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Short communication

Short communication: A milk replacer aversion model in calves to test flavour-masking effects

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HIGHLIGHTS

- A milk replacer aversion model was developed by adding a mixture of feed additives with a non-palatable/bitter taste.
- Milk refusal and/or eating rate may be indicators for milk replacer aversion.
- A sensorial additive was able to revert the aversion caused by the model.

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ABSTRACT

This study aimed to evaluate the availability of two sensory additives (SAs) to masque non-palatable ingredients commonly present in milk replacers (MRs). Both SAs were based on a synergistic mixture of non-nutritive flavouring substances with four taste categories (sweet, umami, sour, and salty); the only difference between them was the inclusion of polyols, which were not included in SA2. Two experiments were conducted for this purpose. In Experiment 1, an MR aversion model was developed using 24 Holstein male calves (7 ± 0.9 d of age and 43.3 ± 1.39 kg of body weight [BW]). In the first two weeks of the study, calves were fed increasing amounts of MRs until the animals were able to consume 8 L/d at 15% dry matter (DM) concentration for two feedings per day. Thereafter, MR aversion was induced in half of the calves by adding a mixture of bitter taste feed additives (Bittermix) from days 14 to 22 of the study (aversion week). The daily MR intake and eating rate were recorded from the two previous days of the challenge and during the aversion week. In Experiment 2, the same model was used with 37 Holstein male calves (6 ± 0.9 d of age and 40.2 ± 1.40 kg of BW). Owing to health issues in this experiment, the aversion week was postponed at 38.5 ± 1.12 d of age until the animals were able to consume at least 90% of 8 L/d at 12.5% DM concentration. The aversion test was performed as follows: no supplementation (CTRL), Bittermix at 30 g/kg of dry MR (BM), BM plus SA1 at 2 g/kg of dry MR (SA1), and BM plus SA2 at 2 g/kg of dry MR (SA2). Data were analysed with a generalised mixed model that accounts for the fixed effects of MR supplementation, the meals during the aversion week, the interaction of the MR supplementation and meals, and a calf as a random effect. In the aversion test of Experiment 1, calves that were fed Bittermix showed an increase in the incidence and amount of MR refusals ($P < 0.05$) compared with CTRL calves. In Experiment 2, the incidence of refusal and time devoted to consuming MR were similar in all treatments. However, the eating rates were greater ($P < 0.01$) in CTRL and SA2 than in BM and SA1. Adding a mixture of bitter tasting feed additives in the MR changed the feed intake parameters of the calves. The addition of a specific sensory additive, namely, SA2, reversed the negative effects caused by the bitter products.

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1. Introduction

Ruminants select their diet via trial and error to avoid plants or food that could make them sick (Provenza and Balph, 1987). Their preferences or aversions are a result of the interactions between nutritional factors such as the requirements of the animal, the post-ingestive feedback (effects of nutrients and toxins on chemo-, osmo-, and mechanoreceptors), the sensory properties of the food (taste, smell, and texture), and the pleasure evoked by the food (hedonic behaviour), which stimulate or depress feed intake (Bach et al., 2012; Baumont, 1996; Provenza et al., 1996). From the different tastes observed in nature (bitter, salty, sweet, sour, and umami), bitter taste seems to have negative effects on palatability and manifests as indifference or weak to strong feed rejection in ruminants (Ginane et al., 2011).

