The Journal of Animal & Plant Sciences, 23(4): 2013, Page: 1067-1071
ISSN: 1018-7081

CHANGES IN BLOOD METABOLITES OF EARLY WEANED NILI-RAVI BUFFALO (*BUBALUS BUBALIS*) CALVES

M. A. Rashid, T. N. Pasha, M. A. Jabbar, and A. Ijaz*

Department of Animal Nutrition, University of Veterinary and Animal Sciences, Lahore, Pakistan
*Department of Physiology, University of Veterinary and Animal Sciences, Lahore-54000, Pakistan.
Corresponding author's e-mail: tnpasha@uvas.edu.pk

ABSTRACT

Study was conducted to evaluate the effect of age and sampling time on blood metabolites of early weaned Nili-Ravi male buffalo (*Bubalus bubalis*) calves. Eight newly born, male buffalo calves were fed on colostrum for the first 3 days; thereafter, whole milk was fed at 10% of BW for 6 weeks and intakes were adjusted according to live BW on a weekly basis. At week 7 and 8 calves were fed at 5% and 2.5% of BW, respectively, and weaned off milk at the end of week 8. Calf starter was offered *ad-libitum* from week 2 through 12 and intakes were recorded on a daily basis. Jugular blood samples were collected 30 minutes before (-30) feeding, at the time of feeding (0), 30, 60 and 90 minutes post morning feeding (+30, +60 and +90). Sampling was conducted at week 4, 8 and 12 of age. Results revealed that blood glucose concentration decreased ($P<0.01$) with advancing age. Blood urea nitrogen (BUN) concentration increased ($P<0.01$) with advancing age and was higher at week 8 and 12 ($P<0.05$) compared with the concentration at 4 week of age. The concentration of plasma NEFA was highest at week 8 and lowest at week 12 ($P<0.05$). Changes in blood metabolites with advancing age in calves weaned earlier at 8 weeks of age indicate the association between rumen fermentation and blood metabolites that can be used as an indicator for rumen development.

Key words: Buffalo calf, Milk feeding, Early weaning, Starter, Blood metabolites.

INTRODUCTION

A newly born calf is functionally mono-gastric, and for an effective transition to a ruminant depends upon the amount and type of feeding. Unlike adult ruminants, young calves are fed on milk or milk replacers along with solid feed (starter concentrate, hay or green forage). Calves during the pre-weaning period fulfill most of their energy needs through intestinal digestion and absorption of milk or milk replacers. However, after weaning from milk, calves fulfill their energy needs from fermentation of solid feeds in rumen. Therefore, in early weaning strategies, calves are fed limited amount of milk along with good quality highly fermentable starter feed (NRC, 2001).

Developments of digestive tract in young calves occur in three phases: pre-ruminant phase, transitional phase, and ruminant phase; (Davis and Drackley, 1998). Rumen function in young calves is initiated through fermentation of carbohydrates and subsequent production of fermentation end products VFA; acetate, propionate, and butyrate as major part of VFA's produced in rumen. Anderson *et al.* (1987) reported that newly born calves have adequate population of rumen bacteria even at day three of their life; however, bacterial population increases with advancing age and starter intake.

To get a successful transition to a ruminant early access and greater consumption of solid feed (calf starter) are of paramount importance. Early weaning of calves allows the establishment of rumen fermentation at an early age through increased consumption of starter feeds. A higher starter intake leads to changes in rumen fermentation patterns that are mirrored in blood metabolites. Quigley III *et al.* (1991) reported a lower blood glucose concentration and increased blood ketone levels in calves weaned earlier (day 28) to those weaned later (day 56). Authors associated the changes in blood metabolites to advancing age and increased starter intake due to weaning at an early age. Whereas, other investigations documented that higher milk intakes result in higher blood glucose concentration (Quigley III *et al.*, 1991; Davis and Drackley, 1998; Khan *et al.*, 2007a). Contrary to blood glucose, the concentration of BUN has been reported to increase with advancing age (Quigley *et al.*, 2006; Khan *et al.*, 2007a). Higher BUN levels have been associated to higher degradation of feed ingredients by rumen microbes and consequent microbial protein synthesis (Hadorn *et al.*, 1997). Work from Quigley III (1996) documented a positive correlation of plasma NEFA concentration with milk replacer intake and vice versa for starter intake.

