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Growth, Blood Metabolites, and Health of Holstein Calves Fed Milk Replacer Containing Different Amounts of Energy and Protein

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ABSTRACT : This study was conducted to compare the effects of feeding high protein and low energy milk replacer (HPR; CP 25%, ME 3.6 Mcal/kg DM) with low protein and high energy milk replacer (HPR; CP 21%, ME 4.2 Mcal/kg DM) on feed consumption, body weight (BW) gain, health and selected blood metabolites in Holstein calves during the pre-weaning period. At each feeding, each milk replacer (MR) was prepared by mixing 0.125 kg of dry MR in 1L of warm (60°C) water. The calves were fed either HPR (n = 10) or HER (n = 10) using mobile plastic bottles fitted with soft rubber nipples. All calves received 1.8L diluted MR at each feeding 3 times daily during the first 4 weeks of age; feeding frequency was reduced to 2 times daily for the next 2 weeks of age and then to once daily during the last week of the experiment. Jugular blood was sampled in calves at day 7, 14, 21, 35 and 49 of age to enumerate selected metabolites. Daily MR, starter and hay intake during the pre-weaning period were similar in calves fed HPR and HER. Consumption of starter, MGH and total DM steadily increased with the age of calves. Final BW, daily BW gain and feed efficiency of calves were not affected by treatments. Serum glucose, cholesterol, creatinine were decreased ($p<0.05$) and blood urea N was increased ($p<0.05$) in calves fed HER or HPR as they grew older. Serum glucose, total protein and albumin concentrations in calves were not affected by treatments. Serum GPT and GOT concentrations were higher ($p<0.05$) in calves on HPR than on HER. Scouring score, days scoured, respiratory score, rectal temperature and general appearance were similar in calves fed HPR and HER. Poor general appearance (dullness and droopy ears) of calves fed either HPR or HER reflected nutritional insufficiency and stress. In conclusion, energy and protein concentrations in MR did not affect feed intake and BW gain in Holstein calves during the pre-weaning period. Poor general appearance and lower BW gain of calves compared to those reported in the literature for milk fed calves prompt a demand for further research to improve the daily nutrient supply to MR-fed calves. (**Key Words :** Milk Replacer, Calf Starter, Growth, Weaning, Calves)

INTRODUCTION

Amount, composition, and feeding method of milk replacer (MR) to neonatal calves have shown effects on their performance, behavior, health, and welfare traits (Brown et al., 2005; Khan et al., 2007ab). During pre-weaning period, intake of nutrients from liquid feeds is usually limited to stimulate early dry feed intake and allow development of ruminal function and early weaning (Bush and Nicholson, 1986). Restricted milk or MR feeding to calves generally depresses their growth (Khan et al., 2007a), health and behavior (Huzzey et al., 2005) because of poor nutrients supply (Khan et al., 2007b). Whereas, *ad libitum* supply of liquid feed to calves delays the initiation of ruminal fermentation and development (Baldwin et al., 2004) due to depressed solid feed intake (Jensen, 2006). In a recent study (Khan et al., 2007a), we have compared pre and postweaning performance of Holstein female calves fed milk conventionally (10% of calves BW for 45 days) and step-down (STEP, calves were fed milk 20% of their BW until 25 d of age and then gradually reduced to 10% of their BW for the remaining pre-weaning period) methods. Significantly greater milk consumption, dry feed intake, BW gain, and feed efficiency were observed in calves on STEP compared with conventionally fed calves.

Conventional MR typically comprises whey proteins and animal fat. Crude protein content is usually 25%, and fatty acid content generally ranges from 8 to 14% (DM basis). Recently, Diaz et al. (2001) demonstrated the effects of energy and protein contents of MR on growth performance of neonatal calves. Their results indicated higher BW gain during pre-weaning in calves fed on MR

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Table 1. Ingredient and chemical composition of milk replacers (MR), calf starter (CS) and mixed grass hay (MGH)

