Ciencia Veterinaria
ISSN: 1515-1883
ISSN: 1853-8495
revista@vet.unlpam.edu.ar
Universidad Nacional de La Pampa
Argentina

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Ciencia Veterinaria, vol. 24, no. 1, 2022, January-June, pp. 1-16
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Sección: Comunicación corta
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Artículo de Melendez P, Riquelme P, Reyes C
CIENCIA VETERINARIA, Vol. 24, Nº 1, enero-junio de 2022, ISSN 1515-1883 (impreso) E-ISSN 1853-8495 (en línea)
DOI: http://dx.doi.org/10.19137/cienvet202224106

Effect of rumen protected thiamine on blood concentration of beta-hydroxyl butyrate in postpartum Holstein cows: a pilot study

Efecto de la tiamina protegida en rumen sobre la concentración sanguínea de beta hidroxibutirato en vacas Holstein posparto: un estudio piloto

Efeito da tiamina protegida no rúmen sobre a concentração sanguínea de beta-hidroxil butirato em vacas holandesas pós-parto: um estudo piloto

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Fecha de recepción: 10/09/2021
Fecha de aceptación para su publicación: 24/11/2021

ABSTRACT

A development of a rumen bypass product containing thiamine might be a valuable alternative on the prevention of ketosis in dairy cattle. The objective of this pilot study was to determine the effects of rumen protected thiamine (RPT) on blood beta-hydroxyl butyrate (BHB) in Holstein postpartum cows. The study was conducted on a dairy herd with 650 cows, randomly assigned to a treatment group (T, n=20) receiving daily for 10 days postpartum, orally, 60 g rumen protected thiamine, and a control group (C, n=20), receiving a placebo. Blood samples were collected on days 3, 7, and 10 after calving. At day 3 and 10 postpartum BHB levels were similar between groups;

however, at day 7, BHB concentrations were different (0.57 and 0.83 mmol/L for T and C respectively, P ≤0.05). It is concluded that a rumen protected thiamine oral product decreased the blood concentrations of BHB during the first 10 days postpartum. Based on this pilot study, this additive deserves further investigation to elucidate its potential mechanism of physiological action as a ketosis preventive agent.

Keywords: thiamine, ketosis, beta-hydroxyl butyrate, Holstein, postpartum, cattle

RESUMEN

El desarrollo de un producto de sobrepaso del rumen que contenga tiamina podría ser una alternativa valiosa para la prevención de la cetosis en el ganado lechero. El objetivo de este estudio piloto fue determinar los efectos de una tiamina protegida de sobrepaso del rumen (RPT) sobre el beta hidroxibutirato (BHB) en sangre en vacas Holstein posparto. El estudio se realizó en un hato lechero con 650 vacas, asignadas aleatoriamente a un grupo de tratamiento (T, n = 20) que recibió diariamente durante 10 días después del parto, por vía oral, 60 g de tiamina protegida de sobrepaso del rumen y un grupo de control (C, n = 20), recibiendo un placebo. Se recolectaron muestras de sangre los días 3, 7 y 10 posparto. En el día 3 y 10 después del parto, los niveles de BHB fueron similares entre los grupos; sin embargo, en el día 7, las concentraciones de BHB fueron diferentes (0.57 y 0.83 mmol / L para T y C respectivamente, P ≤0.05). Se concluye que un producto oral de tiamina protegido en el rumen disminuyó las concentraciones sanguíneas de BHB durante los primeros 10 días posparto. En base a este estudio piloto, este aditivo merece una mayor investigación para dilucidar su mecanismo potencial de acción fisiológica como agente preventivo de la cetosis.

Palabras clave: tiamina, cetosis, beta hidroxibutirato, Holstein, posparto, ganado lechero

