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The effect of slow-release milk replacer feeding on health and behaviour parameters in dairy breed calves

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Research Article

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Abstract

The aim of this research paper was to evaluate the effect of a slow-release milk replacer on health and behaviour of neonatal dairy calves. This was done with the potential benefits to welfare during transport in mind. A total of 15 calves were randomly divided into three groups of 5, namely, a control group fed twice in 24 h with 3 l of a conventional milk replacer, a slow-release group fed once in 24 h with 2 l of conventional milk replacer and 1 litre of a specialised micro-encapsulated feed and an enriched-replacer group fed once in 24 h with 3 l of milk replacer enriched with micellar casein. Blood samples were taken before feeding and 6, 12, 18 and 24 h after and analysed for acid–base parameters, electrolytes, glucose, haemoglobin, cortisol, insulin, cholecystokinin and adiponectin. Calf behaviour was recorded between 6 and 14 h after feeding. There was a significant increase in blood pH 6 h after feeding in all groups, but the glucose, HCO_3^- and base excess increased significantly in the slow-release group only, whereas sodium increased significantly in the enriched group only. Glucose levels remained significantly higher in the slow-release group, relative to the control, at 6, 12, and 18 h after feeding. Insulin levels changed significantly over time in the enriched and control group but remained constant in the slow-release group. Insulin levels were significantly higher in the control group when compared to the slow-release group after feeding. Adiponectin changed significantly over time after feeding in the control group only, but no significant changes were observed between the feeding groups. Behavioural patterns were similar in control and slow release groups but less favourable (less lying time, more vocalisations) in the enriched group. In conclusion, once-daily feeding of slow-release milk replacer demonstrated favourable patterns of blood variables related to satiety and hunger as well as behavioural patterns that did not differ from conventional twice-daily feeding.

Good feeding is one of the pillars of animal welfare and a major contributing factor to calf health. Feeding satisfaction, or lack of it (i.e. hunger), has not been well studied in calf welfare systems. Hunger, as a result of food deprivation or restriction has been associated with reduced welfare and increased stress responses in cattle (Chen *et al.*, 2015; Bourguet *et al.*, 2011). Certain behavioural changes in calves in response to hunger have been established (Thomas *et al.*, 2001), however, we have not been able to identify any holistic approach to the evaluation of hunger in calves. Feeding calves infrequent meals is associated with an increased serum insulin to glucose ratio (Miller-Cushon and Devries, 2015), which could cause insulin resistance resulting in poor growth rates, disease and possible mortality (Bach *et al.*, 2020). Hormones and metabolites can be used as biomarkers for acute hunger and stress, and several have been studied as satiety markers in humans (Gibbons *et al.*, 2019). Some have previously been measured in calves in relation to colostrum intake (Kesser *et al.*, 2015) and non-nutritive suckling (de Passillé *et al.*, 1993). However, there is currently a paucity of information on the satiety cascade in calves either before or after feeding (pre- and post-prandial levels, respectively).

There is large scope to enhance the welfare of calves through adoption of satiety-based feeding protocols that will give calves feeding satisfaction in a conventional feeding system, as well as in specific circumstances, such as during transport. Non-replacement dairy calves are those that are born on dairy farms, but which are either male or surplus to replacement heifer requirements. With increase in dairy production, the number of these 'unwanted' calves is also increasing. In the EU for example, the number of unweaned male and female calves that are not kept for replacement are around 1.5 million/year (Eurostat, 2019). These calves are often transported for a prolonged period of time (>8 h) to be used for veal or beef production. The EU legislation outlines certain criteria when it comes to transport of calves. The maximum duration of travel is 19 h, with a mandatory rest-stop after 9 h, where calves

are fed if necessary (European Union, 2005) but the welfare benefit of these rest-stops appears to be limited (Marti *et al.*, 2017; Meléndez *et al.*, 2020). Additionally, the necessity to feed the calves is left in the hands of individuals.