Ingredients (% DM)	HPR[1]	HER[2]	CS	MGH[3]
Whey powder	34	28	-	-
Fat powder	19	30.5	-	-
Wheat powder	15	11.5	-	-
Whey protein concentrate	10	7	-	-
Lactose	10	10	-	-
Skim milk powder	4	2	-	-
Soy protein concentrate	3	6	-	-
Dicalcium phosphate, 18.5%	2.0	2.0		
Minerals and vitamin premix[4]	2.6	2.6	-	-
Feed additives	0.1	0.1	-	-
Salt	0.3	0.3		
Chemical composition (% DM)			-	-
DM	94.3	94.7	89.0	90.8
Crude protein	25.0	21.0	21.4	9.8
Fat	10.3	17.9	3.1	-
Ash	10.6	8.8	3.7	7.10
Crude fiber	0.51	1.1	-	-
Neutral detergent fiber	-	-	11.5	53.5
Total digestible nutrients	-	-	72.3	57.0
ME[5] (Mcal/kg of DM)	3.6	4.2	-	-

[1] High protein and low energy MR (a commercial preparation).
[2] Low protein and high energy MR.
[3] Mixed grass hay contained 40% orchard grass, 40% tall fescue, and 20% white clover on dry matter basis.
[4] Vitamin and mineral premix provided per kilogram: lysine, 60 g; methionine, 50 g; choline chloride, 20.0 g; vitamin A, 5,102 kIU; vitamin D3, 850 kIU; vitamin E, 12.0 kIU; vitamin K, 280 mg; vitamin C, 3.0 g; thiamin, 170 mg; riboflavin, 420 mg; pantothenic acid, 1,127 mg; niacin, 1,400 mg; pyridoxine, 400 mg; biotin, 4.5 mg; folic acid, 35 mg; vitamin B12, 3.5 mg; magnesium, 13.2 g; manganese, 2,050 mg; iron, 4,000 mg; copper, 800 mg; cobalt, 40.1 mg; zinc, 3.5 g; iodine, 150 mg; selenium, 10.5 mg.
[5] ME was calculated using equations given in NRC (2001).

containing higher amount of both protein and fat than those on conventional MR. Conventional MR generally contains higher protein and lower fat contents than whole milk on DM basis. This nutrient imbalance is one of the important reasons for reduced growth in MR-fed calves compared with those on whole milk feeding (Quigley et al., 2006). Furthermore, higher protein may adversely affect liver and kidney functions in pre-weaned calves (Kurz and Willett et al., 1991; Lohakare et al., 2006; Khan et al., 2007a). Scientific literature regarding the effects of energy and protein contents in MR on solid feed consumption, growth, blood metabolites and health parameters in pre-weaned Holstein calves is limited.

This study was conducted to compare the effects of feeding high protein and low energy MR (HPR) with low protein and high energy MR (HPR) on feed consumption, BW gain, health and selected blood metabolites in Holstein calves during pre-weaning period.

MATERIALS AND METHODS

Calves, management, feeding and treatments

All experimental procedures were reviewed and approved by the ethics committee on the use of animals in research, National Institute of Animal Science, South Korea. Holstein calves (n = 20) born during November 2006 to December 2006 were separated from their mothers within 2 h of birth, weighed, and moved into individual pens (1.5× 2.5 m; bedded with wood shavings) where they were fed colostrum at 10% of their BW for the first 3 d. The individual pens were interspersed evenly throughout the calf barn. Pens had solid iron rod sides, with openings in the front and rear to allow calves free access to calf starter and chopped mixed grass hay (MGH) from feeding buckets. The HPR contained 25% CP and 3.6 Mcal/kg DM ME whereas HER contained 21% CP and 4.2 Mcal/kg DM ME. Chemical composition of MR, calf starter and MGH is given in Table 1. Calves were provided free access to water from a bowl drinker in each pen.

At each feeding, each MR was prepared by mixing 0.125 kg of dry MR in 1 L of warm (60°C) water. The calves were fed either HPR (n = 10) or HER (n = 10) using mobile plastic bottles (2 L capacity) fitted with soft rubber nipples. A steel bottle stand was attached to an iron rod at the front side of individual pen at 70 cm above the floor. At each feeding, a bottle containing MR was fitted into the stand and removed after feeding. The bottles were washed using an iodine detergent after each feeding, dried and stored in the inverted position. All calves received 1.8 L diluted MR at each feeding three times a day (at 0800, 1300 and 1800 h) during the first four weeks of age; feeding frequency was reduced to twice daily (at 0800 and 1800 h)

Table 2. Average milk replacer (MR), solid feed consumption, body weight (BW) gain, feed efficiency and health of Holstein calves fed MR containing different amount of energy and protein

