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Effects of Weaning Age on Growth and Blood Parameters of Replacing Holstein Calves Fed on a Restricted Step Up and Down Milk Feeding Program

Research Article

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ABSTRACT

The objective of this study was to investigate the effects of different early weaning programs on growth performance and blood parameters of dairy calves. To meet this objective, thirty Holstein calves with initial body weight of 36 ± 5 kg were randomly assigned at birth to one of the three weaning programs. Experimental groups were: 1) starter diet and whole milk from birth to 6 weeks and weaning at 42 d old (6WW)), 2) starter diet and whole milk from birth to 9 weeks and weaning at 63 d old (9WW) and 3) starter diet and whole milk from birth to 12 weeks and weaning at 84 d old (12WW). Starter diet and water were available *ad libitum* throughout the experiment. Daily starter intake and growth performance of calves were measured until d 90 of life for all groups. Body weight (BW) and starter intake in all measured periods (on d 28, 56 and 84) did not differ between experimental groups. Calves weaned at 42 d (6WW) had higher average daily gain (ADG) in second month than 9WWand 12WWgroups (P<0.05). Calves reared in 6WW group had significantly greater total ADG values than calves weaned on 63 and 84 d (P<0.05). Total feed conversation ratio affected by experimental treatments and was lowest in 6WWcalves (P<0.05). Body length, heart girth and withers height of calves did not differ among treatments at 28, 56 and 84. Plasma cholesterol concentration of calves weaned in 6WWgroup was lower (P<0.05) than calves weaned in 9WW and 12WW. Under the conditions of this study, the results suggest that calves weaned at 42 d were more efficient at utilizing dry matter intake for body weight (BW) gain and growth.

KEY WORDS blood parameters, calves, growth performance, weaning programs.

INTRODUCTION

Calf rearing is one of the most important and sensitive management plans in dairy cattle production systems. Therefore, it is principally important to apply proper approaches to rear the replacing calves in order to improve growth and health (Anderson *et al.* 1987). Some of these approaches are faster transmission from liquid feeding to solid diet, early starter feeding and encouraging rumen development of calves by fermentation of solid feeds (Khan *et al.* 2011).

Dairy calves are born as pseudo-monogastrics with a non-functional rumen and they initially rely on milk to receive their required energy for maintenance and growth (Baldwin *et al.* 2004). Unfortunately, dairy calves are at a great risk of morbidity and mortality during the milk feeding period (Khan *et al.* 2016). Traditionally, weaning period take a long about 10 months when calves reared with their dams. In commercial dairy production systems, feeding milk is costlier than feeding solid feed and shifting calves from milk to solid feed as early as possible has traditionally seemed as a way to diminish feed costs. Time of weaning is a significant point in dairy calf rearing and weaning age can influence calf performance and health parameters directly. Calves on liquid-based diets are more susceptible to diarrhea and other digestive problems and reducing this period may decrease the incidence of digestive problems of suckling calves (Khan *et al.* 2007). There are scientific reports showing that early weaning can increase the concentration of short-chain fatty acids in the rumen by encouraging solid feed intake and stimulate rumen development physically and morphologically (Anderson *et al.* 1987; Eckert *et al.* 2015).

Martin *et al.* (1959) indicated that calves are able to use volatile fatty acids as energy sources at 3 weeks of age. With the development of rumen, the neonatal calves become dependent on the products of rumen fermentation and volatile fatty acids become their main energy source (Paez Lama *et al.* 2014). Calves fed milk replacer and weaned early had higher and earlier starter intake and lower glucose, possibly as an indicator of rumen development (Cheema *et al.* 2016). However, if early weaning program is performed with poor management, it may lead to decrease growth and poor calf health (Winter, 1985). Moreover, any decrease in weaning age could enhance calf's health, lessen diarrhea and thus improve performance. Rashid *et al.* (2013) reported that buffalo calves can be weaned successfully at 8 weeks without compromising their growth performance. Tao *et al.* (2018) investigated the effects of weaning age on growth performance, feed efficiency, nutrient digestibility and blood-biochemical parameters in Drought master crossbred beef calves and showed that the weaning of calves at 6 weeks of age gave positive results.

