JÚLIA TRAVASSOS DA SILVA

EFFECT OF VITAMIN SUPPLEMENTATION ON THE INGESTIVE, DIGESTIVE, AND RUMINAL PARAMETERS OF NELLORE CATTLE

Dissertation submitted to the Animal Science Graduate Program of the Universidade Federal de Viçosa in partial fulfillment of the requirements for the degree of *Magister Scientiae*.

Adviser: Sebastião de Campos Valadares Filho

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BIOGRAPHY

Júlia Travassos da Silva, daughter of Ailton Moreira da Silva and Mônica Maria de Melo Travassos da Silva, was born in Três Rios, Rio de Janeiro – Brazil on May 26, 1995.

She started a bachelor's degree in Animal Science at the Universidade Federal de Viçosa in 2013 and obtained a Bachelor of Science in Animal Science in July of 2018. Also, she interned with a scholarship at the University of Florida from August 2017 to February 2018.

In August of 2018, she started her Master's degree with a major in ruminant nutrition and beef cattle production at the Universidade Federal de Vicosa.

ABSTRACT

SILVA, Júlia Travassos, M.Sc., Universidade Federal de Viçosa, July, 2020. **Effect of vitamin supplementation on the ingestive, digestive, and ruminal parameters of Nellore cattle**. Adviser: Sebastião de Campos Valadares Filho.

Rumen cannulated Nellore bulls (n = 4) were used in a 4 × 4 Latin square design to evaluate the effects of vitamin supplementation on 1) ingestive, digestive, and ruminal parameters; 2) serum concentrations of 25-hydroxyvitamin D (**25(OH)D)**; and 3) *in situ* degradability of complete diets. The following treatments were applied: No vitamin supplementation (**CTL**), supplementation of a B vitamin blend (biotin, niacin, and thiamine; **Vit B blend**), supplementation with a fat-soluble vitamin blend (**Vit ADE**), or a combination of these two blends (**Vit B blend+ADE**). The basal diet (30:70 roughage:concentrate ratio) was formulated to achieve an average daily gain of 1.2 kg/day. Vitamin blends were premixed into the concentrate and the levels of each vitamin per kg of DM were: 3.3 mg of biotin, 111.1 mg of niacin, 28.9 mg of thiamine, 6666.7 IU of vitamin A, 5111.1 IU of vitamin D_3, and 70 IU of vitamin E. Each experimental period lasted 25 days, with 14 days of adaptation to the diets and 11 days for data collection. Dry matter and nutrient intake, as well as ruminal, intestinal, and total-tract digestibility, were not affected ($P \geq 0.072$) by any of the vitamin supplementations. Further, the treatments did not affect ruminal kinetics (omasal flow (g/d) of total N, NH_3-N, Non ammonia N, microbial N, and Non ammonia non microbial N) ($P \geq 0.234$), the efficiency of microbial protein synthesis ($P \geq 0.197$) and nitrogen balance ($P \geq 0.228$). Ruminal parameters (pH, NH_3-N, individual and total fat volatile acids (VFA) concentration) were not affected ($P \geq 0.133$) by vitamin supplementations. As expected, concentrations of serum 25(OH)D increased ($P = 0.001$) in Nellore bulls fed diets with Vit ADE and Vit B blend+ADE supplementation. None of the *in situ* parameters (readily soluble fraction, potentially degradable fraction in the rumen (*b*) or in the rate constant for degradation of *b*) were affected ($P \geq 0.434$) by vitamin supplementation. In conclusion, supplementing Nellore bulls with 25(OH)D_3 was a successful strategy for increasing circulating concentrations of 25(OH)D. However, supplementation of a B-vitamin blend, a fat-soluble vitamin blend, or a combination of these two blends in high concentrate-diets did not improve ruminal fermentation, *in situ* degradability of complete diets, ingestive and digestive parameters in Nellore bulls.

