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Estimation of Nitrogen Requirements of Holstein Suckling Calves at Different Ages

Research Article

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ABSTRACT

The aim of this study was to estimate the nitrogen requirements of Holstein suckling calves at 2, 6 and 8 weeks of age. Newborn healthy calves, randomly divided into 2 groups (n=16 per group), were fed with either a starter based on corn or barley. The calves received pasteurized cow's milk in accordance to the herd schedule. The calves were housed in individual metabolic cages and urinary and fecal nitrogen contents were measured in samples collected for four days at 2, 6 and 8 weeks of age. Nitrogen requirement was estimated using the regression analysis. Nitrogen requirement for maintenance at week 2 significantly (P=0.037) differed between the starters, but not at 6 and 8 weeks. However, the nitrogen requirement for maintenance at 8 weeks of age tended to be differ between male and female calves (P=0.075), but not at weeks 2 and 6. Daily nitrogen requirements for maintenance based on the metabolic weight decreased as the calves aged. Nitrogen requirement for growth was affected by the type of grain in the diet at weeks 2 (P=0.068), 6 (P<0.05) and 8 (P<0.05). No effect of sex was found on nitrogen requirements for growth (P>0.05).

KEY WORDS growth, Holstein calves, maintenance, nitrogen, requirements.

INTRODUCTION

One of the challenges in dairy husbandry is how to improve calf performance and health, especially before weaning. This is achievable by feeding balanced diets at minimum prices, but requires accurate information on calf nutritional requirements and feed nutrients. There is insufficient information on the energy and protein requirements of calves under 100 kg body weight, as emphasized by NRC (2001). Few studies have recently been published aimed at predicting the nutrient requirements of young calves (Bartlett *et al.* 2006; Labussiere *et al.* 2008; Rodrigues *et al.* 2016). Protein, or nitrogen, needed for optimum health and performance, is the most expensive constituent of the diet (Davis and Drackley, 1998). In suckling calves, more accurate estimation of nitrogen requirements for maintenance and growth is of considerable importance to allow for genetic improvement, and different environments and management practices. Older research did not consider the environmental impact of nitrogen excretion, and the longer term effects of nitrogen excess or deficiency (Marai and Haeeb, 2009; Paengkoum *et al.* 2013; Daniela, 2016). Furthermore, the requirement for a particular nutrient may not be the same throughout the preweaning period; i.e., it may change as the calf grows and becomes older. Nitrogen requirement may also be affected by the calf sex, and grain type in the diet, such as barley or corn that are commonly used in the starters. Therefore, the purpose of the current research was to estimate the nitrogen requirements of Holstein suckling calves for maintenance and growth at 2, 6, and 8 weeks of age, and the effects of the carbohydrate source (barley and corn grains) and calf sex on the nitrogen requirement.

MATERIALS AND METHODS

Newborn healthy calves (16 males and 16 females) were allocated at random into two groups and kept in individual cages. They were bottle- fed colostrum at 10% of body weight three times during the first 6 hours of life, followed by pasteurized colostrum until the third day. Thereafter, fresh-pasteurized cow's milk was fed at 6:00 and 15:00 hour according to the herd feeding schedule (4 kg/day until two weeks, 5 kg/day 2-6 weeks and 4 kg 6-8/day weeks of age). Water was freely available throughout the experiment. Calves were fed with their respective starters (barley- or corn-based) from the third day of life. The starter composition is shown in Table 1. Alfalfa hay, of high quality, was available from two weeks of age. Calves were weighed at the beginning of the experiment and then weekly until the end of the experiment. The daily and weekly consumption of alfalfa hay and starter was calculated for each calf by weighing the offered feed and orts. Individual dry matter (DM) intake was calculated by using the data on daily intake of starter, alfalfa hay and milk on dry matter (DM) basis (AOAC, 1990). Milk samples were taken in the last 4 days of the three stages before weaning (0 to 2, 2 to 6, and 6 to 8 weeks of age). Urine and feces were measured in the last four days of each period and at the end of the fourth day. After mixing the samples, a sub-sample was taken for each calf: Urinary pH was adjusted to below 2 for storage in a cold environment (4 ˚C). Nitrogen contents of the starter, alfalfa, urine and feces were measured using the Kjeldahl method (code number 954/01; AOAC, 1990). Nitrogen content of milk was measured by using Milko-Scan 133 B Infraanalyzer Foss Electric, Denmark. The total nitrogen consumed from milk, starter and alfalfa, and that excreted in urine and feces were calculated for each period. The amount of nitrogen lost in hair, dandruff and skin was estimated as 0.018 g/kg body weight (NRC, 2001) for calculation of total retained nitrogen. Nitrogen requirement for maintenance was calculated by regressing the nitrogen intake on retained nitrogen (corrected for zero point):

$$RN = a + b \times MNI \qquad \text{(Equation 1)}$$

Where:
RN: retained nitrogen (g/kg $BW^{0.75}$ per day).
a and b: regression parameters.
MNI: metabolized nitrogen intake (g/kg $BW^{0.75}$ per day).

