransferase), ALKP (Alkaline phosphatase), GGT (Gamma-glutamyl transferase), total protein, triglycerides, albumin, total bilirubin, cholesterol, blood urea nitrogen, creatinine, sodium (Na), potassium (K),

calcium (Ca) and blood glucose were analysed by fully automatic Vitros DT 350 Chemistry system using the kits from Ortho clinical Diagnostics, Johnson and Johnson Company. Osmolality was calculated by the formula (Rasouli, 2016):

$$\text{Posm} = 2 [\text{Na}^+] + \text{Glucose}(\text{mg/dL})/18 + \text{BUN}(\text{mg/dL})/2.8$$

Total immunoglobulins were precipitated by ammonium sulphate according to Oser (1965) and proteins estimated as per Lowry *et al.* (1951).

Statistical analysis

Statistical analysis of data was done by general linear model using SPSS (ver. 26) software. Comparison of group means was done by Tukey's post hoc test. Results were expressed as Mean $\pm$ S.E. and difference between groups were checked for significance ($P < 0.05$).

RESULTS AND DISCUSSIONS

Growth performance

The data revealed significantly higher average weekly weight gain (Table 3), height (Table 4), length (Table 5) and heart girth (Table 6) of Group 3 calves supplemented with (Lysine + Sodium Butyrate) as compared to Group 1 calves (Control). Our results are in concurrence with rice (2017) which stated that sodium butyrate had a positive effect on overall body weight gain in heifers.

Sodium butyrate supplementation was found to improve the growth performance and antioxidant function of pre-weaned dairy calves (Liu *et al.*, 2021). Supplementation with sodium butyrate in milk replacer enhanced growth rate and improved feed conversion into body weight

gain in young calves (Guilloteau *et al.*, 2009).

The mean body weight gain and conversion efficiency of different nutrients were significantly higher in butyric acid fed calves (Vidyarthi and Kurar, 2000). Supplementation of milk replacer with sodium butyrate increased the pancreatic exocrine secretions, probably due to the stimulation of secretagogue gut regulatory peptides, which in turn resulted in the better digestion efficiency and enhanced growth of the animal. Sodium butyrate can improve the growth of calves, enhance feed digestion and nutrient absorption in the small intestines, decrease inflammation, improve the antioxidant and immune capacity, increase feed intake and daily gain and improve feed conversion ratio in piglets and calves (Liu *et al.*, 2021). Lysine has been identified as an amino acid which can limit the milk protein synthesis and growth in growing heifers (Li *et al.*, 2019). It has been shown to improve hepatic lipid metabolism by providing lipoprotein precursors (Kong *et al.*, 2021) and have positive effect on tissue protein synthesis.

The data for leg circumference (Table 5) revealed no significant variation in all the three groups.

Rectal temperature and respiration rate

There was no variation in rectal temperature (Table 6) and respiration rate (Table 7) with the supplementation of lysine and sodium butyrate in buffalo calves.

Hb, PCV and plasma osmolality

No significant difference ($P < 0.05$) was observed for the values of Hb concentration, PCV and osmolality (Table 8) among the buffalo calves fed calf starter supplemented with lysine and sodium butyrate as compared to Control group.

Liver function tests

The present data revealed no significant variation in the values of Bilirubin, ALT, AST, ALKP and GGT (Table 9) during the study period in the Groups 2 (lysine) and 3 (lysine and sodium butyrate) in comparison to Group 1 (Control).

GGT is recognized as an enzyme throughout the body, with its highest concentration in the liver, this enzyme can be connected to many diseases and health-related issues (Koenig and Seneff, 2015). Alkaline phosphatase is an enzyme used as the marker of liver damage or diseases of skeleton, but not all its functions are known other than that it might be involved in bone calcification and transport of phosphate into epithelial cells (Sharma *et al.*, 2014). In cattle, ALP concentration in serum is greater at a young age and decreases as the animals get older (Kaneko, 1997). Since, there was no significant change observed in the liver function tests, this means the supplementation of lysine and sodium butyrate had no adverse effect on the functioning of liver in buffalo calves.