The exact requirement for feeding during transport is unknown, but might be higher than on farm because of increased energy demands from being on a moving vehicle (Velarde *et al.*, 2021). The benefits of feeding encapsulated amino acids (lysine and methionine) and protected fats (phospholipids) have been studied in cattle (Mazinani *et al.*, 2020; Shaidorova *et al.*, 2020) but not in pre-ruminant milk-fed calves.

The objective of this exploratory study was to evaluate the effect of a slow-release, micro-encapsulated milk replacer, in comparison with a conventional milk replacer, on health and behaviour of neonatal calves, with a view of enabling further studies assessing the appropriateness of its use to improve welfare of calves during transport where prolonged periods of acute hunger are common.

Materials and methods

This controlled randomised pilot study was carried out on male Friesian/Friesian-cross calves. They were sourced from multiple dairy farms in the Republic of Ireland and housed on the research farm in Teagasc Moorepark (Co. Cork, Ireland), where the study was carried out. Prior to the commencement of the trial, the calves were randomly allocated to 3 different feeding groups, consisting of 5 calves each. The overall average age was 28 d (range 19–42 d), with the age balanced amongst the three groups with average ages of 27.2, 27.6 and 28.1 d. The sample size was determined on the basis of an a priori power analysis (G-Power version 3.1.9.2) using blood glucose data obtained from Fisher *et al.* (2014), which yielded a statistical power of 91%. The calves were housed in individual pens with straw bedding with visual and tactile contact to their peers and put in different sections of the same barn based on their group. The study was approved by the Teagasc Animal Ethics Committee (TAEC 2020/264) and all procedures were authorised and carried out in accordance with the Health Products Regulatory Authority of Ireland (AE19132/P120).

Group SR (slow release) was fed the slow-release milk formula, group ER (enriched replacer) was fed casein-enriched milk replacer and the control group (C) was fed with 3 l of conventional milk replacer (Volac International Ltd, Hertfordshire, United Kingdom, 26% crude (whey) protein, reconstituted at 125 g/l) at 10am (T0) and again at 6pm (T8), as per routine feeding protocol on the farm. SR were fed 2 l of the same conventional milk replacer mixed with 1 litre of slow-release milk formula (Epsilion Ltd, Cork, Ireland), containing micro-encapsulated carbohydrate, micro-encapsulated fat, micro-encapsulated whey protein and micellar casein, at timepoint 0 only, whereas ER were fed 3 l of milk replacer enriched with micellar casein at timepoint 0 only. Once-off feeding of the specialised formulae was chosen to evaluate its potential viability (relative to conventional twice daily feeding) if fed prior to transport and simulate the conditions for calves during transport, when feeding is often not carried out for a prolonged period of time (up to 19 h, according to the legislation). All of the ingredients were reconstituted with warm water (37°C) immediately prior to feeding and all were administered with a teat bucket. The calves had continuous access to fresh water and the water consumption was evaluated throughout the study by measuring the volume of water consumed every 6 h. After the trial the calves were kept in group pens, where they were fed milk replacer as per routine management on farm and had free access to water and concentrates. The calves were weighed at the start of the trial and then again 6 and 24 d later to obtain daily average weight gains.

Measurements were made over a period of 24 h following feeding. Each calf was blood sampled pre- (T0) and four times post-feeding (at T6, T12, T18 and T24) by jugular venipuncture using 21 G needles. Samples were collected into heparinised syringes for the purposes of rapid blood gas analysis using a Siemens Rapidpoint 500 (Cruinn Diagnostics, Dublin, Ireland). Blood parameters reported by the analyser included pH, bicarbonate ion ($HCO3^-$), base excess (BE), strong ion difference (SID), anion gap (AG), sodium (Na^+), potassium (K^+), chloride (Cl^-), calcium (Ca^{2+}), haemoglobin (Hb) and glucose. Additionally, a serum sample was collected before (T0) and twice after feeding (at T12 and T24) using a Vacuette 6-ml serum clot activator tube (Greiner Bio-One GmbH, Kremsmünster, Austria). The samples were centrifuged with a Sorvall ST40 centrifuge (Thermo Fisher Scientific, Langenselbold, Germany) at 3000 rpm for 10 min and the serum was stored at −20°C until it was analysed for bovine cholecystokinin (CCK), bovine adiponectin, bovine insulin, and bovine cortisol using commercially available ELISA kits, validated for bovine serum (AssayGenie, Dublin, Ireland and for cortisol Cusabio, Houston, TX). The procedures were carried out as per manufacturers' guidelines. Insulin to glucose (I/G) ratio was calculated from the results obtained.

