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Metabolic and immune status of pre-weaning crossbred calves reared on different dietary regimes in Kerala[#]

Manju Sasidharan[1*], A. Kannan[2], K. Shyama[3], K. Raji[4], C. Sunanda[5]
S. Harikumar[6] and Joseph Mathew[7]
Department of Livestock Production Management
College of Veterinary and Animal Sciences, Mannuthy, Thrissur - 680651
Kerala Veterinary and Animal Sciences University
Kerala, India

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Abstract

Seventy two new born crossbred calves were selected and grouped in three dietary treatments-C, T_1 and T_2. Calves in the group T_1 were fed ad libitum milk twice daily for one hour along with calf starter and green fodder. The T_2 group were fed on the dietary protocol as T_1 but was fed calf starter supplemented with essential oils. The dietary treatment of control group (C) was as per the Package of Practices Recommendations, KVASU. Blood samples were collected from the calves at the time of birth, second day and monthly intervals for up to three months for biochemical analysis. The plasma glucose concentrations of calves in dietary treatments C, T_1 and T_2 after one month were 5.69±0.166, 6.26±0.174 and 6.15±0.166 mM respectively. The glucose levels of calves in dietary treatments of C, T_1 and T_2 at three months were 4.22±0.062, 4.50±0.061 and 4.33±0.063, respectively and differed significantly ($p<0.01$) between C and T_1. The serum concentration of Beta-hydroxy butyric acid (BHBA) for dietary treatments C, T_1 and T_2 increased with age and reached 0.418±0.025, 0.403±0.026 and 0.410±0.025 mM respectively and did not differ significantly between treatments. Significantly higher ($P<0.05$) serum cholesterol concentration of 2.82±0.090 and 2.86 ± 0.086 mM were recorded in calves in T_1 and T_2, respectively. Serum concentration of triglycerides, total protein and albumin were similar in all treatment groups. Lower BUN values

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1. *Assistant Professor*
2. *Associate Professor*
3. *Professor, Department of Animal Nutrition*
4. *Associate Professor, Department of Veterinary Physiology*
5. *Statistical Consultant, Ushus, Indira Nagar,Mannuthy, Thrissur-680651*
6. *Associate Professor*
7. *Senior Professor*

**Corresponding author: manjukvasu.ac.in, Ph.9446422328*

of 8.96±0.64 and 8.89±0.64 mM were seen in T_1 and T_2, respectively while C group had serum BUN concentration of 10.33±0.63 mM. Increased levels of serum BHBA indicated rumen development where there is a shift in the precursor of energy from glucose to volatile fatty acids. Ad libitum colostrum feeding resulted in significantly higher (P<0.001) level of IgG from the second day improving the immune status of new-born calves.

Keywords: *Calves, glucose, BHBA, total protein, albumin, BUN, immunoglobulin G*

The first four months in the life of a dairy calf provide the foundation for future production and health. Therefore, it is critical that calves are managed to optimise both health and growth, to maximise their potential. Transferring sufficient IgG to the new born calf via the colostrum is critical to confer adequate immunological protection and disease resistance. Diet manipulation in pre-weaned calves would therefore lead to an alteration in immune function. However, this has yet to be determined. In addition, given the suspected differences in metabolic reserves available to calves on different diets, their ability to respond to an immune challenge could be compromised. This study was conducted in order to analyse the levels of various biochemical parameters in different stages of pre weaning calves namely, first day second day and at monthly intervals up to three months. Earlier research in kids incorporating ayurvedic byproducts in kid starter did not result in significant difference in blood biochemical parameters (Roshma *et al*., 2020). Haemato-biochemical parameters of crossbred calves were studied by Rani *et al*. (2019) after conducting feeding trials incorporating hydroponic fodder maize in calf starter and reported similar values. The study aimed to find out whether biochemical parameters are influenced by different dietary strategies. The plasma or serum concentration of various biochemical indices indirectly denotes the growth, development and health status of calves.

