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Effect of the number of daily distributions of solid feed on veal calves' health status, behaviour, and alterations of rumen and abomasa

Marta Brscic[a], Luisa Magrin[a], Paola Prevedello[a], Andrea Pezzuolo[b], Flaviana Gottardo[a], Luigi Sartori[b] and Giulio Cozzi[a]

[a]Dipartimento di Medicina Animale, Produzioni e Salute (MAPS), Università di Padova, Legnaro (PD), Italy; [b]Dipartimento Territorio e Sistemi Agroforestali (TESAF), Università di Padova, Legnaro (PD), Italy

ABSTRACT
The research aimed at evaluating the effect of three *versus* two daily distributions of solid feed on veal calves' health, behaviour, and rumen and abomasal mucosa alterations with the rationale that three distributions might improve calves' health and welfare. The study was carried out in two veal calf farms that provided different amounts of solid feed (farm A 200 kg DM/calf; farm B 150 kg DM/calf) during the fattening in addition to liquid milk-replacer. It involved 342 calves in farm A and 108 calves in farm B. The change from two to three solid feed distributions/day started for half calves/farm after the third month of fattening when farm A was feeding 800 g and farm B 600 g DM/calf/day. Health status, blood haemoglobin, and behaviour were assessed on farm at different times. Calves' carcass weight was recorded and rumens and abomasa were inspected *post mortem*. Increasing solid feed distributions did not improve calves' health but it reduced non-nutritive oral behaviours (4.8 *versus* 3.2 ± 0.4% for two and three distributions/day, respectively) by prolonging the time spent eating solid feed. Carcass weight was similar between treatments. Three daily solid feed distributions did not reduce the prevalence of rumen mucosa hyperkeratinisation and abomasal alterations, worsening the frequency of rumen plaques. As the partition of daily dose of solid feed in three distributions is more time and labour consuming, its benefits addressing only the reduction of non-nutritive oral behaviours seem not sufficient to justify the routinely adoption of this practice by veal producers.

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Behaviour; gastrointestinal alteration; solid feeding; veal calf; welfare

Highlights

- Three daily solid feed distributions reduced calves' non-nutritive oral behaviours.
- Increasing the number of daily solid feed distributions did not improve rumen and abomasa mucosa alterations.
- Beneficial effect on non-nutritive oral behaviours does not support the greater time and labour required for the third daily solid feed distribution.

Introduction

Veal calf production is mainly based on the fattening of male calves of dairy breeds that are unsuitable for beef production. In recent years, this sector achieved relevant animal welfare improvements through major changes in the traditional housing and feeding systems (Cozzi et al. 2009). In particular, the European Directives for the protection of calves 91/629/EC, 97/2/EC, and 08/119/EC (European Council 1991, 1997, 2008), made mandatory the grouping of calves in multiple pens as alternative to the housing in individual crates, as well as the provision of solid feeds in addition to the liquid milk-replacer (MR) diet. Solid feeding has become a core research topic in veal calves' production over the last twenty years, covering a wide range of issues from the testing of diverse types of feed sources to improve growth performance (Morisse et al. 2000; Cozzi et al. 2002; Suárez et al. 2006) to big challenges such as developing feeding strategies to reduce economic and environmental impacts (Mollenhorst et al. 2016) and antibiotic use (Pardon et al. 2014). Further research driven feeding strategies aimed at promoting calves' welfare in terms of gastro-intestinal health (Prevedello et al. 2012; Brscic et al. 2014; Berends et al. 2015), blood haemoglobin concentration (Prevedello et al. 2009), and non-nutritive

CONTACT Dr Luisa Magrin luisa.magrin@studenti.unipd.it Dipartimento di Medicina Animale, Produzioni e Salute (MAPS), Università; di Padova, Legnaro (PD), Italy

oral behaviours (Veissier et al. 1998; Webb et al. 2012) with different levels of success.

In this scenario, a negative outcome of several studies on calf's welfare was the high prevalence of abomasal lesions in the pyloric area. The high occurrence of these digestive alterations that involved over 70% of calves is a great concern for the veal industry as it was considered a consequence of intensive feeding system used in most fattening units (Bähler et al. 2010; Brscic et al. 2011a). The current provision of large volumes of MR in just two daily meals followed by the ingestion of the solid feed has been hypothesised as potential cause for abomasal lesions. Both solid feeds highly rich in fibrous particles and those highly rich in amylaceous substrates might be responsible for ulcerations on abomasal mucosa. The former type of solid feed could cause a mechanical friction on the pyloric antrum or a partial obstruction of the pyloric exit (Mattiello et al. 2002; Bähler et al. 2010). The latter type might lead to anomalous fermentation of amylaceous substrates and consequent extended periods of abomasal pH hyperacidity (Ahmed et al. 2002). The rationale behind this study is that increasing solid feeding frequency might improve calves' health and welfare and decrease the prevalence of gastrointestinal mucosa alterations. In particular, the partition of the daily amount of solid feed in more meals reduced in volumes might enhance the mechanical load of the stomach through a better digestibility, and a minor abrasive effect of the solid particles on the abomasal mucosa. Therefore, this research aimed at comparing health, behaviour, and prevalence of rumen and abomasal mucosa alterations of veal calves fed different feeding plans that received the daily dose of solid feed in three meals rather than the usual two.

Materials and methods

Farms, housing system and animals

The study was carried out from August 2014 to April 2015 in two commercial veal calf farms (named A and B, for privacy reasons) located in the provinces of Verona and Mantova, North-Eastern Italy that belonged to the same stockbreeder through agistment contracts. The study did not require any approval by the ethical board since no additional calf manipulations were needed. Calf haemoglobin was assessed on the blood samples routinely collected by the farm vet to comply with the minimum threshold value set by the European Directives on calf protection. In both farms, calves were housed in small group pens of four to seven animals each on fully slatted wooden floors with a space allowance fulfilling the minimum requirement of 1.8 m^2/calf (European Council 1991, 1997, 2008). Farm A had housing facilities for a total of 3000 calves and this study used 342 Italian Holstein calves, belonging to the same batch, prevalently males (<3% of females), with an initial age and body weight (mean ± SD) of 20.9 ± 0.42 days and 54.4 ± 0.26 kg, respectively, that were housed in 56 pens of two contiguous barns (28 pens/barn). Farm B had housing facilities for a total of 400 calves and this study used 108 Italian Holstein male calves, belonging to the same batch, with an initial age and body weight of 23.8 ± 1.90 days and 44.9 ± 0.43 kg, respectively, that were housed in 20 pens of two contiguous barns (10 pens/barn).

