Thyroxine and triiodothyronine serum concentration in newborn calves from cows supplemented with barium selenite

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Concentración sérica de tiroxina y triiodotironina en terneros recién nacidos de madres suplementadas con selenato de bario

Concentração de tiroxina e triiodotironina sérica em bezerros recém-nascidos de mães suplementadas com selenato de bário

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Abstract

Background: Barium selenate is an inorganic source of selenium (Se) that has been used in prolonged-release preparations to correct the state of selenium deficiency in bovines. Objective: To evaluate the serum concentrations of triiodothyronine (T₃) and thyroxine (T₄) hormones in

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newborn calves from mothers supplemented during prepartum with barium selenate. **Methods:** Six black Frisian cows with 7 months of gestation were supplemented with barium selenate subcutaneously two months before expected calving date, and other six cows remained as controls. All cows were subjected to a diet with a Se contribution lower than the requirements, constituted by hay from natural pasture and commercial concentrate lacking in Se. The Se balance was measured through the activity of erythrocyte glutathione peroxidase (GPx) and the T₃ and T₄ serum concentrations in calves was determinate by electrochemiluminescence. **Results:** The prepartum Se supplementation increased the GPx activity in cows from day 45 post supplementation (p<0.05). Calves from supplemented mothers showed an average serum Se concentration was higher than calves from non-supplemented mothers. The average concentration of T₃ in the calves from supplemented mothers, was lower in the first hour of life (p<0.05) when compared with calves from mothers of the non-supplemented group. At 7 days of age, there was a decrease (p<0.05) in the serum concentrations of T₄ in both groups. **Conclusions:** The administration of barium selenate to cows in prepartum generates a decrease in the serum concentration of T₃ in the first hour of life of the calf.

**Keywords:** bovine; barium selenate; calves; selenium; supplementation; triiodothyronine; thyroid; thyroxine.

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**Resumen**

**Antecedentes:** selenato de bario es una fuente inorgánica de selenio (Se) utilizada en preparaciones de liberación prolongada para corregir el estado de carencia de Se en bovinos. **Objetivo:** Evaluar las concentraciones séricas de triyodotironina (T₃) y tiroxina (T₄) en terneros recién nacidos de madres suplementadas durante el preparto con selenato de bario. **Métodos:** seis vacas frisón negro con 7 meses de gestación fueron suplementadas vía subcutánea con selenato de bario dos meses previos a la fecha de parto. Otras seis vacas permanecieron como controles. Todas las vacas se mantuvieron con una dieta cuyo aporte de Se fue inferior a los requerimientos y consistió en heno de pradera natural y concentrado comercial sin Se. El balance de Se se midió usando la actividad eritrocitaria de glutación peroxidasa (GPx) y las concentraciones de T₃ y T₄ en terneros mediante electroquimioluminiscencia. **Resultados:** la suplementación con Se aumentó la actividad de GPx en vacas desde el día 45 post suplementación (p<0.05). Los terneros de madres
suplementadas mostraron una concentración sérica promedio de Se mayor que los terneros de madres no suplementadas. La concentración promedio de $T_3$ de terneros de madres suplementadas fue menor en la primera hora de vida ($p<0,05$) que en terneros de madres no suplementadas. A los 7 días de edad hubo una disminución ($p<0,05$) en las concentraciones séricas de $T_4$ en ambos grupos. **Conclusión:** la administración de selenato de bario en vacas preparto genera una disminución en la concentración sérica de $T_3$ en la primera hora de vida del ternero.

**Palabras clave:** bovino; selenato de bario; selenio; suplementación; terneros; tireóide; tiroxina; triyodotironina.

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**Resumo**

**Antecedentes:** o selenato de bário é uma fonte inorgânica de selênio (Se) usada em preparações de liberação prolongada para corrigir o status de deficiência de Se em bovinos. **Objetivo:** avaliar as concentrações séricas de triiodotironina ($T_3$) e tiroxina ($T_4$) em bezerros recém-nascidos de mães suplementadas durante o pré-parto com selenato de bário. **Métodos:** seis vacas fríesianas negras aos 7 meses de gestação foram suplementadas com selenato de bário por via subcutânea dois meses antes do parto. Seis outras vacas permaneceram como controle. Todas as vacas foram mantidas em uma dieta cuja contribuição de Se foi inferior aos requeridos e consistiram em feno natural da pradaria e concentrado comercial sem Se. O balanço de Se foi medido usando a atividade eritrocitária das concentrações de glutatoni peroxidase (GPx) e $T_3$ e $T_4$ em bezerros por eletroquimiluminescência. **Resultados:** a suplementação com atividade de GPx aumentou em vacas a partir do dia 45 após a suplementação ($p<0,05$). Os bezerros de mães suplementadas apresentaram uma concentração sérica média de Se maior que os bezerros de mães não suplementadas. A concentração média de $T_3$ dos bezerros das mães suplementadas foi menor na primeira hora de vida ($p<0,05$) do que nos bezerros das mães não suplementadas. Aos 7 dias de idade houve uma diminuição ($p<0,05$) nas concentrações séricas de $T_4$ nos dois grupos. **Conclusão:** a administração de selenato de bário em vacas de parto gera uma diminuição na concentração sérica de $T_3$ na primeira hora de vida do bezerro.

