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Short communication

# Delayed weaning of Holstein bull calves fed an elevated plane of nutrition impacts feed intake, growth and potential markers of gastrointestinal development

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ABSTRACT

Feeding calves an elevated plane of nutrition pre-weaning improves growth. However, some controversy remains about the optimal time of weaning required to minimize the decline in growth commonly associated to milk withdrawal. This experiment aimed to determine the influence of weaning age on performance of calves fed milk replacer on an elevated plane of nutrition (8 L $d^{-1}$, 150 g $L^{-1}$, 1.2 kg $calf^{-1}$ $d^{-1}$) and to further understand the impacts of weaning age on gastrointestinal development. To meet these objectives, 108 Holstein bull calves (18.7 ± 2.69 d old) were blocked by BW and randomly assigned to one of three treatments: step-down weaning at 8, 10 or 12 weeks of age. Daily milk replacer supply and refusals, and weekly starter and hay intakes were recorded throughout the 10 week experiment. Average daily gain (kg $d^{-1}$) and BW (kg) were measured weekly. Digestive tract development was assessed by sampling blood and fecal matter at 5, 7, 9, 11 and 13 weeks of age. Blood was analysed for βHBA as a marker of rumen development (ruminal ketogenesis) and fecal starch content was used to evaluate its total tract digestibility. Intakes of starter were higher in calves weaned at 8 weeks of age, from week 7 onwards, translating into differences in total starter consumption across the 10 week trial ($P < 0.01$). Weekly differences occurred in ADG ($P < 0.05$) during the weeks of weaning, resulting in differences in final BW and total weight gain between calves weaned at 8 and 12 weeks of age. No differences were observed in fecal starch content ($P > 0.05$), yet, βHBA presented smaller spikes at weaning with increasing weaning age ($P < 0.01$), indicating that older animals may experience a less abrupt activation of rumen function. Overall, the results suggest that delaying weaning of calves fed an elevated plane of nutrition may be beneficial to sustain growth performance and avoid stress during weaning.



## 1. Introduction

Debate regarding the optimal nutrition of dairy calves supports either accelerated ruminal development and reduced costs of feeding in early weaned calves (Kertz et al., 1979; Quigley and Caldwell, 1991), or improved growth and reduced welfare concerns in calves fed elevated planes of nutrition prior to weaning (Khan et al., 2011). As the ingestion of

*Abbreviations:* BW, body weight; ADG, average daily gain; βHBA, beta hydroxybutyrate.

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solid feed results in metabolic and morphological adaptations in the rumen of calves (Baldwin et al., 2004), the strategy and timing of weaning becomes increasingly important when higher milk volumes are fed. The use of a step-down strategy has been suggested to stimulate starter consumption resulting in a faster development of the fore-stomach (Khan et al., 2007). Yet, despite the benefits to performance and health, feeding calves elevated planes of nutrition pre-weaning is still not widely implemented, likely reflecting the lack of studies published regarding the optimal age of weaning using this method for calves fed elevated planes of nutrition prior to weaning.

Increasingly in Europe, male dairy calves are being weaned for veal production (Berends et al., 2015) an industry which requires rapid growth rates. With concerns regarding animal welfare at the fore-front, feeding strategies that address both of these aspects are more likely to be widely adopted. Currently, there is considerable variation among European regions and farms with respect to dairy weaning practices, especially age of weaning, and very little information is available regarding weaning age of young beef calves. North American dairy herds commonly wean between 6 and 8 weeks, but can extend to over 10 weeks of age, as is common in European countries (USDA, 2010; Vasseur et al., 2010). Additionally, there is a paucity of information describing how age of weaning impacts energy balance, feed intake patterns and rumen development. It has recently been shown that calves fed elevated levels of milk and are weaned later in life, experience less weight loss and behavioral signs of hunger or stress during the weaning period (de Passillé et al., 2011). Thus, the overall objective of this experiment was to characterize the effect of weaning age on dietary intakes, growth and development, as we hypothesized that calves fed elevated planes of nutrition and weaned later in life using the step-down strategy, would have higher growth rates and improved gut development as a result of minimising abrupt changes which occur during weaning.