Feeding plan and solid feed distribution treatments

The two farms used different feeding plans that, according to the European Directives for the protection of calves (European Council 1991, 1997, 2008), consisted of a combination of reconstituted MR and solid feed. In farm A, MR was delivered in two daily meals at 07:30 – 08:00 and 18:30 – 19:00 h in a collective trough and its amount was gradually increased from the initial 300 g DM/calf per day to 2160 g DM/calf per day at the end of the fattening cycle. The provision of solid feed started after 20 days from calves' arrival at the fattening unit and its daily amount was increased gradually from 40 g DM/calf at the beginning to 1920 g DM/calf at the end of the fattening cycle, for a total of 200 kg DM/calf. In farm B, MR was delivered in two daily meals at 07:00 – 07:30 and 17:00 – 17:30 h in a collective trough and its amount was gradually increased from the initial 235 g DM/calf per day to 2330 g DM/calf per day at the end of the fattening cycle. The provision of solid feed started after 25 days from calves' arrival at the farm and its daily amount was increased gradually from 35 g DM/calf at the beginning to 1650 g DM/calf at the end of the fattening cycle, for a total of 150 kg DM/calf. According to information of previous fattening cycles gathered from the farm books, the duration of the fattening cycle in both farms was expected to be on average 200 days. As shown in Table 1, the farms differed also for the type of solid feeds used during the fattening.

Regarding the daily schedule of solid feed distribution (SFD), calves of both farms received the solid feed twice a day, 30 min to 1 h after the distribution of the MR, in the first part of fattening. The experimental

Table 1. Feed ingredient and chemical composition (mean ± SE) of the solid feeds provided to the calves during the fattening cycle in the two farms.

	Farm A		Farm B	
Period of fattening (mean calves' age)	I (29–75 day)	II (76 day–end)	I (21–169 day)	II (170 day–end)
Ingredient, as fed basis				
Corn grain, %	44.0	67.0	60.0	43.0
Barley and corn based commercial mixture[a], %	44.0	–	–	16.0
Corn grain based commercial mixture[b], %	12.0	33.0	40.0	22.0
Corn silage based commercial mixture[c], %	–	–	–	18.0
Chemical composition				
Dry matter (DM), %	88.3 ± 0.6	87.2 ± 0.6	87.0 ± 0.4	76.9 ± 0.3
Crude protein (CP), % DM	12.4 ± 0.2	9.1 ± 0.2	9.3 ± 0.1	10.0 ± 0.2
Ether extract (EE), % DM	3.7 ± 0.2	3.5 ± 0.1	3.5 ± 0.2	3.5 ± 0.1
Starch, % DM	58.6 ± 0.8	62.5 ± 1.9	60.6 ± 0.4	53.8 ± 0.3
Neutral detergent fibre (NDF), % DM	13.6 ± 1.0	15.5 ± 1.1	16.6 ± 0.9	21.1 ± 0.5
Non-fibrous carbohydrate[d], % DM	66.1	68.9	62.1	68.1
Ash, % DM	4.2 ± 0.2	3.0 ± 0.1	2.5 ± 0.3	3.3 ± 0.2
Iron, ppm	25.5 ± 0.7	21.8 ± 1.2	22.7 ± 1.5	26.0 ± 0.8

[a]Commercial mixture containing mainly barley, flaked corn, oat, corn grain, lupin bean, corn gluten, and soybean proteins; [b]Commercial mixture containing mainly corn grain, corn gluten, and wheat straw; [c]Commercial mixture containing mainly corn silage, corn grain, and ammonium propionate; [d]Non-fibrous carbohydrate content calculated as: 100 − (NDF + CP + EE + ash)

differentiation of the number of SFD started after the third month of fattening. In farm A the comparison started when calves ate about 800 g DM/calf per day: 170 calves housed in the same barn kept on receiving the solid feed in two meals per day (2×) after each MR meal, whereas for 172 calves housed in an identical contiguous barn the daily amount of solid feed was fractioned in three daily distributions (3×) at 8:30, 10:30, and 19:30 h. In farm B, the different SFD started when calves ate about 600 g DM/calf per day; solid feed was fractioned in three daily distributions (3×) at 8:00, 10:00, and 18:00 h and it was provided to 54 calves housed in the same barn. The remaining 54 calves housed in an identical contiguous barn did not change the usual 2× SFD right after each MR meal throughout the fattening. Both farms provided drinking water to the calves in the collective trough twice a day. Sample of solid feeds were collected monthly in both farms and frozen at −18 °C until laboratory analysis. Thawed samples were analysed for dry matter (DM), crude protein (CP), ether extract (EE), ash, and starch content according to the methods of the Association of Official Analytical Chemists (AOAC 1990) and for neutral detergent fibre (NDF) content as proposed by Van Soest et al. (1991). The non-fibrous carbohydrate content (NFC) was calculated as: 100 − (NDF + CP + EE + ash).

In vivo health status of calves

In vivo individual examination of the calves' health status was carried out in both farms by the same experienced vet during four observation days at different time stages of the fattening. The first observation was performed in the week preceding the start of experimental differentiation of SFD (T 0) and the following ones at 30 days interval: 1 (T 1), 2 (T 2), and 3 (T 3) months after the beginning of the different SFD. During each visit the vet visually inspected every calf from the feeding alley and recorded the number of calves in each pen showing signs (presence/absence as a binary measure) of gastrointestinal, respiratory, integumentary, or locomotory disorders or of a generally compromised health status (Table 2). Haemoglobin concentration was assessed by the farm vet to comply with the threshold value set by the European Directives. Haemoglobin was measured on blood samples collected at three following time points of the fattening cycle in both farms: before the differentiation of SFD (Sample 1) and within the fourth and fifth month of fattening (Samples 2 and 3). Individual blood samples were taken from the jugular vein after the morning feeding using Vacutainer tubes containing K_3 EDTA (Delta Chemie Biotechnology, Napoli, Italy). On the basis of results of the haemoglobin checks, anaemic calves or calves with a high risk of becoming anaemic were treated with an injection of iron dextran (Endofer 100 mg/mL, FATRO S.p.A., Ozzano Emilia, BO, Italy). Iron treatments were expressed as proportions of calves injected with iron over the total number of calves checked at the haemoglobin monitoring. Data regarding mortality and early culling of calves were gathered through the farm books starting at T 0.