Although, average weaning is 9 weeks of age, dairy farmers commonly prefer longer weaning ages, especially for replacing female calves. Early weaning methods are performed by encouraging solid feed intake and restricting milk feeding levels. Little information exists on the effects of different weaning ages in restricted step-up and step-down milk feeding programs. Therefore, the aim of this study was to determine the effects of different early weaning programs on the growth performance, and blood parameters of replacing female Holstein calves fed on restricted step-up and step-down milk feeding program.

MATERIALS AND METHODS

Animals, housing and diet

The current study was carried according to the guidelines of the Iranian Council of Animal Care (1995). Thirty newborn Holstein female calves with average birth weight 36 ± 5 kg and age 1-7 days were selected from Moghan Agro-Industrial and Animal Husbandry dairy herd and allocated to the experimental groups randomly. All calves received approximately 4 kg of colostrum initially and reared by one of three weaning programs. Experimental groups were: 1) starter diet and whole milk from birth to 6 weeks and weaning at 42 d old (6WW), 2) starter diet and whole milk from birth to 9 weeks and weaning at 63 d old (9WW) and 3) starter diet and whole milk from birth to 12 weeks and weaning at 84 d old (12WW). In 6WW group, calves were fed with 4 kg of whole milk per day in two meals for the first two weeks, 6 kg per day in two meals for third and fourth weeks, 4 kg per day for fifth week in two meals and 2 kg per day in one meal for sixth week (Figure 1).

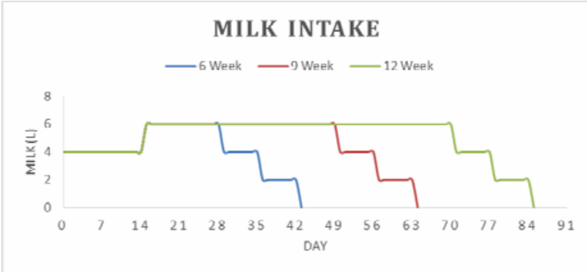

Figure 1 Milk feeding programs in three weaning methods

In 9WW group, calves received 4 kg of whole milk per day in two meals for the first two weeks, 6 kg per day in two meals from third to seventh week, 4 kg per day for eighth week and 2 kg in one meal for ninth week. Calves in 12WW group were offered with 4 kg of whole milk per day in two meals for the first two weeks, 6 kg in two meals from third to tenth week, 4 kg per day for eleventh week in two meals and 2 kg in one meal for twelfth week. Calves were housed in individual pens (2×1.2 m) bedded with chopped straw that were replenished every day. Calves received milk in two equal meals daily at 08:00 and 18:00 from buckets and had continuous *ad libitum* access to water and a calf starter mix (90% starter feed and 10% chopped alfalfa hay). The nutrient compositions and chemical analysis of the starter feed were shown in Table 1.

Data collections and measurements

Throughout the experiment, calves were weighed biweekly in the morning individually without prior deprivation of feed and water and weight change was calculated by subtraction. During the experiment, starter intake was measured daily by difference between feed offered and feed refused. Metabolizable energy (ME) intake of calves from milk and starter was considered as total ME intake. The ME intake to gain ratio was calculated by dividing the ME intake on average daily gain of each calf.

All calves were measured for growth biometric parameters including body length, heart girth, and wither height from birth to 84 d, monthly. Wither height was measured by using a sliding-scale height stick.