## 2. Materials and methods

### 2.1. Experimental design, animals and treatments

All experimental procedures and animal care were conducted in accordance with animal welfare legislation, and were approved by the animal experimentation committee (DEC Dierexperimentencommissie, Utrecht, approval #2013.III.11.120). One hundred and twelve male Holstein Friesian calves, with an average bodyweight (BW) of 49.5 ± 1.84 kg at 18.7 ± 2.69 d of age, were purchased from a local livestock dealer. Shortly after arrival to the calf experimental farm of Nutreco R&D (Winssen, the Netherlands), calves were weighed and their health was examined, with those failing to meet health standards being excluded from the trial, leaving a total of 108. Calves were blocked by BW and penned according to a randomized complete block design including 6 blocks of 3 pens each (with 5 calves per pen) and 2 blocks of 3 pens each (with 3 calves per pen). Within each block pens were randomly allocated to one of three weaning treatments (8, 10 or 12 weeks of age) such that there were equal numbers of calves in each treatment. All calves were then fed twice with 2.0 L of an electrolyte solution (20 g $L^{-1}$ water, Emix, Sloten) in addition to 2.0 L of milk replacer (260 g $kg^{-1}$ crude protein and 160 g $kg^{-1}$ crude fat; DM basis; Sloten B.V., Deventer, The Netherlands) at 39–40 °C. From d 1 onwards milk replacer was fed individually with a pail in two meals of equal volume $d^{-1}$ (at 07:00 and 15:00 h). The volume of milk replacer was increased 0.5 L $meal^{-1}$ every three days over a two week step-up period until a total of 8.0 L was being fed, such that from d 1 to 3, 4.0 L $d^{-1}$ of milk replacer was fed, from d 4 to 6 the volume of milk replacer fed was 5.0 L $d^{-1}$ and from d 7 to 9 6.0 L $d^{-1}$ was fed. On d 10 volumes were increased to 7.0 L $d^{-1}$ and further increased to 8.0 L $d^{-1}$ on d 14. All calves continued to receive 8.0 L $d^{-1}$ until their respected time of step-down weaning. There were no refusals of milk. The group weaned at 8 weeks of age was stepped-down to 4.0 L $d^{-1}$ on d 49 and 0 L on d 56; the group weaned at 10 weeks was stepped-down to 4.0 L $d^{-1}$ on d 63 and 0 L on d 70; and the group weaned at week 12 was stepped-down to 4.0 L $d^{-1}$ on d 77 and 0 L on d 84. Calves also received *ad libitum* calf starter concentrate (pellet, 3 mm, 180 g $kg^{-1}$ DM crude protein, 49 g $kg^{-1}$ DM crude fat and 205 g $kg^{-1}$ DM starch; AgruniekRijnvallei, Wageningen, The Netherlands) and chopped wheat straw from d 1. Calf starter and straw were fed in a trough (one per pen), where starter and straw were physically separated by a partition. Calves had free access to water at all times through a drinking nipple in each pen.

### 2.2. Intake and growth measurements

Calves were weighed at the beginning of the experiment and weekly thereafter to determine weekly BW and ADG. Intakes of starter and straw were recorded daily and orts measured weekly. Metabolizable energy (ME) content of starter, milk replacer and straw were estimated using NRC equations (NRC, 2001). Intakes of ME were calculated by dividing intake of each feed (starter, straw of milk replacer) by its ME content.

### 2.3. Measurement of potential markers of gut development

To assess undigested starch in faeces, fecal samples (50 g) were collected from 24 calves per treatment group (*i.e.*, 3 calves per pen) at 5, 7, 9, 11, and 13 weeks of age, frozen immediately and stored at −20 °C until analysis. In brief, calves were rectally stimulated with a sterile glove to facilitate the collection of a fecal sample. Fecal samples were dried at 60 °C for 48 h prior to grinding to a 1.0 mm particle size. The starch content in fecal samples was analyzed at Masterlab (Boxmeer, the Netherlands), using enzymatic starch technique (NEN-ISO 15914-2004). Means within weaning groups were obtained by averaging individual values.