RESUMO

O desenvolvimento de um produto derivado do rúmen contendo tiamina pode ser uma alternativa valiosa na prevenção da cetose em gado leiteiro. O objetivo deste estudo piloto foi determinar os efeitos da tiamina protegida no rúmen (RPT) sobre o beta-hidroxil butirato (BHB) sanguíneo em vacas holandesas no pós-parto. O estudo foi

conduzido em um rebanho leiteiro com 650 vacas, aleatoriamente designadas a um grupo de tratamento (T, n = 20) recebendo diariamente por 10 dias pós-parto, por via oral, 60 g de tiamina protegida no rúmen e um grupo de controle (C, n = 20), recebendo um placebo. Amostras de sangue foram coletadas nos dias 3, 7 e 10 pós-parto. Nos dias 3 e 10 pós-parto, os níveis de BHB foram semelhantes entre os grupos; entretanto, no dia 7, as concentrações de BHB foram diferentes (0,57 e 0,83 mmol / L para T e C respectivamente, P ≤ 0,05). Conclui-se que um produto oral com tiamina protegida no rúmen diminuiu as concentrações sanguíneas de BHB durante os primeiros 10 dias pós-parto. Com base neste estudo piloto, este aditivo merece uma investigação mais aprofundada para elucidar o seu potencial mecanismo de ação fisiológica como agente preventivo da cetose.

Palavras-chave: tiamina, cetose, butirato de beta-hidroxila, Holstein, pós-parto, gado

Introduction

During the transition period the dairy cow must undergo several adaptive changes, including the reduction of dry matter intake, rumen adaptation to diets high in starch, decrease the risk and severity of hypocalcemia, oxidative stress and immunosuppression. Consequently, the cow may become more susceptible to several diseases, which represent high economic losses for the dairy industry [1, 3]. Peripartum diseases are closely related to each other, where cows with ketosis, for example, are more likely to develop displacement of the abomasum, impaired fertility and reduced milk production. Therefore, prevention of ketosis indirectly reduces the risk of other postpartum disorders [4, 7].

Ketosis is a metabolic disease associated with the typical negative energy balance experienced by dairy cows during the postpartum period. The mechanism of exacerbated ketogenesis lies in the low dry matter intake that cows have around parturition and the sudden onset of milk production just after calving. As a result, the cow begins to mobilize adipose tissue in the form of non-esterified fatty acids. At the same time, glucose is redirected to the mammary gland and spared to produce lactose. Non-esterified fatty acids are taken by the liver and oxidized to acetyl-coA. Because of lack of glucose (used for the milk synthesis), oxaloacetate production is reduced, then acetyl-co A cannot continue its oxidation process in the Krebs cycle, resulting in an increase rate of ketogenesis. Ketone bodies are the acetoacetate, acetone

and ß-hydroxybutyrate (BHB), being the last the most important to be tested in blood. [6, 8].The clinical manifestation of ketosis is characterized by reduced dry matter intake and milk production, which may be accompanied by the presence of neurological signs, while the subclinical condition apparently shows no evident signs and is established when blood BHB is ≥ 1.2 mmol/L. [4,6]. The reported cost of subclinical ketosis is widespread, ranging from US$ 77 to US$ 289 per case in the United States [3].

Ketosis has been a focus of intense research during the last decades. [3, 5-7]. In fact, studies carried out by the Cornell University in the USA, determined that cows with prepartum blood levels of non-esterified fatty acids > 0.3 mEq/L and postpartum BHB > 1.2 mmol/L were 2 to 4 times more likely to experience periparturient diseases, reduce pregnancy rate and decrease milk yield than normal cows. [5, 7]. As mentioned above, ketosis occurs in response to the lack of gluconeogenic precursors. [8]. Therefore, many additives, including niacin, monensin, propylene glycol, glycerol, and propionate have been studied as potential nutritional strategies to prevent ketosis in dairy cows [2]. However, certain key enzymes in the intermediate metabolism at the hepatocyte level deserve much more attention. Thiamine pyrophosphate is the physiologically active form of vitamin B1, participating as a cofactor of key enzymes of intermediate metabolism, such as pyruvate dehydrogenase. This is a multi-enzyme complex, where under certain anaerobic conditions it decarboxylates pyruvate to acetyl Co-A. Thiamine also participates within the citric acid cycle in the decarboxylation of alpha ketoglutarate to succinyl Co-A, favoring the oxidation of glucose to obtain ATP [8]. All these reactions, in theory, should favor the formation of oxaloacetate that assists the entry of acetyl Co-A into the Krebs cycle, avoiding ketogenesis [8]. However, the contribution of thiamine in the diet of dairy cows, if not protected from the action of microorganisms of the rumen, may not have the desired effect. Therefore, the technological development of a rumen bypass product containing thiamine pyrophosphate (RPT) might be a valuable alternative on the prevention of ketosis in dairy cattle. Thus, the hypothesis of this pilot study was that the supplementation of a RPT in dairy cows reduces the concentration of BHB in the blood. Hence, the objective of the present investigation was to evaluate the effect of an oral RPT supplemented during the first 10 days postpartum on the blood concentrations of BHB and accumulated milk production up to 100 days in milk in Holstein cows.