Total protein, albumin and total immunoglobulins

There was no significant ($P < 0.05$) alteration in the concentrations of total proteins, albumin and total immunoglobulins (Table 12) in response to the supplementation with lysine and sodium butyrate in Groups 2 and 3 as compared to Group 1.

Renal function tests

The renal function test parameters (BUN and Creatinine) also were unaffected ($P < 0.05$) by the supplementation of lysine and sodium butyrate (Table 13) in both the Groups 2 (lysine) and 3 (lysine and sodium butyrate) in comparison to Group 1 (Control). Similar results were seen by

Table 1. Dietary composition of experimental groups.

Group	Number of animals	Composition
1	5	*Calf starter with 20% CP
2	5	*Calf starter with 20% CP + **Lysine 100 g/100 kg calf starter
3	5	*Calf starter with 20%CP + **Lysine 100 g/100 kg calf starter + ***Sodium butyrate - 0.3%

*Calf starter with 20% CP (Zothanpuui *et al.*, 2015).

**Lysine - 100 g/100 kg calf starter (Mudgal *et al.*, 2018).

***Sodium butyrate (Acidifier) - 0.3% (Guilloteau *et al.*, 2009).

Table 2. Weekly Temperature-Humidity Index (THI).

Week	Temperature (°C)	Humidity (%)	THI Index
1	17.2	97	63.07
2	19.2	87	66.13
3	19.5	87	66.63
4	17.4	98	63.46
5	16.1	98	61.14
6	15.0	66	58.60
7	17.0	82	60.22
8	20.5	88	68.36
9	20.4	65	66.82
			Mean THI = 63.82

Table 3. Average weekly weight gain (Kg) of buffalo calves supplemented with lysine and sodium butyrate in calf starter during winter (mean THI = 63.34) (mean $\pm$ S.E.).

Week	Group 1 (Control)	Group 2 (Calf starter + lysine)	Group 3 (Calf starter + lysine + sodium butyrate)
1	0.98 ^b $\pm$ 0.17	1.14 ^a $\pm$ 0.12	1.5 ^a $\pm$ 0.10
2	1.16 $\pm$ 0.21	1.18 $\pm$ 0.14	1.66 $\pm$ 0.11
3	1.12 $\pm$ 0.17	1.28 $\pm$ 0.11	1.28 $\pm$ 0.10
4	0.90 ^b $\pm$ 0.13	1.84 ^a $\pm$ 0.37	0.92 ^b $\pm$ 0.12
5	0.86 ^b $\pm$ 0.21	1.18 ^a $\pm$ 0.07	1.42 ^a $\pm$ 0.16
6	1.32 $\pm$ 0.16	1.22 $\pm$ 0.16	1.42 $\pm$ 0.13
7	0.96 $\pm$ 0.17	1.36 $\pm$ 0.13	1.42 $\pm$ 0.22
8	0.82 ^b $\pm$ 0.13	1.32 ^a $\pm$ 0.11	1.52 ^a $\pm$ 0.11
9	0.82 ^b $\pm$ 0.17	1.08 ^a $\pm$ 0.17	1.4 ^a $\pm$ 0.16
Total weight gain (Difference 0 - 9 week)	8.94 ^b $\pm$ 0.70	11.6 ^a $\pm$ 0.59	12.54 ^a $\pm$ 0.48

Table 4. Height (inches) of buffalo calves supplemented with lysine and sodium butyrate in calf starter during winter (mean THI = 63.34) (mean $\pm$ S.E.).

