

## ORIGINAL ARTICLE

# Peripartal calcium homeostasis of multiparous dairy cows fed rumen-protected rice bran or a lowered dietary cation/anion balance diet before calving

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## Summary

Milk fever is one of the most important metabolic diseases in dairy cattle. Reducing the dietary cation/anion balance (DCAD) with anionic salts is a common prevention strategy. However, many small European farms cannot use total mixed rations (TMR) in the close-up period. Including anionic salts in compound feeds can result in feed refusals and moderate inclusions to preserve feed palatability results in insufficient DCAD reduction. Rumen-protected rice bran induces the adaptation of Ca metabolism in dairy cows by a reduction of Ca intake and by a reduction of the availability of dietary Ca. In the presence of a negative control, rumen-protected rice bran (2.8 kg/day) was compared with a lowered DCAD diet (from 269 to 4 meq/kg DM) in their effect to prevent milk fever. In a randomized block design, 45 multiparous Holstein cows joined the trial sequentially from 21 days before the expected calving date and were observed until the 8th week of lactation. Feed and nutrient intakes were recorded, and Ca, P, Mg in serum and urine, urine pH, serum NEFA and milk production in early lactation were compared. Feeding rumen-protected rice bran before calving improved the recovery of calcaemia after calving and had a positive effect on DMI after calving. The moderately low DCAD diet did not positively influence serum Ca at calving. Calcaemia recovered even later than in control, and cows showed reduced DMI post-calving and higher NEFA levels in the first 36 h after calving. This moderate reduction of DCAD did not provide an intermediate prevention level indicating that DCAD needs to be reduced to the recommended levels to prevent milk fever. Rumen-protected rice bran may be a suitable feed to reduce hypocalcaemia post-partum and can be included in pre-calving compound feeds representing a palatable alternative to anionic salts.

**Keywords** calcium homeostasis, phytic acid, milk fever, rice bran, DCAD

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## Introduction

Milk fever is one of the most relevant production diseases in dairy cows. It has a substantial impact on animal welfare and it mainly affects dairy production by indirectly increasing the incidence of other production diseases (Curtis et al., 1985).

Clinical milk incidence of fever is difficult to measure because it depends on the subjective evaluation of unspecific symptoms at calving. In the United States, 84% of all farms report cases, and about 5% of all animals are thought to develop milk fever at calving (USDA, 2008). In turn, hypocalcaemia can be measured objectively as a negative deviation of serum Ca from reference values. Subclinical hypocalcaemia, defined as serum Ca levels < 2.0 mM, occurs in 25%

of the heifers and in 40 to 55% of multiparous cows (Reinhardt et al., 2011).

Rumen-protected rice bran has been proposed as a means to induce homeostatic adaptation of Ca metabolism in dairy cows in the pre-calving period (Martín-Tereso et al., 2010a). This adaptation is mediated by a reduction of Ca supply, because of the low Ca content in the product, and by a reduction of availability of dietary Ca caused by binding of dietary Ca to the phytic acid in rice bran (Martín-Tereso et al., 2011). Ultimately, the purpose of such application is the prevention of milk fever in dairy cows. Recently, a prospective cohort study found strong indications that rumen-protected rice bran fed before calving improved the recovery of calcaemia after calving (Martín-Tereso et al., 2010b).

The most common practice for dietary prevention of milk fever is the reduction of the dietary cation/anion balance (DCAD) (Goff, 2006). This method of prevention, although proven effective to reduce milk fever incidence, can present practical difficulties of application. In areas with high levels of K in the diets, high levels of anionic salts are necessary to reduce DCAD to recommendations, and this can cause a reduction in voluntary feed intake (Vagnoni and Oetzel, 1998). This can in turn negatively affect energy balance in early lactation (Bertics et al., 1992).

In production conditions where farm sizes do not permit the applications of total mixed rations in the close-up period, as in most regions of Western Europe, anionic salts are often included in compound feeds that are fed on the top of forages. This implies that the concentration of anionic salts in the feed is several fold that needed in a TMR. This can result in compound feed refusals. For this reason, these compound feeds are difficult to formulate with DCAD levels below  $-1000$  meq/kg DM.

To understand how rumen-protected rice bran feeding compares with moderate low DCAD diet as dietary strategies to prevent milk fever, these two pre-calving diets were tested in the presence of a negative control. This trial aimed to evaluate how rumen-protected rice bran and a moderate low DCAD diet influence Ca homeostasis around calving. A secondary objective was to study the effect of these diets on feed intake and energy metabolism in the transition period.

## Materials and methods

### Animals, housing and feeding

This experiment included 45 multiparous Holstein cows from the experimental farm of Nutreco in Boxmeer, the Netherlands. These animals joined the trial sequentially from 21 days before the expected calving date onwards and were observed until the 8th week of lactation. The experiment took place between September 2009 and April 2010. The ethical suitability of the protocol was approved by the Committee of Animal Experimentation of the University of Nijmegen, the Netherlands.