Materials and methods

The study was conducted under the ethical guidelines of Chilean government for the use of animals in research.

DAIRY AND ANIMALS

This is a pilot study with the intend of using the results for further controlled large-scale investigations. The study was carried out on a Holstein dairy (Pahuilmo dairy farm), with 1,300 total cattle, and 650 milking cows in the central area of Chile (Latitude: -33.58. Longitude: -71.12). The mean annual precipitation and lowest and highest temperature were 235 mm, 2.2 ° C and 25 ° C, respectively [9]. Cows were confined in an open barn free-stall system with sand beds, shade, and concrete floor. Cows were milked 3 times a day, in a rotatory parlor, with a Mature Equivalent 305-day milk yield of 12,000 kg. Milk yield was recorded daily in a computerized milking record system (Afimilk, Kibbutz Afikim, 1514800, Israel).

Cows were fed a total mixed ration three times a day. Cows were dried-off between 45 and 70 days before expected parturition. At 4 weeks before expected parturition, cows were moved to a prepartum lot and were fed a total mixed ration with a dietary cation-anion difference of -80 mEq/kg dry matter to prevent clinical hypocalcemia. Prepartum total mixed ration was fed twice a day. Cows calved in individual maternities and moved to a postpartum group until 21 days in milk. Calf was removed from the dam immediately after birth. Diets were formulated to meet or exceed the nutritional requirements of the Cornell Net Carbohydrate and Protein System (CNCPS) [(10)], using a commercial software (NDS, RUM&N Sas, Reggio Emilia, Italy).

During the postpartum period cows were subjected during the first 10 days in milk to a health monitoring protocol every other day, measuring rectal temperatures, presence of retained fetal membranes, abnormal uterine discharges, presence of abnormal milk for the diagnosis of clinical mastitis, evaluation of left paralumbar fossa to determine rumen fill and the presence of left displacement of abomasum or any other evident clinical condition. If cows were diagnosed with any pathological disorder, they were treated according to standard operating procedures established by the farm veterinarian.

Subsequently, at 38 ± 3 days in milk cows were subjected to a synchronization of ovulation protocol (Presynch-Ovsynch) and Timed-Artificial Insemination or breeding on heat detection. Pregnancy was diagnosed by ultrasound at 34 ± 3 days post service.

EXPERIMENTAL DESIGN

In order to find a difference in blood BHB concentration of 0.2 mmol / L (Standard Deviation = 0.20) between a treated group (1.0 mmol/L) and a control group (1.2 mmol/L; cut-off value for subclinical ketosis [6] [7], with 95% of confidence and 80% of power, a minimum sample size of 15 cows per group was calculated. Animals were randomly assigned at parturition to a treatment group (n=20) receiving 60 g/day of a rumen-bypass thiamine pyrophosphate (Glukogen, Nutritech, Agua Termal 100 Col., Agua Blanca Sur, Zapopan Jalisco, Mexico) dissolved in 100 ml of water, and offered orally with a syringe after the morning milking from day 1 to 10 postpartum. The control group (n=20) received a placebo of 100 ml of water. Both treated and control cows were housed in the same postpartum pen, received the same total mixed ration and were exposed to the same environmental and management conditions. Ingredients and nutritional composition of diets are shown in Table 1.