For behavioural analysis, the undisturbed behaviour of the calves was recorded in the afternoon (4–6pm; T6-T8; between blood sampling and second feeding for the control group) and again at night (10.30–11.30pm; after blood sampling at T12) using a video camera (GoPro, San Mateo, CA). Lying (sternal or lateral recumbency), standing (body supported by legs), licking fixtures (muzzle in contact with any fixtures of the pen) and self-grooming (tongue in contact with own skin or hair) were scored by instantaneous sampling at 5 min intervals (Færevik *et al.*, 2008). In addition, calf vocalisations were observed and recorded on a group basis, using a newly proposed vocalisation score, which takes into account the duration and intensity/loudness of vocalisations (detailed in online Supplementary Table S1). This was done *via* direct observation 1–2 h after any interactions of personnel with the calves and was carried out by the same research veterinarian, who was placed outside of the view of the calves so as not to disturb them. Each group of calves was observed for 5 min, during which time the number of vocalisation bouts per calf were counted. The loudness of vocalisation was subjectively assessed by the research veterinarian and categorised into one of the three categories (mild, moderate or loud). The overall vocalisation score was calculated by multiplying the two parameters (total score = frequency × intensity).

A comparative assessment of the effect of feeding group, both within each group over time and between groups at each timepoint, was undertaken using a repeated measure mixed model approach. In total, 16 models were generated for the variables pH, HCO_3^-, BE, AG, SID, Na^+, K^+, Cl^-, Ca^{2+} Hb, and glucose, as well as cortisol, insulin, CCK, adiponectin and I/G ratio. Each model accounted for the effects of time, the within-subject effects of feeding group, calf age category (<28, 28 d or older) and calf identity nested within feeding group, in addition to the interaction effects between feeding group and time. For glucose and calcium variables, where a significant pre-feeding (baseline) group effect was observed, data were standardised by subtracting pre-treatment

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values from subsequent data points for the between-groups analysis only. In the case of the lying behaviour variable, the binary response was pooled into two time points (evening and morning observations of lying behaviour) and analysed using a within-and between-group analysis, using the mixed model approach as described above. In all cases, preliminary investigations into the most appropriate covariance structure for the final models (unstructured, autoregressive (first order) or exchangeable), based on the lowest value of the Akaike's and Schwarz's Bayesian information criteria, determined that unstructured structure was the most appropriate. Normality of the residuals was confirmed through kernel density estimate plots. Post-hoc pairwise comparison estimations of each final model were conducted, and post-estimate predictions calculated, and reported where statistically relevant ($P < 0.05$). In the case of water intake and average daily weight gains, group comparisons were completed using ANOVA procedures using a Tukey's post hoc test for multiple comparisons. Data management and graphical representations were completed using Microsoft Excel (Microsoft Office v2022, Microsoft Corporation, Redmond, WA, USA). All statistical analyses were performed using Stata/SE v12.1 (StataCorp, TX, USA).

Results

Blood gas

Blood gas results are presented in Fig. 1 and online Supplementary Table S2 (where the significance levels of changes across time are elaborated). Blood pH increased significantly 6 h after feeding for all three feeding groups and then decreased significantly 6 h later for SR and C. BE and HCO_3^- increased significantly 6 h after feeding in SR group only with a further significant increase at 24 h ($P < 0.001$ in all cases). These changes were not significant in the other two groups. When comparing feeding groups, the mean blood pH was significantly lower at T6 and BE and HCO_3^- were significantly lower at T6 and T12, all in ER relative to C or to both other groups, respectively.