Keywords: B vitamin. Fat-soluble vitamin. Digestibility. Rumen.

RESUMO

SILVA, Júlia Travassos, M.Sc., Universidade Federal de Viçosa, julho de 2020. **Efeito da suplementação vitamínica nos parâmetros ingestivo, digestivo e ruminais de bovinos Nelore.** Orientador: Sebastião de Campos Valadares Filho.

Quatro machos Nelore não castrados fistulados no rúmen foram utilizados em um quadrado latino 4×4 para avaliar os efeitos da suplementação vitamínica 1) parâmetros ingestivos, digestivos e ruminais; 2) concentrações séricas de 25-hidroxivitamina D (25(OH)D) e 3) degradabilidade *in situ* de dietas completas. As seguintes dietas foram avaliadas: sem suplementação vitamínica (CTL), suplementação com blend (biotina, niacina e tiamina) de vitaminas B (Vit B blend), suplementação com blend de vitaminas lipossolúveis (Vit ADE) e suplementação com a combinação desses dois blends (Vit B blend+ADE). A proporção volumoso:concentrado foi de 30:70 (base na MS) e as dietas foram formuladas para atender às exigências nutricionais dos animais, para ganho médio diário de 1,2 kg/dia. Os blends de vitaminas foram incluídos aos concentrados, de modo que os níveis de cada vitamina em g/kg MS da dieta fossem: 3,3 mg de biotina, 111,1 mg de niacina, 28,9 mg de tiamina, 6666,7 UI de vitamina A, 5111,1 UI de 25 (OH) D_3 e 70 UI de vitamina E. Cada período experimental teve duração de 25 dias, sendo 14 dias de adaptação às dietas e 11 dias para coleta de dados. O consumo de matéria seca e dos demais nutrientes, bem como a digestibilidade ruminal, intestinal e total, não foram afetados ($P \geq 0,072$) pela suplementação vitamínica. Além disso, os tratamentos não afetaram a cinética ruminal (fluxo omasal (g / d) de N total, N-NH_3, N não amoniacal, N microbiano e N não microbiano não amoniacal) ($P \geq 0,234$), a eficiência da síntese de proteína microbiana ($P \geq 0,197$) e balanço de N ($P \geq 0,228$). Os parâmetros ruminais (pH ruminal e concentrações ruminais de N-NH_3, concentração individual e total de ácidos graxos voláteis) não foram afetados ($P \geq 0,133$) pelas suplementações vitamínicas. Como esperado, as concentrações de 25 (OH) D sérico aumentaram ($P = 0,001$) em novilhos Nelore alimentados com dietas suplementadas com Vit ADE e Vit B blend+ADE. Nenhum dos parâmetros de degradação *in situ* (fração prontamente solúvel, fração potencialmente degradável no rúmen (*b*) ou a constante de taxa de degradação de *b*) foram afetados ($P \geq 0,434$) pela suplementação vitamínica. Em conclusão, a suplementação com 25 (OH) D_3 foi uma estratégia bem-sucedida para aumentar as concentrações circulantes de 25 (OH) D em bovinos Nelore. No entanto, a suplementação com blend de vitaminas do complexo B, blend de vitaminas lipossolúveis ou uma combinação desses dois blends em dietas com alto nível de concentrado não melhoraram a fermentação ruminal, a degradabilidade ruminal in situ das dietas completas, a ingestão e digestão de nutrientes em machos Nelore não castrados.

Palavras-chave: Vitamina B. Vitaminas lipossolúveis. Digestibilidade. Rúmen.