There are several situations in which a bitter taste can be present in ruminant feed rations. Some plant-derived ingredients are extensively used as raw materials in ruminant diets that often contain anti-nutritional factors, which are usually non-toxic; however, these ingredients have been reported to decrease feed intake (i.e., glucosinolates in rapeseed meal, tannins in sorghum, and saponins in soybeans). Furthermore, the high pressure to reduce the use of antibiotics in animal production has increased the demand for and use of alternatives, such as phytogenics, butyrates, or medium-chained fatty acids; this situation has conferred positive benefits on animals (Katsoulus et al., 2017; Kolling et al., 2018; Reddy et al., 2020). However, these compounds can depress feed intake because of their bitterness (Ahmed et al., 2009; Kolling et al., 2016). The addition of sensory additives (SAs) is a common strategy for increasing the consumption of milk replacers (MRs) and starter feed (Fathi et al., 2009; Montoro et al., 2011), and it might be used as a strategy to counteract the negative effects on intake of non-palatable ingredients commonly present in MRs. We hypothesised that MR aversion could be induced by adding a combination of products that cause bitter taste, and this model could be useful in testing the efficacy of SAs to revert these aversion effects. The objectives of the present study were to develop a model that can cause MR intake aversion in pre-weaned calves by using feed additives with bitter palatability (Experiment 1) and to test the effects of SAs on bitterness (Experiment 2).

2. Materials and methods

Calves were raised in the facilities of bonÀrea Agrupa at Granja Nial (Guissona, Spain) and were managed under common animal management conditions with the supervision of IRTA technicians and the approval of the Animal Care Committee of the Government of Catalonia (authorisation code 11,074).

2.1. Experiment 1: assessment of an MR aversion model

A total of 24 Holstein male calves (7 ± 0.9 d of age and 43.3 ± 1.39 kg of body weight [BW]) from a single farm were enroled in this study. Calves were individually housed in outdoor hutches, bedded with straw, and provided one bucket for water and one bucket for concentrate. During the whole study, calves were fed an MR (21% whey protein concentrate, 39% skimmed milk protein, 39% fatted whey, 1% premix, 24.1% CP, and 20.6% fat) at 39 °C–40 °C with a bucket teat. Moreover, calves had ad libitum access to water and pelleted concentrate feed (17.6% CP, 15.7% NDF). During the first two weeks of the study, the calves were adapted to consume more MR by increasing MR gradually: from day 1 to day 7, calves were offered 6 L/d of MR divided into two meals at a rate of 125 g/L; from day 8 to day 13, MR was increased until 8 L/d was reached at a rate of 125 g/L. On day 14 of the study, animals continued to receive 8 L/d of MR but at a rate of 150 g/L. On day 14, when the aversion test started, the animals were allocated by considering BW and age in two treatment groups: half of the calves started to receive a mixture of known additives with a bitter taste (referred to in the text as Bittermix, which contains butyrates, medium chain fatty acids, and plant essential oils) to cause aversion in their MR (BM; N = 12), and the other half of the animals were in the control treatment (CTRL; N = 12) with no additives added in their MR. On days 14 and 15 of the experiment, BM calves received 15 g BM/kg of dry MR; however, given that their MR feeding parameters were not affected, the BM dose was doubled on day 16 (30 g/kg of dry MR) until day 22 of the study.

During the first two weeks of the study, the daily MR refusals and weekly concentrate feed intake were measured. From days 12 to 22 of the study, MR refusals, concentrate intake, and time devoted to consuming MR were recorded (if calves refused to consume MR, they were gently forced once again to eat the MR; if they refused to eat, the timer was stopped). All calves were weighed weekly. The overall success of the MR aversion model was evaluated by recording the MR intake and eating rate (litres of MR consumed divided by the time devoted to consuming the MR offered). Throughout the study, health disorders and veterinary treatments were recorded daily.