Before the start of the trial, cows were housed with the replacement stock during the far-off period. When cows reached 3 weeks before calving, they were transferred to a close-up cubicle stable, where they could freely walk and were fed with an automatic feeding system, which provided free access to forage. This system recorded voluntary feed intake. In a separate feeding box, an electronic system provided

the cows with a daily allowance of pelleted compound feed.

When the first signs of calving were observed, cows were moved to an adjacent calving pen, where they could lay in straw and had access to forage, of which the consumption was also electronically recorded. Directly after calving, all cows were offered a suspension of 45 g of Ca in 20 l of warm water. The reason for this was to reduce the need for intravenous infusions of Ca in specific cases of clinical milk fever.

Soon after calving, cows were transferred to the main lactation stable, where animals could walk freely and had cubicles available for resting. Also at this stable, forage was provided *ad libitum* and intakes were recorded automatically. A daily allowance of compound feed was then supplemented electronically, and its intake or any eventual refusal was recorded by the system.

### Experimental design and diets

The study was organized in a randomized block design to test three pre-calving diets. Treatments were; a negative control diet (Control), a rumen-protected rice bran diet (Rice bran) and a moderately low DCAD diet (DCAD). This last diet was formulated with a moderate dose of anionic salts. Cows were blocked by parity and by expected calving date and assigned randomly to the three dietary treatments. The forage base was common for all animals, consisting of a mixture of maize silage, ray-grass silage and rye-grass hay, mixed at a 40–30–30% DM weight ratio. The treatments differed in the supply of three different pelleted compound feeds, which were supplemented on the top of the forage base with an electronic compound feed dispenser at 4 kg per day until calving. In the first week of compound feed supply, the allowance progressively increased daily from 1 kg on the first day, up to 4 kg after 7 days.

Nutrient profiles of the compound feeds are given in Table 1. The rice bran feed was formulated to include 700 g/kg of defatted rice bran. The rice bran had been treated with formalin (370 g/l formaldehyde) at a level of 3000 ppm fresh weight. This was performed to increase rumen escape of phytic acid as described by Martín-Tereso et al. (2009). The low DCAD feed was formulated to a DCAD of  $-1000$  meq/kg, with ammonium chloride (39 g/kg) and magnesium sulphate (18 g/kg). Furthermore, it included supplemental limestone to obtain a final Ca content of 20 g/kg. Total dietary composition and nutrient intakes varied with the variation of voluntary forage intake, and with any

**Table 1** Nutrient analyses of the experimental compound feeds used in the trial

	Control	Rice bran	Low DCAD
DM	903	889	894
Ash	78	85	115
CP	206	224	231
Starch	143	245	112
Sugars	74	98	78
CF	165	81	119
NDF	352	236	268
Ca	7.4	5.5	22.1
P	4.5	11.5	5.8
Mg	4.8	7.5	9.2
Na	2.5	2.4	2.6
K	10.9	15.2	8.6
Cl	3.2	2.9	29
S	3.6	4	8.7
DCAD	73	162	-1041

Nutrients expressed as g/kg DM except for DCAD meq/kg DM.

incomplete consumption of the compound feed allowance.

After calving, all cows had *ad libitum* access to a new forage mixture, composed by maize silage, ryegrass silage, corn cob meal and ryegrass hay, mixed at a 55–30 to 10–5% based on DM weights. Intakes of this forage were recorded automatically. The compound feed was fed by means of electronic feeders with a daily allowance that increased with days in lactation.

