

## ***Solanum glaucophyllum* in pregnant cows. Effect on colostrum mineral composition and plasma calcium and phosphorus levels in dams and newborn calves**

par R. ROUX, Marie-Jeanne DAVICCO, B. J. CARRILLO \*, J.-P. BARLET (1)

Laboratoire des Maladies métaboliques, I.N.R.A.  
Theix, St Genès Champanelle, 63110 Beaumont

\* C.I.C.V., I.N.T.A., Castelar, Argentina.

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**Summary.** Two successive experiments were carried out on cows at the end of gestation. For several days before calving they were orally fed dried ground leaves of *Solanum glaucophyllum* (Sg) (a solanaceous plant of South America) containing a sterolglycoside of 1,25-dihydroxycholecalciferol, the biologically active metabolite of vitamin D. In Experiment I, Sg was administered for 7 days to 4 Jersey cows (34 g per animal per day) from days 8 to 2 before calving. This treatment significantly inhibited the drop in calcium and phosphate levels associated with parturition in cows. Colostrum levels of calcium ( $3.8 \pm 0.6$  g/l), mineral phosphorus ( $1.97 \pm 0.24$  g/l) and magnesium ( $0.61 \pm 0.04$  g/l) sampled in the 4 cows studied during the first milking after calving were significantly higher than those sampled in the same conditions in 4 controls (calcium :  $2.2 \pm 0.3$  g/l ; mineral phosphorus :  $0.69 \pm 0.05$  g/l ; magnesium :  $0.21 \pm 0.02$  g/l). In Experiment II, Sg was distributed in the concentrated ration for 4 days (days 6 to 3 before calving) to 19 Holstein  $\times$  Friesian cows (25 g per animal per day). This treatment significantly depressed the hypocalcemia and hypophosphatemia associated with parturition in cows. Moreover, the calcium level at birth in the 19 calves of treated cows ( $12.2 \pm 0.3$  mg/dl) was significantly higher than that in the 15 control calves ( $10.6 \pm 0.5$  mg/dl).

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Various steroids have been identified in higher plants. Hence the roots and the leaves of *Dactylis glomerata* possess an antirachitic activity connected with their vitamin D content (Raoul *et al.*, 1968). *Trisetum flavescens*, a graminacea abundant in some pastures in the Bavarian and Austrian Alps, induces a syndrome known as « enzootic calcinosis » in grazing cattle. This is characterized by an important loss of weight, hyperphosphataemia, abherent calcifications in the cardiovascular system, the lungs, the kidneys and the flexor tendons of limbs, and by distortions of the joints (Dirksen *et al.*, 1975). This syndrome seems to be due to the presence in *Trisetum flavescens* of an analog to 1,25-dihydroxycholecalciferol (Peterlik, Regal and Köhler, 1977) (1,25-(OH)<sub>2</sub>-D<sub>3</sub>), the biologically active metabolite of vitamin D<sub>3</sub> (De Luca, 1974). The family of Solanaceae seems to be well provided in derivatives of this vitamin. Thus *Solanum sodomaeum* has been mentioned in connection with « Naahelu disease »

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(1) Correspondence to : J.-P. Barlet, I.N.R.A., Theix, 63110 Beaumont, France.