Table 1 Ingredients and chemical composition of the starter feed

Ingredients (% of DM)		Chemical composition (% of DM)	
Ground corn	44	Dry matter	89
Ground barely	14	Metabolizable energy (Mcal/kg)	3.26
Ground wheat	2	Crude protein	18.1
Soybean meal	37.8	Ether extract (EE)	3.3
Salt	0.2	Acid detergent fibre (ADF)	5.8
Limestone	1	Neutral detergent fibre (NDF)	12.9
Calf mineral/vitamin premix[1]	1	Ash	6.2
-	-	Calcium	0.7
-	-	Phosphorus	0.4

[1] Mineral and vitamin premix provided per kg of diet: Cu: 3300 mg/kg; Fe: 100 mg; Zn: 16500 mg/kg; Mn: 9000 mg; Co: 90 mg/kg; Se: 90 mg/kg; vitamin A: 200000 IU; vitamin D_3: 300000 IU; vitamin E: 10000 IU; vitamin K: 2 mg and Anti-oxidant: 1000 mg/kg.

Heart girth and body lengths were measured using a strip meter (Dairy Innovations, Attica, NY).

Blood samples were taken from the jugular vein on the end of the trial (d 84) before morning feeding into heparinized tubes. Plasma was collected by centrifuging blood samples at 3500 × g for 15 min at 4 ˚C, and stored at -20 ˚C until analysis.

Plasma concentrations of glucose, cholesterol, total protein, albumin and globulin were measured calorimetrically by using the commercial kits (Pars Azmoon co, Tehran, Iran).

Feed samples were dried in a forced-air oven at 65 ˚C for 48 h and grounded by a laboratory mill (1 mm) and analyzed for ash and Kjeldahl N by AOAC (1997) methods and for neutral detergent fibre (NDF) and acid detergent fibre (ADF) based on Van Soest *et al.* (1991).

Statistical analysis

Data of feed intake, average daily weight gain, and body growth parameters were analyzed in a completely randomized design as repeated measurements using the MIXED procedure of SAS statistical software (SAS, 2003). The model included the fixed effects of treatment, time and their interactions and the random effect of calves within treatment according to the following model:

$$Y_{ijkl} = \mu + W_i + T_j + TW_{ij} + A_k(W_i) + E_{ijkl}$$

Where:
Y_{ijk}: dependent variable.
μ: mean.
W_i: effect of weaning age (treatment).
T_j: effect of the time.
TW_{ij}: interaction effect of weaning by time.
$A_k(W_i)$: random effect of calves within treatment.
E_{ijkl}: residual error.

Least squares means were determined for each treatment and the Tukey-Kramer test of SAS was selected to declare the differences among treatments.

Initial body measurements were considered as covariates for the final weight and growth analysis. Data of blood metabolites were analyzed using general linear method (GLM) produce of SAS in a completely randomized design and significant differences were declared at $P \leq 0.05$ and trend to be significant was defined at $0.05 < P \leq 0.15$.

RESULTS AND DISCUSSION

Calves performance data are presented in Table 2. BW at 14, 28, 42, 56, and 70 d did not differ between treatments whereas final BW at d 84 was significantly higher in 12 weeks weaned calves (P<0.05). Average daily gain (ADG) of calves during different time periods were not significantly different among different weaning ages. However, ADG of calves from 56 to 84 d tended to be affected by weaning time, so, calves in 12WW group have higher ADG than calves reared in 6WW and 9WW groups (P<0.15). Average starter intake did not differ among experimental treatments till 42 d and thereafter calves weaned at d 42 had higher starter intake than other calves, significantly (P<0.05). Except at d 70, calves in 9WW and 12WW had similar starter intake. Calves reared in 6WW group reduced their total ME intake when offered milk stepped down after d 35 (Figure 2). Thereafter, they could compensate their ME intake by increasing their starter intake. Calves on 9WW treatment could not compensate their ME intake from milk by solid starter till 77 d.

Mean body length, heart girth and wither height at birth, 28, 56 and 84 d of calves weaned at different times are shown in Table 3. There were no significant effects of different weaning ages on growth measurement of calves.