Week	Group 1 (Control)	Group 2 (Calf starter + lysine)	Group 3 (Calf starter + lysine + sodium butyrate)
0	30.96 $\pm$ 0.42	30.56 $\pm$ 0.51	31.20 $\pm$ 0.40
1	31.30 $\pm$ 0.53	30.86 $\pm$ 0.54	32.24 $\pm$ 0.37
2	31.56 ^{ab} $\pm$ 0.57	31.38 ^b $\pm$ 0.34	32.72 $\pm$ 0.30 ^a
3	31.64 ^b $\pm$ 0.55	31.62 ^b $\pm$ 0.30	33.32 ^a $\pm$ 0.37
4	31.74 ^b $\pm$ 0.32	32.28 ^b $\pm$ 0.43	34.02 ^a $\pm$ 0.40
5	31.82 ^b $\pm$ 0.32	32.36 ^b $\pm$ 0.45	34.70 ^a $\pm$ 0.51
6	32.10 ^b $\pm$ 0.33	32.50 ^b $\pm$ 0.40	35.28 ^a $\pm$ 0.50
7	32.48 ^b $\pm$ 0.32	33.30 ^b $\pm$ 0.38	35.52 ^a $\pm$ 0.55
8	32.80 ^c $\pm$ 0.37	33.52 ^b $\pm$ 0.38	37.16 ^a $\pm$ 0.35
9	33.14 ^b $\pm$ 0.39	33.76 ^b $\pm$ 0.38	37.46 ^a $\pm$ 0.33
Mean	31.95 ^b $\pm$ 0.41	32.21 ^b $\pm$ 0.41	34.36 ^a $\pm$ 0.40

Table 5. Length (inches) of buffalo calves supplemented with lysine and sodium butyrate in calf starter during winter (mean THI = 63.34) (mean $\pm$ S.E.).

Week	Group 1 (Calf starter)	Group 2 (Calf starter + lysine)	Group 3 (Calf starter + lysine + sodium butyrate)
0	25.48 $\pm$ 0.93	23.58 $\pm$ 0.24	25 $\pm$ 0.52
1	25.7 ^a $\pm$ 0.86	23.7 ^b $\pm$ 0.24	25.92 ^a $\pm$ 0.56
2	25.98 $\pm$ 0.81	24.08 $\pm$ 0.19	26.26 $\pm$ 0.89
3	26.14 ^a $\pm$ 0.82	24.2 ^b $\pm$ 0.20	27.36 ^a $\pm$ 0.59
4	26.36 ^a $\pm$ 0.82	24.62 ^b $\pm$ 0.40	27.92 ^a $\pm$ 0.56
5	26.62 ^a $\pm$ 0.74	24.78 ^c $\pm$ 0.36	28.72 ^a $\pm$ 0.46
6	26.94 ^b $\pm$ 0.73	25.24 ^b $\pm$ 0.32	29.16 ^a $\pm$ 0.39
7	27.48 ^b $\pm$ 0.85	26.32 ^b $\pm$ 0.25	29.62 ^b $\pm$ 0.37
8	27.76 ^b $\pm$ 0.85	26.64 ^b $\pm$ 0.28	30.76 ^a $\pm$ 0.33
9	28.08 ^b $\pm$ 0.85	26.92 ^c $\pm$ 0.27	31.00 ^a $\pm$ 0.33
Mean	26.65 ^b $\pm$ 0.82	25.00 $\pm$ 0.27	28.17 ^a $\pm$ 0.5

Table 6. Heart girth (inches) of buffalo calves supplemented with lysine and sodium butyrate in calf starter during winter (mean THI = 63.34) (mean $\pm$ S.E.).

Week	Group 1 (Calf starter)	Group 2 (Calf starter + lysine)	Group 3 (Calf starter + lysine + sodium butyrate)
0	30.16 $\pm$ 0.68	30.46 $\pm$ 0.74	31.4 $\pm$ 0.29
1	30.40 ^b $\pm$ 0.73	30.84 ^b $\pm$ 0.78	32.4 ^a $\pm$ 0.24
2	30.82 ^b $\pm$ 0.73	31.16 ^b $\pm$ 0.68	33.46 ^a $\pm$ 0.24
3	31.10 ^b $\pm$ 0.78	31.38 ^b $\pm$ 0.65	34.06 ^a $\pm$ 0.21
4	32.33 ^b $\pm$ 0.27	32.34 ^b $\pm$ 0.71	34.56 ^a $\pm$ 0.21
5	32.50 ^b $\pm$ 0.26	32.60 ^b $\pm$ 0.67	35.40 ^a $\pm$ 0.49
6	32.92 ^b $\pm$ 0.24	33.36 ^b $\pm$ 0.48	35.76 ^a $\pm$ 0.56
7	33.26 ^b $\pm$ 0.24	33.86 ^b $\pm$ 0.45	36.90 ^a $\pm$ 0.77
8	33.64 ^b $\pm$ 0.22	34.20 ^b $\pm$ 0.41	37.80 ^a $\pm$ 0.57
9	34.04 ^b $\pm$ 0.20	34.60 ^b $\pm$ 0.43	38.12 ^a $\pm$ 0.63
Mean	32.11 ^b $\pm$ 0.43	32.48 ^b $\pm$ 0.6	34.98 ^a $\pm$ 0.42