Materials and methods

Seventy-two new-born crossbred calves were selected from two farms of Kerala Veterinary and Animal Sciences University (KVASU) namely; University Livestock Farm and Fodder Research and Development Scheme (ULF & FRDS) Mannuthy and Cattle Breeding Farm (CBF), Thumburmuzhy. The calves were grouped in three treatments, namely control (C), treatment-1 (T_1) and treatment-2 (T_2) uniformly as possible with regard to birth weight, age and sex. They were maintained under identical management conditions as per Package of Practices Recommendations, KVASU (2016). The different dietary treatments followed by the three groups were as follows:

C – Calves were managed as per Package of Practices Recommendations, KVASU (2016)

T_1 – The animals in this group were fed *ad libitum* colostrum using calf feeders three times a day for four days. From day five onwards they were fed with as much milk they consume for one hour in calf feeders twice daily, at 7 am in the morning and 3 pm in the evening. Calf starter (22-24 per cent CP and 70 per cent TDN) and green fodder were fed *ad libitum.*

T_2 – This group followed the same protocol as T_1 but was fed with calf starter supplemented with a blend of essential oils. The blend was prepared by mixing the essential oils of *Zingiber officinale* (ginger), *Mentha x Piperita* (peppermint) and *Illicium verum* (anise) and contained Trans-caryophenyllene, Deoxycholic acid and Anethole as active ingredients respectively in the ratio 1:1:1. This was incorporated in the calf starter at the rate of 300 mg per kg calf starter (Jeshari *et al*., 2016).

Blood samples were collected from the experimental calves at the time of birth, second day, and monthly intervals for three months for biochemical studies. Plasma glucose (m*M*) serum cholesterol (m*M*) triglycerides (m*M*), serum protein (g/L), albumin (g/L), blood urea nitrogen (BUN) (m*M*) and Beta-hydroxy butyric acid (BHBA) (m*M*) were analysed (Schäff *et al*., 2016) in a fully automated biochemical analyser (SELECTRA PRO Site). Serum immunoglobulin G (IgG) (g/L) was analysed using ELISA kit (Sunlong) (Cordero-Solorzano *et al*., 2022) that utilises sandwich ELISA. The optical density

(OD) was measured spectrophotometrically at a wavelength of 450nm. The concentration of IgG was calculated by comparing the OD of the samples to the standard curve.

Results and discussion

The plasma glucose concentrations on the day of birth and at monthly intervals for dietary treatments C, T_1, and T_2 group calves are presented in Table 1. Plasma glucose concentrations of calves in dietary treatments C, T_1 and T_2 group calves were similar. The plasma glucose concentration of calves in all the treatment groups decreased with age up to third month. After the first and third month of dietary treatments calves in dietary treatment T_1 group had significantly ($p<0.01$) higher plasma glucose levels compared to calves in dietary treatment C. However, the difference in glucose values of calves in dietary treatment T_2 and C group calves was not significant. Plasma glucose concentration of calves increased significantly ($P<0.001$) in the second day than the first day in C, T_1 and T_2 groups. The results were consistent with the reports of Hammon *et al*. (2002), Quigley *et al*. (2006), Khan *et al*.(2007) and Maccari *et al*. (2015). The plasma glucose concentration of all the treatment groups decreased after one month as reported in earlier studies by Quigley *et al*. (1991). The significantly lower glucose values of calves in dietary treatment C indicate a shift in the precursor for energy from glucose to volatile fatty acids indicating alimentary ketogenesis and rumen development (Daniels *et al*., 2008). Hence it could be assumed that calves in dietary treatment C had better rumen development followed by T2 and T1. T_2 group calves had lower glucose values than T_1 but the difference was statistically non-significant. Feeding essential oils could improve rumen development in calves as reported by Liu *et al*. (2017) while there was no significant difference in this study.