**Table 1**
Effects of weaning age of calves fed high planes of nutrition pre-weaning on intakes (kg $d^{-1}$) and growth.

| Parameter[1] | Weaning age (weeks) | | | SEM | *P*-value |
|---|---|---|---|---|---|
| | 8 | 10 | 12 | | |
| Initial BW (kg) | 49.5 | 49.6 | 49.4 | 1.61 | 0.99 |
| BW at week 7 (kg) | 76.8 | 76.0 | 76.7 | 1.61 | 0.94 |
| Final BW (kg) | 122.6$^{b}$ | 125.7$^{ab}$ | 127.8$^{a}$ | 1.61 | 0.09 |
| Average total gain (kg) | 73.1$^{b}$ | 76.1$^{ab}$ | 78.3$^{a}$ | 1.50 | 0.06 |
| ADG until week 7 (kg) | 0.98 | 0.94 | 0.97 | 0.041 | 0.83 |
| ADG week 8 (kg) | 0.68$^{b}$ | 1.30$^{a}$ | 1.25$^{a}$ | 0.061 | <0.001 |
| ADG week 9 (kg) | 0.99 | 0.97 | 0.94 | 0.061 | 0.851 |
| ADG week 10 (kg) | 1.17$^{b}$ | 1.00$^{c}$ | 1.41$^{a}$ | 0.061 | <0.001 |
| ADG week 11 (kg) | 1.25 | 1.27 | 1.23 | 0.061 | 0.892 |
| ADG week 12 (kg) | 1.26$^{a}$ | 1.32$^{a}$ | 1.02$^{b}$ | 0.061 | 0.001 |
| ADG week 13 (kg) | 1.19$^{b}$ | 1.24$^{b}$ | 1.45$^{a}$ | 0.061 | 0.006 |
| Average starter intake (kg $d^{-1}$) | 1.87$^{a}$ | 1.31$^{b}$ | 1.27$^{b}$ | 0.045 | <0.001 |
| Average straw intake (kg $d^{-1}$) | 0.14 | 0.15 | 0.13 | 0.009 | 0.20 |

a–c Within row, means without common superscript differ (*P* <0.05).
1 Body weight, BW; average daily gain, ADG.

Blood samples (5 ml of plasma) of 24 calves per treatment group out of a total of 36 (*i.e.*, 3 calves per pen) were collected via jugular venipuncture using 12-ml (plain vacuum tubes) vacutainers (Becton Dickinson, Franklin Lakes, NJ) 3 h after morning feeding of milk at 5, 7, 9, 11, and 13 weeks of age. The blood samples were kept in tempex boxes with cooling elements and transported to the Animal Health Service (GD; Deventer, the Netherlands) within 2 h after collection. The samples were then centrifuged (1690 × *g* for 10 min at 20 °C) to obtain serum, which was immediately analyzed for β-hydroxybutyrate (βHBA) using spectrophotometry.

### 2.4. Statistical analysis

Data were analyzed as repeated measures using a mixed-effect model in SAS (PROC MIXED, SAS 9.3, SAS Inst., Inc., Cary, NC) that accounted for the fixed effects of Treatment, Week, and their interaction, plus the random effect of Pen within block. Week entered the model as a repeated measure and an autoregressive type 1 covariance structure was used based on Bayesian criterion. When an interaction between treatment and week was detected, contrasts were applied to enable the discrimination between treatments at different time points. Differences were declared significant when $P < 0.05$, and trends reported if $0.05 < P \leq 0.1$.

## 3. Results

### 3.1. Intake and growth

A treatment × week interaction was observed ($P < 0.001$), where starter intakes were similar ($P > 0.05$) across all treatments prior to weaning, but in each week thereafter, weaned calves showed greater intakes than those yet to be weaned (Fig. 1a). Consequently, total consumption of starter (kg $d^{-1}$) over the entire trial was greater in calves weaned at 8 weeks of age. However, due to an increase in starter consumption in week 13 in calves weaned at 12 weeks of age, above that of calves weaned at 10 weeks of age ($P < 0.001$), overall starter intakes did not differ between calves weaned after week 8 ($P > 0.05$; Table 1).