Behaviour

Behavioural observations were carried out by four assessors who had a joined training on farm prior to the beginning of SFD differentiation. Three observations were carried out on the same days of health status visits at T 1, T 2, and T 3. In both farms,

Table 2. Description of the animal-based measures used in the assessment protocol for the *in vivo* examination of calves' health status during the week before the beginning of the experimental differentiation of the number of solid feed distributions, and 1, 2, and 3 months after the beginning of the experimental differentiation of the number of solid feed distributions.

Measure	Description
Gastrointestinal disorder	
Poorer body condition	average weight and condition behind for more than 30% compared to the mid-range of the batch
Bloated rumen	obviously tensed abdomen, more convex than the shape of the ribs
Diarrhoea	liquid manure on calf body
Respiratory disorder	
Abnormal breathing	difficult or laboured breathing, calf performing belly movements, increased respiratory frequency over 40/min
Coughing	visible, sudden and noisy expulsion of air
Nasal discharge	clearly visible flow/discharge from the nostrils, transparent to yellow/green and often of thick consistency
Integumentary alteration	
Hard skin or hyperkeratinisation	thickened skin (wrinkled with loss of hair), often at the shoulders/withers
Chewing wounds	damaged tail/ear with/without presence of fresh blood or scab
Urine drinking signs	wet, yellow snout (sucklers), swollen hairless prepuce (victims)
Skin lesion or swelling	alopecic sites; irregular skin due to mycoses, parasites, viruses; skin redness; or swelling
Locomotory disorder	
Lameness	abnormality of movement, from reduced ability to use one or more limbs in a normal manner to complete inability to bear weight
Joint lesion	thickening of the joint due to accumulation of synovia, swelling
Claw lesion	inflammation with red and swollen skin at the claw edge
Generally compromised health status	
Sick appearance	an apathetic calf or showing hanging ears or a body position indicating pain or any combination of these signs
Wet calf	wet coat along the entire back
Dull fur	entire coat being dull, not shiny or glossy, sometimes appearing as dry or long or both

Table 3. Least means and 95% confidence intervals of prevalence of veal calves observed with health status problems during the four health status observation days according to the number of daily solid feed distributions (2× and 3×) and to the farm (A and B).

	Solid feed distributions (SFD)		Farm (F)		*p* Value			
	2×	3×	A	B	SFD	F	SFD × F	Observation
Pens observed (*n*)	28	28	56	20				
Calves observed[a] (*n*)	216	222	330	108				
Clinical signs[b], % of calves								
Bloated rumen	2.4 (1.2–4.7)	3.8 (2.4–6.0)	3.4 (2.3–5.1)	2.6 (1.3–5.1)	.230	.446	.416	.001
Coughing	3.4 (2.3–5.0)	5.5 (3.9–7.6)	3.7 (2.7–5.0)	4.9 (3.2–7.6)	.058	.349	.343	.220
Skin lesion or swelling	8.1 (6.3–10.3)	6.2 (4.5–8.7)	7.5 (6.1–9.2)	6.7 (4.4–10.4)	.140	.631	.745	.003
Joint lesion	3.7 (2.4–5.7)	5.3 (3.7–7.5)	5.5 (4.6–6.5)	3.6 (1.9–6.6)	.153	.096	.869	.019

[a]Average number of calves observed during the 4 health status observation days; [b]Only the occurrences of clinical signs having an overall prevalence above 5% of calves are processed using a multivariate regression model and presented in this Table.

behavioural observations were carried out considering 10 pens per treatment (2× and 3×). Each observation session lasted 5 h, from 1 h before (H -1) to 4 h after (H 0, H 1, H 2, and H 3) the morning MR meal. Two assessors observed from the feeding alley five pens each belonging to the same treatment for the entire observation session using the scan-sampling technique (Martin and Bateson 2007) with a 5-min interval between scans. The same observers moved in a rotational manner among pens every observation sessions to reduce bias caused by observer effect. At each scan, observers recorded the number of calves per pen that were standing or lying, and the number of calves involved in: eating solid feed, ruminating, grooming (sum of auto- and allo- grooming), non-nutritive oral behaviours (calf performs oral behaviours including tongue rolling, tongue playing, and stereotyped licking of pen as described by Leruste et al. (2014)), manipulating pen facilities (calf licks, nibbles, sucks, or bites at the fence, wall, bucket, trough, floor or objects in the pen not specifically in a stereotypical way), other (calf shows any behaviour which is not described above or is not visible to the observer). Posture and behaviours within pen at each hour of observation were expressed as percentage of calves performing these behaviours.

Post mortem inspection

Calves were slaughtered at the same abattoir when they reached a suitable finishing status according to the standard of the Italian veal market. Individual carcass weight of calves was recorded and rumens and abomasa were inspected. Forestomaches were resected from the intestines, divided (reticulorumen, omasum, and abomasum), and reticulorumens were opened and emptied by the slaughterhouse staff. Presence of papillary epithelial hyperkeratinisation and plaques on the rumen wall were recorded as described by Brscic et al. (2011a). Abomasa were opened by a longitudinal cut made by the observer who evaluated the presence of lesions on the mucosa of the torus pyloricus (binary, presence/absence) and in the pyloric area where they are prevalent according to previous studies (Veissier et al. 2001; Brscic et al. 2011a). Lesions in the pyloric area were counted from 0 (no lesions) to a censored maximum of 4 (presence of 4 or more lesions) within each of three size classes (small: lesions less than 0.5 cm^2 in diameter, medium: 0.5 to 1 cm^2 diameter lesions, and large: lesions larger than 1 cm^2 in diameter) and a weighted lesion mean score was calculated according to Brscic et al. (2011a).