Parameters	Calf starters		SE
	HPR[1]	HER[2]	
Daily MR intake (L/day)	4.01	4.09	0.38
Daily starter intake (g/day)	427	433	36.0
Daily MGH[3] intake (g/day)	71	70	4.9
Daily total DM intake[4] (g/day)	999	1,014	60.2
Initial BW (kg)	40.8	39.8	3.10
Final BW (kg)	53.2	51.5	3.4
Daily BW gain (g)	253	239	17.7
Feed efficiency[5]	0.25	0.24	0.03
Scouring score	3.6	3.8	0.20
Days scoured	5.1	5.5	0.42
Respiratory score	1.1	1.1	0.09
Rectal temperature	38.5	35.5	0.12
General appearance	1.7	1.6	0.05

[1] High protein and low energy MR (n = 10).
[2] Low protein and high energy MR (n = 10).
[3] Mixed grass hay.
[4] Total DMI = MR, calf starter and hay DM.
[5] Feed efficiency = kg of BW gain/kg of total DMI.
Scour scoring: 1 = normal, 2 = soft to loose, 3 = loose to watery, 4 = watery, mucous, slightly bloody, 5 = watery, mucous, and bloody. Scour day considered if score >3.
Respiratory scoring: 1 = normal, 2 = slight cough, 3 = moderate cough, 4 = moderate to severe cough, 5 = severe and chronic cough.
General appearance scoring: 1 = normal and alert, 2 = ears drooped, 3 = head and ears drooped, dull eyes, slightly lethargic, 4 = head and ears drooped, dull eyes, lethargic, 5 = severely lethargic.

for next 2 weeks of age and then to once daily (at 0800 h) during the last week of the experiment. This MR feeding method was used to encourage the solid feed consumption by calves as demonstrated previously by Khan et al. (2006ab).

Sampling and analysis

Body weight of calves, starter and MGH intakes were recorded weekly for 7 weeks during the pre-weaning period. Polyethylene sheets were attached around each feeding bucket to account for wastage of calf starter and hay. Each week calf starter, MGH and their refusals were sampled and analyzed for DM by the method of AOAC (1990).

Jugular blood samples were collected in evacuated tubes (10 ml) without any anticoagulant 30 min before the morning feeding (07:30) when calves were 7, 14, 21, 35 and 49 days of age. These samples were centrifuged at 1,000×g for 20 min and serum was partitioned into aliquots and stored at -20°C until analyzed for glucose, total protein, total cholesterol, blood urea nitrogen (BUN), glutamyl-oxaloacetic transaminase (GOT) glutamyl-pyruvic transaminase (GPT), phosphorus and creatinine by serum analyzer (Arco PC, Biotenica Instruments, SPA, USA).

Health of calves was monitored using the procedure described by Heinrichs et al. (2003). Scoring was as follows: for scour scoring, 1 = normal, 2 = soft to loose, 3 = loose to watery, 4 = watery, mucous, slightly bloody, 5 = watery, mucous, and bloody; for respiratory scoring, 1 = normal, 2 = slight cough, 3 = moderate cough, 4 = moderate to severe cough, 5 = severe and chronic cough; and for general appearance scoring, 1 = normal and alert, 2 = ears drooped, 3 = head and ears drooped, dull eyes, slightly lethargic, 4 = head and ears drooped, dull eyes, lethargic, 5 = severely lethargic. A scour day was considered if the scour score was >3. Scours were also treated with electrolyte therapy (Eltradd, 3 g/L in drinking water; Bayer Animal Health Co., Suwan, South Korea).

Statistical analysis

Weekly feed consumption and BW of calves were presented as mean (±SD). Treatment differences in feed consumption, BW gain, feed efficiency, and incidence of scouring data were evaluated by Student's *t*-test. For time and treatment differences, concentrations of serum metabolites were evaluated using the RANDOM and REPEATED methods of the MIXED procedure of SAS (SAS, 1994). Treatment (i.e., HPR and HER) and time were used as fixed effects and the individual calves were used as random effects. For analyses of differences in time pattern between groups, the interaction (treatment×time) was included in the model. Treatment differences at specific time points were tested by Bonferroni *t*-test ($p<0.05$).