The serum BHBA was absent in calves on first day and second day after birth in all dietary treatment groups. The level of serum BHBA in calves gradually increased from first month onwards but the difference among the treatment groups was non-significant (Table 2). Beta-hydroxy butyric acid increased with increase in grain intake and in early weaned than those weaned late (Quigley *et al*., 1991). Since group C calves were given restricted quantities of milk they consumed more concentrates than T_1 and T_2 calves earlier thereby increasing their grain intake and higher concentration of serum BHBA than the other treatment groups. *Ad libitum* milk feeding resulted in lower BHBA concentration (Schaff *et al*., 2016). T_1 and T_2 group calves that were given *ad libitum* milk were weaned earlier and began concentrate consumption similar to C group calves and hence the difference in concentration of serum BHBA was non-significant between groups. Supplementation of essential oils in calf starter of T_2 group calves along with higher plane of milk feeding did not produce any significant difference in BHBA concentration. This was contrary to the results of Liu *et al*. (2017).

Table 1. Mean plasma glucose concentration (m*M*) of calves in different dietary treatments

Treatment	Mean plasma glucose ± SE					F-value (p-value)
	Day 1	Day 2	After one month	After two months	After three months	
C	5.78 ± 0.228^{B}	7.19 ± 0.151^{A}	5.69 ± 0.166^{bB}	5.10 ± 0.158^{C}	4.22 ± 0.062^{bD}	58.12** (<0.001)
T_1	5.95 ± 0.238^{B}	7.43 ± 0.158^{A}	6.26 ± 0.174^{aB}	5.21 ± 0.163^{C}	4.50 ± 0.061^{aD}	44.34** (<0.001)
T_2	5.31 ± 0.228^{C}	7.36 ± 0.151^{A}	6.15 ± 0.166^{abB}	5.60 ± 0.155^{C}	4.33 ± 0.063^{abD}	38.57** (<0.001)
F-value (p-value)	2.031^{ns} (0.137)	0.641^{ns} (0.529)	3.238* (0.044)	2.839^{ns} (0.064)	5.194** (0.007)	

Means having different small letter as superscript differ significantly within a column

*Means having different capital letter as superscript differ significantly within a row (** p<0.01,* p< 0.05)*

The effect of different feeding strategies on serum cholesterol concentrations among the three treatments was non-significant in the first two months. The serum cholesterol concentration of calves in T_1 and T_2 was significantly higher than in C when the calves were 3 months old (Table 3). The concentration of serum cholesterol was lowest at birth with a mean of 0.79±0.20 m*M* and increased to 1.26±0.30 m*M* in the second day. The results were consistent with the reports of Hammon *et al*. (2002), Maccari *et al*. (2015) and Rani *et al*. (2019) and the values fall in the normal range reported by other researchers. The effect of different feeding strategies on serum cholesterol concentrations among the three treatments was non-significant in the first two months. However, when the calves were three months old, the serum cholesterol concentration of T_1 and T_2 was significantly higher than in C group. Higher plane of milk feeding resulted in higher concentration of serum cholesterol. This higher concentration could also be due to reduced metabolism of cholesterol in *ad libitum* fed calves. Rani *et al*. (2019) reported similar triglyceride concentration in three month old calves. The results were not in agreement with the findings of Schaff *et al*. (2016) where higher levels of cholesterol were recorded in calves given restricted milk.

The effect of different feeding strategies in the serum concentration of triglycerides was non-significant (Table 4). The results were consistent with the findings of Hammon *et al*. (2002), Khan *et al*. (2007) and Maccari *et al*. (2015). The mean triglyceride concentrations of one month old calves in group C, T_1 and T2 were 0.912±0.051, 0.941±0.054 and 0.868±0.051 and the difference in triglyceride concentration was non-significant. The results were not in agreement with the findings of Schaff *et al*. (2016). However the concentration of triglycerides was significantly lower for all calves during the first day.

The serum total protein concentration was not affected by different dietary treatments (Table 5). The serum total protein concentration increased in all dietary treatments as the age of calves increased but was within the normal range. The result was similar to the findings of Schaff *et al*. (2016).