Table 1. Ingredients and nutritional composition of postpartum total mixed ration (kg/d, dry matter basis)

Ingredient	% Dry Matter	Dry Matter (kg)
Alfalfa silage	52.0	5.10
Corn silage	36.4	8.25
Barley brewers, wet	24.5	1.22
Corn grain ground	88.9	3.31
Soybean meal (48% Crude Protein)	89.5	2.32
Wheat bran	88.7	1.33
Bypass-fat	98.5	0.20
Sugar	98.0	0.39
Mineral/Vitamin premix	99.0	0.54
Total	48.9	22.7

Nutrient	% Dry Matter Basis
Dry Matter (%)[1]	48.93
Crude Protein[1]	17.64
Soluble Protein[1]	5.92
Degradable Protein[2]	41.22
ADF[1, a]	20.10
aNDFom[1, b]	29.80
peNDF [2, c]	19.30
NFC [1, d]	40.32
Starch[1]	24.60
Soluble Sugars[1]	6.38
Ether Extract[1]	3.60
Ash [1]	8.69
Ca [1]	0.73
P [1]	0.38
Mg [1]	0.24
Net Energy of Lactation (Mcal/kg dry matter)[2]	1.58

[1] Laboratory analysis
[2] From formulas
[a] Acid Detergent Fiber
[b] Neutral Detergent Fiber from organic matter treated with amylase
[c] Physically Effective Neutral Detergent Fiber
[d] Non-fiber carbohydrates

BLOOD SAMPLING AND MILK PRODUCTION

After the morning milking, experimental cows were sorted out by an electronic computerized gate system and then placed in a chute with a headlock for treatment and placebo oral supplementation and blood collection. Samples were obtained from the tail plexus and were collected at 3, 7, and 10 days postpartum to assess BHB concentrations using a portable meter device (FreeStyle Optium®, Abbott Diabetes Care Inc., Alameda, CA). The handheld device has a sensitivity of 94.8% (95% CI: 92.6-97.0), and a specificity of 97.5% (95% CI: 96.9-98.1). [(11)]. After finishing the daily treatment supplementation and blood sampling protocol, cows were returned to their group. Treatment and blood sampling lasted for no more than 5 minutes per cow. Body condition score at calving was measured by the same evaluator using a scale 1-5 [(12)]. Milk production was measured and recorded daily, and cumulative milk production up to 100 days in milk was analyzed.

STATISTICAL ANALYSIS

Data analysis was performed using the software SAS 9.4 [13]. The concentrations of blood BHB were analyzed using a mixed model analysis for repeated measures, considering the effect of treatment (RPT, control), day of sampling (3, 7, 10 days postpartum), lactation (primiparous, multiparous), body condition score at calving and their potential interactions as explanatory variables. The cow was considered as random effect nested within treatment and the interaction treatment x day of sampling was considered the most important variable of the model, since it measures the parallelism of the BHB concentration curves between both groups over time. The best model fitting was obtained using the Schwarz Bayesian Criterion, according to the best covariance structure matrix between the observations (auto-regressive, uncorrelated, and symmetric) [14]. The level of significance was established at $P \leq 0.05$. A tendency was considered with a P-value between 0.15 and 0.05. Cumulative milk production up to 100 days in milk was compared between groups by an analysis of variance, considering as independent effects the parity number; body condition score at calving, and treatment, using the PROC GLM of SAS 9.4 [13]. Least square means were reported.

Mixed models for repeated measures were defined as:

$$Y_{ijklm} = \mu + G_i + Cow\ (G_i)_j + Time_k + (G * Time)_{ik} + BCSC_l + Par_m + e_{ijklm}$$

Where:

Y_{ijklm} = Plasma metabolite concentration or milk yield
μ = population mean
G_i = fixed effect of group (Thiamine, control)
$Cow\ (G_i)_j$ = random effect of cow nested within group
$Time_k$ = fixed effect of time (3, 7 and 10 days in milk)
$(G * Time)_{ik}$ = interaction group by time
$BCSC_l$ = effect of body condition score at calving
Par_m = effect of parity
e_{ijklm} = random error term

Results

Mixed model for repeated measures considered as significant variables the effect of day postpartum ($P = 0.0022$) and the interaction of treatment x day ($P = 0.037$). Lactation and body condition score at calving were non-significant variables. (Table 2). Covariance parameters

demonstrated that the auto-regressive covariance structure was the best for goodness of fit. This indicates that the BHB concentrations curves between both groups were non-parallel and therefore differed over time (Figure 1). BHB concentrations were similar between groups at day 3 postpartum (> 0.7 mmol/L); however, at day 7 postpartum BHB concentrations decreased drastically in the RPT group (< 0.6 mmol/L) while increased drastically in the control group (> 0.8 mmol/L). At day 10 postpartum, both groups had similar BHB concentrations (< 0.6 mmol/L).