The results for blood metabolite concentrations are presented in Table 4. Glucose concentration was similar among treatments whereas blood cholesterol was affected significantly by weaning age, as late weaned calves (12WW) had higher cholesterol concentration (P<0.01). Calves in 12WW tended to had higher total protein (P=0.06) and globulin concentrations (P=0.09).

No difference was observed in blood albumin concentration among experimental treatments.

Table 2 Performance data of calves in different weaning groups

Item	Weaning groups*			SEM	P-value
	6WW	9WW	12WW		
Number of calves	9	10	10		
Body weight (kg)					
At birth (kg)	36.00	35.00	36.00	0.99	0.68
At d 14	40.66	40.70	42.30	0.86	0.31
At d 28	45.7	46.30	49.0	1.37	0.22
At d 42	54.10	53.10	56.30	1.67	0.37
At d 56	62.50	60.10	63.70	2.26	0.49
At d 70	74.70	72.70	78.30	2.21	0.18
At d 84	86.60[ab]	84.80[b]	92.70[a]	2.51	0.05
Average daily gain (kg/d)					
At d 14	0.308	0.382	0.393	0.04	0.30
At d 28	0.332	0.365	0.434	0.05	0.47
At d 42	0.562	0.473	0.499	0.04	0.39
At d 56	0.562	0.473	0.499	0.04	0.38
At d 70	0.811	0.823	0.966	0.05	0.11
At d 84	0.814	0.810	0.974	0.48	0.13
Starter intake (kg/d)					
At d 14	300.3	331.1	266.2	41.58	0.55
At d 28	519.3	474.7	559.8	41.06	0.35
At d 42	778.4	739.9	814.2	75.12	0.78
At d 56	2512.6[b]	2873.2[a]	2296.0[b]	78.33	0.001
At d 70	3698.6[a]	3689.3[a]	3573.8[b]	34.52	0.03
At d 84	4962.6[a]	4782.1[b]	4729.6[b]	59.63	0.02
ME:gain					
At d 14	17.79	15.88	16.98	0.94	0.29
At d 28	19.73	19.40	18.37	2.56	0.93
At d 42	7.12[b]	13.77[a]	13.79[a]	1.07	0.002
At d 56	14.00[b]	25.89[a]	23.22[a]	2.15	0.001
At d 70	14.15	14.52	15.71	1.10	0.61
At d 84	18.99	18.88	16.75	1.28	0.41
ME intake (Mcal/d)					
At d 14	4.71	4.75	4.63	0.13	0.81
At d 28	6.08	5.48	6.78	0.44	0.16
At d 42	4.67[b]	6.06[a]	6.35[a]	0.23	0.001
At d 56	7.79[b]	11.19[a]	10.96[a]	0.28	0.001
At d 70	11.46[b]	11.39[b]	14.68[a]	0.11	0.001
At d 84	15.38[ab]	14.86[b]	15.85[a]	0.18	0.003

* The interaction between times at weaning was not significant for any of the evaluated data.
6WW: starter diet and whole milk from birth to 6 weeks and weaning at 42 d old; 9WW: starter diet and whole milk from birth to 9 weeks and weaning at 63 d old and 12WW: starter diet and whole milk from birth to 12 weeks and weaning at 84 d old.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