Although studies in cattle calves have highlighted the changes in blood metabolites; however, in majority of investigations calves were sampled at 2 hours post-morning feeding. Investigation by Quigley and Bernard (1992), reported marked changes in blood metabolites of cattle calves when sampled at 0 and 2 hours post-morning feeding. To best of our information no published literature is available on changes in blood

metabolites of milk fed early weaned buffalo claves. Further, in Pakistan, rearing of buffalo calves is through conventional system where they are kept with the dam and are allowed to suckle a little amount of milk and weaned around the age of one year (Khan *et al.*, 2007b). Therefore, objectives of current experiment were to investigate the effect of age and sampling time on changes in concentration of blood metabolites (blood glucose, BUN and NEFA) in early weaned male buffalo calves.

MATERIALS AND METHODS

The experiment was conducted at the Buffalo Research Institute (BRI), Pattoki, Pakistan. The experiment protocol was approved by the Animal Research Committee, Department of Animal Nutrition, University of Veterinary and Animal Sciences, Lahore, Pakistan.

Animals and Feeding Managements: Newly born Nili-Ravi male buffalo (*Bubalus bubalis*) calves (n=8) were procured during the months of August and September 2008. According to farm protocols; calves were ear tagged and kept with their dams for first three days of their life to ensure adequate colostrum intake. After that, calves were weighed, weaned off dam and brought to calf sheds bedded with rice straw. Individual milk feeding was carried out twice daily at 8:00 am and 05:00 pm, using stainless steel buckets fitted with rubber nipples (De Laval, Netherlands). Milk feeding buckets were placed in an iron stand, fixed against the wall 68 cm above the ground. Buckets and nipples were washed with detergent, rinsed with hot water and dried after morning and evening milk feeding.

Calves were vaccinated against Hammeorhagic septicemia using a s.c. injection of killed *Pasteurela mulotcida* vaccine (Veterinary Research Institute, Lahore) at week 4 of age. Sick calves were treated according to veterinary practices at the farm. Sick calves with body temperature > 39.5^{o}C were treated using ceftiofur sodium (Excenel, Pfizer, Exton, PA) as an antibiotics for at least 3 days.

Calves were kept in the study for a period of 12 weeks; after the colostrum feeding were fed individually on whole milk at 10% of their live BW from week 1 through 6 and individual intakes were adjusted according to live BW on a weekly basis. At week 7 and 8 milk allowance was reduced to 5% and 2.5% of BW, respectively. Calves were weaned off milk at the end of week 8 of the experiment. Calf starter concentrate was offered *ad-libitum* from week 2 through 12 and individual intakes were recorded daily. Similarly, all calves were given *ad-libitum* access to water in plastic buckets through the entire experiment.

Milk, Feed Sampling and Analysis: Whole milk samples were collected in the morning and evening on a weekly basis, composited for each collection and transported to laboratory. Milk samples were analyzed using a lacto-scan (Milkotronic Analyzer, MCC, Bulgaria). The average nutrient composition (%) of whole milk is given in Table 1.

Samples of calf starter were taken fortnightly, dried at 55°C in a forced air oven. Starter samples were ground to a 1mm size using a Willy mill (Arthur H. Thomas, Philadelphia, USA) and dry matter was determined at105°C for 3 hours using hot air oven The CP contents were determined as N x 6.25 using a Kjeldahl apparatus (AOAC, 1990), and crude fat contents were determined using a Soxhlet Petroleum ether extractor (AOAC, 1990). Ash determination was carried out at 550°C for 3 hours using a muffle furnace. The NDF and ADF contents were determined using the method of Van Soest *et al.* (1991). The chemical composition of calf starter feed is presented in Table 2.

Jugular blood samples were collected at week 4, 8, and 12 using K_3EDTA coated tubes. Calves were sampled at 30 minutes before feeding (-30), at the time off feeding (0), 30, 60 and 90 minutes post morning feeding (+30, +60, +90, respectively). Plasma was harvested by centrifugation (3000 x *g* for 20 minutes), divided into aliquots and stored in a refrigerator at -20 °C till the further analysis. Before the final analysis, samples were thawed completely to make sure that there were no ice chunks. Plasma samples were analyzed for blood glucose, blood urea nitrogen (BUN) using colorimetric kits (Cresent diagnostic, Saudi Arabia) and plasma non-esterified fatty acids (NEFA) was performed using Wako chemical kit (Wako chemicals, Japan).

Statistical Analysis: Data were subject to Komologrov Smirnov's to evaluate the normal distribution of data. Data were analyzed using repeated measures ANOVA. The model included sampling age (4, 8, 12 weeks) as fixed factors and sampling time as repeated measure as well as age x time interaction. Results are presented as least square means ± SEM and declared statistically significant at $P<0.05$. The statistical analysis was carried out using statistical package SPSS (Version 13.0 SPSS Inc., Chicago, IL).