2.2. Experiment 2 (Exp. 2): testing of different flavours using the MR aversion model

A total of 37 male Holstein calves (6 ± 0.9 d of age and 40.2 ± 1.40 kg BW) from several farms were divided in three different batches. Animals were raised under similar conditions as the calves in Experiment 1, with the only difference being the duration of the adaptation phase, which had to be prolonged for some animals because of the appearance of respiratory problems. This prolonged adaptation resulted in a delay of one week on the onset of the aversion test with respect to Experiment 1 because of the need to wait for the calves to recover their consumption of MR to at least 90% of the MR offered. Calves were adapted to high levels of MR intake by increasing the levels of MR from 4 to 8 L/d of MR at a rate of 125 g/L. This level was maintained throughout the experiment without reaching the same level as that of Experiment 1 to ensure MR intake. Daily MR refusals and concentrate intake were measured throughout the study; during the last week of the adaptation period (before starting the MR aversion test), the time devoted to consuming MR in the morning meal was also recorded. The aversion test was performed in three batches of calves, and all treatments were represented in each batch: no supplementation in the MR (CTRL; N = 9), Bittermix added at 30 g/kg of dry MR (BM; N = 9), BM plus SA1 at 2 g/kg of dry MR (SA1; N = 10), and BM plus SA2 at 2 g/kg of dry MR (SA2; N = 9). Both SAs were based on a synergistic mixture of non-nutritive flavouring substances, including four taste categories (sweet, umami, sour, and salty); the only difference between them was the inclusion of polyols, which were not included in SA2 (Lucta S.A., Madrid, Spain). During the aversion week, the procedure described in Experiment 1 was followed for one week. Throughout the study, all calves were weighed weekly, and health disorders and veterinary treatments were recorded daily.

2.3. Statistical analysis

In both experiments, data were analysed using SAS software (version 9.4, Institute, Cary, NC, USA). Performance and intake data were analysed using a mixed-effect model, with the calf considered a random effect. In Experiment 1, data from the aversion test were considered from days 16 to 22, which were the days when calves received a higher dose of BM. The model then accounted for BM addition, meals throughout the aversion test duration, and their interaction as fixed effects. In Experiment 2, the model accounted for additive treatments in the MR, meals throughout the aversion test duration, and their interaction as fixed effects. In both experiments, the average per meal of each MR intake parameter and the days before starting the aversion test were considered covariates. In Experiment 2, the batch of calves involved during the aversion week was considered a block, and BW and age during the aversion test week were considered covariates. In both cases, meals given during the aversion test were entered in the model as a repeated measure by using an autoregressive covariance matrix (the structure with the lowest Bayesian criterion).

Additionally, in both experiments, the MR refusal data contained many zeros. To solve the zero-inflated problem for MR refusals, it was considered a binary variable ("0," refuses to drink part of the MR; "1," consumes all MR) and then analysed with a generalised mixed model for each experiment by considering the same fixed effects described above for each experiment. Thereafter, calves that consumed all MR were excluded from subsequent analysis, and the amount of MR refusal was analysed as previously described by using a mixed-effects model. These values did not follow a normal distribution and were subjected to square root transformation. The least square means and the standard error of the mean presented herein correspond to the non-transformed data, and the P-values correspond to the results with transformed data. For Experiment 2, a mixed model accounting only for calves and meals with refusals could not be performed because not all treatments and meals had animals with refusals, and the least squares means were not estimated for those missing treatments and meals. Therefore, data on the incidence of refusal presented in Table 2 include all calves that were involved in the aversion test analysed with a chi-square test. All analyses were performed either by considering morning and afternoon meals together or separately.

2.4. *Experiment 1: assessment of an MR aversion model*

During the entire milk aversion test (days 14 to 22 of the study), no differences in calf growth or concentrate DM intake were observed between the treatments (Table 1). Milk feeding parameters were analysed by considering only the days when the Bittermix dose was higher and caused aversion (30 g/kg of dry MR from days 16 to 22). The incidence of MR refusal in the afternoon meals was lower ($P < 0.05$) in CTRL calves than in BM calves (Table 1). The analysis results for calves that refused to consume part of the MR indicated that greater ($P < 0.05$) amounts of MR were refused in BM calves than in CTRL calves. This difference ($P < 0.01$) was observed only during the afternoon meal (Table 1). The effect was less evident in the morning meals probably because the inter-meal interval was longer from the afternoon meal to the morning meal than vice versa, and the animals were hungrier in the morning than in the afternoon. The MR eating rate increased, and the time devoted to consuming the MR decreased throughout the aversion test meals (data not shown). Only a numerical decrease in eating rate was observed between treatments when Bittermix was added to the MR ($P = 0.16$; Table 1). As expected, BM addition changed the MR feeding behaviour because a greater amount of MR refusal was observed in afternoon meals. This finding was probably due to the nature of bitter compounds—a bitter taste is often associated with the presence of toxins in feed and results in a rather negative hedonic response (Ginane et al., 2011). The aversion effect of BM was dose dependant and hunger dependant and caused some changes in the MR intake; therefore, the dose had to be doubled. The effects were only observed during afternoon meals.