Metabolic fecal nitrogen was calculated by regressing the nitrogen intake on apparent digestible nitrogen intake (Kohn *et al.* 2005):

$$ANI = a + b \times NI \qquad \text{(Equation2)}$$

Where:
ANI: apparent digestible nitrogen intake (g/kg $BW^{0.75}$ per day).
a and b: regression parameters.
NI: nitrogen intake (g/kg $BW^{0.75}$ per day).

Endogenous urinary nitrogen was calculated by subtracting the metabolic fecal nitrogen and nitrogen released from the body surface area from the nitrogen requirement for maintenance.
Nitrogen requirement for growth was estimated as:

$$NPg = a \pm SE + (\text{Ration CP} \times W^{b \pm SE}) \qquad \text{(Equation 3)}$$

Where:
NPg: nitrogen requirement for growth (g/kg $BW^{0.75}$ per day).
Ration CP: percentage of ration crude protein.
a and b: regression parameters.
SE: standard error.
W: body weight (kg).

Statistical analysis

The study was arranged as a 2 × 2 factorial experiment (two starter feeds and two sexes) in a completely randomized design, with eight repetitions and replications in time (3 age intervals spaced unequally). Data were analyzed by using the Proc GLM, and the mean separation was performed by the least squares means procedure adjusted for the Tukey's test (SAS, 2004). The level of significance was set at $P < 0.05$, and a tendency at $0.05 < P < 0.15$).

RESULTS AND DISCUSSION

The least squares means of dry matter and nitrogen intake, retained nitrogen in the body, metabolic fecal nitrogen, and endogenous urinary nitrogen as affected by the starter type and calf sex at various ages are reported in Table 2. Dry matter intake (DMI) at weeks 2, 6 and 8 of age did not differ between the diets based on corn and barley grains ($P>0.05$). Dry matter intake at two weeks of age tended to be higher for male (4.6%) than female calves ($P=0.074$). There was no difference in DMI between male and female calves at weeks 6 and 8 of age ($P>0.05$). There was no interaction between calf sex and starter type in DMI.

Table 1 Ingredients (g per kg as-fed) and composition of the starter, milk and alfalfa

Ingredients	Starter		Alfalfa hay	Milk
	Corn-based	Barley-based		
Corn	620	-		
Barley	-	620		
Rapeseed meal	30	30		
Soybean meal	260	260		
Soybean	50	50		
Calcium carbonate	9	9		
Sodium carbonate	8	8		
NaCl	5	5		
Mineral mix[1]	10	10		
Vitamin mix[2]	8	8		
Chemical and nutritional composition (g per kg DM)				
Dry matter (g/kg)	930.6	930.7	941.1	116.6
Ash	76.8	80.0	81.9	67.0
Crude protein (CP) (N×6.25)[4]	197.1	200.4	127.5	257.3
Crude fat	57.3	51.9	49.7	320.8
Non-fiber carbohydrates (NFC)	518.3	502.7	221.2	344.8
Neutral detergent fiber (NDF)	150.5	165.0	519.7	-
Metabolizable energy (MJ/kg)	16.820	16.862	8.912	22.133

[1] Mineral supplied per kilogram of mixture: Calcium: 64 mg; Phosphorus: 30 mg; Magnesium: 44 mg; Manganese: 4 mg; Zinc: 4.6 mg; Iron: 10.5 mg; Copper: 1 mg; Iodine: 0.025 mg; Selenium: 0.037 mg and Cobalt: 0.01 mg.

[2] Vitamin supplied per kilogram mixture: vitamin A: 1350000 IU; vitamin E: 6700 IU; vitamin B_{12}: 9.3 µg; vitamin B_6: 0.873 mg; vitamin B_5: 29.65 mg; vitamin B_3: 1.7 mg; vitamin B_1: 0.8 mg; Cholecalciferol: 80000 IU; Riboflavin: 0.85 mg; Choline chloride: 7.5 mg; Biotin: 0.013 mg and Folic acid: 0.88 mg.