**Palavras-chave:** bovino; bezerros; selenato de bário; selenio; suplementação; tireóide; triiodotironina; tiroxina.
Introduction

The hormones thyroxine (T₄) and triiodothyronine (T₃) are synthesized exclusively by the thyroid through a series of reactions linked to the histological organization of the gland (Carvalho and Dupuy, 2017). Thyroid hormones have an effect on growth, development, differentiation, reproduction and in various routes of the intermediary metabolism. Although T₃ is the biologically active hormone, T₄ is considered a pro-hormone that requires conversion to T₃ (Suttle, 2010). The deiodination process is catalyzed by one of the three deiodinase enzymes currently described which are distributed mainly in extrathyroidal tissues (Beckett and Arthur, 2005). All deiodinases contain a residue of selenocysteine in their active site (Stadtman, 2000), therefore an adequate metabolic balance of selenium (Se) is essential for synthesis, activation, metabolism, and secretion of thyroid hormones. An insufficient supply of Se in ruminants compromises the metabolism of thyroid hormones affecting the conversion of T₄ to T₃ (Contreras et al., 2002; 2005; Voudouri et al., 2003; Rowntree et al., 2004) and that calves born to mothers with negative metabolic balances of Se do not only have compromised their Se status but also the neonatal synthesis of thyroid hormones (Awadeh et al., 1998; Guyot et al., 2011). To correct or control clinical signs of deficiency, it is common to resort to inorganic Se sources (e.g. sodium selenite) or organic sources (e.g. selenized yeasts; Davis et al., 2008). In this regard, the metabolic path of Se is dependent on the source employed (Ortman and Pehrson, 1999; Juniper et al., 2008; Slavik et al., 2008), with selenite being more easily metabolized to the immediate precursors of selenocysteine in comparison with the organic forms of Se. The barium selenate (BaSeO₄) is an inorganic source that has been used in the formulation of prolonged-release preparations to provide an adequate Se status for at least 7 months (Leyán et al., 2004; Judson and Badidge, 2010), generating a high and sustained concentration of Se in plasma and liver compared to other inorganic forms (Davis et al., 2008).

The hypothesis of this work is that the blood concentration of thyroid hormones in newborn calves from Se deficient mothers can be improved by supplementing the mothers with barium selenate as a source of Se. The objective of this study is to evaluate the blood concentration of T₃ and T₄ in newborn calves from mothers supplemented in prepartum with barium selenate.
Materials and methods

Ethical considerations

The trial was developed according to the bioethics protocols for the use of animals in research of the Universidad Austral de Chile (Fondecyt 119-09939).

Animals

In the area of Valdivia, Chile (39°48'LS; 73°15'LO), 12 clinically healthy black Friesian cows were used for the study. The animals were distributed in 2 groups of six animals, homogeneous in terms of age (6.5 years), number of deliveries (3.5 deliveries), body weight (594 kg), gestational age (230 days) and milk production in the last lactation (6,406 L).

Experimental design

The animals were housed throughout the experimental period, in individual stalls on a concrete floor and straw bed. All the cows were fed a diet with a low intake of Se (Se <0.05 ppm) consisting of 9.5 kg of natural pasture hay [Se = 0.02 ppm of dry matter — DM) and 1 kg of commercial concentrate (Cosetan®, Biomaster IANSA-Chile; Se = 0.12 ppm DM), in individual feeders and water ad libitum. One of the groups of animals was selected at random and was supplemented with Se (Se-S) and the other group remained as a control without supplementation (Se-D). After calving, which occurred in the month of August (late winter), the calves received colostrum from their mothers in a volume of 4 L during the first 24 hours and then by staying with them for three days. Afterward, they were placed in individual pens where they continued to receive whole milk in a volume of 4 L/d.

Supplementation with selenium

The cows of the Se-S group were supplemented subcutaneously with 1 mg Se/kg using barium selenate (Depose®, Young Animal Health Ltd) in a single dose of 1 mL/50 kg/BW, administered 60 days before expected calving date.
Samples

Blood samples from each cow were collected into heparin-coated and noncoated tubes by coccygeal venipuncture prior to supplementation (day 0), and then every 15 days for 90 days. Blood samples were collected from the calves into noncoated tubes immediately after birth, prior to the ingestion of colostrum and at 7 days post birth. The blood plasma and serum were maintained at -20 °C until analysis.