Experiment 2 (Exp. 2): testing different flavours using the MR aversion model

There were no differences in BW, average daily gain, and DM intake amongst the four treatments throughout the study. The week before starting the aversion test, milk intake parameters were measured, and all four treatments had similar initial values (Table 2). In accordance with Experiment 1, the aversion test lasted for 7 days (14 meals). All calves in the study that refused meals recovered their intake levels after the seven-day test and overcame the negative effects of BM supplementation, except for one calf in the SA1 treatment. Contrary to Experiment 1, there were no differences in the prevalence of MR refusals either for the morning meal or in the afternoon meal. However, the MR eating rate was greater in CTRL and SA2 than in BM and SA1 ($P < 0.01$) (Table 2), particularly in the afternoon meals ($P < 0.01$). Fig. 1 shows how all calves in the different treatments gradually increased the MR eating rate throughout the experiment ($P < 0.001$; for time effect). This finding was probably a consequence of the adaptation of BM-fed calves to a less palatable feed (Provenza and Balph, 1987), and an age effect in all calves was observed (Miller-Cushon et al., 2013). Although there was a general increase in eating rate in all treatments up to the 10th meal, changes in eating rate throughout meals showed a significant increase in eating rate from the afternoon to the morning meal but a decrease in eating rate from the morning to the afternoon meal (Fig. 1). Flavour supplementation had no interactions with time, thus indicating that SA2 calves and

Table 1
Effect of Bittermix on performance during the whole aversion test (14 to 22 day of study), and on milk feeding parameters during the successful milk aversion test performed from 16 to 22 d of study (Exp. 1).[a]

	Aversion treatment[b]			P-values[c]		
	CTRL (n=12)	BM (n=12)	SEM[d]	BM	meal	BM x meal
Initial age, d	7.2	7.6	0.86	0.73	-	-
Initial BW, kg	42.7	43.2	1.42	0.80	-	-
Aversion test week (from 14 to 22 d of study)						
Performance						
Age 14 d of study, d	21.2	21.6	0.86	0.73	-	-
BW 14 d of study, kg	49.8	47.7	2.28	0.23	<0.001	0.26
BW 21 d of study, kg	56.1	52.8	2.28	0.23	<0.001	0.26
ADG 14–21 d of study, kg/d	0.47	0.41	0.079	0.13	<0.001	0.64
DM intake, g/d						
Milk replacer DM intake	1098	1029	36.0	0.18	<0.001	0.13
Concentrate DM intake	164	127	19.9	0.19	<0.001	0.98
Milk intake parameters (from 16 to 22 day of study)						
Total incidence MR refusal, %	0.62	19.6	51.2	0.98	0.86	0.99
Incidence MR refusal am, %	0.009	12.5	5.75	0.99	0.98	0.99
Incidence MR refusal pm, %	15.6	29.3	4.6	0.04	0.90	0.91
MR total refusal, g/meal[e,f]	83	217	76.7	0.0003	0.27	0.25
MR refusal am, g/meal[e,f]	60	91	10.6	0.81	0.34	0.27
MR refusal pm, g/meal[e,f]	100	298	46.8	0.005	0.70	0.20
Time devoted to consume MR[g]	404	429	34.4	0.61	0.002	0.62
Time devoted to consume MR am[g]	413	409	34.0	0.94	0.02	0.24
Time devoted to consume MR pm[g]	394	426	35.5	0.54	0.01	0.39
MR eating rate, L/min	0.71	0.59	0.057	0.16	0.004	0.27
MR eating rate am, L/min	0.68	0.67	0.047	0.81	0.008	0.62
MR eating rate pm, L/min	0.69	0.59	0.064	0.28	0.11	0.34