Table 2 Least squares means of dry matter intake (DMI), and nitrogen intake (NI), retained nitrogen (RN) in the body, metabolic fecal nitrogen (MFN) and endogenous urinary nitrogen (EUN) as affected by calf sex and starter type at different calf ages

Title	Unit	Age (week)																	
		2						6						8					
		Starter based on		P-value	Calf sex		P-value	Starter based on		P-value	Calf sex		P-value	Starter based on		P-value	Calf sex		P-value
		Corn	Barley		Male	Female		Corn	Barley		Male	Female		Corn	Barley		Male	Female	
DMI	g/d	537	533	0.742	547	523	0.074	1150	1152	0.982	1144	1158	0.879	1488	1504	0.883	1527	1466	0.578
NI	g/kg $BW^{.75}$	1.307	1.288	0.552	1.319	1.277	0.192	1.940	1.982	0.739	1.922	2.000	0.531	2.113	2.178	0.551	2.141	2.149	0.941
RN	g/kg $BW^{.75}$	0.221	0.290	0.116	0.295	0.215	0.069	1.086	1.190	0.457	1.114	1.161	0.737	0.990	1.132	0.237	1.173	0.948	0.067
MFN	g/kg $BW^{.75}$	0.084	0.069	0.124	0.077	0.077	0.978	0.033	0.067	0.261	0.046	0.055	0.772	0.035	0.066	0.314	0.060	0.041	0.533
EUN	g/kg $BW^{.75}$	0.632	0.568	0.086	0.577	0.623	0.211	0.577	0.478	0.353	0.522	0.513	0.907	0.356	0.258	0.333	0.198	0.416	0.038

Nitrogen intake was unaffected by starter type, calf sex, calf age, or a starter-sex interaction at various age classes. The amount of retained nitrogen at two weeks of age was 31.2% higher in calves receiving the barley-based compared to the corn-based starter (P=0.116). Nitrogen retention at two and eight weeks of age was 37.2 and 23.7% higher in males than in female calves (P=0.07). There was no interaction between diets and calf sex in the amount of nitrogen retained in the body at different ages. Metabolic fecal nitrogen in 2-week old calves feeding on the barley-based starter was 21.7% higher than those on the corn-based starter (P=0.124). No difference was recorded between starters at the age of 6 and 8 weeks.

There was no effect of calf sex or its interaction with starter type on metabolic fecal nitrogen loss in any age class. The endogenous urinary nitrogen loss in 2-week old calves tended (P=0.086) to be higher (9.7% higher) in those feeding on the corn-based starter. Endogenous urinary nitrogen loss at the 8th week of age in female calves was about twice that in male calves. There was no interaction between diet and calf sex on endogenous urinary nitrogen excretion in any age class.

Nitrogen requirement for maintenance (Table 3) at two weeks of age was 11.9% higher (P=0.037) in calves that received the corn-based compared with the barley-based starter.

Table 3 Least squares means of nitrogen requirement for maintenance (g N/kg BW$^{.75}$/d) as affected by calf sex and starter type

Calf age	Starter based on		P-value	Calf sex		P-value
	Corn	Barley		Male	Female	
2 week	0.734	0.656	0.037	0.672	0.717	0.209
6 week	0.609	0.563	0.601	0.586	0.585	0.989
8 week	0.408	0.341	0.536	0.276	0.474	0.075

The mean nitrogen requirement for maintenance at the age of 8 weeks was 71.7% higher (P=0.075) in female than in male calves.

At two weeks of age, nitrogen requirement for maintenance tended to be affected by the starter –sex interaction; being different between male calves receiving the corn and barley starters (P=0.075) on the one hand, and between female calves on corn starter and male calves on barley starter (P=0.096) on the other hand (Table 4). Nitrogen requirement for growth (Table 5) at two weeks of age tended (P=0.068) to be higher (0.7%) in calves that were fed on the barley-based starter. Nitrogen requirement for growth was unaffected by calf sex.

The interaction effect of treatment and calf sex on nitrogen requirement for growth at two weeks of age tended to be different (P=0.097) between male calves on the corn starter and female calves on the barley starter (Table 6). At 6 weeks of age, a starter by calf sex interaction on nitrogen requirement for growth indicated significant differences (P=0.007) between male and female calves receiving the corn-based starter, and male calves on the barley starter. It was also different between male calves on the corn starter and female calves on the barley starter (P=0.007). At 8 weeks of age, nitrogen requirements for growth differed between male calves receiving corn and barley starters.

No significant differences were found in DMI between calves on different starter diets or between male and female calves (P>0.05). In the present study, with *ad libitum* starter feeding, DMI at 2, 6 and 8 weeks of age was 1.33, 2.10 and 2.29% of body weight, respectively (data not tabulated).