Analysis of the within-group comparisons for changes in glucose concentrations over time indicated a significant increase at T6 in SR with no further significant change in that group. Glucose concentration increased significantly at T12 (4 h after their second feed) in C and decreased significantly at T18 ($P = 0.05$), whereas the ER group demonstrated a significant decrease between 6 and 12 h ($P = 0.01$). Between-group comparison at each of the timepoints indicated that standardised glucose levels for group SR were consistently higher when compared to C at T6, T12 and T18 ($P < 0.05$ or better), but no significant difference was noted at any of the timepoints relative to the ER group. With a mean concentration of 67 g/dl, the glucose concentration was below the normal reference range (Dillane *et al.*, 2018) in group SR at T0 (before feeding) but would not be considered clinically as hypoglycaemia (Roadknight *et al.*, 2021).

Sodium concentrations were low but within normal reference ranges (lower limit of 133.3 mM) for all groups with the exception of ER prior to feeding (T0: 131.7 mM) and the control group at timepoint T24 (132.5 mM). Within-group changes over time (increase followed by decrease) were significant ($P < 0.05$ or better) for ER and C but not SR (Fig. 1). SR exhibited significantly higher sodium than C (T6) and ER (T6 and T12). All other electrolyte concentrations (K^+, Cl^-, Ca^{2+}) were within the normal reference ranges (Dillane *et al.*, 2018) throughout the study regardless of the feeding group.

Within-group changes in haemoglobin levels over time indicated a significant ($P < 0.05$) decrease at T12 followed by a significant increase at T24 ($P = 0.02$) for ER, with values significantly lower than C at T12 and T18. SR had significantly higher haemoglobin than the other groups at T24.

Hormones

Hormone results are shown in Fig. 2 with additional statistical evaluation in online Supplementary Table S2. Cortisol levels did not change significantly over time regardless of the feeding group. However, between-group comparison indicated that the mean cortisol concentration was significantly ($P < 0.05$) higher in group SR when compared to C at T12.

Within-group changes in insulin showed a significant increase at T12 in C followed by a decrease at T24. By contrast, insulin decreased significantly in ER as early as T12 but remained relatively constant over time in SR. As a consequence, insulin was significantly higher in C than in ER at T12 and T24. There were no significant differences in G/I ratios between the groups or over time. Adiponectin increased at T12 and then decreased at T24 in C but did not change significantly in either ER or SR. Between group differences were non-significant throughout. There was a moderate positive correlation between adiponectin and insulin ($r = 0.44$ overall), somewhat higher in C ($r = 0.50$) and ER ($r = 0.56$) than SR ($r = 0.38$). There was also a moderate correlation between cortisol and glucose in C ($r = 0.39$), which was not evident in ER or SR.

Behaviour

Behaviour data are in Table 1. Calves in C, SR and ER spent 78. ± 5, 87 ± 5 and 59 ± 7% (mean ± SEM) of their time lying down (respectively, % of total observations). In the afternoon, ER spent significantly ($P < 0.05$) less time lying down than either C or SR and at night this difference became more significant ($P < 0.001$) because C and SR increased their lying time significantly ($P < 0.001$ for both groups). In addition, younger calves (<28 d) spent significantly more time lying down ($P < 0.05$), regardless of the feeding group. Other behaviours (licking fixtures and self-grooming) were too infrequent to yield a meaningful analysis.

Vocalisation scores are summarised in Table 2. Overall, calves were vocalising more loudly and more often as time after feeding increased, with a positive correlation ($r = 0.74$) between the vocalisation score and time, across all feeding groups. The overall vocalisation score for SR was numerically (non-significantly) lower than for ER but not C.

Growth

There were no significant differences in water consumption or average daily weight gains between the three groups.