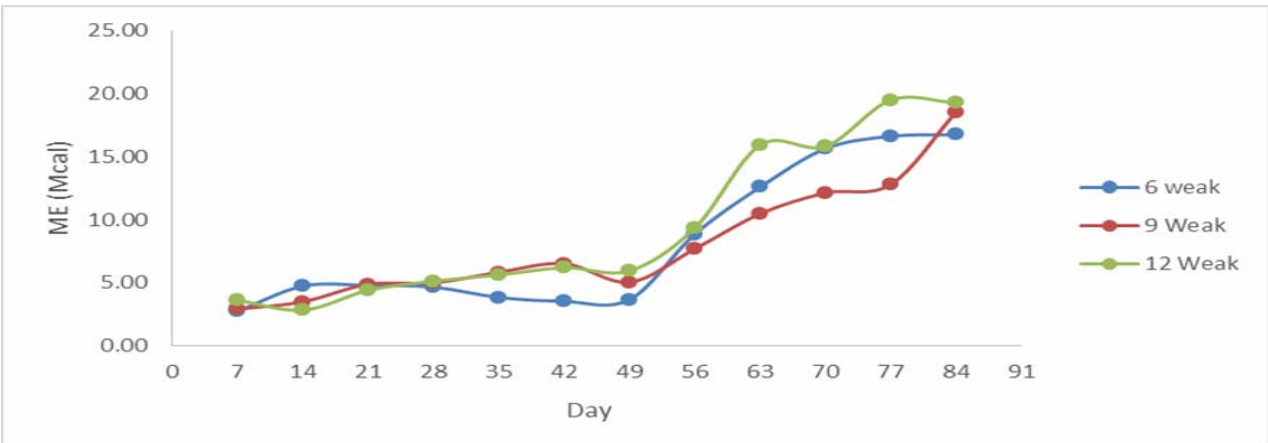

Figure 2 Estimated daily ME intake of calves weaned in three weaning methods

To obtain maximum growth and daily gain of replacing calves, dairy farmers prefer traditionally to feed their calves with milk for long periods. However, early weaning has been recommended in the dairy industry to accelerate rumen development, reducing costs, labours and diarrhea incidence (Kertz *at al.* 1979; Quigley and Caldwell, 1991). There are two main weaning method including starter intake based and age based weaning methods (Greenwood *et al.* 1997; Khan *et al.* 2007).

In the current study, the effects of three age dependent weaning programs on growth performance of replacing female calves were examined. Calves reared in three weaning groups had statistically similar ADG and shortening the milk feeding period from 84 to 42 days did not affect weight gain of calves. Also, in the present study, the average daily gain of late weaned calves was higher than in the early weaned calves. However, Rashid *et al.* (2013) reported that buffalo calves weaned at the 8th, 10th and 12th week of their life displayed similar growth rates (387-401 g/day) up to 12 weeks of age. Abdullah *et al.* (2013) reported that the average daily gain of buffalo calves fed whole milk at 10% of body weight up to day 56 and weaned at 120 days was 457 g/day. Azim *et al.* (2011) has reported an average growth rate of 520 g/day in buffalo calves weaned at day 45 and offered early weaning diets.

Eckert *et al.* (2015) observed that calves weaned at 8 weeks had higher ADG than calves weaned at 6 weeks. However, they fed the calves with a higher plane of milk nutrition than our study (8 L versus 6 L per day). Weaning is the shift from away the liquid to solid feed and a smooth transition allows calves to consume and digest sufficient solid feed to support growth during and after weaning. Restricted and step down milk feeding strategies were used to encourage enough starter intake and smooth transition in age based weaning methods (Eckert *et al.* 2015; Khan *et al.* 2016). In this study, all calves consumed enough starter feed to wean at d 42 based on starter intake dependent weaning method (Greenwood *et al.* 1997) so, calves in 6WW, 9WW and 12WW groups ate 1.44, 1.39 and 1.45% of their BW, respectively. Greenwood *et al.* (1997) demonstrated that calves fed low plane of nutrition could be successfully weaned when starter intake reaches 1% of BW.

De Passill *et al.* (2011) reported that starter intake was low in Holstein calves before weaning due to high milk feeding. Studies on rumen development of calves indicate that concentrate feeding helps in earlier rumen papillae development than seen with hay; however, the physical form and chemical composition of starter diet are also important (Khan *et al.* 2016).