## Samples and analyses

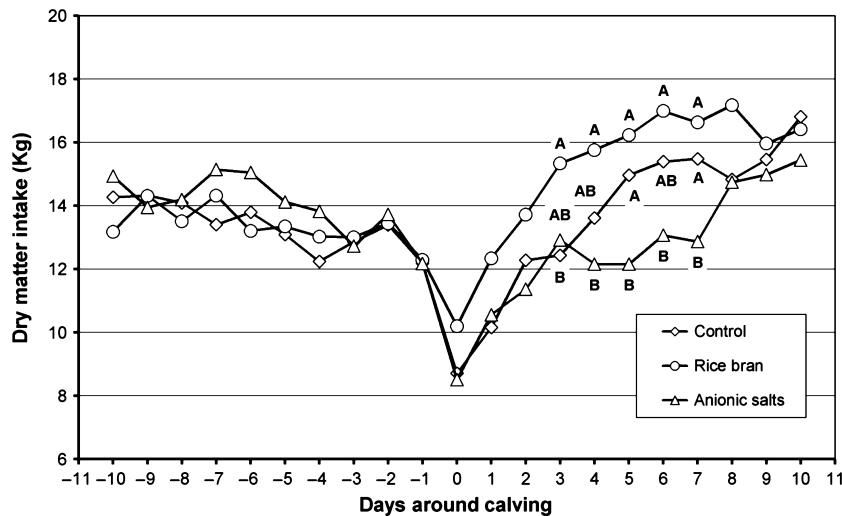
Each of the forage silos used in the diets was sampled from several representative locations in the silage clamps and pooled by silo, and these pooled samples were analysed. Samples of hay and compound feed were taken upon arrival to the farm and later analysed. Concentrate samples were analysed for DM, ash, ether extract (EE), crude protein (CP), sugars (European Commission, 2009) and starch (NEN3574/C1, 1979). Neutral detergent fibre (NDF) was determined with alpha amylase (Van Soest *et al.*, 1991) without sodium sulphite and expressed without residual ash. In the forages, these nutrients were analysed by Near Infrared Spectroscopy (NIRS) at BLGG, Oosterbeek, the Netherlands. Calcium, Mg, Na and K and were determined by calcination at 500 °C, digested with hydrochloric acid and later measurement by atomic absorption spectrophotometry (Perkin Elmer AAnalyst 800, Norwalk, CT, USA). Phosphorus was analysed by spectrophotometry (AOAC method 4.8.14) and Cl by trimetric titration (AOAC method 969.10) (AOAC, 2005). Sulphur was analysed by dual range sulphur carbon analysis (Leco SC-144DR; Leco Corporation, St Joseph, MI, USA). Dietary cation/anion difference (DCAD) (Block, 1984) was calculated from Na, K, Cl and S adding cation equivalents and subtracting anion equivalents.

**Table 2** Feed intakes and nutrient content of the feed intakes (Lsmeans)

	Close-up (from day -10 to calving)				Early lactation (from day 1 to 10)			
	Control	Rice bran	Low DCAD	SEM	Control	Rice bran	Low DCAD	SEM
DMI (kg)	12.94	13.09	13.48	0.372	ab 14.05	a 15.64	b 13.03	0.564
Forage DMI (kg)	9.95	9.97	10.38	0.355	a 11.44	a 12.61	b 10.15	0.448
Concentrate DMI (kg)	2.99	3.12	3.11	0.127	2.63	3.02	2.88	0.237
Ash	a 69	b 70	c 78	0.4	64	64	64	0.3
CP	a 124	b 130	b 130	1.8	120	121	124	1.5
Starch	a 160	b 185	c 145	4.0	228	227	226	6.2
Sugars	a 30	b 37	a 32	1.3	a 28	a 28	b 32	1.4
CF	a 230	b 208	a 222	3.0	183	182	178	4.8
NDF	a 451	b 420	c 436	4.7	369	368	359	8.1
Ca	a 4.0	a 4.2	b 7.7	0.33	4.45	4.52	4.67	0.10
P	a 3.1	b 4.9	c 3.5	0.08	3.2	3.3	3.4	0.05
Mg	a 2.3	b 3.0	c 3.4	0.07	2.4	2.4	2.5	0.05
Na	1.3	1.3	1.3	0.02	1.2	1.2	1.2	0.02
K	a 17.3	b 18.3	c 16.9	0.12	16.8	16.7	16.7	0.12
Cl	a 3.4	a 3.4	b 9.6	0.15	3.1	3.1	3.2	0.03
S	a 2.2	a 2.3	b 3.4	0.05	1.9	2.0	2.0	0.02
DCAD	a 269	a 287	b 4	8.5	272	269	269	3.0

Nutrients expressed as g/kg DM except for DCAD meq/kg DM.

Different letters indicate differences with a  $p < 0.05$ .



**Fig. 1** Daily DMI from 10 days before calving to 10 days after calving. Difference in letters indicates significant differences with  $p < 0.05$ .

**Table 3** Calving observations of calcaemia, odds ratio for calving problems and colostrum production

	Control	Rice bran	Low DCAD	SEM
Ca nadir (mm)	1.67	1.76	1.75	0.077
OR Ca infusion at calving	0.33	0.29	0.33	0.562*
OR assisted calving	0.40	0.64	0.53	0.534*
OR oedema	0.27	0.43	0.40	0.550*
Colostrum production (l)	7.61	6.91	5.90	1.107

OR, Odds ratio.

No significant differences between the treatments.

$\text{logit}(p) = \log[p/(1 - p)]$ .

\*SEM is reported in the logit transformed because confidence intervals are not symmetric.