RESULTS AND DISCUSSION

Blood Glucose: Least square means of blood metabolites are presented in the Table 3. In a developing rumen of young calf, blood metabolites are the indicator of fermentative changes. In the current investigation, blood glucose concentration decreased ($P<0.01$) with advancing age; and was lowest ($P<0.01$) at week 12 compared with concentration at week 4 of age. Sampling time and age x time had no effects on blood glucose concentration.

Glucose is a primary source of energy, playing a vital role in metabolism of animal body. Contrary to monogastric animals, ruminants fulfill majority of their glucose requirements through the process of gluconeogenesis. However, young calves during their pre-ruminant stage rely heavily on milk as major source of energy. The decrease in concentration was more prominent at week 8 of age as well as at the end of experiment (week 12). The decrease in blood glucose concentration at week 4 and 12 may be attributed reduced milk intake and higher starter intake. In a previous investigation Quigley III *et al.* (1991), reported a lower blood glucose in cattle calves weaned earlier and associated the change with increase in calf starter consumption during the post-weaning period. Further, this decrease in blood glucose may be associated to the higher ruminal fermentation due to higher starter intake and switching of energy reliance on VFA as a main source (Hammon *et al.*, 2002; Khan *et al.*, 2007a). A sharp decrease in blood glucose concentration at weaning (week 8) may be attributed to the sudden reduction in milk intake which is digested and absorbed at a faster rate as compared to the rumen fermentation end products i.e. VFA of starter rations (Baldwin VI *et al.*, 2004). However, other research suggested that decline in blood glucose was in response to the reduction in milk provision which is digested and absorbed (Davis and Drackley, 1998; Baldwin VI *et al.*, 2004) from intestine directly at a faster rate than fermentation end products of the rumen resulting in higher blood glucose levels (Quigley III *et al.*, 1991).

Blood Urea Nitrogen (BUN): Contrary to blood glucose, the concentration of BUN increased with advancing age ($P<0.01$). Blood urea nitrogen was higher at week 8 and 12 ($P<0.05$) compared to the concentration at 4 weeks of age. Furthermore, the age x time interaction was also significant for BUN concentration ($P<0.01$). Adult ruminants fulfill their protein needs either from protein contents present in the feed stuff escaping ruminal degradation as well from microbial crude protein synthesized in the rumen. However, in young calf the potential changes occur in microbial protein synthesis in rumen. Higher levels of BUN at week 8 and 12 might be related to the higher starter feed consumption and probably a more efficient functioning of the rumen (Khan *et al.*, 2007a). However, Hadorn *et al.* (1997) associated the higher BUN concentration with increased CP intake and consequent higher degradation rates of starter. During the current experiment, we observed relatively higher values of BUN than those documented in cattle calf experiments (Quigley III *et al.*, 1991; Quigley and Bernard, 1992; Lesmeister and Heinrichs, 2004). A difference in species could be a possible explanation for higher BUN values in buffalo calves. Our results are in line with results of Sultan *et al.* (2009) who also documented a higher BUN concentration (37 mg/dl) in growing buffalo calves. Although, higher BUN could cause renal dysfunction but despite the higher BUN levels at week 8 and 12 no days functioning was observed. These findings could be an indicator that buffalo as a ruminant can tolerate higher BUN concentration or might have a different method of nitrogen utilization. A similar trend of increased BUN concentration with advancing age and early weaning has been documented by other researchers (Quigley III, 1996; Quigley *et al.*, 2006). Whereas, in a recent investigation Kehoe *et al.* (2007) reported no difference in BUN levels of calves weaned at 3,4 5,6 week of ages at the end of 8 week weaning experiment in cattle calves indicating that reducing the weaning age has no effect on BUN concentration.

Table 1. Chemical composition of buffalo milk.

Composition %	Milk components ±SE
Total solid	12.59 ±0.59
Fats	4.37 ±0.34
Solid not fats	8.21 ±0.27
Proteins	3.45 ±0.53
Lactose	4.34 ±0.14
Ash	0.77 ±0.03

[1] Every week, morning and evening bulk tank milk samples composited for analysis

Table 2. Composition and analysis of calf starter diet on dry matter basis.