Discussion

The objective of a microencapsulated nutrient is to facilitate a delayed release and absorption and with this provide a prolonged feeding satisfaction (Mangan *et al.*, 2019). Our study aimed to assess if this would be the case in pre-weaned calves, with a view to establish potential benefits for calves during transport, when usual feeding practices are not in place. To achieve this,

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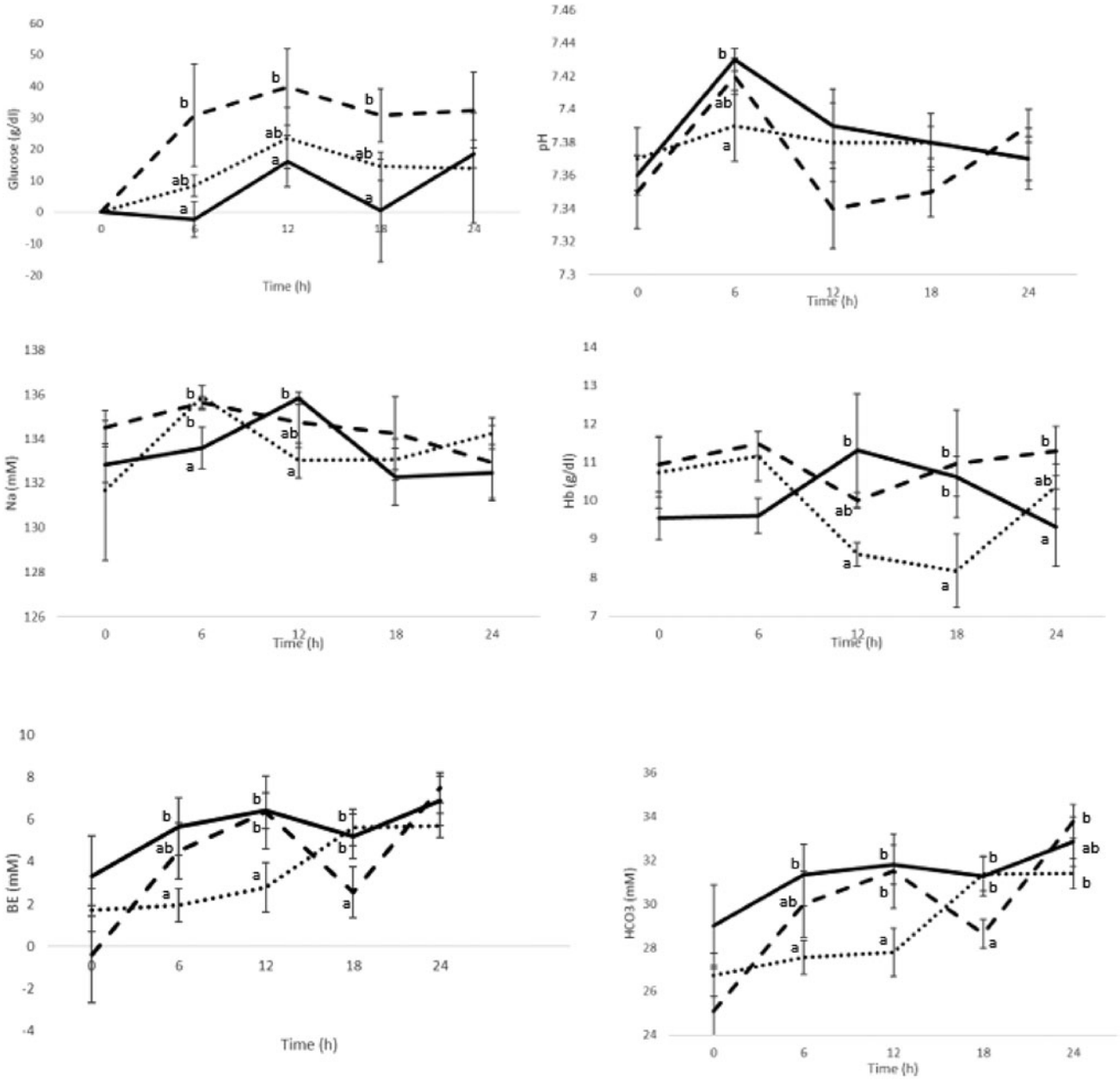