which affects cattle in Hawaii (Ross *et al.*, 1971), and whose clinical and biochemical symptoms are similar to those observed in alpine enzootic calcinosis (Lynd *et al.*, 1965). In Florida, *Cestrum diurnum* causes hypercalcaemia and tissue calcinosis in cattle (Krook *et al.*, 1975a) and horses (Krook *et al.*, 1975b). This domestic shrub contains an active principle which, like  $1,25-(\text{OH})_2\text{-D}_3$ , prevents the inhibition of intestinal calcium absorption induced by strontium (Wasserman, Corradino and Krook, 1975). In the same way, the wasting disease of grazing animals, known as « Enteque seco » in Argentina (Worker and Carillo, 1967) and as « Espichamento » in Brazil (Döbereiner *et al.*, 1971), results from the ingestion of *Solanum glaucophyllum* (Sg) (also known as *Solanum malacoxylon*). The leaves of Sg contain a steroid whose chemical properties and biological effects are very similar to those of  $1,25-(\text{OH})_2\text{-D}_3$ . Thus, in chicks, Sg increases intestinal calcium absorption by stimulating the duodenal synthesis of calcium-binding protein, even in birds given a strontium-rich diet (Wasserman, 1974). It has been demonstrated that strontium inhibits renal  $1\ \alpha$ -hydroxylase activity, allowing the sole derivatives, already hydroxylated on carbon 1, to retain a biological activity (Omdahl and De Luca, 1971). Sg is equally active in the nephrectomized (Walling and Kimberg, 1975a) or diabetic rat (Schneider, Wasserman and Schedl, 1975). These two observations confirm that the biological activity of the vitamin analog present in the plant is not linked with the hydroxylation of carbon in position 1. An active aqueous extract from Sg leaves treated with  $\beta$ -glycosidase liberates a soluble substance into organic solvents. This substance migrates with  $1,25-(\text{OH})_2\text{-D}_3$  on chromatography columns and, after ultra-violet absorption and mass spectrometry, emerges as identical to this metabolite (Peterlik *et al.*, 1976; Wasserman *et al.*, 1976); it inhibits renal  $1\ \alpha$ -hydroxylase activity in the chicken as intensively as  $1,25-(\text{OH})_2\text{-D}_3$  (Procsal *et al.*, 1976). Furthermore, *in vitro* an aqueous extract from Sg leaves stimulates duodenal calcium transport (Corradino and Wasserman, 1974) and bone resorption (Puche and Locatto, 1974). Thus the calcinogenic factor present in Sg is now considered to be  $1,25-(\text{OH})_2\text{-D}_3$ -glycoside (Wasserman *et al.*, 1976).

In the bovine, the intramuscular injection of  $1,25-(\text{OH})_2\text{-D}_3$  induces significant hypercalcaemia and hyperphosphataemia (Capen, Hoffsis and Norman, 1977). Similar results have been obtained with  $1\ \alpha$ -hydroxycholecalciferol (Barlet, 1975), a synthetic derivative obtained from cholesterol, whose structure differs from that of  $1,25-(\text{OH})_2\text{-D}_3$  only by the absence of an hydroxyl group in position 25 (Holick *et al.*, 1973). Furthermore, when injected into the cow before calving,  $1\ \alpha$ -hydroxycholecalciferol prevents the fall in plasma calcium and phosphate levels occurring in this animal at the time of parturition (Sansom *et al.*, 1976; Barlet, 1977; Gast *et al.*, 1977; Sachs *et al.*, 1977). It therefore seemed interesting to study the influence of Sg (given orally to the cow at the end of gestation) on plasma calcium and phosphate levels in the dam and the newborn calf at the time of parturition, and on the mineral composition of colostrum. We report the results here.

## Materials and methods.

Two successive experiments were carried out. In experiment I we studied the effect of a daily dose of Sg (35 g of dried powdered leaves mixed with 1 l of water)

on the plasma calcium, magnesium and phosphate levels and on the colostrum mineral composition of 4 Jersey cows in 3rd or 4th lactation. The Sg was given *per os* from days 8 to 2 before parturition ; the 4 controls received 1l of water by the same way. The 8 animals were fed hay and grain concentrate. Thus, the approximate daily intake of each cow was 50 g of calcium, 55 g of inorganic phosphorus and 35 g of magnesium.

In experiment II, from days 6 to 3 before parturition, we fed Sg (25 g of dried powdered leaves given as a mixture with the ration concentrate) to 19 Holstein × Friesian cows in 3rd, 4th or 5th lactation, and observed its effect on the plasma calcium, magnesium and phosphate levels in the dams and newborn calves. These 19 cows and 15 randomly chosen controls received a daily ration providing each one with 80 g of calcium, 75 g of inorganic phosphorus and 55 g of magnesium. The total dry matter ingested by the 34 animals was measured daily.

During both experiments, each cow was mechanically milked immediately after parturition. The daily milk production (milk containing 4 p. 100 fat from 2 daily milkings) was measured (table 1). In experiment I a colostrum sample was collected from the first 6 milkings after parturition. In all cows blood samples were taken daily at 9 a. m. and at the time of parturition. In the 34 newborn calves used in experiment II, blood samples were collected at birth and 24 hrs after delivery. Each blood sample was taken with a heparinized syringe from the external jugular vein and centrifuged. The plasma was frozen until analysis. Calcium and magnesium were measured by atomic absorption spectrophotometry (Perkin Elmer 420) in plasma and in colostrum. Inorganic phosphorus was determined by colorimetry (Technicon autoanalyser).