RESULTS AND DISCUSSION

Average daily MR, calf starter and MGH consumption are presented in Table 2. Daily MR, starter and hay intake during pre-weaning period were similar in calves fed HPR and HER. Initiation of solid feed consumption in calves is generally related with the physical and metabolic development of rumen to ferment organic matter and to absorb its end products. Generally, amount and feeding method of MR rather than its composition effect the solid feed consumptions in calves during pre-weaning and early post-weaning periods (Quigley et al., 2006). Restricted MR or milk feeding to calves generally depressed their growth (Jasper and Weary, 2002), health and behavior (Huzzey et al., 2005) because of poor nutrients supply (Khan et al., 2007a). Whereas *ad libitum* MR or milk feeding to calves delayed the initiation of ruminal fermentation and development (Baldwin et al., 2004) due to depressed solid feed intake (Hammon et al., 2002; Jensen, 2006; Quigley et al., 2006). In the present study, lack of differences in solid feed consumption between calves on HPR and HER may be ascribed to similar amount and method of MR feeding during pre-weaning period. Consumption of starter, MGH and total DM was steadily increased with the age of calves (Figure 1). A marked increase in solid feed DM consumptions was noticed after 21 days of age. Similar to

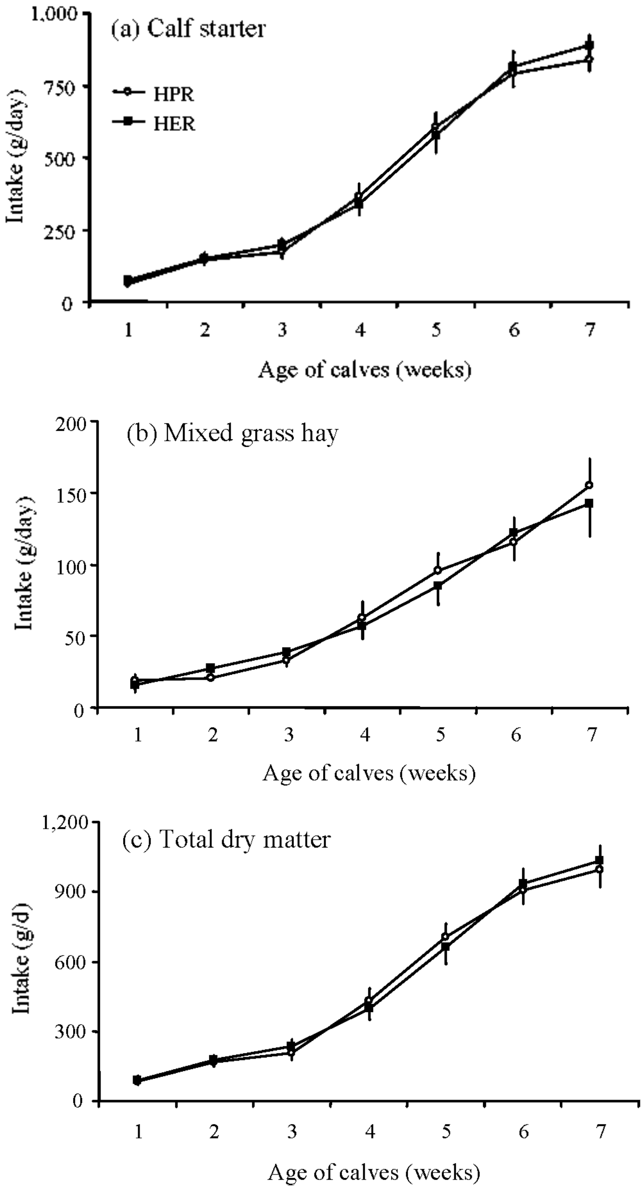

Figure 1. Average (±SD) weekly consumption of calf starter (a), mixed grass hay (b) and total solid feed DM (c) during pre-weaning period in Holstein calves fed milk replacer (MR) containing different amount of energy and protein. HPR = High protein and low energy MR (n = 10). HER = Low protein and high energy MR (n = 10). Mixed grass hay contained 40% orchard grass, 40% tall fescue, and 20% white clover on dry matter basis.

present results, a slow increase in solid feed consumption with age during pre-weaning period has been demonstrated by other workers (Albright and Arave, 1997; Khan et al., 2007ab). Khan et al. (2007b) described a rapid surge in solid feed consumption and reduced blood glucose levels in Holstein calves with reduced milk supply. Similarly, Abdelsamei et al. (2005) indicated that a decrease in milk replacer consumption linearly increased the intake of hay by Holstein calves during pre-weaning period.