Selenium metabolic balance evaluation

The metabolic balance of Se in cows was evaluated by the blood activity of glutathione peroxidase (GPx) using a commercial reagent (Ransel®, Randox), according to Contreras et al. (2002). The activity of GPx was expressed in U/g of hemoglobin (Hb).

Determination of the serum concentration of $T_3$ and $T_4$ in calves

The determination of serum concentrations of hormones $T_3$ and $T_4$ was carried out by the electrochemiluminescence technique in an Elecsys 1010 device (Roche). The values were expressed nmol/L. The serum ratio of $T_3/T_4$ was calculated by their quotient on serum concentration values.

Determination of serum selenium concentration in calves

It was carried out using an atomic absorption spectrophotometry device (Thermo® Series AA Solaar), at 196 nm, with hydride generation in a Thermo Scientific model VP100, after acid digestion of the sample as indicated by Muñiz-Naveiro et al. (2005). The values were expressed in mg/L.

Statistical analysis

Data were expressed as means and standard deviation. The normality of the data was established by the Shapiro-Wilk test. The significance of the differences in the Se balance of the supplemented and non-supplemented cows was evaluated by means of a variance analysis of repeated measures.
followed by Tukey's multiple comparisons test. The differences in the concentration of thyroid hormones in the calves were evaluated by Student's "t" test. The analysis was performed with the GraphPad© Prism 3 software (GraphPad Software, USA) and it was considered significant with a value of p<0.05.

**Results**

The mean initial values of the GPx activity in the Se-D and Se-S groups were 111 ± 52 U/g Hb and 118 ± 29 U/g Hb (p>0.05), respectively. Subsequent measurements in the Se-D group showed a reduction in the blood activity of GPx up to values considered marginal (<100 U/g Hb) at day 30 and following days (Figure 1). In contrast, in the Se-S group, an increase in blood activity of GPx with respect to the initial value was observed. The differences between groups were detected from day 45 until the end of the experiment (p<0.05).
The average concentration of T3 was lower in the first hour of life in the calves from mothers of the group Se-S, compared with calves from mothers of the group Se-D (p<0.05; Table 1). The Se-D values presented at day 7 were similar to the concentrations shown by the group Se-S (p>0.05). On the other hand, the T4 concentrations during the first hour of life were similar between groups (p>0.05). On day 7, both groups presented a decrease that was only significant in the group Se-S (p<0.05), nevertheless, the T3:T4 ratio was similar between groups in the first hour of life (p>0.05) and on day 7. However, the ratio between periods was increased in group Se-D (p<0.05; Table I).

Table 1. Serum concentrations (mean ± SD in nmol/L) of T3, T4 and the T3:T4 ratio in calves at 1 hour (h) and 7 days after birth from cows maintained fed ration with low selenium and supplemented during the prepartum with barium selenate (Se-S) and non-supplemented (Se-D).

<table>
<thead>
<tr>
<th></th>
<th>T3</th>
<th>T4</th>
<th>T3:T4 ratio</th>
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<tbody>
<tr>
<td></td>
<td>1 h</td>
<td>7-day</td>
<td>1 h</td>
</tr>
<tr>
<td>Se-S</td>
<td>5.92 ± 0.6</td>
<td>4.64 ± 0.94</td>
<td>181 ± 51</td>
</tr>
<tr>
<td>Se-D</td>
<td>8.64 ± 2.46*</td>
<td>4.23 ± 1.16*</td>
<td>188 ± 31</td>
</tr>
</tbody>
</table>

*p<0.05 between groups; *p<0.05 between periods.

Discussion

According to the NRC (2001), the Se requirement for dairy cattle corresponds to 0.3 ppm. The diet used in this experiment contained 0.04 ppm, which established that the non-supplemented animals presented a negative metabolic balance for this mineral. Consistent with this, the blood activity of GPx in the cows of the group Se-D remained within the range considered low marginal (<100 U/g Hb; Ceballos and Wittwer, 1996). Although it is described that the minimum concentration of Se in the diet of grazing cattle should be greater than 0.05 ppm in order not to show alterations in the health and production of cattle (Suttle, 2010), in this study no clinical signs attributable to this lack were observed during the trial period. On the other hand, supplementation with barium selenate significantly increased the activity of GPx in the cows of the group Se-S from day 45 post-supplementation, reaching values higher than 200 U/g Hb during the period of deliveries which are considered appropriate for lactating cows (Ceballos and Wittwer, 1996). This result is in
agreement with reports by other authors using different Se sources (Awadeh et al., 1998; Guyot et al., 2011). According to Gunter et al. (2013), the magnitude of this increase in the concentration of Se in the blood of calves of supplemented mothers is dependent on the source of Se employed.