[a] Values are least square means.
[b] CTRL: calves not supplemented during the MR aversion test; BM: calves supplemented with Bittermix during the MR aversion test (15 g/kg of dry MR from 14 to 15 day of study and 30 g/kg of dry MR from 16 to 22 day of study).
[c] BM: effect of Bittermix supplementation in the MR; meal: effect of the meal number during the aversion week; BM x meal: effect of the interaction of Bittermix supplementation and meal number.
[d] standard error of the mean.
[e] least square means from those calves that refused to consume part of the MR offered.
[f] p-values correspond to root-square transformed values.
[g] seconds/meal.

Table 2
Effect of sensory additives on performance and milk feeding parameters before and during milk aversion test (Exp.2).[a].

	Treatments[b]					P-values[c]		
	CTRLn = 9	BMn = 9	SA1n = 10	SA2n = 9	SEM[d]	T	meal	T x meal
Initial age, d	5.4	5.9	5.9	7.0	0.91	0.68	-	-
Initial BW, kg	39.1	39.6	40.4	40.1	1.42	0.92	-	-
Previous data								
number of calves with refusal	1	1	2	1	11.02	0.92	-	-
Time devoted to consume MR, s	313	349	347	293	33.8	0.60	-	-
MR eating rate, L/min	0.79	0.71	0.79	0.83	0.069	0.68	-	-
Aversion test week (from 25 to 32 of study)								
Performance								
Age 25 d of study,	30.1	30.3	31.6	33.0	1.52	0.53	-	-
BW 25 d of study,	53.2	52.6	56.1	53.6	1.56	0.45	-	-
BW 32 d of study,	59.3	58.1	61.7	58.4	1.79	0.45	-	-
ADG, kg/d	0.88	0.88	0.87	0.83	0.068	0.95	-	-
DM intake, g/d								
Milk replacer	940	920	930	910	21	0.81	-	-
Concentrate	160	190	210	180	43	0.88	-	-
Milk intake parameters (from 25 to 32 of study)								
Total incidence MR refusal, %	4.8	11.9	10.0	10.3	2.51	0.23	-	-
Incidence MR refusal am, %	1.6	4.8	7.1	6.3	2.60	0.48	-	-
Incidence MR refusal pm, %	7.9	19.1	12.9	14.3	4.19	0.34	-	-
MR total refusal, g/meal[5]	70	182	92	200	-	-	-	-
MR refusal am, g/meal[e]	20	111	65	181	-	-	-	-
MR refusal pm, g/meal[e]	120	252	119	219	-	-	-	-
Time devoted to consume MR, s	254	288	316	249	23.0	0.15	<0.001	0.18
Time devoted to consume MR am, s	248	280	316	253	23.1	0.16	0.001	0.32
Time devoted to consume MR pm, s	260	295	317	247	29.9	0.36	0.027	0.23
MR eating rate, L/min	1.08[a]	0.82[b]	0.83[b]	1.00[a]	0.056	0.002	<0.001	0.46
MR eating rate am, L/min	1.09[a]	0.85[b]	0.85[b]	1.0[ab]	0.06	0.02	<0.001	0.47
MR eating rate pm, L/min	1.08[a]	0.79[b]	0.81[b]	1.01[a]	0.064	0.002	<0.001	0.35

[a] Values are least square means.
[b] CTRL: no supplementation in the MR; BM: Bittermix at 30 g/kg of dry MR; SA1: BM plus sensory additive 1 at 2 g/kg of dry MR; SA2: BM plussensory additive 2 at 2 g/kg of dry MR.
[c] T: treatment effect; meal: effect of the meal number of the aversion week; T x meal: effect of the interaction between treatment and meal number.
[d] SEM: standard error of the mean.
[e] average from those calves that refused to consume part of the MR offered.