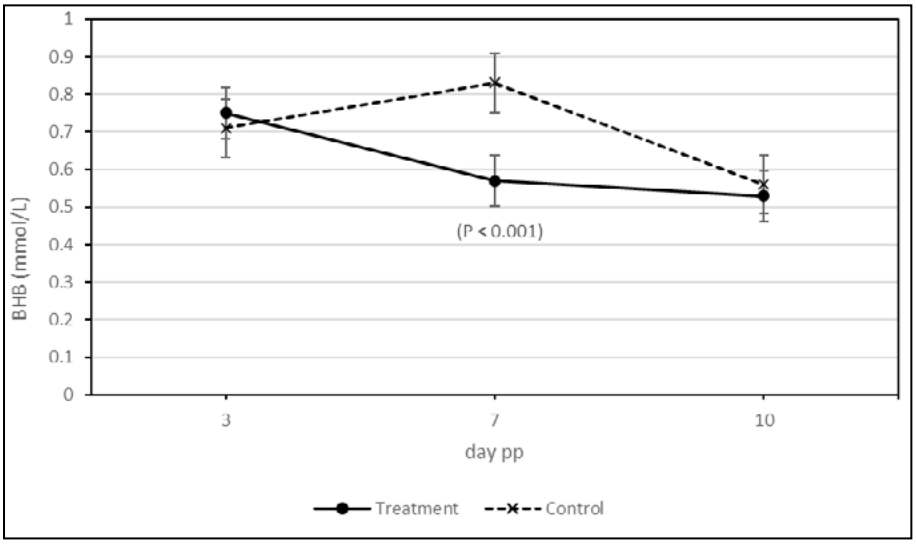
Cumulative milk production at 100 days in milk were 4626.6 kg for the control group and 5208.8 kg for the treated group, tending to be different (Standard Deviation = 535 kg) (P = 0.11).

Figures and Tables

Table 2. T Mixed model for repeated measures. Type III Tests, F-statistics and probabilities for fixed effects for blood BHB concentrations (mmol/L) in cows supplemented with rumen protected thiamine.

Fixed Effects Type III Tests		
Effect	F-Statistic	P-Value
Group	0.44	0.5127
Day	7.05	0.0022
Group * Day	3.54	0.0377
Lactation	0.66	0.4203
Body Condition Score at calving	0.68	0.3905

Figure 1. Blood concentrations of BHB (mmol/L) in cows treated with rumen protected thiamine and controls at day 3, 7 and 10 postpartum. Interaction day x treatment (P =0.037). Higher concentration of BHB at day 7 postpartum for control than treatment group (P < 0.001).



Discussion

Ketosis is a typical metabolic disorder of dairy cows during the early postpartum period. It is an alteration associated to the metabolism of glucose and fatty acids that is closely related to the typical negative energy balance during the onset of lactation. Therefore, the highest incidence of ketosis occurs within the first 7 days postpartum [5, 6]. According to the results of the present investigation, our hypothesis was accepted since BHB concentrations were lower in the treated group than the control group, especially at 7 days postpartum. We have found no scientific publication reporting the impact of any product based on any potential oral rumen protected thiamine on the levels of BHB in Holstein dairy cows.

Although, in both groups, BHB concentrations were below the cut-off value to categorize cows as having subclinical ketosis (> 1.2 mmol / L) [6, 7] the reduction of BHB in the treated group suggests a factual benefit for the dairy cow. Higher production of ketone bodies can inevitably lead to a vicious cycle of lower dry matter intake and higher BHB production in the liver, which is undesirable for cows during the early postpartum period [15]. Ketosis is a costly disease that is strongly associated, as a risk factor, to displacement of the abomasum. In addition,

displacement of the abomasum is a periparturient disease with the highest economic cost reported for dairy cattle in the US (US$ 650) [3], consequently, by preventing a case of ketosis, the economic benefit for the dairy producer is evident and extremely important.