**Table 4** Serum parameters (mm)

	Ca	P	Mg	NEFA
Close-up (3 weeks before calving)				
Control	AB 2.48	AB 2.16	0.972	0.143
Rice bran	A 2.45	A 2.26	0.953	0.154
Low DCAD	B 2.51	B 2.12	0.963	0.179
SEM*	0.012	0.011	0.0043	0.0149
Hours after calving (calving – 48 h after)				
Control	A 1.93	1.51	0.977	AB 0.546
Rice bran	B 2.08	1.63	0.983	A 0.477
Low DCAD	AB 1.99	1.61	0.965	B 0.638
SEM*	0.014	0.010	0.0078	0.0249
Early lactation (4 weeks after calving)				
Control	A 2.47	1.80	0.989	0.445
Rice bran	A 2.48	1.74	0.956	0.468
Low DCAD	B 2.39	1.74	0.927	0.434
SEM*	0.013	0.006	0.0069	0.0196

Difference in capital letters indicates significant differences with  $p < 0.10$ . No differences were found with a  $p < 0.05$ .

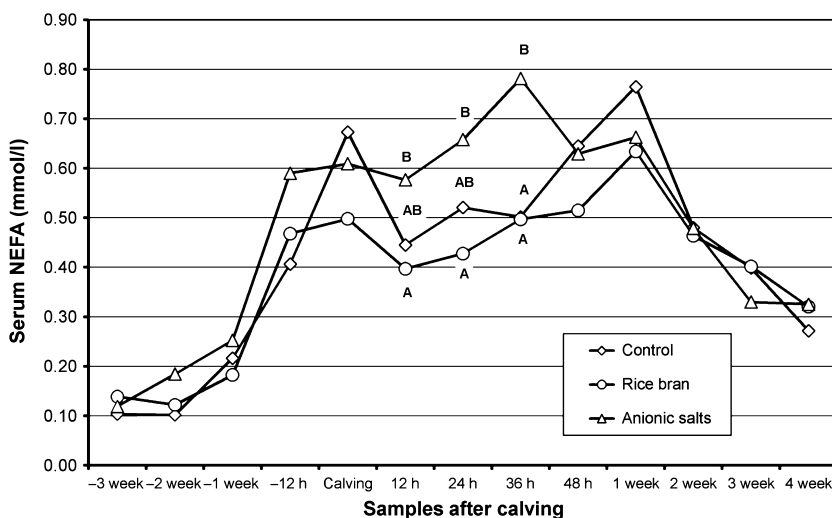
\*SEM reported mathematically transformed as confidence interval is not symmetric.

During the pre-calving period, every Wednesday, urine and blood samples were taken from all animals in the close-up group. Urine was sampled from mid-stream urination with a clean plastic cup upon spontaneous urination or with the aid of stimulation of the perineum. Within minutes after obtaining the sample, pH was measured with a calibrated pH meter, and smaller samples were kept at  $-20\text{ }^{\circ}\text{C}$  for later analyses. Blood samples were taken from the tail vein with vacuum syringes, and serum was separated by centrifugation and stored frozen at  $-20\text{ }^{\circ}\text{C}$  for later analyses.

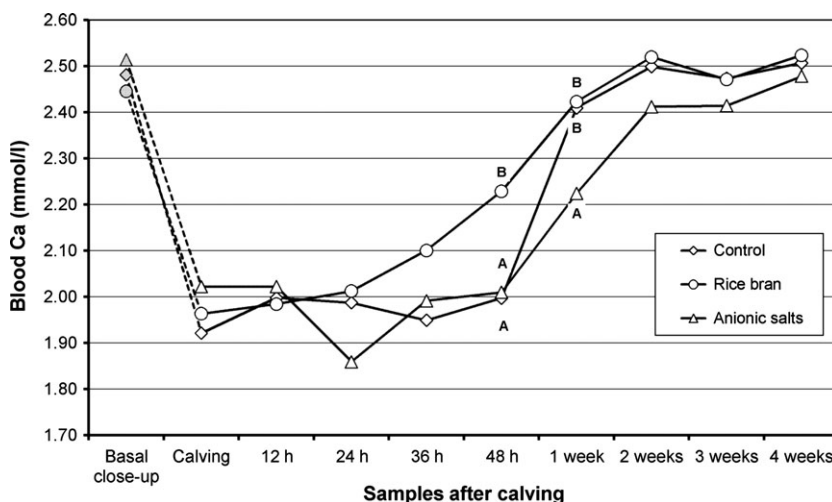
From the time when the first signs of the coming calving were observed, every 12 h (at 7:00 and 19:00), blood samples were taken from the cows until 48 h after calving. Additionally, a blood sample was taken immediately after calving, before the Ca suspension was offered. Beyond these 48 h of periparturient period, weekly samples were taken on Wednesdays for the first 4 weeks of lactation.

Urine samples were analysed for Ca, P and Mg. Creatinine was also analysed to study determine urinary mineral excretion from the mineral/creatinine ratios. Serum samples were analysed for Ca, P, Mg and for non-esterified fatty acids (NEFA).