Ingredients	Inclusion %
Maize ground	45.46
Rice polish	12.47
Sugar cane molasses	4.88
Soybean meal	17.27
Canola meal	11.17
Vegetable oil	3.95
Mineral mixture[1]	2.26
Lime	2.54
Nutrient composition	
DM	88.6
CP	17.6
Fat	8.2
ME[2], M.cal/kg	3.1
NDF	11.7
ADF	6.1
Ash	6.1

[1]Each kg contains DCP 70.81%, salt 18.91%, magnesium sulfate 8.64%, ferrous sulfate 8.64%, manganese sulfate 0.49%, zinc sulfate 0.22%, copper sulfate 0.03%, potassium iodide 0.009%, cobalt chloride 0.009%, sodium selenate 0.0015%.
[2]Metabolizable energy was calculated using equation of NRC (2001).

Plasma Non-esterified Fatty Acids (NEFA): The concentration of plasma NEFA was highest at week 8 and lowest at week 12 (P<0.05). However, sampling time and age x time had no effect on plasma NEFA concentration. Non-esterified fatty acids are the source of energy in ruminants and lactating dairy cattle, elevated plasma NEFA levels indicate negative energy balance (Cameron *et al.*, 1998). The higher NEFA values for calves at 8 weeks might be due to increased gluconeogenic activity in liver metabolism to fulfill higher energy demands for growth (Baldwin VI *et al.*, 2004). In another experiment, a lower plasma NEFA concentration was documented in cattle weaned earlier (day 28) to those weaned later during the post-weaning (Quigley III *et al.*, 1991). Furthermore, authors documented a decrease in the plasma NEFA concentration with the advancing age. In current experiment, increased levels of NEFA at week 8 may be related to increased intake of starter with high fat contents (Thomas, 2006). Higher fat levels in the starter were due to the inclusion of high fat rice polish. Other research in Jersey calves reported a negative correlation with the starter intake (Quigley III (1996). Authors reported that plasma NEFA concentration was positively correlated with milk replacer intake; whereas, negatively correlated with starter intake.

Table 3. Effect of advancing age and sampling time on blood glucose, blood urea nitrogen and non-esterified fatty acids of Nili-Ravi buffalo calves.

Traits	Age in weeks		Time[1]					SEM	*P*-value		
			-30	0	30	60	90		Age	Time	Age x time
Glucose,	4	106.08[a]	84.49	97.31	115.65	110.04	122.92				
mg/dl	8	83.167[ab]	77.79	76.56	81.76	93.90	85.82	6.27	0.001	0.088	0.343
	12	57.49[b]	55.21	53.36	63.10	58.91	56.92				
BUN,	4	14.61[b]	14.31[b]	14.36[b]	15.01[b]	15.98[b]	13.39[b]	2.19	0.001	0.477	0.002
mg/dl	8	33.17[a]	36.12[a]	29.53[a]	32.67[a]	35.02[a]	32.51[a]				
	12	34.34[a]	30.87[a]	34.19[a]	34.09[a]	33.70[a]	38.87[a]				
NEFA,	4	0.28[ab]	0.32	0.36	0.21	0.28	0.25				
mEq/L	8	0.35[a]	0.50	0.37	0.35	0.26	0.26	0.05	0.048	0.812	0.421
	12	0.15[b]	0.23	0.09	0.18	0.13	0.13				

[a-b] For each variable values without a common superscript within a column differ significantly (P<0.05).

[1]Blood samples were carried out at week 4, 8, and 12. Further, at each sampling week samples were taken at 30 minutes before feeding (**-30**), at the time off feeding (**0**), 30, 60 and 90 minutes post morning feeding (**+30, +60, +90**, respectively). Calves after colostrum feeding were fed whole milk at 10% of BW for the first 6 week and intakes were adjusted weekly. Thereafter, individual milk allowances were reduced to 5% and 2.5% of BW during the week 7 and 8, respectively. Calf starter was provided *ad-libitum* from week 2 through to week 12. Water was also made available free choice throughout the experiment.

Conclusion: In current experiment, with the advancement of age concentration of blood glucose decreased whereas that of BUN increased. Changes in blood metabolites were more evident during the pre-weaning (week 8) and post-weaning period (week 12). Further, these changes were associated with lesser milk intake and increased starter intake. Study also revealed that young buffalo calves can tolerate higher levels of BUN. However, further research is required to evaluate the effect of these changes on calf health and their relationship with rumen development in buffalo calves.

Acknowledgements: Authors would like to thank Higher Education Commission (HEC) of Pakistan for providing the financial support. We are also thankful to Buffalo Research Institute staff for calf care and milk provision, and Yaseen Tipu for assistance in laboratory analysis, and Kenneth F. Kalscheur and Meredith Brent (South Dakota State University, USA), Khalid Mahmood Anjum and Muhammad Naveed-ul-Haque (UVAS, Lahore, Pakistan) for their contribution in manuscript writing and editing.