Weekly BW of calves fed HPR and HER is presented in

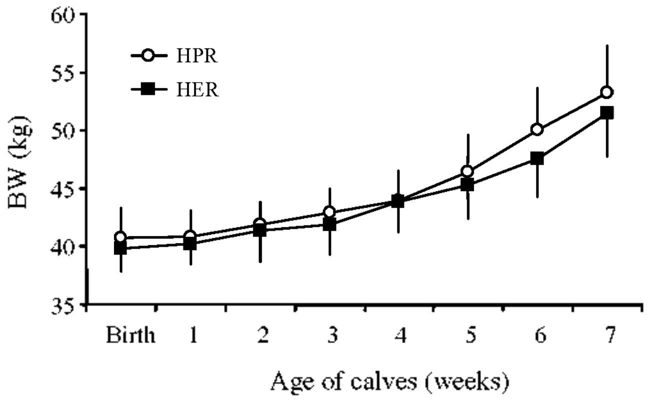

Figure 2. Average (±SD) weekly body weight of Holstein calves fed milk replacer (MR) containing different amount of energy and protein. HPR = High protein and low energy MR (n = 10). HER = Low protein and high energy MR (n = 10).

Figure 2. Initial BW was similar in calves on HPR and HER. Final BW, daily BW gain and feed efficiency of calves were not affected by treatments (Table 2). Daily BW gain was quite low in calves either fed HPR or HER compared with those reported by others (Diaz et al., 2001; Quigley et al., 2006; Khan et al., 2007ab) in calves fed whole milk or MR using restricted, *ad libitum* or STEP feeding methods. Lower BW gain in present study than those reported by others may be ascribed to the differences in daily nutrients supply to calves from liquid feed (milk or MR). In present study, poor general appearance (dullness and droopy ears) of calves fed either HPR or HER also reflected nutritional insufficiency and stress. Before the initiation of solid feed consumption and establishment of rumen fermentation neonatal calves completely rely on nutrients supplied by liquid feed to derive their energy needs. Therefore the composition and amount of MR supplied to calves has shown significant effects on BW gain of calves during pre-weaning period (Diaz et al., 2001). It may be suggested from present results that nutrients supplied to calves by commercial MR using conventional feeding (in a restricted amount) system are not enough to support good growth in pre-weaned calves. A higher supply of both protein and energy along with other micro nutrients may be required for better growth performance of pre-weaned calves. Further, usage of several growth factors and hormones recently demonstrated in milk (Blum and Baumrucker et al., 2002) may also be considered to improve BW gain in pre-weaned calves.

Scouring score, days scoured, respiratory score, rectal temperature and general appearance were similar in calves fed HPR and HER (Table 1). High incidence of diarrhea is usually related with the sanitary, management housing conditions and immune status of the calves rather than their daily amount of milk or MR intake (Hammon et al., 2002; Quigley et al., 2006; Khan et al., 2007b). All calves in

Table 3. Blood serum concentrations of metabolites in Holstein calves fed milk replacer (MR) containing different amount of energy and protein

Traits	Treatments	Age of calves (days)					SE	Fixed values, p		
		7	14	21	35	49		Treatment	Time	Treatment ×time
Glucose (mg/dl)	HPR[1]	101.1	94.5	83.5	85.4	82.1	6.12	0.11	0.02	0.15
	HER[2]	104.5	92.2	88.6	85.2	81.2				
Total protein (mg/dl)	HPR	7.5	6.9	6.8	7.2	6.9	0.18	0.20	0.13	0.10
	HER	7.2	6.7	6.7	7.1	7.1				
Albumen (g/dl)	HPR	4.1	4.3	4.7	4.8	4.7	0.12	0.24	0.05	0.17
	HER	4.0	4.1	4.6	4.5	4.5				
Blood urea nitrogen (mg/dl)	HPR	9.1	10.7	12.4	13.2*	14.1	0.65	0.04	0.04	0.21
	HER	8.2	10.0	11.5	11.4	12.8				
Total cholesterol (mg/dl)	HPR	105.8*	98.4*	96.7*	90.2*	91.9*	5.82	0.02	0.03	0.08
	HER	113.7	121.2	105.6	120.3	103.5				
GOT[3] (μl)	HPR	94.4*	73.1*	75.4*	69.4*	65.5*	3.99	0.03	0.04	0.18
	HER	74.3	62.2	66.4	60.7	58.4				
GPT[4] (μl)	HPR	77.4*	52.5*	29.1	39.3	33.5	2.44	0.04	0.03	0.20
	HER	67.8	44.4	31.8	35.5	32.1				
Creatinine (mg/dl)	HPR	2.2*	1.9	1.9	2.1*	1.7	0.11	0.05	0.04	0.09
	HER	1.9	1.7	1.8	1.6	1.5				