TABLE 1

*Division of the animals, treatments, milk production and ingestion of dry matter*

Breed	Experiment I		Experiment II	
	Jersey		Holstein × Friesian	
Number of animals	4 treated	4 controls	19 treated	15 controls
Body weight post partum (kg ; mean ± SEM)	379 ± 17	374 ± 21	639 ± 31	647 ± 27
Daily dose of Sg (g)	35	0	25	0
Dry matter ingested during the 7 days before and after calving (kg/day ; mean ± SEM)	—	—	10.2 ± 0.3	11.1 ± 0.4
Daily milk production during the 7 days after calving (kg/day ; mean + SEM)	11.3 ± 1.7	10.9 ± 1.3	19.1 ± 1.4	20.9 ± 1.9

**Results.**

In the 23 treated cows, the ingestion of Sg significantly inhibited the hypocalcaemia and hypophosphataemia occurring at parturition (fig. 1 and 2). In the Holstein × Friesian cows, from 24 hrs after the first dose of Sg until the 6th day after the last

treatment, plasma calcium and phosphate concentrations in the 19 treated animals were significantly higher than those of controls (fig. 2). Similarly, plasma calcium and phosphate levels in the 4 treated Jersey cows were significantly higher than those measured in controls from the 48th hr after the beginning of the treatment until the 4th day after its termination (fig. 1).

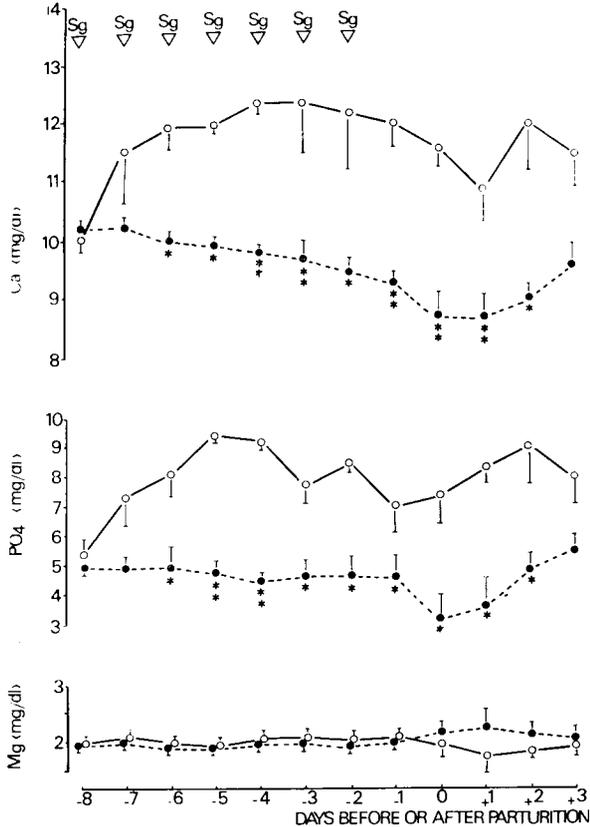


FIG. 1. — Influence of the ingestion of *Solanum glaucophyllum* (Sg) (35 g/animal/day for 7 days) on plasma calcium (Ca), phosphate (PO<sub>4</sub>) and magnesium (Mg) levels in 4 pregnant Jersey cows (o—o) (●—● controls) (mean ± SEM). The Student's *t* test was used to compare simultaneously each parameter measured in the treated and control cows (\* *P* < 0.05 ; \*\* *P* < 0.01).

At birth, the plasma calcium level ( $12.2 \pm 0.3$  mg/dl) of the 19 calves born from the Holstein × Friesian treated cows was significantly higher than that ( $10.6 \pm 0.5$  mg/dl) of the 15 calves born from the controls. This difference between the two groups of calves disappeared 24 hrs after birth. Plasma phosphate and magnesium levels in newborn calves were not significantly changed when the cows ingested Sg (fig. 2).

At the first milking after parturition the level of calcium in the colostrum ( $3.8 \pm 0.6$  g/l) from the 4 treated Jersey cows was significantly higher than in the colostrum of the 4 controls ( $2.2 \pm 0.3$  g/l). During the first two milkings the levels of inorganic phosphorus in the colostrum of the treated animals ( $1.97 \pm 0.24$  and  $1.57 \pm 0.13$  g/