Statistical analysis

Statistical analyses of data were carried out using SAS/STAT version 9.3 (SAS Inst. Inc., Cary, NC, USA). Pen was the experimental unit for data regarding *in vivo* health status and behaviour. Individual calf was the experimental unit for blood haemoglobin concentration and carcass weight. Health status data were expressed as percentages of calves that were involved by a given problem over the calves observed at pen level. Only the occurrences of clinical signs having an overall prevalence above 5% of calves were analysed with a multivariate regression model that considered the effects of treatment (2× *vs.* 3× SFD), farm (A *vs.* B), observation day, and the interactions treatment × farm, treatment × observation day, and farm × observation day using Proc Genmod of SAS with the Poisson distribution and log link options. Haemoglobin concentration data were analysed using a hierarchical mixed effects model with the Proc Mixed procedure of SAS. The model considered the fixed effects of treatment, farm, sampling day as repeated measure and calf within farm as subject, and treatment × farm, treatment × sampling day, and farm × sampling day interactions. Iron injections were expressed as percentage of treated animals over the animals sampled at each haemoglobin sampling day within treatment. These percentages were compared using the *k* proportions test with XLSTAT to assess differences by treatment and by farm within sampling day. Carcass weight data were analysed with the Proc GLM procedure of SAS that considered the effects of treatment, farm, and their interaction, and the initial body weight as covariate.

Behavioural data was gathered post training during the test observation on farm were used to test the agreement between each of the four assessors and the experienced veterinarian (silverstandard) with the Cohen's Kappa test using Proc Freq of SAS. Intra class correlation coefficients (ICC) were calculated also for behavioural data gathered during the three observation sessions to assess agreement between observers *a posteriori* and they were graded according to Cicchetti (1994). Behavioural data were analysed using hierarchical mixed effects models with the Proc Mixed procedure of SAS that considered the fixed effects of treatment, farm, observation day, treatment × farm and treatment × time from milk interactions, and time from milk as repeated measure and pen within farm as subject. *Post mortem* data were expressed as percentages of affected organs (within each of three size classes in case of affected abomasa) over the total observed at barn level. These percentages were compared using the k proportions test with XLSTAT to assess differences by treatment and by farm. Data regarding the abomasal lesion score were analysed with a non-parametric Kruskal–Wallis test using XLSTAT to assess differences by treatment, farm, and their interaction.

Results

In vivo health status of calves

According to the current diets fed to veal calves, the solid feeds provided to the calves were particularly rich in starch (Table 1) and the daily starch intake at the end of the fattening was about 1200 and 900 g/calf in farm A and farm B, respectively. Mortality rate recorded during the experiment was very low (0.6% in farm A and none in farm B) and in farm A, it was equally distributed between 2× and 3× SFD. Moreover, regardless of farm and SFD, the occurrences of most disorders investigated at the health status visits (Table 2) had an overall prevalence below 5% of calves. Clinical signs of bloated rumen, coughing, skin lesion or swelling, and joint lesion were recorded for a greater percentage of animals (Table 3) but they were not affected by SFD and farm ($p > .05$). No significant treatment × farm interactions were recorded for these clinical problems. There was a significant effect of the observation day ($p < .001$) on bloated rumen with a similar prevalence at T 0 and T 1, increasing at T 2 and was the highest at T 3 (Figure 1, top left and right). Prevalence of skin lesion or swelling and of joint lesion was also affected by the observation day ($p < .05$) with different trends over time. Skin lesions or swellings were higher at T 1 and T 2 compared to T 0 and T 3, while joint lesions were higher at T 3 compared to the other observations. Blood

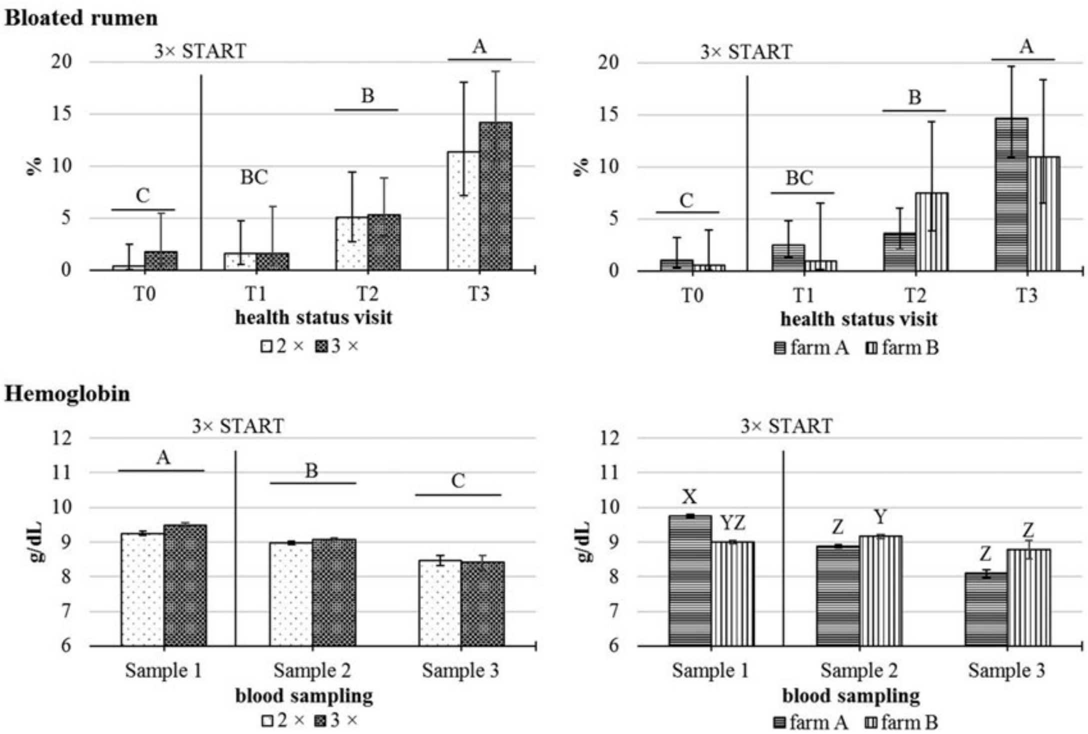

Figure 1. Prevalence of veal calves observed with bloated rumen (Least square means ±95% confidence intervals) (top figures) and haemoglobin concentration (Least square means ± SEM) (bottom figures) according to the interaction between treatment and observation or blood sampling day (left figures) and between farm and observation or blood sampling day (right figures). Different letters (A–C) indicate significant differences for observation or sampling day (p <.001). Different letters (X–Z) indicate a significant farm × blood sampling day interaction (p <.001).

