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Serum Protein of Female Holstein Calves Fed Milk through Step-Down and Conventional Methods

Research Article

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ABSTRACT

The serum proteinograms of 10 female Holstein calves fed milk either through conventional (COV) or step-down (STP) milk feeding methods at different ages were evaluated. Calves fed using the COV method were fed colostrum during the first 24 hours that they stayed with their respective mothers and received transition milk for another 2 days and later, and then milk at four liters/day for 59 days. Calves fed using the STP method were fed colostrum and then milk as follows: Six liters of milk/day from days six to 25 after birth, four liters of milk/day from days 26 to 45, and two liters of milk/day from days 46 to 59 after birth. Blood sampling was performed at 7, 21, 28, 42, 49 and 56 days of age. For calves fed using either of the two milk feeding methods, the serum concentration of albumin increased, while the serum concentration of β-globulin decreased with increasing calf age. Calves fed using the COV method had higher serum total protein, globulin, β-globulin, and γ-globulin concentrations, while the concentrations of A/G, α1-globulin, and α2-globulin were higher for calves in the STP group. These results are important for establishing the serum protein profile of calves, which is significantly influenced by milk feeding methods and calf age.

KEY WORDS artificial feeding, dairy cattle, electrophoretic serum protein profile, ruminants.

INTRODUCTION

In the conventional method (COV) of milk feeding dairy calves, the liquid diet is supplied in a constant quantity equivalent to 10% of the animal's body weight, divided into two meals per day. However, this system meets little more than maintenance requirements, provides low weight gain and food efficiency, and results in behavior suggestive of hunger (Gomes *et al*. 2014).

The step-down milk feeding method (STP) provides greater milk volume than the COV method. Consequently, feeding calves using the STP method improved the gain, feed efficiency, and prevented the problems associated with depressed solid feed intake compared to the COV method (Azevedo *et al*. 2014; Ozkaya, 2014). However, these methods did not influence the dry matter ingestion and performance of calves during the pre-weaning and post-weaning periods (Daneshvar *et al*. 2015).

The milk feeding method can promote changes in the metabolic, endocrine, and immunological status of dairy calves (Khan *et al*. 2016), which would influence the profile of serum protein fractions (PSPF). The analysis of proteinograms by electrophoresis is a useful tool to diagnose the nutritional and immunological status of animals (Schade *et al*. 2016; Lisbôa *et al*. 2016). However, the PSPF parameters in young calves need to be better established, as

well as the influence of artificial milk supply or age on these parameters (Tóthová *et al*. 2016). Therefore, we evaluated the serum proteinograms of female Holstein calves fed using conventional or step-down milk feeding methods at different ages.

MATERIALS AND METHODS

Animals, housing, and management

The procedures adopted in this study were approved by the ethics committee for the use of animals CEUA of the Federal University of Minas Gerais, Brazil, under protocol number 178/2011. Female Holstein calves (n=10) with an average birth weight of 37.53 kg (±2.36 kg), were separated from their mothers 24 h after birth, randomly transferred to individual pens (1.2×2.0 m) and fed colostrum *ad libitum* for the first two days. Pens had solid iron rod sides, with openings to allow calves free access to starter ration, grass hay, (*Cynodon* sp.) and water.

Experimental procedures

The calves were distributed in the treatments in a completely randomized design. They were adapted to the treatments from the third to the fifth day after birth. The liquid diet was supplied at 8:00 and 16:00 in two equivalent daily meals. Before feeding, the milk was warmed in a water bath to raise its temperature to 39 ± 0.5 ˚C. All calves were fed milk using mobile plastic bottles (2 L capacity), fitted with soft rubber nipples.

For the COV feeding method, the calves were fed four liters of milk daily for the entire period of 59 days. For the STP feeding method, six liters of milk/day were provided from days six to 25 after birth, four liters of milk/day were provided from days 26 to 45 after birth, and two liters of milk/day were provided from days 46 to 59 after birth. Milk samples were analyzed using the official methods described in the Instruction Regulations Number 68, of April 14, 2006 (Brazilian Ministry of Agriculture, Livestock and Food Supply, 2006), and milk samples were found to contain an average of 3.0% crude protein, 4.7% fat, 4.4% lactose, and 12.0% total solids. Calves were weighed at birth, at the beginning of the experiment, and then at 7-d intervals throughout the study. Milk consumption, starter ration, and hay intakes were measured daily.