The serum albumin concentrations of calves in the three dietary treatments increased with age and were within the normal limits. The different treatments did not alter the serum albumin concentration in calves (Table 6). The difference in albumin concentration among the three treatment groups was non-significant and the results were not in agreement with the findings of Schaff *et al*. (2016) where the albumin concentration was lowered by *ad libitum* milk feeding.

The serum BUN concentration was lowest at the time of birth and increased from second day onwards (Table 7). There was no significant difference in BUN concentration

Table 2 . Mean serum BHBA concentration (m*M*) of calves in different dietary treatments

Treatment	Mean serum BHBA ± SE			F-value (p-value)
	After one month	After two months	After three months	
C	0.100 ± 0.005^{C}	0.250 ± 0.015^{B}	0.418 ± 0.025^{A}	95.48** (<0.001)
T_1	0.107 ± 0.005^{C}	0.237 ± 0.016^{B}	0.403 ± 0.026^{A}	82.83** (<0.001)
T_2	0.107 ± 0.005^{C}	0.231 ± 0.015^{B}	0.410 ± 0.025^{A}	58.34** (<0.001)
F-value (p-value)	0.680^{ns} (0.509)	0.441^{ns} (0.645)	0.092^{ns} (0.912)	

Means having different capital letter as superscript differ significantly within a row

*(** p<0.01, p > 0.05, difference within a column is non-significant*

Table 3. Mean serum cholesterol concentration (m*M*) of calves in different dietary treatments

Treatment	Mean serum cholesterol ± SE					F-value (p-value)
	Day 1	Day 2	After one month	After two months	After three months	
C	0.793 ± 0.032^{D}	1.259 ± 0.047^{C}	2.93 ± 0.088^{A}	2.84 ± 0.061^{A}	2.55 ± 0.086^{bB}	334.1** (<0.001)
T_1	0.773 ± 0.033^{C}	1.273 ± 0.049^{B}	2.95 ± 0.092^{A}	2.84 ± 0.064^{A}	2.82 ± 0.090^{aA}	204.2** (<0.001)
T_2	0.802 ± 0.032^{C}	1.244 ± 0.047^{B}	2.68 ± 0.088^{A}	2.86 ± 0.061^{A}	2.86 ± 0.086^{aA}	228.8** (<0.001)
F-value (p-value)	0.205^{ns} (0.815)	0.092^{ns} (0.912)	2.870^{ns} (0.062)	0.049^{ns} (0.952)	3.776* (0.0.027)	

Means having different small letter as superscript differ significantly within a column (*P<0.05*)

Means having different capital letter as superscript differ significantly within a row (*P<0.01*)

Table 4. Mean serum triglyceride concentration (m*M*) of calves in different dietary treatments

Treatment	Mean serum triglyceride ± SE					F-value (p-value)
	Day 1	Day 2	After one month	After two months	After three months	
C	0.452 ± 0.03^{B}	0.883 ± 0.038^{A}	0.912 ± 0.051^{A}	0.906 ± 0.04^{A}	0.822 ± 0.038^{A}	28.01** (<0.001)
T_1	0.454 ± 0.032^{B}	0.843 ± 0.040^{A}	0.941 ± 0.054^{A}	0.852 ± 0.04^{A}	0.852 ± 0.04^{A}	18.89** (<0.001)
T2	0.440 ± 0.030^{B}	0.757 ± 0.038^{A}	0.868 ± 0.051^{A}	0.808 ± 0.04^{A}	0.828 ± 0.038^{A}	19.03** (<0.001)
F-value (p-value)	0.061^{ns} (0.940)	2.902^{ns} (0.060)	0.487^{ns} (0.616)	1.551^{ns} (0.218)	0.165^{ns} (0.848)	

p >0.05, difference within a column is non-significant

Means having different capital letter as superscript differ significantly within a row (*P<0.01*)

Table.5. Mean serum total protein (g/L) of calves in different dietary treatments