Table 7. Leg circumference (cm) of buffalo calves supplemented with lysine and sodium butyrate in calf starter during winter (mean THI = 63.34) (mean $\pm$ S.E.).

Week	Group 1 (Control)	Group 2 (Calf starter + lysine)	Group 3 (Calf starter + lysine + sodium butyrate)
0	20.20 $\pm$ 0.26	20.34 $\pm$ 0.20	20.66 $\pm$ 0.27
1	20.46 $\pm$ 0.26	20.56 $\pm$ 0.19	20.86 $\pm$ 0.27
2	20.70 $\pm$ 0.25	20.84 $\pm$ 0.17	21.12 $\pm$ 0.24
3	20.90 $\pm$ 0.25	21.12 $\pm$ 0.16	21.40 $\pm$ 0.24
4	21.16 $\pm$ 0.24	21.46 $\pm$ 0.17	21.58 $\pm$ 0.25
5	21.56 $\pm$ 0.24	21.76 $\pm$ 0.17	21.98 $\pm$ 0.22
6	21.84 $\pm$ 0.24	22.00 $\pm$ 0.19	22.20 $\pm$ 0.20
7	22.16 $\pm$ 0.26	22.46 $\pm$ 0.18	22.58 $\pm$ 0.23
8	22.56 $\pm$ 0.26	22.74 $\pm$ 0.18	22.92 $\pm$ 0.24
9	22.80 $\pm$ 0.23	23.02 $\pm$ 0.18	23.14 $\pm$ 0.22
Mean	21.43 $\pm$ 0.24	21.63 $\pm$ 0.17	21.84 $\pm$ 0.23

Table 8. Rectal temperature ($^{\circ}$ F) of buffalo calves supplemented with lysine and sodium butyrate in calf starter during winter (mean THI = 63.34) (mean $\pm$ S.E.).

Week	Group 1 (Control)	Group 2 (Calf starter + lysine)	Group 3 (Calf starter + lysine + sodium butyrate)
0	100.76 $\pm$ 0.19	100.22 $\pm$ 0.19	100.34 $\pm$ 0.19
1	100.44 $\pm$ 0.11	100.38 $\pm$ 0.11	100.20 $\pm$ 0.11
2	100.18 $\pm$ 0.09	100.30 $\pm$ 0.09	100.28 $\pm$ 0.09
3	100.26 $\pm$ 0.06	100.36 $\pm$ 0.06	100.72 $\pm$ 0.06
4	100.52 $\pm$ 0.19	100.74 $\pm$ 0.19	100.16 $\pm$ 0.19
5	100.50 $\pm$ 0.13	100.28 $\pm$ 0.13	100.28 $\pm$ 0.13
6	100.26 $\pm$ 0.10	100.16 $\pm$ 0.10	100.36 $\pm$ 0.10
7	100.36 $\pm$ 0.07	100.60 $\pm$ 0.07	100.64 $\pm$ 0.07
8	100.52 $\pm$ 0.11	100.34 $\pm$ 0.11	100.36 $\pm$ 0.11
9	100.66 $\pm$ 0.19	100.56 $\pm$ 0.19	100.36 $\pm$ 0.19

Table 9. Respiration rate (breaths/min) of buffalo calves supplemented with lysine and sodium butyrate in calf starter during winter (mean THI = 63.34) (mean $\pm$ S.E.).