REFERENCES

Anderson, K. L., T. G. Nagaraja, J. L. Morrill, T. B. Avery, S. J. Galitzer, and J. E. Boyer (1987). Ruminal microbial development in conventionally or early-weaned calves. J. Anim. Sci. 64(4):1215.

AOAC. (1990). Official methods of analysis.15th edition. Association of Analytical Chemists, Arlington, Virginia, USA.

Baldwin VI, R. L., K. R. McLeod, J. L. Klotz, and R. N. Heitmann (2004). Rumen development, intestinal growth and hepatic metabolism in the pre- and postweaning ruminant. J. Dairy Sci. 87, Supplement(0):E55-E65.

Cameron, R. E. B., P. B. Dyk, T. H. Herdt, J. B. Kaneene, R. Miller, H. F. Bucholtz, J. S. Liesman, M. J. Vandehaar, and R. S. Emery (1998). Dry cow diet, management, and energy balance as risk factors for displaced abomasum in high producing dairy herds. J. Dairy Sci. 81:132–139.

Davis, C. L. and J. K. Drackley. (1998). The development, nutrition, and management of the young calf. Iowa State University Press. Ames, IA.

Hadorn, U., H. Hammon, R. M. Bruckmaier, and J. W. Blum (1997). Delaying colostrum intake by one day has important effects on metabolic traits and on gastrointestinal and metabolic hormones in neonatal calves. J. Nutr. 127(10):2011-2023.

Hammon, H. M., G. Schiessler, A. Nussbaum, and J. W. Blum (2002). Feed intake patterns, growth performance, and metabolic and endocrine traits in calves fed unlimited amounts of colostrum and milk by automate, starting in the neonatal period1. J. Dairy Sci. 85(12):3352-3362.

Kehoe, S. I., C. D. Dechow, and A. J. Heinrichs (2007). Effects of weaning age and milk feeding frequency on dairy calf growth, health and rumen parameters. Livest. Sci. 110(3):267-272.

Khan, M. A., H. J. Lee, W. S. Lee, H. S. Kim, S. B. Kim, K. S. Ki, J. K. Ha, H. G. Lee, and Y. J. Choi (2007a). Pre- and postweaning performance of Holstein female calves fed milk through step-down and conventional methods. J. Dairy Sci. 90(2):876-885.

Khan, Z. U., S. Khan, N. Ahmad, and A. Raziq (2007b). Investigation of mortality incidence and managemental practices in buffalo calves at commercial dairy farms in Peshawar city. J. Agr. Biol. Sci. 2(3):16-22.

Lesmeister, K. E. and A. J. Heinrichs (2004). Effects of corn processing on growth characteristics, rumen development, and rumen parameters in neonatal dairy calves. J. Dairy Sci. 87(10):3439-3450.

NRC. (2001). Nutrient requirements of dairy cattle. 7th revised ed. National Academy Press, Washington, D.C.

Quigley III, J. D. (1996). Influence of weaning method on growth, intake, and selected blood metabolites in Jersey calves. J. Dairy Sci. 79(12):2255-2260.

Quigley III, J. D., L. A. Caldwell, G. D. Sinks, and R. N. Heitmann (1991). Changes in blood glucose, nonesterified fatty acids, and ketones in response to weaning and feed intake in young calves. J. Dairy Sci. 74(1):250-257.

Quigley, J. D. and J. K. Bernard (1992). Effects of nutrient source and time of feeding on changes in blood metabolites in young calves. J. Anim. Sci. 70(5):1543-1549.

Quigley, J. D., T. A. Wolfe, and T. H. Elsasser (2006). Effects of additional milk replacer feeding on calf health, growth, and selected blood metabolites in calves. J. Dairy Sci. 89(1):207-216.

Sultan, J. I., A. Javaid, M. Nadeem, M. Z. Akhtar, and M. I. Mustafa (2009). Effect of varying ruminally degradable to ruminally undegradable protein ratio on nutrient intake, digestibility and n metabolism in nili ravi buffalo calves (bubalus bubalis). Livest. Sci. 122(2-3):130-133.

Thomas, M. (2006). Growth, rumen development, and metabolism of Holstein calves fed distillers grains. M. S. Thesis. in Deptt. of Dairy Sci. South Dakota State University, Brookings, SD.

Van Soest, P. J., J. B. Robertson, and B. A. Lewis (1991). Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74(10):3583-3597.