Ca in serum and urine was analysed by photometric colour test (Ca-oCPO complex). Serum and urine Mg were analysed by photometric colour test and P with a photometric UV test. Creatinine in serum and urine was analysed with a kinetic colour test (Jaffé method). All serum and urine analyses were carried out using equipment from Olympus Diagnostica GmbH (Hamburg, Germany) at Synlab.vet (Augsburg, Germany).



**Fig. 2** Evolution of serum NEFA from 3 weeks before calving to 4 weeks after calving. Difference in letters indicates significant differences with  $p < 0.05$ .



**Fig. 3** Evolution of serum Ca from calving to 4 weeks after calving. Difference in letters indicates significant differences with  $p < 0.05$ .

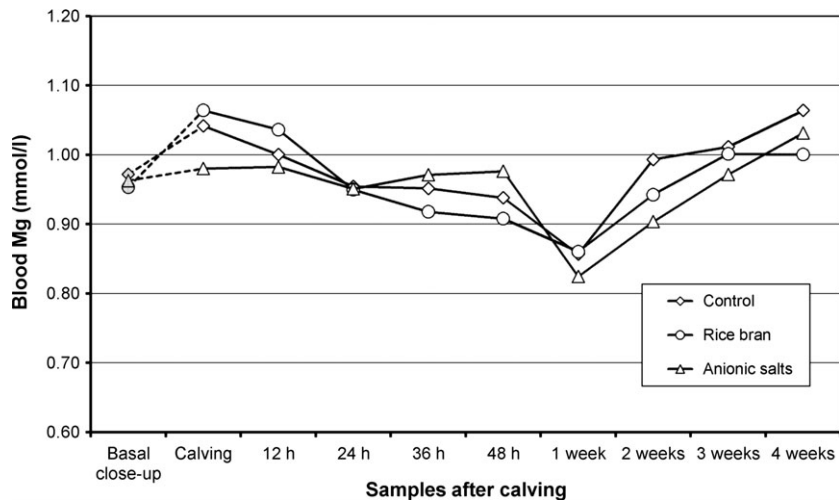
**Statistical analysis**

Daily feed intakes and nutrient composition were computed for every animal with data obtained from day -10 to calving and for the first 10 days of lactation. Analyses of variance were performed on these repeated observations on the subject cow using PROC MIXED of SAS (version 9.1; SAS Institute, Cary, NC, USA). The model included 'block', 'treatment' and the interaction between 'day' and 'treatment'.

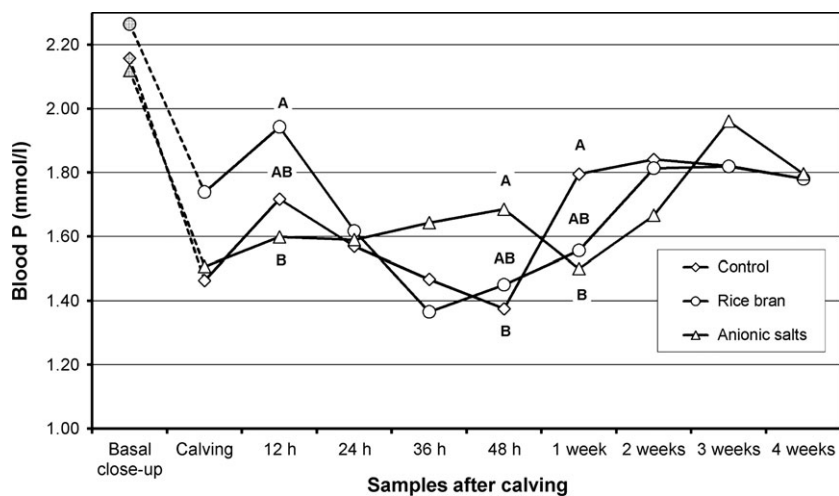
Colostrum production and serum Ca nadir, as non-repeated observations, were analysed with PROC MIXED including 'block' and 'treatment' and also with 'parity' number as covariable. The application of Ca infusions, incidence of udder oedema, and the need for assistance at calving were analysed with PROC GENMOD of SAS, as binomial observations using logistic transformation.

Data on serum Ca, P, Mg and serum NEFA were checked for normality. Serum Ca and P presented negative skewness and were transformed with  $Ca' = 1/(4 - Ca)$  and  $P' = 1/(4.5 - P)$ . Serum Mg and NEFA had positive skewness and were transformed with  $X' = (1 + X)$ . These repeated measures were separated as weekly samples before calving, samples around calving and weekly samples post-calving. Observations were analysed as repeated measures with PROC MIXED including 'block', 'treatment', 'sampling time' and 'parity' as covariable.