The effect of supplementation with organic and inorganic Se on different organic systems in cattle, including the function of the thyroid gland, has been reported by different authors (Awadeh et al., 1998; Rowntree et al., 2004; Koenig and Beauchemin, 2009; Guyot et al., 2011; Rose et al., 2012); however, few studies refer to the effect generated in newborns from mothers supplemented with Se. In this study, the results showed that the average concentration of T₃ was lower in the first hour of life (p<0.05) in the calves from mothers of the group Se-S (Table 1).

Although it was not possible to find studies conducted under similar conditions, other authors report values of thyroid hormones in the newborn by applying different experimental models that differ from those indicated in this study (Davicco et al., 1982; Stojić et al., 2002; Kirovski et al., 2008). These differences can be attributed not only to factors inherent to the methodologies used for the determination of the hormones but also to the potential influence of the environment on the animals subjected to experimentation. In this regard, Stanko et al. (1991) indicated that calves born during the winter period have higher concentrations of T₃ and T₄ than those born in other seasons of the year because the cold exerts a stimulating effect on the synthesis and secretion of these hormones in the newborn. The intake of colostrum is another factor that has been related to the concentrations of thyroid hormones in the neonate (Grongnet et al., 1985). In this study, the calves received colostrum ad libitum from their mothers, who presented a different metabolic status of Se (Figure 1); however, some studies have indicated that iodine transferred transplacentally from the mother to the fetus would be more relevant than iodine transferred by colostrum (Davicco et al., 1982; Guyot et al., 2011).

The decrease in the concentration of hormones as time elapses from birth is consistent with that reported for ruminants by other authors (Awadeh et al., 1998; Takahashi et al., 2001; Stojić et al., 2002; Guyot et al., 2011; Rose et al., 2012). In this sense, the highest initial concentration of T₃ in the newborn is related to the synthesis of thermogenin, a protein necessary for the production of heat in brown adipose tissue (Carstens, 1994). When analyzing the effect of Se between groups, it
is observed that the concentration of \( T_3 \) was lower in the group of calves coming from mothers supplemented with Se. This unexpected effect could be attributed to the administration of barium selenate, a salt of low solubility formulated for subcutaneous deposition of Se that is released over a period of one year. The uncontrolled release can reach values that, in the conditions of this test, could be considered supra-nutritional, influencing a lower conversion of \( T_4 \) to \( T_3 \) in newborn calves. This paradoxical effect, with a similar source of Se, has been observed in heifers supplemented with barium selenate, in which the erythrocyte GPx activity exceeded by far the value that is considered metabolically adequate, causing a decrease in the cellular immune response (Leyán et al., 2006). The hypothesis of an effect associated with the formulation of Se administered is reinforced by studies conducted using different sources of Se. In this regard, Awadeh et al. (1998) reported that the concentration of \( T_3 \) in calves from mothers supplemented with 60 ppm of Se, in the form of organic Se, was higher than in calves from mothers supplemented with the same amount of inorganic Se, with the highest \( T_3:T_4 \) ratio being showed by calves from mothers who received salts containing 120 ppm Se. However, in other trials, no effect on the concentration of thyroid hormones in newborns was obtained by supplementing prepartum cows with selenized yeast or selenite (Rowentree et al., 2004; Koenig and Beauchemin, 2009).

There was also no effect on the thyroid hormone concentrations of newborns when using intraruminal boluses formulated on the basis of Se, iodine and cobalt, and supplied two months prior to delivery (Rose et al., 2012). The effect of different formulations of Se (selenite and selenium yeast) on the concentration of thyroid hormones in adult animals has also been studied, and although they increase the activity of GPx and the concentration of Se in blood they do not cause differences in the concentration of \( T_3 \), \( T_4 \), and the \( T_3:T_4 \) ratio with respect to non-supplemented animals (Gunter et al., 2013). These results were attributed to a compensatory response of non-supplemented animals, in which the expression of the enzyme iodothyronine deiodinase type I in the thyroid would be increased.

The studies related to the effect of the Se on the function of the thyroid gland have preferably used organic Se, selenite salts and elemental selenium with varied results. This is the first study that uses slow-release barium selenate in cows during prepartum to assess its effect on the concentration of thyroid hormones in calves, which also shows results that do not allow to establish criteria for practical use. The analysis of the results obtained in this study raises questions about
the adequate balance of Se in the prepartum cow and the consequences of supranutritional contributions that could cause negative metabolic responses in the newborn. Faced with this scenario, further studies are required on this subject.

**Declarations**

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**Conflicts of interest**

The authors declare they have no conflicts of interest with regard to the work presented in this report.

**Author contributions**

Víctor R. Leyán: Study design of the study, sample processing and manuscript production.

Ricardo H. Chihuailaf: Data processing and manuscript production He was responsible for

Fernando G. Wittwer: Study design of the study, sample processing and manuscript reviewing and edition.

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