Week	Group 1 (Control)	Group 2 (Calf starter + lysine)	Group 3 (Calf starter + lysine + sodium butyrate)
0	27.600 $\pm$ 0.80	28.20 $\pm$ 0.80	27.20 $\pm$ 0.80
1	28.200 $\pm$ 0.87	26.00 $\pm$ 0.87	26.80 $\pm$ 0.87
2	26.800 $\pm$ 0.70	26.00 $\pm$ 0.70	25.40 $\pm$ 0.70
3	25.800 $\pm$ 1.46	26.00 $\pm$ 1.46	25.60 $\pm$ 1.46
4	27.000 $\pm$ 1.27	25.60 $\pm$ 1.27	27.80 $\pm$ 1.27
5	25.400 $\pm$ 0.72	26.80 $\pm$ 0.72	25.40 $\pm$ 0.72
6	26.400 $\pm$ 0.72	25.60 $\pm$ 0.72	25.20 $\pm$ 0.72
7	25.200 $\pm$ 1.59	25.20 $\pm$ 1.59	26.60 $\pm$ 1.59
8	28.400 $\pm$ 1.14	25.80 $\pm$ 1.14	27.00 $\pm$ 1.14
9	26.600 $\pm$ 0.84	25.00 $\pm$ 0.84	26.00 $\pm$ 0.84

Table 10. Hb, PCV and plasma osmolality of calves supplemented with lysine and sodium butyrate in calf starter during winter (mean THI = 63.34) (mean $\pm$ S.E.).

Days	Group 1 (Control)	Group 2 (Calf starter + lysine)	Group 3 (Calf starter + lysine + sodium butyrate)
Haemoglobin (g/dl)			
0	12.44 $\pm$ 0.56	11.7 $\pm$ 0.50	11.3 $\pm$ 0.26
30	12.46 $\pm$ 0.84	13.2 $\pm$ 0.36	11.9 $\pm$ 0.38
60	12.86 $\pm$ 0.51	12.8 $\pm$ 0.48	12.3 $\pm$ 0.40
PCV (%)			
0	36.44 $\pm$ 1.18	34.40 $\pm$ 1.53	33.84 $\pm$ 1.19
30	36.66 $\pm$ 2.03	38.56 $\pm$ 0.70	35.78 $\pm$ 1.26
60	38.40 $\pm$ 1.51	38.18 $\pm$ 1.34	36.88 $\pm$ 1.19
Mean	37.16 $\pm$ 0.50	37.04 $\pm$ 0.36	35.5 $\pm$ 0.48
Osmolality (mOsmol)			
0	281.81 $\pm$ 7.76	275.36 $\pm$ 6.71	288.85 $\pm$ 5.03
30	286.12 $\pm$ 4.96	286.53 $\pm$ 5.08	294.63 $\pm$ 4.85
60	296.82 $\pm$ 4.55	288.88 $\pm$ 6.17	292.66 $\pm$ 6.40

Table 11. Liver function tests in buffalo calves supplemented with lysine and sodium butyrate in calf starter during winter (mean THI = 63.34) (mean $\pm$ S.E.).