Blood sample collection and analysis

Jugular blood samples were collected 30 min before morning feeding on days 7, 21, 28, 42, 49, and 56 in evacuated tubes (5 mL), which were immediately transported at 4 ˚C to the laboratory. These samples were centrifuged at 1500 rpm for 15 minutes, followed by removal of serum, which was stored in eppendorf tubes at -20 ˚C.

The total serum protein concentrations were determined by the biuret method using a UV spectrophotometer (NOVA® 1600/1800UV). The serum albumin (ALB) concentrations were measured using commercial kits (Bioclin®–QUIBASA QUÍMICA BÁSICA Ltda, Belo Horizonte, MG, Brasil) and the concentration of globulin (GLO) was calculated as the difference between total protein and ALB. The ALB/GLO ratio was calculated according to Kaneko *et al*. (2008). The PSPF were obtained by horizontal electrophoresis on 12% agarose gel (CELMGEL®) and with TRIS buffer, for 30 minutes. The gels were stained for 5 minutes in 200 mL of 0.1% starch black, after de-staining in acetic acid (7%) and ethanol until the bottom of the gel was clear. The gels were read with CELM SE-250® software. Relative protein concentrations within each fraction were determined as the optical absorbance percentage, and absolute concentrations (g/dL) were calculated using the total protein concentration. The major protein fractions were divided from cathode to anode as albumin, alpha 1, alpha 2, beta, and gamma-globulins, respectively, according to the manufacturer's instructions.

Data analysis

The blood parameters were analyzed in subplots for two methods of milk feeding (COV and STP) and six ages (7, 21, 28, 42, 49, and 56 days). The means of quantitative variables (ages) were submitted to polynomial regression analysis, using the system for statistical and genetic analysis (SAEG) version 9.1, 2007.

RESULTS AND DISCUSSION

In this study the COV and STP feeding methods respectively promoted abrupt or gradual weaning of dairy calves and the starter ration intake, hay intake, total dry matter intake and weight gain were not significantly affected (P>0.05) by these milk feeding methods, or by the interaction between these methods and age (Table 1). These results are similar to those reported by Ozkaya (2014) when comparing two different breastfeeding schemes for Dutch calves from birth to weaning at 56 days of age. This author noted that calf performance and health were not affected by breastfeeding. In the present study, STP calves did not increase feed intake or hay with a reduction in milk supply from 46 days of age. A linear increase in solid diet intake was expected, as the supply of liquid diet was reduced according to Eckert *et al*. (2015); however, the results found may be justified by the amount of liquid diet offered, since this together with the solid diet was sufficient to meet the demand of the animals, as evidenced by the absence of hunger-inducing behaviors such as vocalization and stress (Khan *et al*. 2016; Silper *et al*. 2014).

Table 1 Starter ration intake (RI), hay intake (HI), dry matter intake (DMI), and total weight gain (TWG) of female calves fed milk either through conventional (COV) or step-down (STP) methods at different ages

Milk feeding method	RI (g DM day^{-1})	HI (g DM day^{-1})	DMI (g DM day^{-1})	TWG (kg)
COV	478.34	147.93	634.90	37.50
STP	550.52	139.12	698.76	38.46
CV (%)	15.73	13.92	14.18	3.63
SEM	80.93	19.98	94.57	1.38

CV: coefficient of variation and SEM: standard error of the means.