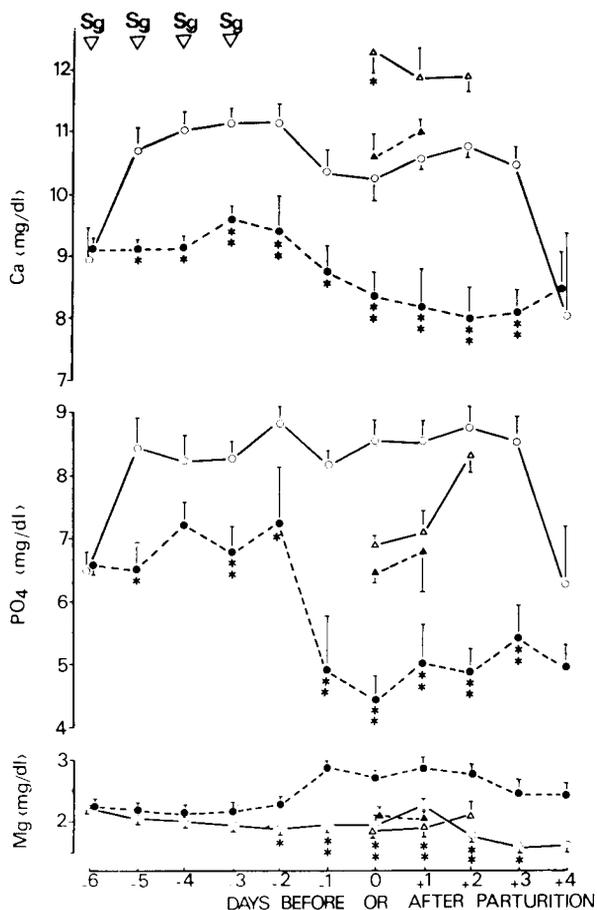


FIG. 2. — Influence of the ingestion of *Solanum glaucophyllum* (Sg) (25 g/animal/day for 4 days) on plasma calcium (Ca), phosphate (PO<sub>4</sub>) and magnesium (Mg) levels in 19 pregnant Holstein × Friesian cows (○—○) and in their newborn calves (△—△) (●—● control cows; ▲—▲ calves of control cows) (mean ± SEM).

The Student's *t* test was used to compare simultaneously each parameter measured in the treated and control cows (or in the calves of the treated cows and those of the controls) (\* *P* < 0.05 ; \*\* *P* < 0.01).

respectively) was significantly higher than in the colostrum of the controls ( $0.69 \pm 0.05$  and  $0.79 \pm 0.07$  g/l) ; the same was true of magnesium levels in the colostrum ( $0.61 \pm 0.04$  and  $0.46 \pm 0.07$  g/l in the treated ones ;  $0.21 \pm 0.02$  and  $0.20 \pm 0.03$  g/l in the controls) (fig. 3).

With the doses used, the ingestion of Sg did not seem to have any toxic effect on the 23 treated animals. During the experimental period, Sg did not alter in any significant way either the daily production of colostrum or the amounts of dry matter ingested (table 1).

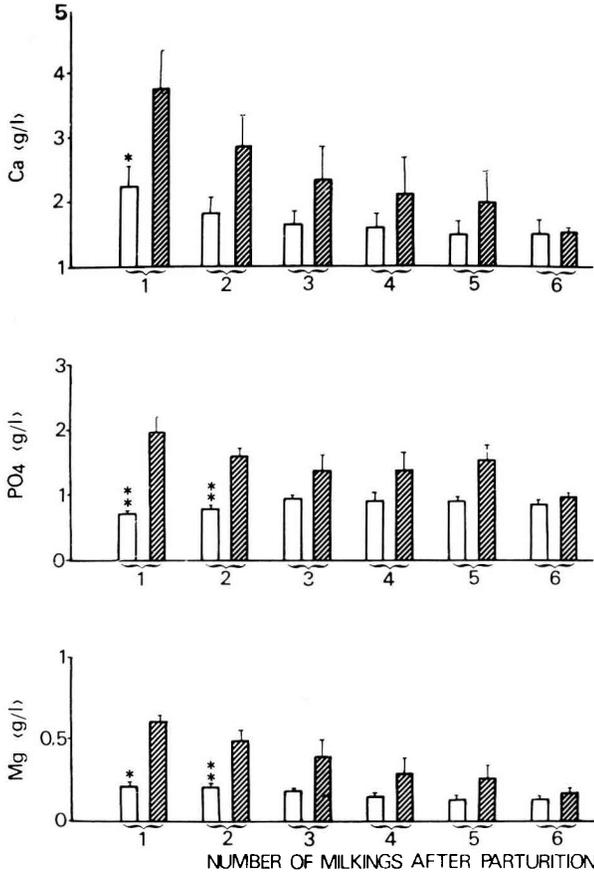


FIG. 3. — Influence of the ingestion of *Solanum glaucophyllum* (35 g/animal/day, from days 8 to 2 before parturition) on the levels of calcium (Ca), phosphate (PO<sub>4</sub>) and magnesium (Mg) in the colostrum of 4 Jersey cows (hatched bars) during the first 5 milkings after parturition (open bars : control cows) (mean ± SEM).