Dry matter intake at 2 weeks of age was 4% higher than NRC (2001) values, and 10.5% higher at 6 weeks of age. However, NRC values do not predict voluntary feed intake. NRC (2001) has assumed that a calf at about 2 week of age would consume, on the average, a diet in which 60% percent of DMI are derived from milk or milk replacer and 40% from the starter; we found that the starter diet accounted for only 9% of DMI at 2 weeks of age. In this regard, Davis and Drackley (1998) predicted that intake of DM from the starter increased from 0.8-1.0% at 3 weeks to 2.8-3.0% percent at 8 weeks of age, which is 26% higher than voluntary DMI at 8 weeks of age in the present study. Despite similar intakes of N, which also depends on DMI, retained nitrogen in the body tended to be significant (P=0.07) at 2 and 8 weeks of age for both male and female calves (Table 2). Nitrogen intake is partitioned into two components; i.e. maintenance and gain. Nitrogen for maintenance is lost in urine and feces, whereas gain pertains to N stored in tissues. For optimal N retention, the diet should be properly balanced for all essential nutrients, with an energy intake sufficient to support protein synthesis. Therefore, N intake should not exceed that required for the expected gain supported by the energy intake (Vermeire, 2005). The challenge is always to determine the required energy for maintenance plus the desired rate of gain and provide adequate feed intake to meet this rate of gain. The diet must provide the amount of nitrogen required to meet this rate of gain at the established feed intake level. Nitrogen retained in the body also increased with increasing calf age, which is predictable due to increased weight gain.

Metabolic fecal nitrogen (MFN) was unchanged statistically by the starter type and calf sex at different ages. This was anticipated because MFN is affected greatly by DMI (NRC, 2001; CSIRO, 2007; Tedeschi *et al.* 2015), even more than that by body weight (Mitchell, 1934). However, Ørskov *et al.* (1970) and Ørskov and Grubb (1979) reported a higher rate of MFN in calves fed a barley-based diet. In the trial reported here, the rate of MFN loss at 2 weeks, but not at 6 or 8 weeks of age, was within the range of values (0.073 to 0.125 g per kg metabolic body weight) in Holstein × Gyr male calves feeding on milk and starter (Silva *et al.* 2016). Donnelly and Hutton (1976) reported a range of 1.9 to 2.7 g MFN per kg DMI in calves fed milk or milk replacer.

In agreement with Roy (1970), endogenous urinary nitrogen (EUN) decreased with increasing calf age. The EUN loss at 8 weeks of age in the present study was close to the values reported by Blaxter and Wood (1952) and Roy (1970) (0.168 to 0.218 grams per kilogram metabolic body weight per day). According to Swanson (1977) and Fox *et al.* (2004), the EUN loss in calves at 6 and 8 weeks of age was 0.43 and 0.44 grams per kg metabolic body weight per day, respectively.

With increased calf age, nitrogen requirement for maintenance decreased. Due to the higher turn-over of protein in young animals (Liu *et al.* 1995), N requirement (as percentage of metabolic body weight) will be higher in younger calves.

Table 4 Interaction effect of starter type and calf sex on the nitrogen requirement for maintenance according to the calf age

Calf age	Starter based on		Calf sex		Amount	Starter based on		Calf sex		Amount	P-value
	Corn	Barley	Male	Female		Corn	Barley	Male	Female		
2 week	*		*		0.735	*			*	0.733	1.000
	*		*		0.735		*	*		0.609	0.075
	*		*		0.735		*		*	0.702	0.910
	*			*	0.733		*	*		0.609	0.096
	*			*	0.733		*		*	0.702	0.932
		*	*		0.609		*		*	0.702	0.280
6 week	*		*		0.597	*			*	0.620	0.998
	*		*		0.597		*	*		0.575	0.998
	*		*		0.597		*		*	0.551	0.980
	*			*	0.620		*	*		0.575	0.984
	*			*	0.620		*		*	0.551	0.943
		*	*		0.575		*		*	0.551	0.997
8 week	*		*		0.261	*			*	0.556	0.221
	*		*		0.261		*	*		0.291	0.997
	*		*		0.261		*		*	0.392	0.814
	*			*	0.556		*	*		0.291	0.332
	*			*	0.556		*		*	0.392	0.713
		*	*		0.291		*		*	0.392	0.911

Table 5 Least squares means of nitrogen requirement for growth (g/kg gain) as affected by calf sex and starter type

Calf age	Starter based on		P-value	Calf sex		P-value
	Corn	Barley		Male	Female	
2 week	30.2	30.4	0.068	30.3	30.4	0.219
6 week	28.3	28.8	0.0009	28.5	28.6	0.363
8 week	27.3	28.1	0.0001	27.7	27.7	0.595