Weekly calf growth was influenced by weaning time (treatment × week $P < 0.001$; Fig. 1b). In calves weaned at 8 weeks, a 46% reduction in ADG occurred during the step-down at week 7, plateauing at 0.68 kg $d^{-1}$, but then recovered to levels similar to un-weaned calves by week 9 ($P > 0.05$). In week 9, a pause in growth was observed in calves weaned at 10 weeks resulting in a trend ($P = 0.07$) for lower ADG compared to un-weaned calves. Calves weaned at 12 weeks maintained an ADG of 1.02 kg $d^{-1}$ during their step-down week (week 11) and consequently, calves weaned at 12 weeks of age had a greater ($P < 0.001$) ADG during weaning, than those weaned at 8 weeks of age. In the week following weaning, ADG of calves weaned at 12 weeks increased above that of earlier weaned calves ($P \leq 0.001$), resulting in calves weaned at 12 weeks of age tending to have a greater total weight gain over the trial period (kg) and their final BW showed a tendency to be 4.2% higher ($P = 0.06$), than those weaned at week 8 (Table 1). Differences in bodyweight occurred during the weeks post weaning for the calves weaned at weeks 8 and 12, but no differences between other treatments for BW were detected for calves weaned at week 10 (Fig. 1b).

### 3.2. Markers of intestinal development

Similar to starter intake, serum βHBA levels showed a treatment × week interaction ($P < 0.001$) such that, serum βHBA levels were similar across all treatments during the first four weeks of the trial (Fig. 1c). Serum βHBA levels increased sharply, but to a similar extent ($P = 0.69$) after weaning of calves at weeks 8 and 10, but displayed less ($P < 0.01$) of an increase in

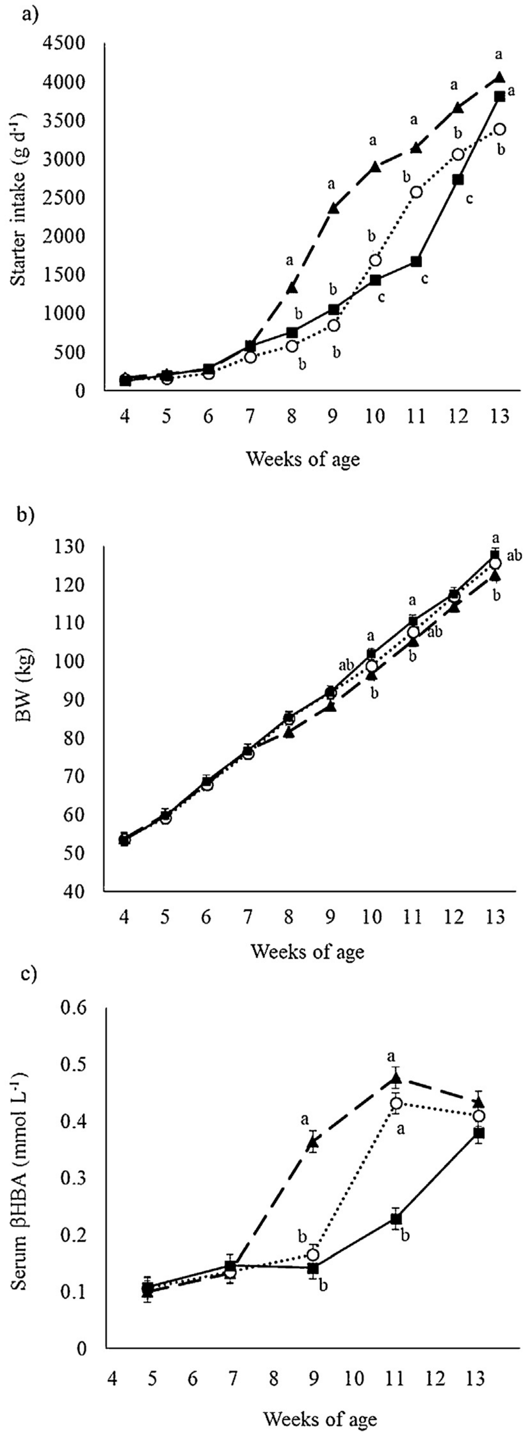


**Fig. 1.** Summary of mean (a) starter intake (g $d^{-1}$); (b) body weight (BW; kg) and (c) serum βHBA (mmol $L^{-1}$) of calves weaned at 8 (—▲—), 10 (···○···) and 12 (—■—) weeks of age. SEM is indicated by bars. Differing superscript letters (a, b, c) indicate weekly means differ between treatments (treatment × week interaction; $P<0.05$).