Days	Group 1 (Calf starter)	Group 2 (Calf starter + lysine)	Group 3 (Calf starter + lysine + sodium butyrate)
Total bilirubin (mg/dl)			
0	0.42 $\pm$ 0.09	0.22 $\pm$ 0.03	0.22 $\pm$ 0.04
30	0.54 $\pm$ 0.06	0.22 $\pm$ 0.03	0.28 $\pm$ 0.05
60	0.62 $\pm$ 0.03	0.56 $\pm$ 0.08	0.36 $\pm$ 0.08
ALT (U/L)			
0	38.8 $\pm$ 4.24	40.4 $\pm$ 3.70	48.4 $\pm$ 4.35
30	43.0 $\pm$ 2.93	40.8 $\pm$ 5.10	51.0 $\pm$ 4.59
60	56.8 $\pm$ 9.69	53.4 $\pm$ 4.00	52.0 $\pm$ 8.86
AST (U/L)			
0	103.60 $\pm$ 2.50	99.6 $\pm$ 1.69	102.2 $\pm$ 2.29
30	102.0 $\pm$ 2.56	140.2 $\pm$ 22.18	98.0 $\pm$ 3.86
60	237.0 $\pm$ 69.18	195.4 $\pm$ 15.21	168.8 $\pm$ 69.03
ALKP (U/L)			
0	121.4 $\pm$ 13.11	122.2 $\pm$ 13.11	134.8 $\pm$ 13.11
30	123.8 $\pm$ 13.3	106.4 $\pm$ 13.3	134.8 $\pm$ 13.3
60	139.0 $\pm$ 15.08	134.6 $\pm$ 15.08	112.8 $\pm$ 15.08
GGT (U/L)			
0	22.4 $\pm$ 1.47	23.6 $\pm$ 2.60	21.8 $\pm$ 2.43
30	17.0 $\pm$ 0.894	27.6 $\pm$ 4.66	22.0 $\pm$ 1.92
60	15.2 $\pm$ 2.35	50.0 $\pm$ 29.88	21.4 $\pm$ 4.45

Table 12. Total protein, albumin and total immunoglobulins in buffalo calves supplemented with lysine and sodium butyrate in calf starter during winter (mean THI = 63.34) (mean $\pm$ S.E.).

Days	Group 1 (Calf starter)	Group 2 (Calf starter + lysine)	Group 3 (Calf starter + lysine + sodium butyrate)
Total proteins (g/dl)			
0	6.68 $\pm$ 0.18	7.96 $\pm$ 0.44	7.54 $\pm$ 0.12
30	7.32 $\pm$ 0.20	8.28 $\pm$ 0.32	8.02 $\pm$ 0.23
60	8.06 $\pm$ 0.10	8.62 $\pm$ 0.26	8.58 $\pm$ 0.19
Albumin (g/dl)			
0	2.36 $\pm$ 0.12	2.34 $\pm$ 0.20	2.46 $\pm$ 0.18
30	2.74 $\pm$ 0.21	2.48 $\pm$ 0.10	2.80 $\pm$ 0.20
60	2.92 $\pm$ 0.14	3.02 $\pm$ 0.12	2.82 $\pm$ 0.22
Total immunoglobulin (g/L)			
0	6.718 $\pm$ 0.19	6.814 $\pm$ 0.23	6.954 $\pm$ 0.25
30	7.174 $\pm$ 0.12	7.322 $\pm$ 0.20	7.538 $\pm$ 0.19
60	8.236 $\pm$ 0.22	8.132 $\pm$ 0.11	8.352 $\pm$ 0.20

Table 13. Renal function tests in buffalo calves supplemented with lysine and sodium butyrate in calf starter during winter (mean THI = 63.34) (mean $\pm$ S.E.).

Days	Group 1 (Calf starter)	Group 2 (Calf starter + lysine)	Group 3 (Calf starter + lysine + sodium butyrate)
BUN (mg/dl)			
0	12.6 $\pm$ 1.40	13.0 $\pm$ 0.94	16.6 $\pm$ 0.51
30	17.8 $\pm$ 3.90	15.8 $\pm$ 0.37	16.6 $\pm$ 1.32
60	16.8 $\pm$ 4.89	18.6 $\pm$ 1.91	19.4 $\pm$ 4.03
Creatinine (mg/dl)			
0	2.26 $\pm$ 0.11	1.96 $\pm$ 1.38	1.88 $\pm$ 0.28
30	1.62 $\pm$ 0.12	1.38 $\pm$ 0.10	1.54 $\pm$ 0.16
60	2.30 $\pm$ 0.09	1.96 $\pm$ 0.24	1.68 $\pm$ 0.10

Table 14. Sodium, potassium and chloride in buffalo calves supplemented with lysine and sodium butyrate in calf starter during winter (mean THI = 63.34) (mean $\pm$ S.E.).