Urine Ca, P and Mg contents were expressed as a ratio with creatinine to estimate daily urinary excretion of these minerals, using creatinine as marker to correct for urine volume variation. Creatinine content, the ratio between Ca, P and Mg and creatinine and pH were checked for normality. Mg creatinine ratio fitted a normal distribution. PH measurements during the close-up period presented negative



**Fig. 5** Evolution of serum Mg from calving to 4 weeks after calving. Difference in letters indicates significant differences with  $p < 0.05$ .



**Fig. 4** Evolution of serum P from calving to 4 weeks after calving. Difference in letters indicates significant differences with  $p < 0.05$ .

skewness and required the transformation  $X' = 1/(10 - X)$ . PH measurements after calving fitted a normal distribution. Creatinine and Ca creatinine ratio required the inverse transformation  $X' = 1/(1 + X)$  to comply with normality. Phosphate creatinine ratio was transformed logarithmically. Urine data from 3 weeks before or after calving were analysed as repeated measures on the subject cow with PROC MIXED. The model included the time point of sampling, 'block' and 'treatment'.

Weekly milk production parameters for the first 8 weeks of lactation were calculated and processed with PROC MIXED including 'block', 'treatment' and the interaction between 'treatment' and 'lactation week'.

Covariance structure assigned to PROC MIXED was 'Autoregressive' except for serum samples for which 'Toeplitz' was used, because observations were not equally spaced in time.

## Results

Before calving, voluntary intake of forage, total dry matter intake and concentrate consumption did not differ significantly between the three treatments (Table 2). After calving, however, feed intakes were different ( $p < 0.05$ ) between cows fed the rice bran and low DCAD groups.

Forage intake after calving was 2.5 kg DM greater in cows fed rice bran before calving. Specific differences in DMI were observed between day 3 and day 7, in which rice bran fed cows ate more feed than those which had the DCAD treatment, with the control cows having an intermediate feed intake (Fig. 1).

The average composition of the ingested feeds during the dry period was different between treatments. The high starch content in the rice bran used in the feed, created a difference of 40 g/kg DM

**Table 5** Urine parameters in the 3 weeks before and after calving

	Creatinine	Ca/creat	P/creat	Mg/creat	pH
Close-up (3 weeks)					
Control	1.46	a 0.65	0.08	7.79	a 8.35
Rice bran	1.39	a 0.61	0.11	8.09	a 8.33
Low DCAD	1.46	b 1.40	0.08	7.83	b 7.87
SEM	0.016*	0.045*	0.119*	0.607	0.018*
Lactation (3 weeks)					
Control	0.98	0.78	5.17	10.44	8.27
Rice bran	1.01	0.57	5.49	9.19	8.25
Low DCAD	1.06	0.53	5.15	9.10	8.27
SEM	0.016*	0.037*	0.023*	1.112	0.045

Creatinine in g/l. Ca, P and Mg in mm.

Difference in letters indicates significant differences with  $p < 0.05$ .

\*SEM is reported mathematically transformed because confidence intervals are not symmetric.

starch with the DCAD treatment and of 25 g/kg DM with the control treatment. Also, cows in the DCAD diet consumed more Ca, Cl and S, in agreement with the dietary strategy of the treatment. This diet had a DCAD of 4 meq/kg DM, which was much lower than in the other two treatments, which had 269 meq/kg DM in the control diet and 287 meq/kg DM in the rice bran diet.

The nadir of serum Ca was not different between the treatments. Furthermore, colostrum production, the odds to be administered a Ca infusion, to require assistance at calving and the occurrence of udder oedema, were all similar between treatments (Table 3).

Serum parameters presented were similar between treatments ( $p > 0.05$ ) (Table 4). Before calving, serum Ca tended to be lower in the cows fed the rice bran than in those fed the low DCAD feed. In the same way, rice bran fed cows had a higher average serum Ca in the hours around calving, and a higher serum Ca than DCAD cows during the first 4 weeks after calving. Also serum NEFA for anionic salt cows around calving tended to be higher than for rice bran cows ( $p < 0.10$ ). However, longitudinal analysis of the data shows significant ( $p < 0.05$ ) differences in NEFA at specific time points. Serum NEFA were significantly higher in the hours after calving for low DACD as compared to rice bran, whereas control presented intermediate values (Fig. 2). Serum Ca in rice bran fed cows was higher at 48 h, and 1 week after calving as compared with anionic salts, and also with controls at 48 h ( $p < 0.10$ ) (Fig. 3). Serum P values were similar in cows with different dietary treatments, despite the apparent higher serum P during rice bran feeding before calving (Table 4). Also some specific differences were found at some specific sampling points (Fig. 4).