haemoglobin concentration was similar between treatments (8.90 ± 0.06 g/dL and 8.99 ± 0.08 g/dL for 2× and 3× SFD, respectively) and farms (8.91 ± 0.10 g/dL and 8.98 ± 0.05 g/dL in farm A and farm B, respectively) and it decreased progressively according to the sampling day ($p < .001$). There was no significant treatment × farm interaction for blood haemoglobin concentrations. A significant farm × sampling day interaction was observed (Figure 1, bottom right) with the highest concentration in farm A at the first sampling followed by the concentration in farm B at the second sampling. Iron treatments to prevent anaemia were followed each blood haemoglobin sampling day. At the first sampling, they differed according to both, treatment (45.0 ± 2.7% and 31.4 ± 2.9% of calves for 2× and 3× SFD, respectively; $p < .001$) and farm (17.2 ± 0.6% and 60.6 ± 1.5% of calves for farm A and farm B, respectively; $p < .001$). There was no treatment effect on the prevalence of iron injected calves after the second haemoglobin sampling (38.4 ± 1.0% and 36.3 ± 2.1% of calves for 2× and 3× SFD, respectively; $p > .05$) but a significant farm effect (25.4 ± 0.6% and 50.0 ± 0.5% of calves for farm A and farm B, respectively; $p < .001$). No iron treatment was performed after the last haemoglobin sampling. Iron injections did not differ for treatment × farm interaction.

Behaviour

During the three observation sessions the agreement between each assessor and the experienced veterinarian was ≥0.81 for all behaviours. Intra class correlation coefficients were good (between 0.61 and 0.73) for all behavioural variables except for eating solid feed that was excellent (0.81) and for ruminating and grooming that were fair (≤ 0.58). Among observed behaviours, SFD treatment affected only non-nutritive oral behaviours being higher for 2× calves (4.8 ± 0.4% *vs.* 3.2 ± 0.4%; $p < .05$). This behaviour progressively increased over time from T 1 to T 3 ($p < .05$). Grooming and manipulating pen facilities differed according to farm resulting more frequent in farm B compared to farm A (8.7 ± 0.29% *vs.* 7.8 ± 0.29%, $p < .05$ for grooming; and 13.0 ± 0.56% *vs.* 11.4 ± 0.56%, $p < .05$ for manipulating pen facilities). Grooming decreased over time with the lowest frequency at T 3 ($p < .05$). The interaction between treatment and farm did not affect non-nutritive oral behaviour, grooming, and manipulating pen facilities activities. Standing increased over time with the highest percentage of calves observed standing at T 3 and similar percentages at T 1 and T 2 ($p < .05$). A significant SFD × farm interaction was observed for standing (75.5 ± 1.69% *vs.* 70.2 ± 1.69% for 2× and 3× calves in farm A respectively; 66.3 ± 1.63% *vs* 72.3 ± 1.63% for 2× and 3× calves in farm B respectively; $p < .05$). The significant interactions between SFD and time from MR distribution observed for standing, eating solid feed and non-nutritive oral behaviour are shown in Figure 2.

Post mortem inspection

Calves from farm A were slaughtered after 206 days of fattening and calves from farm B after 211 days of fattening. Carcass weights of calves differed by farm, being higher in farm B than those in farm A (147.3 ± 2.3 kg *vs.* 140.8 ± 1.2 kg, respectively; $p < .05$). The different SFD did not affect calves' carcass weight (142.6 ± 1.6 kg *vs.* 145.6 ± 1.5 kg for 2× and 3× SFD, respectively; $p > .05$). The interaction SFD × farm did not affect carcass weights of calves. Results of the *post mortem* rumen and abomasal mucosa evaluations are reported in Table 4. There was a tendency by 3× SFD calves to reduce the prevalence of hyperkeratinized rumen mucosa compared to those receiving the two daily distributions. Plaques were observed more frequently on rumen mucosa of calves fed 3× SFD. Presence and severity of abomasal lesions were similar between SFD treatments, while they worsen in farm A compared to farm B either in terms of frequency on torus pylorus or of medium and large lesions in the pyloric area.