Figure 1. Mean values for changes in blood gas variables over time, relative to pre-treatment values, in 15 calves after being fed with casein-enriched milk replacer (····), slow-release milk replacer (---) and conventional milk replacer (▬▬). The calves were fed at timepoint 0 and the second feed was 8 h later for conventional milk replacer only. [a,b] Letter superscripts not in common represent significant ($P < 0.05$) unit change difference between the feeding groups at each timepoint.

we compared once-daily feeding with treatment formulae, (firstly slow release formula, SR, containing micro-encapsulated carbohydrate, micro-encapsulated fat, micro-encapsulated whey protein and micellar casein and secondly micellar casein-enriched formula, ER) to twice daily feeding with normal formula (C). Some of the metabolic, hormonal and behavioural parameters indicate that the feeding of microencapsulated nutrient containing milk replacer once in 24 h is comparable to twice/day feeding with a conventional milk replacer. On the other hand, certain parameters suggest different metabolic patterns in calves fed with the slow-release formula.

Blood glucose levels increased most when calves were fed with a slow-release milk replacer. There is a caveat, however, since the mean glucose level in this group was significantly lower than in the other two groups before the start of this trial. The significant increase may represent, in part, a correction of baseline glucose values, although levels did then remain relatively constant throughout the day, a feature less obvious in the other two groups where significant changes over time were observed. Similarly, insulin concentration increased significantly after feeding with the conventional and casein-enriched milk replacer, but did not change over time in calves fed with the slow-release formula. However, the first post-prandial measurement took place 6 h after feeding, whereas glucose (and insulin) would generally increase in the first 1–2 h after feeding and start returning to base levels again at around 4 h after feeding (Vicari *et al.*, 2008;

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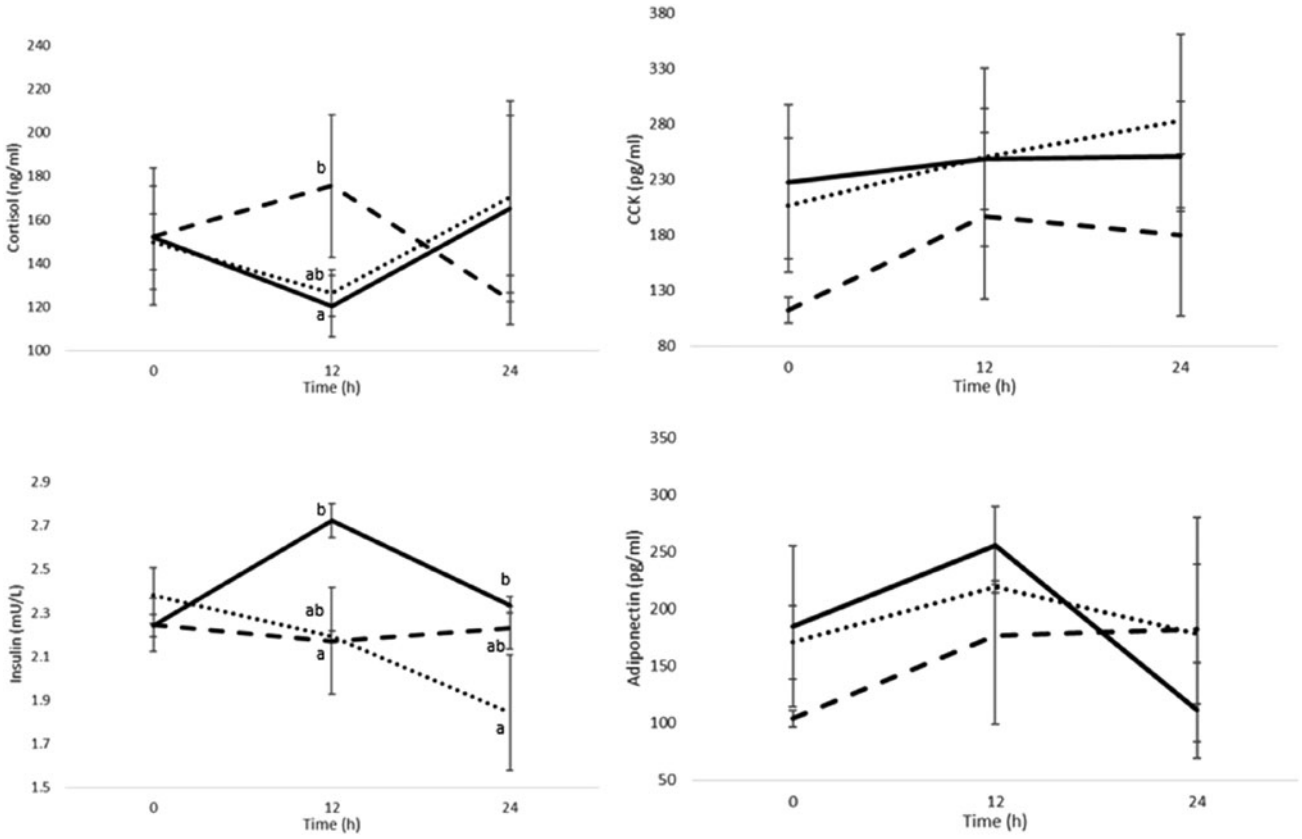