At present study, due to restricted milk that offered, all calves consumed noticeable starter from the first weeks of the life and similar intake was observed till 42 d. After d 42, calves in 6WW group due to weaning and in 9WW group due to start stepping down of milk had higher starter intake. However, the total ME intake was lower in calves in 6WW program at 42 d that was due to their deprivation from milk. These calves, also, had higher starter intake at the end of experiment when all calves had been subjected to weaning. At first weeks of the life, calves rely exclusively on milk to meet their nutrients requirements and they have to be encouraged to consume solid feed as early as possible to wean successfully (Khan *et al.* 2016). The onset of solid feed intake and the amount of solid feed that consumed can influence rumen development. Highly palatable starter feeds, containing easily fermentable carbohydrates, are thought to stimulate physical and metabolic development of the rumen and coincide with development of salivary apparatus, rumination behaviour, and several physiological adjustments at the gut, hepatic and tissue levels (Baldwin *et al.* 2004).

In the present study, because all calves had enough starter intakes from first weeks, shortening the milk feeding periods from 12 weeks to 6 weeks did not influence the gain performance of calves. Kehoe *et al.* (2006) reported that calves weaned at 5 or 6 weeks of age had lower starter intake than calves weaned after 3 or 4 weeks whereas similar ADG and structural measurements were observed from weeks 1 to 8 among different weaning age groups. Bjorklund *et al.* (2013) reported that ADG for weaning groups was lower for the early weaned calves (weaned at 4 weeks of age; 0.51 kg/d) in comparison to the mid (weaned at 6 weeks of age; 0.63 kg/d) and late weaned calves (weaned at 8 weeks of age; 0.75 kg/d). However, other researchers, in agreement with our results, reported no difference in ADG of calves weaned earlier. Appleman and Owen (1974) reported that weaning at 6 or 8 weeks of age had no effect on daily gain of calves. In addition, Winter (1985) observed similar growth measurements among calves weaned at 3, 5 or 7 weeks. However, Kehoe *et al.* (2006) concluded that calves weaned earlier require more attention during weaning to stimulate starter intake than calves weaned later.

Encouraging earlier and higher starter intake is a key management tool to have a successful early weaning program. Previous studies have reported that restricted milk feeding program encourages solid feed intake (Appleby *et al.* 2001; Jasper and Weary, 2002) and this has been considered a key contributor to the rumen development (Baldwin *et al.* 2004).

In addition, stepping down the amount of milk fed during last weeks of suckling periods enhances solid feed intake resulting in stimulation of rumen development and improvement of growth characteristics (Sweeney *et al.* 2010; Khan *et al.* 2007).

Table 3 Body measurements of calves in different weaning groups

Item	Weaning groups*			SEM	P-value
	6 WW	9WW	12WW		
Number of calves	9	10	10		
Body length (cm)					
At birth	65.0	64.6	63.1	0.96	0.38
At 28 d	71.5	72.5	67.0	1.50	0.11
At 56 d	76.8	78.1	79.5	1.90	0.17
At 84 d	88.7	90.1	91.6	1.72	0.79
Change from birth to 84 d	23.7	25.5	28.5	1.60	0.64
Heart girth (cm)					
At birth	77.8	77.5	77.7	1.20	0.86
At 28 d	88.7	89.5	85.8	2.14	0.63
At 56 d	99.3	98.7	101.7	2.08	0.27
At 84 d	107.7	106.4	106.4	1.35	0.77
Change from birth to 84 d	29.8	28.9	28.7	1.75	0.87
Withers height (cm)					
At birth	65.0	64.6	61.3	1.90	0.73
At 28 d	71.5	72.5	68.6	1.91	0.14
At 56 d	76.8	78.1	79.5	1.85	0.99
At 84 d	88.7	90.1	91.6	1.10	0.92
Change from birth to 84 d	19.6	18.9	19.6	1.11	0.85

* The interaction between times at weaning was not significant for any of the evaluated data.
6WW: starter diet and whole milk from birth to 6 weeks and weaning at 42 d old; 9WW: starter diet and whole milk from birth to 9 weeks and weaning at 63 d old and 12WW: starter diet and whole milk from birth to 12 weeks and weaning at 84 d old.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 4 Plasma concentration of glucose, cholesterol, total protein, albumin and globulin of calves in different weaning groups