calves after weaning at 12 weeks, compared to those weaned at 10 weeks and a tendency ($P=0.06$) for a smaller increase, compared to those weaned at 8 weeks. Increases were such that after weaning, βHBA levels were greater ($P<0.001$) than those of un-weaned calves, irrespective of the time of weaning. Starch content of the starter was 20.5 g 100 $g^{-1}$ DM. Fecal starch content (g 100 $g^{-1}$ feces) was similar between treatment groups ($P>0.05$), averaging 0.11, 0.16 and 0.17 g 100 $g^{-1}$ in week 5, 7 and each week from week 9 onwards, respectively. No treatment × time interaction was observed.

## 4. Discussion

In agreement with previous studies (Jasper and Weary, 2002; Quigley et al., 2006), intakes of starter and straw were negligible in the first four weeks of life. Average overall pre-weaning intakes for calves weaned at 8 weeks of age were similar to a previous study by Terré et al. (2007) where male Holstein calves were fed an elevated level of nutrition (up to 7 L milk $d^{-1}$; 250 g $kg^{-1}$ CP; 0.31 *vs.* 0.36 kg $d^{-1}$, respectively), but were lower than later weaned calves (av. 0.40 and 0.63 kg $d^{-1}$ for calves weaned at 10 and 12 weeks, respectively) despite the higher milk volumes fed in the current study. Additionally, starter consumption rose in all animals from 5 weeks of age, until a surge at weaning, which is consistent with findings from other weaning strategies (de Passillé and Rushen, 2012; Hill et al., 2012; Khan et al., 2007; Sweeney et al., 2010).

Calf growth (ADG, kg $d^{-1}$) decreased at weaning, regardless of the time of weaning, but these decreases were not as pronounced as those previously reported (Sweeney et al., 2010; Jasper and Weary, 2002) and were less severe the later the calves were weaned (Fig. 1b), which agrees with previous reports (de Passillé and Rushen, 2012). A growth advantage was evident in BW between the calves weaned at 8 and 12 weeks of age, after week 10 of the experiment indicating that calves may benefit from a delayed weaning when fed an elevated plane of nutrition pre-weaning (Fig. 1b).

Increasing portal blood βHBA concentrations are considered to reflect increasing ruminal mass and cell number, as the ketogenic capacity per cell remains relatively constant prior to weaning age (Lane et al., 2000, 2002). Our results support this, as serum βHBA was low and treatment had no effect before weaning. However, sharp increases in serum βHBA and starter intake were observed following the step-down week of weaning and the increase occurred earlier for calves weaned at a younger age, suggesting an earlier activation of the fore-stomach. This activation occurred more gradually in later weaned calves, reflected by the lower post-weaning βHBA spike, having potentially beneficial effects on calf intakes and growth during the weaning period. Fecal starch content has recently been shown to correlate with total tract digestibility (Fredin et al., 2014), where a reduction in starch content represents an increase in feed efficiency in lactating dairy cows (Firkins, 2008); however, no difference was observed in the current study. The methods employed for starch determination are relatively new and as such, require further work to determine their optimal level of sensitivity and potential use for starch determination from fecal samples.

## 5. Conclusion

These results suggest that delaying age at weaning reduces the transient reduction of average daily gain and the rise in βHBA at weaning, while improving overall weight gain in calves fed an elevated plane of nutrition prior to weaning. Overall, this indicates that feeding an elevated plane of nutrition can beneficially improve production performance whilst minimising the welfare concerns associated with pre-weaned calves.

## Conflict of interest

S.J. Meale and M.A Steele have no conflicts of interests. L.N. Leal and J. Martín-Tereso work for Nutreco, a company with commercial interests in the field of calf nutrition.


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