Table 6 Interaction effect of starter type and calf sex on nitrogen requirement for growth according to the calf age

Calf age	Starter based on		Calf sex		Amount (g/kg gain)	Starter based on		Calf sex		Amount (g/kg gain)	P-value
	Corn	barley	Male	Female		Corn	Barley	Male	Female		
2 week	*		*		30.2	*			*	30.3	0.801
	*		*		30.2		*	*		30.3	0.488
	*		*		30.2		*		*	30.5	0.097
	*			*	30.3		*	*		30.3	0.973
	*			*	30.3		*		*	30.5	0.583
		*	*		30.4		*		*	30.5	0.815
6 week	*		*		28.2	*			*	28.4	0.481
	*		*		28.2		*	*		28.8	0.007
	*		*		28.2		*		*	28.8	0.007
	*			*	28.4		*	*		28.8	0.257
	*			*	28.4		*		*	28.8	0.295
		*	*		28.8		*		*	28.8	0.998
8 week	*		*		27.3	*			*	27.3	0.946
	*		*		27.3		*	*		28.1	< 0.0001
	*		*		27.3		*		*	28.1	< 0.0001
	*			*	27.3		*	*		28.1	< 0.0001
	*			*	27.3		*		*	28.1	< 0.0001
		*	*		28.1		*		*	28.1	0.997

In the present study, N requirement for maintenance at 8 weeks of age in calves feeding the barley-based starter was close to the value of 0.35 g per kg metabolic body weight per day in calves (AFRC, 1993). However, data on N requirement for the maintenance of suckling calves are scarce.

Rodrigues *et al.* (2016) reported that the N requirement for maintenance of Holstein calves up to 87 days of age in tropical regions was 0.587 g per kg $BW^{0.75}$ per day; the value during the whole experimental period in the present experiment (approximately 0.552) was in line with their data.

According to Silva *et al.* (2016), the N requirement for maintenance in crossbred male calves (Holstein×Gyr) over a 64-day period was 0.373 g per kg $BW^{0.75}$ per day. This is similar to our data at the age of 8 weeks (0.374), regardless of the starter type and calf sex. Generally, protein requirement for maintenance is relatively low, but the requirement for gain is relatively high, with the opposite being true for the energy.

During the first 2-3 weeks of age, protein digestion in the gastro-intestinal tract (GIT) of calves is often low (Arieli *et al.* 1995; Terosky *et al.* 1997), including that of high quality of milk proteins. It means that grains with lower availability of nutrients, such as corn, will probably be less well and more slowly digested than wheat and barley grains. The physical form of starch, its relation to proteins, and the cellular integrity of starch containing units affect grain nutrient digestibility (Theurer *et al.* 1999). However, with increasing age and GIT development, calves are able to mitigate any discordance of nutrient digestibility. In contrast to the present results, Khan *et al.* (2007) reported that N balance (as a percentage of N intake) was greatest in calves fed corn, followed by wheat, barley and oat.

Nitrogen requirement for growth at 2 weeks of age tended to be higher (P=0.07) for calves fed with the barley-based starter, and by the age of 6 and 8 weeks, the difference was significant (P<0.05). Silva *et al.* (2016) reported a net protein requirement of 119.1 ± 33.9 g per kilogram weight gain in crossbred Holstein × Gyr male calves over a 64-day period. However, they did not include the dietary protein concentration in the suggested equation. It is clear that body gain composition and performance are influenced by changes in protein and energy intake (Donnelly and Hutton, 1976; Bartlett, 2001), therefore, in the present study, the protein concentration in starter diet was included in the predicted equation (equation No. 3). The present results are in line with those of Blome *et al.* (2003) who reported that the average additive daily gain, weight gain efficiency and final weight of calves increased as the dietary protein content increased. According to the present results, as the calf age increased, N requirement for gain decreased. Because of decreased dietary protein concentration with increasing calf age (2 weeks 25%, 6 weeks 22.8% and 8 weeks 21.8%), the decrease in N requirement for weight gain was anticipated (Rahmani, 2019).

CONCLUSION

Nitrogen is an essential nutrient for the health and performance of young animals. In suckling calves, accurate estimation of nitrogen requirements for maintenance and growth is of considerable importance in allowing formulation of balanced diets. This avoids the provision of excessive or insufficient nutrients to the animal. The present results showed that either barley or corn grains can be included in starter diets without undesirable consequences. No differences were found between male and female calves in N requirements during the suckling period. However, further studies using different protein concentrations and grain types in starter diets are needed. The ratios of protein and energy, and their composition are important. Other aspects to be considered include, stress factors, and for male animals destined for slaughter carcass composition and palatability.

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