Calves fed using the COV method had higher concentrations of total serum protein and GLO, (P<0.05) than calves fed using the STP method (Table 2). Both groups were fed a similar amount and period of colostrum, so these total serum protein and GLO results may be related to other factors such as success in passive immunity transfer because it depends on the concentration of immunoglobulins in colostrum, ingested volume, time interval between birth and ingestion, sanitary quality of colostrum and absorption capacity. Being that the quality of the colostrum is related to the volume produced, previous contact of the cow with pathogens and the duration of the dry period may have had an influence, just as the absorptive capacity depends on the good physiological condition of the calf at birth. In this way, the differences observed in the current work should be related to the longer time spent by the calf to raise and suck the colostrum. Moreover, the microbiological quality of colostrum is also an important factor, because bacteria in colostrum can reduce the intestinal absorption of macromolecules such as immunoglobulins (Biburger *et al*. 2014).

The calves having total serum proteins above 5.5 g/dL (Kaneko *et al*. 2008) is considered a sufficient intake of colostrum, and the animals of the VOC treatment presented a sufficient colostrum intake when compared to the STP animals be tested against the values of total serum protein according Table 2.

For both calf groups, a linear increase with age (P<0.05, Table 2) was observed for the serum ALB concentration, which can be related to the increase of solid diet intake in calves fed with both methods. This result is in agreement with other studies which reported a progressive increase in serum ALB concentrations in calves from birth until 24 weeks of age (Tóthová *et al*. 2016).

In order to maintain the osmotic blood balance, the ALB and GLO concentrations are inversely proportional (Kaneko *et al*. 2008), which explains the increasing ALB and decreasing β-globulin concentrations with increases in calf age observed in this study (Table 2). The values of the variables analyzed are within the range of normal variation for calves from birth to 45 days of life. Albumin is synthesized in the liver and its concentration can be altered by the protein in the diet liquid or feed, and the same is true for urea.

However, albumin is a useful indicator when the protein deficit is longer, since the albumin deficit is later and less intense when compared to urea (Delfino *et al*. 2014). In this way, the results found show no protein deficit in the offered diets.

The ALB/GLO ratios were higher in calves fed using the STP method (Table 2). This was most likely due to lower globulin concentrations in these calves, as the albumin concentration did not differ between the milk feeding methods.

After birth, many animal species have low concentrations of plasma proteins due to the minimal amounts of GLO and low concentrations of ALB (Kaneko *et al*. 2008; Seifzadeh *et al*. 2019). When newborn mammals consume colostrum, a rapid increase in immunoglobulins occurs as a result of the absorption of colostrum immunoglobulins, yet the increase in ALB concentration is due to the ingestion of protein in the diet. The reduction of ALB promotes a decrease in blood osmotic pressure, and can be observed in the case of inflammatory processes and infectious disease (Teixeira *et al*. 2014), although these did not occur in the present study.

Calves fed using the COV method had higher concentrations of serum β-globulin and γ-globulin (P<0.05) than calves fed using the STP method (Table 2). In calves fed using the COV method, serum concentrations of β-globulins decreased in a linear trend as calf ages increased (P<0.05, Table 2). The reduction of β-globulins recorded in the present study does not indicate infection or liver problems, but may be a sign of stress caused by changes in diet or due to degradation of passively transferred immunoglobulins.

These results can be justified by the absorptive capacity, since this depends on the physiological condition of the calf at birth. The differences observed in the present study should be related to the longer time spent by the calf to raise and suck colostrum, as a similar result was observed in the concentrations of total proteins and globulins.

A significant increase in colostrum intake is observed when calves present total serum proteins above 5.5 g/dL (Kaneko *et al*. 2008), therefore the animals of the VOC treatment presented a sufficient intake of colostrum when compared to the PPS animals because they presented higher concentrations than those recommended.

Table 2 Serum concentrations of total protein, albumin, globulin, albumin/globulin ratio, α_1-globulin, α_2-globulin, β-globulin, and γ-globulin (g/dL) of female calves fed milk either through conventional (COV) or step-down (STP) methods at different ages