* Means are significantly ($p<0.05$) different between treatments.
[1] High protein and low energy MR (n = 10). [2] Low protein and high energy MR (n = 10).
[3] Glutamyl-oxaloacetic transaminase. [4] Glutamyl-pyruvic transaminase.

present study were fed 10% colostrum of their BW within 2 h of their birth. This method and level of colostrum feeding were described to be sufficient to achieve satisfactory level of serum IgG (10 mg/ml) in calves to combat infectious diseases during early weeks of life (Woodward, 1998; Nonnecke et al., 2003; Samanta et al., 2006).

Serum glucose was decreased ($p<0.02$) in calves fed HER or HPR as they grew older (Table 3). This change has been attributed to the physiological shift in the primary energy source from glucose to volatile fatty acids (VFA) when the rumen in young calves becomes functional (Hammon et al., 2002). In neonatal calves due to the reflexive closure of the reticular groove and the lack of short-chain fatty acids in the ruminal lumen, the primary source of energetic substrate is glucose derived from intestinal absorption (Khan et al., 2007b). With the initiation of solid feed consumption, ruminal fermentation proceeds and VFA starts replacing glucose as an energy source (Baldwin et al., 2004). Serum glucose, total protein and albumen concentrations in calves were not affected by treatments. Treatment × time interactions for glucose, total protein and albumen in calves during 49 days were not significant.

Blood urea N was increased ($p<0.04$) with the advancing age of calves (Table 3). Higher concentrations of BUN in older calves may be ascribed to higher CP consumption from solid feed and its subsequent degradation in the rumen. Similar to present results, other workers (Baldwin et al., 2004; Kumar and Dass, 2006; Khan et al., 2007ab) also demonstrated increasing concentration of both ruminal ammonia and BUN in calves with their advancing age. Blood urea N concentration was higher in calves on HPR than those fed HER which could be attributed to higher concentration of protein in the former. Higher concentration of BUN is also an index of the renal dysfunction, however the creatinine concentration in the calves fed through both methods was in the safe range (Hadorn et al., 1997; Hammon et al., 2002), declined as calves grew older, and did not differ between treatments, suggesting that normal liver and renal functions in calves. Furthermore, declined serum GPT and GOT concentrations with the age in calves also indicated normal liver functions. Serum GPT and GOT concentrations were higher ($p<0.05$) in calves on HPR than in those on HER. Treatment×time interaction for GOT and GPT in calves during 49 days was not significant. Higher serum GPT and GOT in calves fed HPR may be ascribed to higher protein intake. Higher concentrations of GOT, and GPT in calves are usually related to the anomalies of liver metabolism and diarrhea, however in the present study, the concentration of these enzymes were within normal and safe range as previously described by Rauprich et al. (2000).

Serum total cholesterol concentration was reduced ($p<0.03$) with the advancing age of calves. Lower concentrations of serum triglycerides and cholesterol in calves with their advancing age were previously demonstrated by Hadorn et al. (1997) and attributed to reduced supply of milk. In present study, a reduced supply of MR to older calves probably reduced the serum cholesterol in older calves. Serum total cholesterol concentration was higher ($p<0.02$) in calves on HER than in those on HPR. Treatment×time interactions for BUN and serum cholesterol in calves during 49 days were not significant. Higher serum cholesterol in calves fed HER may be ascribed to higher supply and absorption of fat.

CONCLUSIONS

Energy and protein concentrations in MR did not affect the feed intake, BW gain in Holstein calves during pre-weaning period. Lower blood glucose and higher BUN in older calves may be attributed to initiation of some ruminal activity. Higher serum GPT and GOT in calves fed HPR may be ascribed to higher protein intake. Significantly higher serum total cholesterol in calves fed HER was the function of higher fat consumption.

REFERENCES

Abdelsamei, A. H., D. G. Fox, L. O. Tedeschi, M. L. Thonney, D. J. Ketchen and J. R. Stouffer. 2005. The effect of milk intake on forage intake and growth of nursing calves J. Anim. Sci. 83:940-947.

Albright, L. L. and C. W. Arave. 1997. The Behaviour of Cattle. CAB International, Wallingford, UK.