Humans with severe diabetic keto-acidosis along with encephalopathy developed concomitantly a severe deficiency of thiamine in the blood. When patients received an intravenously thiamine-based product, they showed an immediate positive response. The authors of that report suggested that the thiamine deficiency had a very consistent association with the ketosis and encephalopathy in the patients [16]. In another prospective study, 15 human patients out of 22 with diabetic keto-acidosis had a blood thiamine deficiency. This study concluded that thiamine deficiency is common in children with diabetic ketosis, and that it worsens when patients are treated with insulin. Therefore, when metabolic acidosis persists despite adequate treatment of diabetic keto-acidosis, other factors such as thiamine deficiency should be considered [17]. Undoubtedly, thiamine has a very consistent association with BHB levels in blood of human diabetic patients, and this relationship may also be present in transition dairy cows, where in the present study RPT supplementation in postpartum cows reduced the concentration of BHB in blood. One of the major drawbacks of this pilot study was the no evaluation of thiamine in blood. Unfortunately, this assay was not feasible to be carried out, but it is considered essential for further studies to evaluate thiamine levels in blood to demonstrate the potential rumen bypass effect.

Thiamine participates as a cofactor of the enzyme pyruvate dehydrogenase, alpha-ketoglutarate dehydrogenase and transketolase, where it also participates within the Krebs cycle in the decarboxylation of alpha-ketoglutarate to succinyl-CoA [8]. Consequently, since all these reactions favor the formation of oxaloacetate and assist the entry of Acetyl Co-A into the Krebs cycle, it is reasonable to suggest that the supplementation of RPT may play certain role in the reduction of ketogenesis.

In relation to milk production, the group treated with RPT tended to have a higher milk yield than the control group (P=0.11) with approximately 500 kg more milk accumulated during the first 100 days in milk. To some extent, similar results were found in a study that reported 3 experiments with thiamine supplementation. In this investigation, thiamine was not rumen bypass and only 1 out of the 3 experiments found higher milk yield. The third experiment found lower milk yield and fat content in the thiamine group when compared with the control group [18]. In addition, in a study conducted in Egypt, cows

supplemented with 340 mg of non-protected thiamine increased milk production, fat and protein yields. Interestingly, serum thiamin concentration was significantly higher in the supplemented group than the control diet, meaning part of the thiamine bypassed the rumen [19]. These studies did not evaluate BHB concentration in blood. Ketosis can be associated with either low or high milk production [3-7]. If thiamine has a positive effect on liver function, making intermediate metabolism more efficient, especially at the level of Krebs cycle, it supports the findings that the synthesis of ketone bodies may be reduced and there might be greater gluconeogenic precursors available to favor milk production.

B-complex vitamins are supposed to be synthesized in sufficient quantities by rumen microorganisms and deficiencies are rarely observed in ruminants. Nevertheless, over the last years, numerous studies have reported that, under certain conditions, subclinical deficiencies of B vitamins may occur in high producing dairy cows. Both, rumen synthesis and degradation of some B vitamins, including thiamine, have been observed in cattle [20]. It is well-known that the excess of dietary sulfur and starch, that may induce rumen acidosis, may trigger thiamine deficiency in ruminants [21]. Consequently; thiamine supplementation may have beneficial effects in dairy cows, increasing milk yields and solids components [19]. In the lights of the present results, further experimental designs are warranted to confirm that RPT is truly rumen bypass, by measuring it in blood, and by evaluating several other intermediary metabolites that participate in the process of ketogenesis, fats, and carbohydrate metabolism. In addition to its role in carbohydrate and energy metabolism, the effects of thiamine on cell regulation, immune function, and oxidative stress should be evaluated. In addition, the additive should be tested in a way that is easy to administer in feed, ideally blended in a total mixed ration, since the administration via oral with an individual syringe is impractical and difficult to carry out in commercial conditions of dairy operations. For the prevention of ketosis, under Chilean conditions, daily cost of supplementation of propylene glycol is US$ 0.54, monesin is US$ 0.25, and for the current tested product (RPT) is US$ 0.24. If the use of this additive proves to be effective, rumen protected thiamine might be considered as an innovative nutritional strategy to improve thiamine status, reduce ketogenesis, increase milk production and improve profitability of high-producing lactating dairy cows.

Conclusions

This pilot study suggests that a potential rumen protected thiamine product decreases the blood concentrations of BHB during the early postpartum period of Holstein cows. The results of this investigation open a window to further evaluate a potential impact of a vitamin B1 rumen bypass product to prevent ketosis and improve milk production in lactating dairy cows.

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