Milk feeding Method	Age (days) 7	21	28	42	49	56	Average	CV (%)
Total protein (g/dL)								
COV	6.68	6.08	6.24	6.16	6.48	6.24	6.31[a]	7.47
STP	5.68	5.36	5.40	5.88	5.54	5.84	5.61[b]	
Albumin[1] (g/dL)								
COV	2.87	3.05	3.02	3.19	3.40	3.26	3.13	12.68
STP	2.73	3.11	2.95	3.03	2.68	3.53	3.00	
Globulin (g/dL)								
COV	3.78	3.02	3.21	2.97	3.07	2.97	3.17[a]	23.03
STP	2.94	2.25	2.44	2.85	2.85	2.30	2.60[b]	
Albumin/globulin ratio								
COV	0.78	1.02	1.03	1.11	1.20	1.15	1.04[b]	30.22
STP	0.96	1.40	1.22	1.22	1.00	1.54	1.22[a]	
α_1-globulin (g/dL)								
COV	0.52[a]	0.46[a]	0.51[a]	0.47[a]	0.36[b]	0.43[a]	0.45[b]	18.55
STP	0.43[a]	0.52[a]	0.54[a]	0.58[a]	0.57[a]	0.48[a]	0.52[a]	
α_2-globulin (g/dL)								
COV	0.50	0.46	0.47	0.52	0.35	0.49	0.46[b]	22.65
STP	0.52	0.56	0.48	0.48	0.49	0.45	0.49[a]	
β-globulin[2] (g/dL)								
COV	0.94	0.84	0.75	0.79	0.79	0.78	0.81[a]	13.79
STP	0.79	0.66	0.75	0.68	0.66	0.69	0.71[b]	
γ-globulin (g/dL)								
COV	1.82	1.25	1.46	1.17	1.56	1.25	1.41[a]	45.48
STP	1.19	0.49	0.66	1.09	0.86	0.67	0.82[b]	

[1] Albumin concentration= 0.0086 × age + 2.7722, R^2= 12.36%.
[2] β-globulin concentration= - 0.0022 × age + 0.8417, R^2= 10.86%.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
CV: coefficient of variation.

The interaction between age and milk feeding methods was significant (P<0.05) for serum α1-globulin concentrations. This concentration was higher for Holstein female calves fed using the STP method at 49 days (Table 2). This result could be associated with the increasing solid diet consumption in calves fed using STP method, which was stimulated by restricting the milk supply to two liters of milk per day. The serum γ-globulin concentrations in calves in the COV group were higher than in calves in the STP group, although no difference was observed between the evaluated ages.

This globulin is associated with the control of bacterial and viral infections or hepatic alterations. However, no visible signs of diarrhea or hepatitis were observed in calves in the COV group. Low levels of serum γ-globulin usually indicate immunological deficiency, which was not verified in this study (Table 2). The present study emphasizes the importance of establishing reference intervals for PSPF of ruminants according to age categories, since these variables reflect the animals' health condition and can be directly influenced by intrinsic and extrinsic factors such as age, breed, sex, food management, sanitary management and environmental conditions (Cheema *et al*. 2016; Leite *et al*. 2017; Paez *et al*. 2014).

CONCLUSION

We observed an adequate nutritional and immunological status in calves fed an artificial milk supply using COV or STP methods, together with an appropriate solid diet. We demonstrated that the type of artificial milk supply method influences the profile of serum protein fractions of calves for the first time, which may contribute to improving the nutritional status and health of these animals.

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REFERENCES

Azevedo R.A.D., Rufino S.R.D.A., Duarte D.V.L., Soares A.C.M. and Geraseev L.C. (2014). Desempenho de bezerros leiteiros em aleitamento artificial convencional ou fracionado. *Rev. Bras. Saúde Prod. Anim.* **15,** 237-247.

Biburger M., Lux A. and Nimmerjahn F. (2014). How immunoglobulin Gantibodies kill target cells: revisiting an old paradigm. *Adv. Immunol*. **124,** 67-94.

Cheema A.T., Bhatti S.A., Akbar G., Wynn P.C., Muhammad G., Warriach H.M., McGill D. and Forthcoming M. (2016). Effect of weaning age and milk feeding level on pre- and post-weaning growth performance of Sahiwal calves. *Anim. Prod. Sci*. **58,** 314-321.