Days	Group 1 (Calf starter)	Group 2 (Calf starter + lysine)	Group 3 (Calf starter + lysine + sodium butyrate)
Sodium (mEq/L)			
0	137.40 $\pm$ 4.00	134.00 $\pm$ 3.39	140.0 $\pm$ 3.3
30	138.60 $\pm$ 2.92	139.00 $\pm$ 2.	142.8 $\pm$ 2.6
60	144.00 $\pm$ 2.81	139.60 $\pm$ 3.1	141.2 $\pm$ 3.1
Potassium (mEq/L)			
0	4.840 $\pm$ 0.16	4.700 $\pm$ 0.33	4.440 $\pm$ 0.26
30	4.880 $\pm$ 0.18	4.620 $\pm$ 0.22	4.760 $\pm$ 0.28
60	5.100 $\pm$ 0.10	5.120 $\pm$ 0.27	4.960 $\pm$ 0.20
Chloride (mEq/L)			
0	106.40 $\pm$ 3.76	102.20 $\pm$ 3.36	100.80 $\pm$ 3.81
30	108.20 $\pm$ 1.65	106.80 $\pm$ 3.55	107.80 $\pm$ 3.30
60	110.00 $\pm$ 1.30	107.80 $\pm$ 3.45	107.80 $\pm$ 2.33

Table 15. Plasma glucose, cholesterol and triglycerides in buffalo calves supplemented with lysine and sodium butyrate in calf starter during winter (mean THI=63.34) (mean $\pm$ S.E.).

Days	Group 1 (Control)	Group 2 (Calf starter + lysine)	Group 3 (Calf starter + lysine + sodium butyrate)
Glucose (mg/dl)			
0	45.36 $\pm$ 2.19	49.04 $\pm$ 1.80	52.86 $\pm$ 1.38
30	46.30 $\pm$ 2.13	52.08 $\pm$ 1.90	55.92 $\pm$ 1.51
60	50.92 $\pm$ 2.21	54.72 $\pm$ 2.06	60.06 $\pm$ 1.72
Cholestrol (g/dl)			
0	55.20 $\pm$ 21.77	63.00 $\pm$ 2.68	61.20 $\pm$ 3.73
30	56.80 $\pm$ 2.57	64.20 $\pm$ 2.55	66.00 $\pm$ 3.96
60	67.60 $\pm$ 2.22	67.20 $\pm$ 4.28	70.80 $\pm$ 3.12
Triglycerides (mg/dl)			
0	26.40 $\pm$ 6.97	27.88 $\pm$ 8.28	35.60 $\pm$ 10.74
30	39.60 $\pm$ 20.73	18.86 $\pm$ 6.03	38.40 $\pm$ 10.93
60	49.60 $\pm$ 11.38	64.80 $\pm$ 10.58	65.40 $\pm$ 7.38

Gavade *et al.* (2019) in which mean values of BUN in calves from Treatment groups given rumen protected lysine showed non-significant effect.

Sodium, potassium and chloride

The levels of electrolytes (sodium, potassium and chloride) did not vary significantly ($P < 0.05$) in the Groups 2 (lysine) and 3 (lysine and sodium butyrate) as compared to Group 1 (Control) (Table 14). The mean values of electrolytes reported in the present study are within the normal reference range for calves. The similar results have been reported by Dillane *et al.* (2018) for sodium, potassium and chloride.

Plasma glucose, cholesterol and triglycerides

The concentrations of biochemical constituents of plasma (glucose, cholesterol and triglycerides) were not affected by the supplementation of lysine and sodium butyrate in the Groups 2 (lysine) and 3 (lysine and sodium butyrate) as compared to the Control group (Table 15). Górka *et al.* (2014) reported differential responses by adding sodium butyrate to the milk replacer or calf starter. Araujo (2015) found no apparent effect of supplementing milk replacer with sodium butyrate on performance and glucose metabolism in calves.

CONCLUSION

The data indicates the positive influence of the acidifier - sodium butyrate and the essential amino acid - lysine on the growth of calves with no detrimental effect on metabolic profile.

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