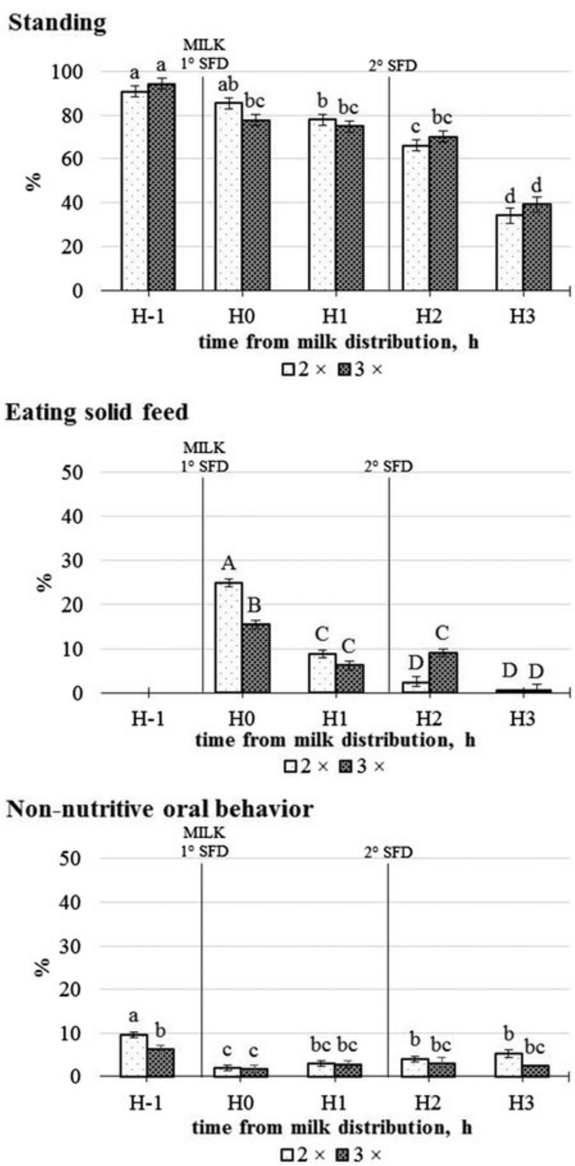

Figure 2. Effect of treatment × time from milk-replacer distribution interaction on the percentage of veal calves observed in standing posture, eating solid feed or performing non-nutritive oral behaviour during the three 5-h behavioural observation sessions (Least square means ± SEM). Different letters indicate significant differences within a given behaviour ($p < .05$ lower case; $p < .001$ capital letters). 1° SFD: 1st solid feed distribution for all calves; 2° SFD: 2nd solid feed distribution only for calves receiving three daily doses of solid feed.

Discussion

Veal production in Europe has very standardised rearing systems (Cozzi et al. 2009) and as reported by several recent studies (Prevedello et al. 2012; Berends et al. 2015; Mollenhorst et al. 2016), the amount of solid feeds provided to the calves has by far been increased above the recommended quantity set by the Directives in force. The rationale behind this research was that increasing the number of solid feed meals might have an impact on calves' health and welfare. The fractioning the daily amount of solid feed in three rather than the usual two meals did not affect calves' mortality as well as the occurrences of severe health problems that were generally low. Based on the similar carcass weights, we could hypothesise that SFD had not relevant effects on calves' growth. However, the three SFD allowed a significant welfare improvement through the reduction of non-nutritive oral behaviours that are known to be indicators of poor welfare (Bokkers et al. 2009). In particular, we observed a reduction of non-nutritive oral behaviours during the hour before feeding when these behaviours are shown to be more frequent as calves crave for the MR (Veissier et al. 1998; Bokkers and Koene 2001). As pointed out by Webb et al. (2012), the relationship between solid feed and non-nutritive oral behaviours in veal calves is rather complex and the feeding strategy plays a key role in reducing these behaviours that are signs of frustration and chronic stress. In this study the occurrence of these behaviours was higher compared to that (on average 1.21% ± 0.45) reported by Brscic et al. (2014), and this

Table 4. *Post mortem* rumen and abomasal mucosa evaluations (mean ± SE) carried out at the slaughterhouse according to the number of daily solid feed distributions (2× and 3×) and to the farm (A and B).

	Solid feed distributions (SFD)		Farm (F)		*p* Value	
	2×	3×	A	B	SFD	F
Inspected rumens (*n*)	209	209	340	74		
Rumen mucosa alteration, % of rumens						
Hyperkeratinisation	36.8 ± 0.03	28.2 ± 0.03	32.8 ± 0.03	31.1 ± 0.05	.099	.874
Plaques	84.7[B] ± 0.02	95.2A ± 0.01	89.8 ± 0.02	90.5 ± 0.03	.001	.999
Inspected abomasa (*n*)	209	205	337	77		
Abomasal lesion, % of abomasa						
Presence on torus pylorus	75.6 ± 0.03	78.5 ± 0.03	80.1[a] ± 0.02	63.6[b] ± 0.06	.552	.008
Presence of at least 1 lesion in pyloric area	88.0 ± 0.02	91.2 ± 0.02	90.8 ± 0.02	84.4 ± 0.04	.367	.206
Small lesions	57.9 ± 0.06	61.0 ± 0.07	60.8 ± 0.05	53.2 ± 0.10	.590	.280
Medium lesions	51.7 ± 0.08	56.1 ± 0.08	57.9[A] ± 0.06	36.4[B] ± 0.14	.421	<.001
Large lesions	72.7 ± 0.08	74.6 ± 0.09	76.6[a] ± 0.07	61.0[b] ± 0.15	.742	.014
Abomasal lesion score[a]	10.7 ± 0.53	11.3 ± 0.58	11.8[A] ± 0.44	7.4[B] ± 0.71	.770	<.001

[a]Score weighed according to the number of lesions (from 0 to maximum of 4, censored) and size category (1 = < 0.5 cm^2; 2 = 0.5 – 1 cm^2; 3 = > 1 cm^2) calculated as [(*n* of lesions size 1 × 1) + (*n* of lesions size 2 × 2) + (*n* of lesions size 3 × 3). Different superscript letters indicate significant differences for $p < .001$ (A-B) and for $p < .05$ (a-b) within treatment and farm effects.

might be due to the higher proportion of concentrates and NFC content, and the lower NDF content of our solid feeds (Table 1) than those of the cited previous study (corn grain: around 80% as-fed basis; average NFC: 62% of DM and NDF: 22.9% of DM). A further positive effect observed on 3× calves was the prolonged ingestive pattern of solid feed. The additional solid feed distribution extended the time spent eating and a peak of intake was recorded in 3× calves about 2 h after the morning milk distribution when they were offered the second solid feed, not delivered to 2× calves. Likewise to the provision of driking water (Gottardo et al. 2002), the additional feed distribution could act as environmental enrichment reducing the need of calves to perform stereotyped activities.

The prevalence of bloated rumen, skin lesions or swellings and their trend over time are in line with previous results of Brscic et al. (2009). The prevalence of joint lesions observed in this study was higher compared to the average values (<1% of calves) reported by Brscic et al. (2009, 2011b). However, all these clinical signs might be directly related to the resources on farm rather than to the change in the number of solid feed distributions. The large amounts of high starch feeds delivered towards the end of the fattening in both farms could act as predisposing factors for anomalous ruminal fermentations of the solids in calves still receiving MR as well as for an inefficient volatile fatty acid (VFA) absorption in the forestomaches (Hinders and Owen 1965). The former hypothesis could be related to a miss-adaptation of the rumen microorganism to the solid feeds consequent to MR leaking in the rumen as a result of a partial failure of the reticular groove reflex (Suàrez et al. 2007). Berends et al. (2015) observed amounts of milk recovery in the rumen on average around 20% of the last MR meal regardless of the level of solid feed and of the roughage-to-concentrate ratio of the solid. The latter hypothesis, regarding inefficient VFA absorption, could find support in the results reported by Brscic et al. (2011a) and in the *post mortem* data of this study, given the high occurrence of rumen mucosa alterations. Hinders and Owen (1965) demonstrated that the amount of ruminal VFA absorption decreased with increasing severity of ruminal hyperkeratosis. Rumen plaques might also create a barrier to VFA absorption predisposing the rumen to bloat (Suàrez et al. 2007).