Daneshvar D., Khorvash M., Ghasemi E., Mahdavi A.H., Moshiri B., Mirzaei M., Pezeshki A. and Ghaffari M.H. (2015). The effect of restricted milk feeding through conventional or step-down methods with or without forage provision in starter feed on performance of Holstein bull calves. *J. Anim. Sci*. **93,** 3979-3989.

Delfino J.L., Barbosa V.M., Gondim C.C., Oliveira P.M., Nasciutti N.R., Oliveira R.S.B.R., Tsuruta S.A., Mundim A.V. and Saut J.P.E. (2014). Perfil bioquímico sérico de bezerros Senepol nos primeiros 120 dias de idade. *Semina, Ciênc. Agrárias.* **35,** 1341-1350.

Eckert E., Brown H.E., Leslie K.E., DeVries T.J. and Steele M.A. (2015). Weaning age affects growth, feed intake, gastrointestinal development, and behavior in Holstein calves fed an elevated plane of nutrition during the preweaning stage. *J. Dairy Sci*. **98,** 1-12.

Gomes I.P.O. and Arruda Córdova H. (2014). Aleitamento intensificado para bezerros da raça holandesa: Desempenho, consumo, conversão alimentar e escore de consistência fecal. *Arch. Vet. Sci.* **19,** 65-71.

Kaneko J.J., Harvey J.W. and Bruss M.L. (2008). Clinical Biochemistry of Domestic Animal. Academic Press, San Diego, California.

Khan M.A., Bach A., Weary D.M. and Keyserlingk M.A.G. (2016). Invited review: Transitioning from milk to solid feed in dairy heifers. *J. Dairy Sci*. **91,** 885-902.

Leite R.F., Baccili C.C., Silva C.P.C., Novo S.M.F., Baldacim V.A.P., Benites N.R. and Gomes V. (2017). Passive immunity transfer in calves fed with colostrum from dams with subclinical mastitis. *Arq. Inst. Biol*. **84,** 1-7.

Lisbôa J.A.N., Sturion T.T., Anjos M.C. and Flaiban K.K.M.C. (2016). Passive transfer of immunity and serum proteinogram during the first 35 days of age in Nelore calves conceived naturally or through *in vitro* fertilization. *Acta Sci. Vet*. **44,** 1420-1427.

Ozkaya S. (2014). Comparison of two different milk feeding schedules on growth performance of Holstein calves during the pre-weaning period. *Indian J. Anim. Res.* **48,** 384-388.

Paez L.S., Grilli D. and Egea V. (2014). Rumen development and blood metabolites of Criollo kids under two different rearing systems. *Livest. Sci*. **167,** 171-177.

Schade J., Rossi R.M., Fonteque G.V., Martins E., Lisbôa J.A.N., Flaiban K.K.M.C., Preti M.C.P. and Fonteque J.H. (2016). Transferência de imunidade passiva e proteinograma sérico em bezerros das raças Crioula Lageana variedade Mocha e Aberdeen Angus (Red Angus) nos primeiros seis meses de vida. *Pesq. Vet. Bras*. **36,** 33-40.

Seifzadeh S., Ramezani M., Seifdavati J., Abdi Benemar H. and Razmazar V. (2019). Effects of weaning age on growth and blood parameters of replacing Holstein calves fed on a restricted step up and down milk feeding program. *Iranian J. of Appl. Anim. Sci*. **9(2),** 197-204.

Silper B.F., Lana A.M.Q. and Carvalho A.U. (2014). Effects of milk replacer feeding strategies on performance, ruminal development, and metabolism of dairy calves. *J. Dairy Sci.* **97,** 1016-1025.

Teixeira W.T., Fonteque G.V., Ramos A.F., Mariante A.S., Egito A.A., Martins V.M.V., Saito M.E. and Fonteque J.H. (2014). Transfer of passive immunity and serum proteinogram in the first six months of life of Criollo Lageano and black and white Holstein calves. *Pesq. Vet. Bras.* **10,** 980-986.

Tóthová C., Nagy O., Seidel H., Kováč G. and Nagyová V. (2016). Changes in the concentrations of serum proteins in calves during the first month of life. *J. Appl. Anim. Res*. **44,** 338-346.