**Table 6** Milk production in the first 8 weeks of lactation

	Control	Rice bran	Low DCAD	SEM
Milk yield (kg)	39.9	38.7	37.7	1.10
Fat corrected milk 4% (kg)	40.4	41.3	38.4	1.14
Protein content (%)	3.21	3.29	3.26	0.039
Fat content (%)	4.11	4.32	4.15	0.084
Lactose content (%)	b 4.50	a 4.59	a 4.58	0.027
Urea content (mg/dl)	17.46	19.69	21.98	3.137
Protein yield (g)	1270	1264	1226	35.6
Fat yield (g)	1616	1652	1536	45.7
Lactose yield (g)	1804	1782	1741	55.2
Daily urea (g)	ab 7.11	b 7.64	a 6.59	0.332

Difference in letters indicates significant differences with  $p < 0.05$ .

Furthermore, serum Mg presented no clear differences between the treatments (Fig. 5).

Urinary excretion of Ca before calving in the low DCAD treatment cows was more than two-fold higher than the other two treatments ( $p < 0.05$ ). Also urinary pH was lower for this treatment (Table 5).

Milk production was similar across treatments. Only lactose was lower for cows fed control before calving and daily urea excretion in milk was higher for rice bran fed cows (Table 6).

## Discussion

Defatted rice bran has a nutrient profile that allows its inclusion in dairy rations, and it can be applied with great flexibility (Grasser *et al.*, 1995). Its high fibre and low fat content limits its use for monogastric species and makes it cost attractive for feeding dairy cows in the regions where it is available.

In this trial, the supplemental compound feed to be fed daily at a rate of 4 kg fresh matter, was formulated with 700 g/kg rice bran without interfering with any main formulation constrain, nor did it present problems for pelleting. The resulting rice bran content in the close-up diet averaged 167 g/kg DM. The level of rice bran in the present experiment was slightly higher than that applied in an earlier experiment (Martín-Tereso *et al.*, 2010b). Although feed formulation intended to result in similar macronutrient intakes across the treatments, the high inclusion of rice bran increased starch content in that treatment (Table 2). Other differences between the diets were found mainly in the mineral profile with higher contents of S, Cl and Ca in the DCAD treatment. Also the naturally high content of P and Mg in rice bran (Resurrection *et al.*, 1979) resulted in differences in mineral profile between this and the other diets.

In contrast with our earlier experiment (Martín-Tereso *et al.*, 2010b), Ca content in the rice bran treatment was equal to that of control. Two modes of action of rice bran on Ca metabolism have been identified which can act on dietary Ca availability: its low Ca content and its high content of phytic acid (Martín-Tereso *et al.*, 2011). In the context of this experiment, only phytic acid can be responsible for interaction with Ca metabolism, because Ca content in the rice bran treatment was similar to that of control.

Anionic salts are known to negatively affect voluntary intake of concentrates (Oetzel and Barmore, 1993), and therefore, the concentration of anionic salts in the concentrate was formulated to reduce its DCAD, while still allowing for complete consumption of the concentrate supplement. This compound feed was formulated to have a DCAD of  $-1000$  meq/kg DM. (Table 1). This feed in combination with a high voluntary forage intake of high DCAD forage, resulted in a final DCAD for the total ration near neutrality (4 meq/kg DM). Bringing DCAD close to 0 has proven to significantly reduce milk fever incidence (Charbonneau *et al.*, 2006), although the hypercalciuria that mediates its milk fever preventive effect is only effectively observed at lower DCAD diets (Martín-Tereso and Verstegen, 2011). For this last reason, the general recommendation in practice is to aim for DCAD values between  $-100$  to  $-150$  meq/kg DM for the total diet. This is done to obtain optimal benefits from anionic salts in close-up diets.

The low DCAD feed also contained Ca carbonate following the recommendation to provide Ca between 8.5 and 10.0 g/kg DM (Goff, 2006). Supplementing Ca in combination with anionic salts is controversial, and some meta-analyses have shown that dietary Ca at those levels can give higher milk fever incidences (Oetzel, 1991; Lean *et al.*, 2006). In practice, however, anionic salts are often combined with Ca supplementation.

Low DCAD diets can reduce feed intake (Vagnoni and Oetzel, 1998; Moore *et al.*, 2000). In the present trial, no differences were observed during anionic salt supplementation, most probably because the dose was intentionally limited to prevent this effect. However, after calving, animals on the low DCAD treatment showed a negative carryover effect resulting in reduced forage intake as compared to control animals (Table 2). This is remarkable, because ration composition in this period was identical for all three treatments. Daily DMI in early lactation (Fig. 1) shows a delayed increase in DMI in the first 10 days after

calving for DCAD cows as compared with control cows and especially with rice bran fed animals. These last animals ate daily 2.5 kg more of DM in the first 10 days of lactation than those that had been on the anionic salt diet. This reduced intake does not seem to be the cause for the numerically greater serum NEFA levels ( $p < 0.10$ ) observed in the DCAD cows within a few hours after calving (Table 4). The difference in NEFA is significant at 12, 24 and 36 h after calving (Fig. 2), and after this period, the three treatments had similar serum NEFA levels.