The Student's *t* test was used to compare simultaneously each parameter in the colostrum of treated and control animals (\* P < 0.05 ; \*\* P < 0.01).

### Discussion.

In the two groups of animals used, the ingestion of Sg during the days before parturition inhibited the hypocalcaemia and hypophosphataemia occurring at this time (fig. 1 and 2). In the chicken, oral administration of Sg or of *Cestrum diurnum* induces a rapid, intense increase in 1,25-(OH)<sub>2</sub>-D<sub>3</sub> plasma level (Haussler *et al.*, 1977). In the rat, a dose of Sg produces a more marked hypercalcaemia and hyperphosphataemia when it is given *per os* than when injected parenterally, as the liberation of the active steroid requires hydrolysis preferentially occurring in the intestine (Ladizesky, Mautalen and Camberos, 1974). For the same reason, an aqueous extract of Sg leaves is much more active after incubation with rumen juice (Boland *et al.*, 1976). Thus, the

liberation of  $1,25\text{-(OH)}_2\text{-D}_3$  by Sg leaves in the digestive tract of the treated cows can easily explain the rise in plasma calcium and phosphate levels observed in the treated animals (fig. 1 and 2).

This hypercalcaemia and hyperphosphataemia may result both from a stimulation of intestinal absorption of calcium (Samson, Vagg and Dobereiner, 1971 ; O'Donnell and Smith, 1973 ; Lawson, Smith and Wilson, 1974 ; Wasserman, 1974 ; Canas *et al.*, 1977) and inorganic phosphorus (Mautalen, 1972 ; Walling and Kimberg, 1975b) and from bone resorption. In Sg-treated rats, studies with  $^{45}\text{Ca}$  have demonstrated a more rapid and intense turnover for calcium (Puche *et al.*, 1976). Furthermore the addition of aqueous extract of Sg to bone cultures *in vitro* induces a rise in the level of calcium and hydroxyproline in the medium (Puche and Locatto, 1974 ; Simonite, Morris and Collins, 1976).

The plasma magnesium level in the Holstein  $\times$  Friesian cows treated with Sg is significantly lower than that of controls during the day before and the 3 days after parturition (fig. 2). In the dog, calciferol ingestion induces hypercalcaemia and hypomagnesemia, resulting essentially from a rise in the intracellular concentration of magnesium (Wallach *et al.*, 1966). In the rat, the ingestion of 5 000 IU of calciferol increases the urinary excretion of magnesium (Lifshitz, Harrison and Harrison, 1967). In the parturient cow there is an inverse relationship between plasma calcium and magnesium levels (Barlet, 1971).

At birth, the plasma calcium level in calves from Sg-treated Holstein  $\times$  Friesian cows was significantly higher than that of calves from control cows (fig. 2). It has already been demonstrated that the ingestion of Sg by the cow during the last month of gestation (50 mg of Sg/kg b.w./day, for 6 days) induces a significant rise in plasma calcium and phosphate levels in both dam and foetus (Barlet *et al.*, 1978). These result indicate that the analog to  $1,25\text{-(OH)}_2\text{-D}_3$  contained in Sg might stimulate the placental transfer of calcium from the mother to the foetus or that  $1,25\text{-(OH)}_2\text{-D}_3$  crosses the placenta and acts directly in the foetus, inducing bone resorption and/or increasing intestinal absorption of calcium from foetal intestine (it has been shown in rats that foetal intestinal calcium-binding protein is vitamin D-dependent ; Garel, personal communication).