Figure 2. Mean values for changes in hormone levels over time, relative to pre-treatment values, in 15 calves after being fed with casein-enriched milk replacer (····), slow-release milk replacer (---) and conventional milk replacer (▬). The calves were fed at timepoint 0 and the second feed was 8 h later for conventional milk replacer only. [a,b] Letter superscripts not in common represent significant ($P < 0.05$) unit change difference between the feeding groups at each timepoint.

MacPherson *et al.*, 2016; Macpherson *et al.*, 2019), which was a limitation of this study.

Feeding calves infrequent meals is associated with an increased serum insulin to glucose ratio (Miller-Cushon and Devries, 2015), but in our study the G/I ratio of calves fed the slow-release formula just once in 24 h was comparable to the G/I ratio of calves fed with conventional milk replacer twice in 24 h. The different profiles in glucose and insulin concentrations indicate that the slow-release milk feeding achieved a sustained and prolonged release of energy, which was the intended effect of such feeding. Protein enrichment has been shown to induce satiety in humans (Batterham *et al.*, 2006) and casein could be considered a naturally slow-releasing protein nutrient, as it will be clotted in the abomasum and absorbed slowly over time. We did not observe this, since limited benefits were noted in calves fed the casein-enriched milk replacer.

Adiponectin is important in controlling glucose and fat metabolism by regulating insulin sensitivity (Meier and Gressner, 2004). Adiponectin concentration increased significantly after feeding with a conventional milk replacer and then decreased significantly at the end of the study, whereas it remained relatively constant in the slow-release and casein-enriched feeding groups, indicating that levels could fluctuate with hunger and satiety. This is further supported by the moderate positive correlation between insulin and adiponectin, which is consistent with other studies (Sauerwein and Häußler, 2016), indicating that adiponectin could potentially be used as a hunger/satiety marker in bovines. It would be useful to evaluate additional hormones, which are

Table 1. Lying behaviour assessed at two different time categories (afternoon and night) for calves in three different feeding groups: Control standard milk replacer (C), slow-release milk replacer (SR) and casein-enriched milk replacer (ER)

	C	SR	ER
Overall lying	78.3 ± 5.4	86.7 ± 5.5	59.4 ± 7.1
Afternoon	67.5 ± 9.4[a]	78.0 ± 9.0[a]	59.0 ± 9.1[b]
Nighttime	94.9 ± 3.8[a]	100.0[a]	60.0 ± 9.1[b]

Values are % of total lying time, mean ± SEM
[a,b]Letter superscripts not in common represent a significant ($P < 0.05$) difference between the feeding groups.