Treatment	Mean serum total protein ± SE					F-value (p-value)
	Day 1	Day 2	After one month	After two months	After three months	
C	39.61 ± 1.15^{C}	48.17 ± 1.04^{C}	51.20 ± 0.93^{B}	55.08 ± 0.87^{AB}	55.04 ± 0.97^{A}	2.68^{*} (0.034)
T_1	40.86 ± 1.20^{C}	49.72 ± 1.09^{B}	51.06 ± 0.97^{AB}	53.17 ± 0.91^{A}	54.52 ± 1.01^{A}	23.42** (<0.001)
T2	38.88 ± 1.15^{C}	49.90 ± 1.04^{B}	51.23 ± 0.93^{B}	54.06 ± 0.87^{A}	54.91 ± 0.97^{A}	32.06** (<0.001)
F-value (p-value)	0.729^{ns} (0.485)	0.820^{ns} (0.444)	0.009^{ns} (0.991)	1.154^{ns} (0.320)	0.075^{ns} (0.928)	

P>0.05, difference within a column is non-significant

Means having different capital letter as superscript differ significantly within a row (*P<0.01*)

among treatments in the first two days, but the concentration was significantly higher in group C than in T_2 during first and second month. In the third month the variation in serum BUN concentration became non-significant among the three groups. The marked rise of blood urea

Table 6. Mean serum albumin (g/L) of calves in different dietary treatments

Treatment	Mean serum albumin ± SE					F-value (p-value)
	Day 1	Day 2	After one month	After two months	After three months	
C	20.32 ± 0.41^{E}	22.87 ± 0.32^{D}	25.23 ± 0.39^{C}	30.44 ± 0.37^{B}	32.56 ± 0.43^{A}	141.91^{**} (<0.001)
T_1	19.96 ± 0.42^{E}	22.72 ± 0.33^{D}	24.99 ± 0.4^{C}	30.41 ± 0.38^{B}	32.48 ± 0.45^{A}	116.35^{**} (<0.001)
T2	20.43 ± 0.41^{E}	23.67 ± 0.32^{D}	25.13 ± 0.39^{C}	29.91 ± 0.37^{B}	33.12 ± 0.43^{A}	98.76^{**} (<0.001)
F-value (p-value)	0.349^{ns} (0.706)	2.556^{ns} (0.083)	0.087^{ns} (0.916)	0.658^{ns} (0.520)	0.643^{ns} (0.528)	

p > 0.05, difference within a column is non-significant

Means having different capital letter as superscript differ significantly within a row (*P<0.01*)

Table 7. Mean serum blood urea nitrogen (m*M)* of calves in different dietary treatments

Treatment	Blood urea nitrogen ± SE					F-value (p-value)
	Day 1	Day 2	After one month	After two months	After three months	
C	7.73±0.24	9.43±0.27	10.44 ± 0.26^{a}	10.13 ± 0.37^{a}	10.33±0.63	1.454^{ns} (0.240)
T_1	7.57 ± 0.25^{B}	9.35 ± 0.28^{A}	10.02 ± 0.27^{abA}	9.73 ± 0.39^{abA}	8.96 ± 0.64^{A}	6.046^{**} (0.003)
T2	7.91 ± 0.24^{C}	8.70 ± 0.27^{B}	9.39 ± 0.26^{bA}	8.72 ± 0.38^{bAB}	8.89 ± 0.64^{AB}	6.869^{**} (0.001)
F-value (p-value)	0.471^{ns} (0.626)	2.229^{ns} (0.114)	4.088^{*} (0.020)	3.738^{*} (0.028)	1.650^{ns} (0.198)	

Means having different small letter as superscript differ significantly within a column

Means having different capital letter as superscript differ significantly within a row

*(** P<0.01 * p< 0.05, ns p>0.05)*

Table 8. Mean serum immunoglobulin G (g/L) of calves in different dietary treatments