The high occurrence of rumen plaques and hyperkeratinisation observed in this study compared to the average values reported by Brscic et al. (2011a) could arise from the high starch load of the diets provided in both farms. The aetiology of these alterations of the rumen wall in veal calves was mainly associated to diets with high starch/fibre ratio (Suàrez et al. 2006; Brscic et al. 2011a) and low abrasive value (Greenwood et al. 1997). In this study, the three daily distributions of solid feed tended to reduce the occurrence of rumen mucosa hyperkeratinisation but they had a detrimental effect on the prevalence of rumen plaques. It is difficult to find a biological explanation for this result since the intake of starch was equal between 3× and 2× calves. Moreover, the three daily distributions of solid feed have not overcome also the problem of abomasal lesions that imply a given degree of poor welfare in calves (Broom 2006), being potentially fatal to cattle (Hund et al. 2015). The aetiology of abomasal lesions in veal calves might be due to the association between the large volumes of MR provided in the two daily meals which over-distend the stomach wall (Wiepkema et al. 1987; Veissier et al. 2001; Webb et al. 2014), and the poorly digested solid feeds that worsen the mechanical load of the stomach through the additional abrasive effect of the solid particles (Bähler et al. 2010; Brscic et al. 2011a).

The outcomes of the present study suggest also a potential association between abomasal lesions and starch intake since calves of farm A that received a greater amount of starch throughout the fattening had the worst prevalence and severity of abomasal lesions. It is likely that this high occurrence of abomasal lesions recorded in calves of farm A could have impaired their growth performance, even if our material is not sufficient to prove this assumption. According to Ahmed et al. (2002), the lower abomasal luminal pH caused by the transit of a fermenting amylaceous substrate may act as risk factor for ulceration of the mucosa. In veal calves, blood haemoglobin concentration is managed through a feeding plan low in iron and it is monitored by regular checks during the fattening targeting a progressive reduction over time (Cozzi et al. 2009). Results of the present study showed that SFD did not affect haemoglobin trend and the significant farm effect observed at the 1st and 2nd sampling arose from their different feeding plan. Outcomes of this study leave space for other revisions of the actual feeding programs for veal calves such as the further daily differentiation of solid feed or its *ad libitum* distribution, or for investigations of innovative feeding programs such as the differentiation of the MR in more daily meals or its *ad libitum* distribution.

Conclusions

The outcomes of the research demonstrated that the partition of the daily amount of solid feed in three meals compared to the usual two ones was not a solution to improve calves' health and particularly to lower the prevalence of gastrointestinal mucosa alterations. As this practice would be more time and labour consuming, its benefits addressing only the reduction of calves' non-nutritive oral behaviours seem not sufficient enough to justify its routinely adoption by veal producers.

Disclosure statement

No potential conflict of interest was reported by the authors.

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ORCID

Marta Brscic http://orcid.org/0000-0001-8366-5961
Luisa Magrin http://orcid.org/0000-0002-2153-6117
Andrea Pezzuolo http://orcid.org/0000-0002-9955-9896
Flaviana Gottardo http://orcid.org/0000-0002-0427-2180
Giulio Cozzi http://orcid.org/0000-0003-0408-1082

References

Ahmed AF, Constable PD, Misk NA. 2002. Effect of feeding frequency and route of administration on abomasal luminal pH in dairy calves fed milk replacer. J Dairy Sci. 85:1502–1508.

AOAC. 1990. Official methods of analysis. 15th ed. Arlington (VA): Association of Official Analytical Chemists.

Bähler C, Regula G, Stoffel MH, Steiner A, von Rotz A. 2010. Effects of the two production programs 'Naturafarm' and 'conventional' on the prevalence of non-perforating abomasal lesions in Swiss veal calves at slaughter. Res Vet Sci. 88:352–360.

Berends H, van den Borne JJGC, Stockhofe-Zurwieden N, Gilbert MS, Zandstra T, Pellikaan WF, van Reenen CG, Bokkers EAM, Gerrits WJJ. 2015. Effects of solid feed level and roughage-to-concentrate ratio on ruminal drinking and passage kinetics of milk replacer, concentrates and roughage in veal calves. J Dairy Sci. 98:5621–9.

Bokkers EAM, Koene P. 2001. Activity, oral behaviour and slaughter data as welfare indicators in veal calves: a comparison of three housing systems. Appl Anim Behav Sci. 75:1–15.

Bokkers EAM, Leruste H, Heutinck LFM, Wolthuis-Fillerup M, van der Werf JTN, Lensink BJ. van Reenen CG. 2009. Inter-observer and test-retest reliability ofon-farm behavioural observations in veal calves. Anim Welfare. 18:381–390.

Broom DM. 2006. Behaviour and welfare in relation to pathology. Appl Anim Behav Sci. 97:73–83.

Brscic M, Gottardo F, Prevedello P, Tessitore E, Cozzi G. 2009. Veal calves' clinical/health status in large groups fed with automatic feeding devices. Ital J Anim Sci. 8:187–189.