AOAC. 1990. Official Methods of Analysis. 15th ed. Association of Official Analytical Chemists, Arlington, VA.

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

Blum, J. W. and C. R. Baumrucker. 2002. Colostral and milk insulin-like growth factors and related substances: Mammary gland and neonatal (intestinal and systemic) targets. Domest. Anim. Endocrinol. 23:101-110.

Brown, E. G., M. J. Vandehaar, K. M. Daniels, J. S. Liesman, L. T. Chapin, D. H. Heisler and M. S. Weber Nielsen. 2005. Effects of increasing energy and protein intake on body growth and carcass composition of heifer calves. J. Dairy Sci. 88:585-594.

Bush, R. S. and J. W. G. Nicholson. 1986. The effects of weaning schedule, duration of milk feeding and fishmeal on calf performance. Can. J. Anim. Sci. 66:691-698.

Diaz, M. C., M. E. Van Amburgh, J. M. Smith, J. M. Kelsey and E. L. Hutten. 2001. Composition of growth of Holstein calves fed milk replacer from birth to 105-kilogram body weight. J. Dairy Sci. 84:830-842.

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. 127:2011-2023.

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 period. J. Dairy Sci. 85:3352-3362.

Heinrichs, A. J., C. M. Jones, L. R. VanRoekel and M. A. Fowler. 2003. Calf Track: A system of dairy calf workforce management, training, and evaluation and health evaluation. J. Dairy Sci. 86(Suppl. 1):115.

Huzzey, J. M., T. J. DeVries, P. Valois and M. A. G. von Keyserlingk. 2006. Stocking density and feed barrier design affect the feeding and social behavior of dairy cattle. J. Dairy Sci. 89:126-133.

Jasper, J. and D. M. Weary. 2002. Effects of *ad libitum* milk intake on dairy calves. J. Dairy Sci. 85:3054-3058.

Jensen, M. B. 2006. Computer-controlled milk feeding of group-housed calves: The effect of milk allowance and weaning type. J. Dairy Sci. 89:201-206.

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 post-weaning performance of Holstein female calves fed milk through step-down and conventional methods. J. Dairy Sci. 90: 876-885.

Khan, M. A., H. J. Lee, W. S. Lee, H. S. Kim, K. S. Ki, T. Y. Hur, G. H. Suh, S. J. Knag and Y. J. Choi. 2007b. Structural growth, rumen development, metabolic and immune response of Holstein male calves fed milk through step-down and conventional methods. J. Dairy Sci. 90:3376-3387.

Kumar, R. and R. S. Dass. 2006. Effect of niacin supplementation on growth, nutrient utilization and blood biochemical profile in male buffalo calves. Asian-Aust. J. Anim. Sci. 19:1422-1428.

Kurz, M. M. and L. B. Willett. 1991. Carbohydrate, enzyme, and hematology dynamics in newborn calves. J. Dairy Sci. 74: 2109-2118.

Lohakare, J. D., A. K. Pattanaik and S. A. Khan. 2006. Effect of dietary protein levels on the performance, nutrient balances, metabolic profile and thyroid hormones of crossbred calves. Asian-Aust. J. Anim. Sci. 19:1588-1596.

Nonnecke, B. J., M. R. Foote, J. M. Smith, B. A. Pesch and M. E. Van Amburgh. 2003. Composition and functional capacity of blood mononuclear leukocyte populations from neonatal calves on standard and intensified milk replacer diets. J. Dairy Sci. 86:3592-3604.

NRC. 2001. Nutrient requirements of dairy cattle. 7th revised edition. National Academy Press, Washington, DC.

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. 89:207-216.

Rauprich, A. B. E., H. M. Hammon and J. W. Blum. 2000. Influence of feeding different amounts of first colostrum on metabolic, endocrine, and health status, and growth performance in neonatal calves. J. Anim. Sci. 78:896-908.

Samanta, A. K., R. S. Dass, M. Rawat, S. C. Mishra and U. R. Mehra. 2006. Effect of dietary vitamin E supplementation on serum α-tocopherol and immune status of crossbred calves. Asian-Aust. J. Anim. Sci. 19:500-506.

SAS User's Guide: Statistics, Version 6.11 Edition. 1994. SAS Inst., Inc., Cary, NC.

Woodward, B. 1998. Protein, calories, and immune defenses. Nutr. Rev. 56:S84-S92.