In the cow, the colostrum is richer in mineral elements than is normal milk. A sharp decrease in the mineral level of the colostrum occurs during the first days after parturition (Guéguen, 1971). In the 4 Jersey cows, Sg does not change the decrease in the levels of calcium, inorganic phosphorus and magnesium in the colostrum during the first 6 milkings (fig. 3). In these 4 animals the levels of calcium, magnesium and inorganic phosphorus in the colostrum of the first milking are much higher than in the colostrum milked at the same time from control cows (fig. 3). The mineral composition of cow's milk, which is mainly determined by genetic factors, does not vary in relation to the diet (Guéguen, 1971). The levels of calcium and phosphorus in the milk of domestic ruminants is not under the control of parathyroid hormone and calcitonin since treatments such as parathyroidectomy or intravenous infusion of chelating agents in the cow (Pischke and Stott, 1964) or calcitonin deficiency induced by thyroidectomy (with thyroxine supplementation) in the goat (Barlet, 1974) do not alter milk composition. To our knowledge, no treatment up to now given to the pregnant cow has changed the mineral composition of colostrum. The mammary glands

of lactating rats maintained on a vitamin D-deficient diet supplemented with radio-labelled cholecalciferol were found to mainly contain the unchanged vitamin 5 days after parturition (Weisman *et al.*, 1976). Furthermore these glands contained a specific binding protein for cholecalciferol. The suggested biological role of this protein is to selectively pick up the sterol from the maternal circulation to ensure its supply via the milk to the newborn (Edelstein, Eizenberg and Harell, 1976). In our experiments, inducing 1,25-(OH)<sub>2</sub>-D<sub>3</sub> glycoside in the dam by Sg increases calcium levels both in the foetal (Barlet *et al.*, 1979) and newborn calf and in the colostrum.

No clinical symptom was observed in either of the 2 groups of Sg-treated cows. The amounts of dry matter ingested and of colostrum produced during the experimental period were not significantly different in treated and control animals (table 1). The absence of toxic effects of Sg might result from the low doses used and from the short time of the treatment (4 to 7 days). In Argentina, cattle suffering from « Entequ seco » quickly recovered when transferred to pastures deprived of Sg (Worker and Carrillo, 1967).

Sg has already been used in therapeutic tests in patients suffering from hypoparathyroidism (Casco *et al.*, 1977) or from renal insufficiency during hemodialysis (Herrath *et al.*, 1975 ; Mautalen *et al.*, 1977 ; De Vernejoul, Mautalen and Miravet, 1978). Thus Sg might be used efficiently in the prophylaxis of parturient hypocalcaemia in dairy cows. This solanaceous plant offers a natural source of water-soluble 1,25-(OH)<sub>2</sub>-D<sub>3</sub>. Furthermore the glycosidic molecules linked to the sterol prevent a too rapid liberation of it, and the intestinal mucosa of the animals ingesting Sg is exposed for a relatively long time to the active metabolite (Miravet *et al.*, 1977). It can therefore be assumed that therapeutic applications of Sg will be widely considered in the future.

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**Résumé.** Au cours de 2 expériences successives des vaches en fin de gestation ont reçu par voie orale, pendant quelques jours avant la parturition, des feuilles séchées et broyées de *Solanum glaucophyllum* (Sg) (Solanée d'Amérique du Sud) qui renferment un stérolglycoside du 1,25-dihydroxycholecalciférol, métabolite biologiquement actif de la vitamine D<sub>3</sub>.

Dans l'expérience I, Sg a été administré pendant 7 jours à 4 vaches Jersiaises (35 g par animal et par jour, du 8<sup>e</sup> au 2<sup>e</sup> jour précédant le vêlage). Ce traitement inhibait de façon significative la baisse de la calcémie et de la phosphatémie associée à la parturition chez les vaches. De plus, la teneur en calcium ( $3,8 \pm 0,6$  g/l), en phosphore minéral ( $1,97 \pm 0,24$  g/l) et en magnésium ( $0,61 \pm 0,04$  g/l) du colostrum prélevé au cours de la première traite effectuée immédiatement après vêlage chez les 4 vaches traitées, était significativement supérieure à celle du colostrum prélevé dans les mêmes conditions chez 4 témoins où elle était respectivement de  $2,2 \pm 0,3$  g/l pour le calcium,  $0,69 \pm 0,05$  g/l pour le phosphore minéral et  $0,21 \pm 0,02$  g/l pour le magnésium.

Dans l'expérience II, Sg a été distribué, mélangé à la ration de concentré, pendant 4 jours (du 6<sup>e</sup> au 3<sup>e</sup> jour précédant le vêlage) à 19 vaches Holstein × Frisonnes (25 g de Sg par animal et par jour). Ce traitement inhibait de façon significative l'hypocalcémie et

l'hypophosphatémie associées à la parturition chez ces animaux. En outre, à la naissance, la calcémie ( $12,2 \pm 0,3$  mg/dl) des 19 veaux issus des vaches traitées était significativement supérieure à celle ( $10,6 \pm 0,5$  mg/dl) des 15 veaux nés de vaches témoins.

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