Table 2. Group vocalisation scores for three feeding groups: Control standard milk replacer (C), slow-release milk replacer (SR) and casein-enriched milk replacer (ER)

	T2	T10	T14	T20	T26
C	0	1	3	2	2
SR	0	0	3	2	2
ER	0	1	3	3	6

The scoring was carried out 2 h after any interventions with the calves.

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commonly associated with hunger/satiety. For example, leptin has been recently identified of particular importance in suckling calves (Hayashi *et al*., 2020) and could be a good candidate for a potential hunger marker.

Cortisol is increased in hunger periods and acts to upregulate gluconeogenesis in ruminants (Forslund *et al*., 2010). In the current study, the correlation between glucose and cortisol concentrations was only evident in the control group. Although serum cortisol levels did not change significantly over time, it is interesting to note that the patterns were different, with calves fed the slow-release formulation peaking at 12 h after feeding and the control group at the end of the study (16 h after second feed). This could correspond with the state of hunger in the control group at the end of the study, whereas cortisol levels decreased in calves on slow-release milk replacer at the end of the study, suggesting they might be in a less pronounced state of hunger.

Blood pH increased over the first 6 h after feeding for all groups and then decreased again, which is in contrast to previous studies (Nagy *et al*., 2003; Vajda *et al*., 2007). It would be beneficial to conduct more frequent measurements, particularly in the first few hours after feeding, both to evaluate the effect of feeding on the acid-base status of these calves, as well as giving a more detailed analysis of glucose status. In spite of some variation and changes within the blood electrolyte concentrations, these generally remained within the normal reference range regardless of the feeding group. Haemoglobin levels also remained within the norm for all groups, and although significant differences between the groups were noted, there was no sign of dehydration seen in any of the feeding groups. Moreover, the water intake was the same regardless of the feeding group, indicating that the novel formulations used in this study had no effect on the thirst/hydration status of these calves.

Literature suggests that calves fed restricted diets spend more time standing (de Paula Vieira *et al*., 2008) and vocalise more frequently (Thomas *et al*., 2001). The behaviour of our calves fed a conventional milk replacer was comparable to that of those fed the specialised slow-release milk replacer, making it a potentially viable option to reduce the negative effects of prolonged fasting on the behaviour and welfare during transport. However, the duration of behavioural observations in this study was relatively short and should be investigated further. Vocalisation as part of animal behaviour can indicate stress in animals and could be used to study emotions in calves (Hwan Jeon *et al*., 2009). The proposed vocalisation scoring system utilised is a simple tool, which could be used as a part of welfare assessment in calves, but it would need to be validated, particularly regarding its specific correlation with hunger. As this scoring system was used on a group base, it could potentially be skewed by particularly vocal individual animals. Furthermore, it cannot distinguish between moderate vocalisation with few repetitions and mild vocalisation with many repetitions, which is another limitation.

Transport is a very stressful event for animals for a variety of reasons, including exposure to prolonged periods of hunger. Neonatal calves are particularly at risk, and have shown decreased levels of glucose, albeit comparable to glucose levels in calves that were fasted but not transported (Fisher *et al*., 2014). In this study, the novel slow-release feeding method was compared to conventional twice/day feeding but in order to draw conclusions on the potential benefits of this novel feed for calves during transport, future trials should be carried out under transport conditions on calves exposed to prolonged fasting. Moreover, this study was only carried out once, whereas a repeated study design would minimise potential random effects.

In conclusion, no negative effects were observed with feeding a slow-release micro-encapsulated milk replacer to calves. On the contrary, there are some suggestions that it could be beneficial to calf health and welfare but further research is required to fully elucidate the potential benefits of such feeding.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S0022029923000560

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