Item	Weaning groups*			SEM	P-value
	6WW	9WW	12WW		
Number of calves	9	10	10		
Glucose (mg/dL)	73.2	73.8	92.4	6.63	0.43
Cholesterol (mg/dL)	65.8^{b}	74^{ab}	88.8^{a}	4.78	0.02
Total protein (g/dL)	4.21	5.63	5.40	0.27	0.06
Albumin (g/dL)	3.71	3.66	3.79	0.08	0.82
Globulin (g/dL)	1.04	1.96	1.60	0.17	0.09

* The interaction between times at weaning was not significant for any of the evaluated data.
6WW: starter diet and whole milk from birth to 6 weeks and weaning at 42 d old; 9WW: starter diet and whole milk from birth to 9 weeks and weaning at 63 d old and 12WW: starter diet and whole milk from birth to 12 weeks and weaning at 84 d old.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Therefore, restricted milk feeding and stepping down milk provision during the weeks leads to weaning are two strategies to encourage solid feed intake. At the present study, calves weaned in different weaning age groups had similar growth performance by using these two management tools. Hopkins *et al.* (1993) fed restricted amount of whole milk (3.8 L per day) to calves that were weaned at 28 or 56 d and observed that 28 d weaned calves consumed more starter feed after d 28 and they had body weight at 90 d similar to 56 d weaned calves. In our study, 6WW and 9WW calves started to consume more starter feed than 12WW calves after d 56 that was coincided with two weeks after weaning of 6WW calves and last step down week in 9WW group. This increase in starter consumption could compensate the removal of ME intake from milk in early weaned calves (calves in 6WW and 9WW) and resulted in similar ME intake among different weaning groups during final week of the experiment.

At present study, glucose concentration of calves was not different across treatments that is in line with the results of Kehoe *et al.* (2006). In suckling calves, the primary source of energy is glucose that generally comes from the intestinal digestion of milk lactose. Initiation of solid feed consumption starts rumen fermentation and therefore volatile fatty acids production that will replace with glucose to support energy demands (Baldwin *et al.* 2004). In weaned calves with developed rumen, more than 50 percent of blood glucose synthesized by gluconeogenesis pathway from non-carbohydrate substrates, especially rumen derived propionate. Calves weaned at 12 weeks of age had higher cholesterol concentration than 6 and 9 weeks weaned calves.

Cholesterol is a lipid molecule and is transported through the blood circulation in lipoprotein particles. Generally, high blood concentration of cholesterol shows its high dietary consumption or high cholesterol biosynthesis due to higher fat consumption and more lipidemic diets (Murray *et al*. 2006). Therefore, higher blood cholesterol of 12WW calves may be attributed to their higher whole milk consumption that is a more lipidemic diet due to its high fat content per dry matter.

In addition, calves in 12WW group had higher blood total protein and globulin concentrations. Globulin can inactivate and degrade antigen proteins in the body and its content can reflect the immune status of animal (Li *et al*. 2008). Chai *et al*. (2017) reported that serum globulin concentration in early-weaned lambs was higher than that in ewe-reared lambs. This may be due to the difference in the source of amino acids supply for calves in different groups. So, 12WW calves obtained some of their amino acid requirements from milk proteins whereas other calves had no access to milk and all of reached amino acids to the intestines were originated from solid feed directly or indirectly from its fermentation in the rumen.

CONCLUSION

The results of this study showed that by using restricted milk feeding regimes and step down weaning as two management tools for performing early weaning programs, calves who weaned at 6, 9 or 12 weeks of age had similar ADG, BW and body dimensions at 84 days of age. Calves weaned earlier on 6 weeks could compensate their ME intake from milk by solid feed 2 weeks after weaning and had more starter feed intake on 84 d. Based on results of this study, early weaning methods can be used successfully for replacing calves by using restricted milk feeding and step-down weaning methods.

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