Treatment	Mean serum immunoglobulin ± SE					F-value (p-value)
	Day 1	Day 2	After one month	After two months	After three months	
C	20.30 ± 1.392^{B}	25.52 ± 1.72^{bA}	28.02 ± 2.39^{A}	26.23 ± 1.74^{bA}	26.04 ± 2.23^{bA}	7.483^{**} (<0.001)
T_1	22.34 ± 1.336^{B}	30.88 ± 1.65^{aA}	32.64 ± 2.29^{A}	33.32 ± 1.67^{aA}	36.17 ± 2.14^{aA}	8.249^{**} (<0.001)
T2	23.28 ± 1.074^{B}	32.83 ± 1.32^{aA}	29.50 ± 1.83^{A}	29.02 ± 1.33^{abA}	31.33 ± 1.71^{abA}	10.824^{**} (<0.001)
F-value (p-value)	1.685^{ns} (0.197)	8.362^{**} (0.001)	1.253^{ns} (0.295)	4.416^{*} (0.018)	4.748^{*} (0.013)	

Means having different small letter as superscript differ significantly within a column

Means having different capital letter as superscript differ significantly within a row (*P<0.01*)

*(** P<0.01 * p< 0.05)*

concentrations from birth onwards possibly mirrored higher rates of protein degradation or amino acid deamination after high protein intake (Hammon *et al*., 2002). Intensive milk fed calves catabolizes less aminoacids, compared to control group calves as indicated by the reduced plasma urea concentration (Bartlett *et al*., 2006, Schaff *et al*., 2016). In the third month the variation in serum BUN concentration became non-significant among the three

groups. The results were consistent with the reports of Maccari *et al*. (2015).

The difference in mean serum immunoglobulin G (g/L) levels of calves after first colostrum feeding among the calves in different treatment groups was non-significant (Table 8). Serum immunoglobulin G (IgG) was absent at the time of birth. The serum IgG concentration increased during second day and was significantly different among treatments. Calves in dietary group C had significantly lower serum IgG concentration than T_1 and T_2 throughout the experiment. The difference in serum IgG concentration after first colostrum feeding among the dietary treatments was non-significant. Many factors, including timing of colostrum intake, volume of colostrum consumed and the immunoglobulin concentration of the colostrum affects the optimisation of absorption (Weaver *et al*., 2000). The supplementation of essential oils in calf starter did not have any effect on serum IgG concentration. Though the serum IgG concentration of all the groups were above the minimum required level of 10g/L that might prevent Failure of Passive Transfer (FPT), the lower concentration of IgG in dietary treatment C group calves may result in a lower immunity level. When ≤ 2L of colostrum was fed in the first feeding after birth as per conventional protocol it may be inadequate to ensure adequate mass of immunoglobulin for passive immunity of calves (Quigley and Drewry, 1998). Most maternal antibodies have a decay half-life of 16 to 28 days (Fulton *et al*., 2004). One month old calves are capable of producing active immune response and hence the difference in IgG levels was non-significant in all the groups. The serum IgG concentration of C group calves remained in the lower range throughout the three months experiment period.

Conclusion

Results of the present study revealed that *ad libitum* milk fed calves had significantly higher plasma glucose concentration throughout the dietary treatment while all the values were in the normal range. The glucose concentration decreased with increase in age and BHBA production indicating rumen development. Due to higher intake of nutrients in *ad libitum* milk fed calves, the catabolism of cholesterol and amino acids might have been lower resulting in higher serum cholesterol and lower BUN values compared to control group. Serum IgG level was higher for *ad libitum* colostrum fed calves indicating good passive transfer of immunity. Other serum biochemical parameters were not significantly affected by *ad libitum* milk feeding. Supplementation of essential oils in calf starter of *ad libitum* milk fed calves did not have any significant effect on the blood biochemical parameters. On summarising the results it could be inferred that *ad libitum* feeding of colostrum and milk during the first five weeks of life could improve the metabolic and immune status of calves.

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Conflict of Interest

The authors declare no conflicts of interest for the work

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