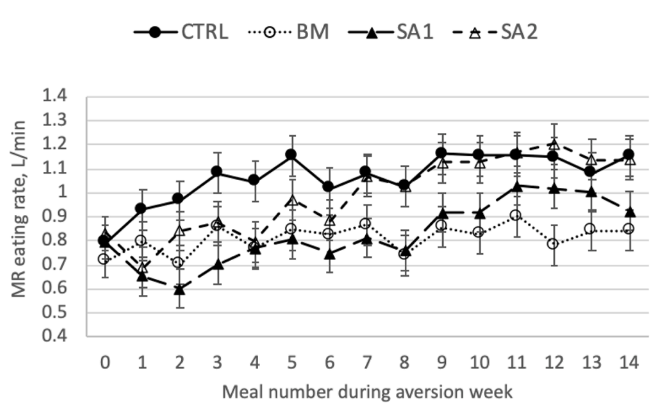

Fig. 1. Evolution of milk replacer eating rate of calves during the aversion test. CTRL calves were neither fed with BM nor aroma MR supplementation; BM treatment contained 30 g BM/kg MR; SA1 contained 30 g BM/kg MR and sensory additive 1 at the dose of 2 g/kg; SA2: contained 30 g BM/kg MR and sensory additive 2 at the dose of 2 g/kg (Exp.2). Time effect $P < 0.001$, from 2nd to 10th meal significant changes between consecutive meals.

CTRL calves have had similar results since day 1 of the aversion test (Fig. 1).

BM caused changes in the MR feeding parameters of both experiments. These changes differed from one experiment to the other, with MR refusal being more significant in Experiment 1 and MR eating rate being more significant in Experiment 2. However, the MR eating rate in Experiment 1 and the MR refusal in Experiment 2 presented the same trend between the CTRL and BM groups but only differed numerically. Owing to health disorders, the calves in Experiment 2 were older and received less MRs than the calves in Experiment 1 when the aversion test was conducted; these modifications may explain the differences found between experiments. The calves in Experiment 1 were 21-days old and received 1200 g/d of dry MR when the aversion test was started. This amount of food may satiate these animals; therefore, they were more likely to leave some milk in the case of aversion. By contrast, the 1000 g/d of dry MR for 30-day old calves in Experiment 2 was not able to completely satiate the animals, and aversion was indicated by a decrease in eating rate. Todd et al. (2018) observed that acidification reduced the palatability of MR, and calves responded by extending their sucking bout duration. In the current study, this finding could be understood as a reduction in the rate of intake. Despite the differences in both experiments, the numerical differences in MR eating rate between CTRL and BM calves in Experiment 1, together with the results in Experiment 2 and literature references (Todd et al., 2018), indicate that the rate of intake is a milk aversion indicator.

3. Conclusion

The addition of bitter taste feed additives in MR changed the feed intake parameters of calves. Although this study was not able to clearly identify the indicators of aversion, milk refusal and eating rate seem to be the most promising indicators, particularly when animals are more satiated because of shorter meal intervals. Nevertheless, the model presented herein was appropriate for identifying the capacity of a specific SA, namely, SA2, to reverse the negative effects caused by bitter products.

CRediT authorship contribution statement

Marta Terré: Conceptualization, Methodology, Validation, Formal analysis, Writing – original draft, Writing – review & editing, Supervision, Project administration. **Marina Tortadès:** Investigation, Writing – original draft. **Sandra Genís:** Investigation. **Roberta Cresci:** Investigation. **Andrea Frongia:** Investigation. **Marçal Verdú:** Methodology, Data curation, Resources. **Marta Blanch:** Conceptualization, Methodology, Validation, Writing – review & editing, Supervision, Funding acquisition.

Declaration of Competing Interest

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