Brscic M, Heutinck LFM, Wolthuis-Fillerup M, Stockhofe N, Engel B, Visser EK, Gottardo F, Bokkers EAM, Lensink BJ, Cozzi G, et al. 2011a. Prevalence of gastrointestinal

disorders recorded at postmortem inspection in white veal calves and associated risk factors. J Dairy Sci. 94:853–863.
Brscic M, Gottardo F, Leruste H, Lensink J, Van Reenen CG, Cozzi G. 2011b. Prevalence of locomotory system disorders in veal calves and risk factors for occurrence of bursitis. Agric Conspec Sci. 76:4.
Brscic M, Prevedello P, Stefani AL, Cozzi G, Gottardo F. 2014. Effects of the provision of solid feeds enriched with protein or nonprotein nitrogen on veal calf growth, welfare, and slaughter performance. J Dairy Sci. 97:4649–4657.
Cicchetti DV. 1994. Guidelines, criteria, and rules of thumb for evaluating normed and standardized assessment instruments in psychology. Psychol Assess. 6:284–290.
Cozzi G, Brscic M, Gottardo F. 2009. Main critical factors affecting the welfare of beef cattle and veal calves raised under intensive rearing systems in Italy: a review. Ital J Anim Sci. 8:67–80.
Cozzi G, Gottardo F, Mattiello S, Canali E, Scanziani E, Verga M, Andrighetto I 2002. The provision of solid feeds to veal calves: I. Growth performance, forestomach development, and carcass and meat quality. J Anim Sci. 80: 357–366.
European Council. 1991. Directive 91/629/EC laying down minimum standards for the protection of calves. Official Journal of the European Communities of 11 December 1991, No. L 340/28.
European Council. 1997. Directive 97/2/EC amending Directive 91/629/EEC laying down minimum standards for the protection of calves. Official Journal of European Communities of the 20 January 1997, No. L 25/24.
European Council. 2008. European Council. 2008. Directive 2008/119/EC laying down minimum standards for the protection of calves (Codified version). Official Journal of the European Union of 18 December 2008, No. L 10/7.
Gottardo F, Mattiello S, Cozzi G, Canali E, Scanziani E, Ravarotto L, Ferrante V, Verga M, Andrighetto I. 2002. The provision of drinking water to veal calves for welfare purposes. J Anim. Sci. 80:2362–2372
Greenwood RH, Morrill JL, Titgemeyer EC, Kennedy GA. 1997. A new method of measuring diet abrasion and its effect on the development of the forestomach. J Dairy Sci. 80:2534–2541.
Hinders RG, Owen FG. 1965. Relation of ruminal parakeratosis development to volatile fatty acid absorption. J Anim Sci. 48:1069–1073.
Hund A, Dzieciol M, Schmitz-Esser S, Wittek T. 2015. Characterization of mucosa-associated bacterial communities in abomasal ulcers by pyrosequencing. Vet Microbiol. 177:132–141.
Leruste H, Brscic M, Cozzi G, Kemp B, Wolthuis-Fillerup M, Lensink BJ, Bokkers EAM, van Reenen CG. 2014. Prevalence and potential influencing factors of non-nutritive oral behaviors of veal calves on commercial farms. J Dairy Sci. 97:7021–7030.
Martin P, Bateson P. 2007. Measuring behaviour: an introductory guide. 3rd ed. Cambridge (UK): Cambridge University Press.
Mattiello S, Canali E, Ferrante V, Caniatti M, Gottardo F, Cozzi G, Andrighetto I, Verga M. 2002. The provision of solid feeds to veal calves: II. Behavior, physiology, and abomasal damage. J Anim Sci. 80:367–375.
Mollenhorst H, Berentsen PBM, Berends H, Gerrits WJJ, de Boer IJM. 2016. Economic and environmental effects of providing increased amounts of solid feed to veal calves. J Dairy Sci. 99:2180–2189.
Morisse JP, Huonnic D, Cotte JP, Martrenchar A. 2000. The effect of four fibrous feed supplementations on different welfare traits in veal calves. Anim Feed Sci Technol. 84:129–136.
Pardon B, Catry B, Boone R, Theys H, De Bleecker K, Dewulf J, Deprez P. 2014. Characteristics and challenges of the modern Belgian veal industry. Vlaams Diergeneeskd Tijdschr. 83:155–163.
Prevedello P, Moro L, Brscic M, Gottardo F, Stefani A. 2009. Trend overtime of total haemoglobin, iron metabolism and trace minerals in veal calves fed high amounts of two different solid feeds. Ital J Anim Sci. 8:184–186.
Prevedello P, Brscic M, Schiavon E, Cozzi G, Gottardo F. 2012. Effects of the provision of large amounts of solid feeds to veal calves on growth and slaughter performance and intravitam and postmortem welfare indicators. J Anim Sci. 90:3538–3546.
Suárez BJ, Van Reenen CG, Beldman G, van Delen J, Dijkstra J, Gerrits WJJ. 2006. Effects of supplementing concentrates differing in carbohydrate composition in veal calf diets: I. Animal performance and rumen fermentation characteristics. J Dairy Sci. 89:4365–4375.
Suárez BJ, Van Reenen CG, Stockhofe N, Dijkstra J, Gerrits WJJ. 2007. Effect of roughage source and roughage to concentrate ratio on animal performance and rumen development in veal calves. J Dairy Sci. 90:2390–2403.
Van Soest PJ, Robertson JB, Lewis BA. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J Dairy Sci. 74:3583–3597.
Veissier I, Ramirez De La Fe AR, Pradel P. 1998. Nonnutritive oral activities and stress responses of veal calves in relation to feeding and housing conditions. Appl Anim Behav Sci. 57:35–49.
Veissier I, Passillé AM De, Rushen J, Van Reenen C, Roussell S, Andanson S, Pradel P. 2001. Calves' response to repeated social regroupiny and relocation. J Anim Sci. 79:2580–2593.
Webb LE, Bokkers EAM, Engel B, Gerrits WJJ, Berends H, Van Reenen CG. 2012. Behaviour and welfare of veal calves fed different amounts of solid feed supplemented to a milk replacer ration adjusted for similar growth. Appl Anim Behav Sci. 136:108–116.
Webb LE, Jensen MB, Engel B, Van Reenen CG, Gerrits WJJ, De Boer IJM, Bokkers EAM. 2014. Chopped or long roughage: What do calves prefer? Using cross point analysis of double demand functions. PLoS One. 9:1–8.
Wiepkema PR, Van Hellemond KK, Roessingh P, Romberg H. 1987. Behaviour and abomasal damage in individual veal calves. Appl Anim Behav Sci. 18:257–268.