The results on DMI and serum NEFA levels are very relevant to the evaluation of rice bran as an alternative to feeds to reduce DCAD levels in close-up diets. A major justification of the efforts to control milk fever incidence is to reduce the associated incidence of other production diseases as ketosis, which has been estimated to increase by 24-fold in the case of milk fever (Curtis *et al.*, 1985). Therefore, it is important that dietary intervention before or after calving does not reduce intakes and thus does not impair energy balance in the transition process. An additional implication is that in small farms, often close-up heifers share the ration with multiparous cows. It is questionable if heifers benefit from low DCAD considering its impact in their DMI (Moore *et al.*, 2000).

The experiment could not verify any difference in the odds to receive a Ca infusion, the need to receive assistance at calving or the risk of udder oedema (Table 3). Small animal numbers and high variability in these observations exclude the possibility to detect significant differences in this trial.

All animals were offered a Ca suspension in warm water immediately after calving, and they all voluntarily drank it. The supply of these 45 g of Ca intended to assure the presence of a sufficient amount of Ca in the GI tract right after calving. This should have allowed for the reflection in serum Ca of any degree of improvement in the activation of Ca absorption.

Serum parameters were similar between treatments (Table 4). However, several differences in serum tended to be different when presented at  $p < 0.10$ . These differences are consistent with the results of a previous experiment performed with larger number of animals (Martín-Tereso *et al.*, 2010b). Serum Ca levels were numerically lower in rice bran fed animals before calving similarly as in the previous trial. This is indicative of a reduced nutritional availability of Ca. Also, in agreement with earlier results, serum Ca was higher in the hours after calving as compared with control. This is in line with the general hypothesis on the action of rice bran on Ca metabolism. Furthermore, in the



first weeks after calving, the low DCAD treatment seemed to present a delayed recovery of calcemia. The evolution of serum Ca in time shows differences between the different treatments (Fig. 3). Serum Ca at calving is similar for all pre-calving diets, just like serum Ca nadir (Table 3). However, the average serum Ca in rice bran fed cows showed a quicker trend for recovery. In the other hand, control and low DCAD fed animals maintained a low serum Ca for the first 48 h after calving. There was a different pattern of serum Ca recovery, in which the rice bran fed animals presented a higher serum Ca for rice bran at 48 h after calving, and the low DCAD group a lower serum Ca for the first week after calving. This is consistent with the first evaluation of this concept, in which serum Ca was higher than controls for the first 3 days after calving. In both trials, rice bran fed cows, initiated the recovery of calcaemia immediately after calving. Controls remained at minimum levels for at least 48 h in this trial, and at least for 12 h in the first trial (Martín-Tereso et al., 2010b).

The results here described for the low DCAD treatment are not consistent with other studies on the use of anionic salts and hypocalcaemia. Nevertheless, other authors have found a lack of responsiveness to anionic salts under specific circumstances (Tucker et al., 1992), or small non-significant differences with controls (Ramos-Nieves et al., 2009). In the present trial, DCAD reduction was moderate as compared with most references, reaching only neutrality. This resulted in a mild, although significant, reduction in urinary pH (Table 5), which remained more than one point above the recommendation for a pH between 6.2 and 6.8 (Goff, 2006). The limited impact of the diet on acid-base balance resulted in a significant but small increase in urinary Ca excretion, which only doubled that of control and rice bran fed animals. In contrast, hypercalciuria associated with anionic salts feeding has been described as greater than six-fold (Roche

et al., 2006) and even greater than 15-fold (Schonewille et al., 1994) compared to that of controls.

Recent findings on the mechanism of Ca reabsorption in the kidney have brought light into the way metabolic acidosis causes hypercalciuria in mammals, and this has been linked to the mode of action of low DCAD diets in the prevention of milk fever (Martín-Tereso and Verstegen, 2011). The re-absorption of Ca is controlled by the Ca channel TRPV5, which fails under acidic conditions, increasing urinary Ca losses (Suzuki et al., 2008). This is explanatory to hypercalciuria observed in cows in association with low DCAD diets. Urinary Ca excretion in relation with DCAD follows a curve that only increases substantially in the negative range of DCAD (Roche et al., 2003). The described low DCAD treatment had limited impact on urinary pH and urinary Ca excretion. This could be the reason this treatment could not elicit a positive response against hypocalcemia.

## Conclusion

Rumen-protected rice bran positively influenced the rate of recovery of calcaemia of cows after calving and had a positive impact on DMI in the first week after calving.

The low DCAD feed contained an insufficient dose of anionic salts, in relation to voluntary forage intake, to have a preventive effect against milk fever. In fact, recovery of calcaemia for this treatment took place even later than in the control. Further, this treatment had a negative impact on DMI post-calving and serum NEFA levels in the first 36 h after calving.

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