SUMMARY

Effect of vitamin supplementation on the ingestive, digestive, and ruminal parameters of Nellore cattle

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ABSTRACT

The objective of the present study was to evaluate the effect of B-vitamin blend (biotin, niacin, and thiamine), fat-soluble vitamin blend (ADE), or a combination with these two blends supplementation on ingestive, digestive, ruminal parameters, serum concentrations of 25-hydroxyvitamin D [25(OH)D] and *in situ* degradability of complete diets in bulls receiving diets with high concentrate. Four rumen cannulated Nellore bulls (BW = 289.4 ± 11.2 kg) were used in a 4 × 4 Latin square design. The following diets were evaluated: diet without vitamin supplementation (CTL), diet with B vitamin blend (biotin, niacin, and thiamine) supplementation (Vit B blend), diet with fat-soluble vitamin blend (Vit ADE) supplementation and combination with these two blends (Vit B blend+ADE) supplementation. Vitamins blends were premixed into the concentrate, and the levels of each vitamin per kg of dry matter (DM) were: 3.3 mg of biotin, 111.1 mg of niacin, 28.9 mg of thiamine, 6666.7 IU of vitamin A, 5111.1 IU of vitamin D_3, and 70 IU of vitamin E. Each experimental period lasted 25 days with 14 days for adaptation and 11 days for data collection. There were no effects ($P > 0.05$) of vitamin supplementation on DM and nutrients intake. Ruminal, intestinal, total-tract digestibility and *in situ* parameters were not affected ($P > 0.05$) by vitamins supplementation. The addition of vitamins blends in the diets not affected ($P > 0.05$) ruminal kinetics. The treatments did not affect ($P > 0.05$) the omasal flow (g/d) of total N, NH_3-N, Non ammonia N (NAN), N microbial, and Non ammonia non microbial N (NANNM). The efficiency of microbial protein synthesis and nitrogen balance was not affected ($P > 0.05$) by vitamin blend supplementation. Results did not show a significant effect ($P > 0.05$) in ruminal pH, NH_3-N, individual volatile fat acids (VFA), and total VFA concentration due to vitamin blend supplementation. Serum 25-hydroxyvitamin D concentrations increased ($P < 0.01$) in Nellore bulls fed diets with Vit ADE and Vit B blend+ADE supplementation. Supplementing Nellore bulls with 25(OH)D_3 was a successful strategy for increasing ($P < 0.05$) circulating

concentrations of 25(OH)D. However, B-vitamin blend, fat-soluble vitamin blend, or a combination with these two blends supplementation in diets with high concentrate level do not improve ($P > 0.05$) ruminal fermentation, *in situ* degradability of complete diets, ingestive and digestive parameters in Nellore bulls.

Key words: B vitamin, fat-soluble vitamin, digestibility, rumen

1. INTRODUCTION

Vitamins are organic compounds needed in small amounts, but essential for life (Combs, 2012). However, vitamin nutrition is still a challenging and dynamic field for ruminant nutritionists around the world. Currently, intensive production systems highlight the importance of vitamins supplementation to fulfill the animals' requirements.

In the 20th century, several studies with B-vitamin led to the general concept that the concentrations found in basal feeds and the ability of ruminal bacteria to synthesize these vitamins are enough to meet ruminant requirements (Wegner et al., 1940; Agrawala et al., 1953; Kon and Porter, 1954). Thus, minimum daily dietary supplementation for these vitamins are either not needed or have not been established for beef cattle.

The requirements for fat-soluble vitamins A, D, and E, were based on the prevention of clinical deficiencies rather than on amounts and ratios appropriate to their physiological stages, animal categories, and growing conditions (NASEM, 2016). Besides that, these requirements might be changed in feedlots situations where greater levels of concentrate are used. Nevertheless, knowledge about the effect of these vitamin supplementations on rumen parameters in beef cattle fed with high concentrate level is still very limited.

According to Santschi et al.(2005), diets with higher concentrate levels would probably influence bacterial population and rumen passage rate, which could impair the B-vitamins ruminal synthesis and usage. Da Costa Gomez et al. (1998), in an *in vitro* evaluation, reported that diets with a high grain content reduce ruminal biotin synthesis. Niehoff et al.

(2013) stated that niacin supplementation is more advantageous for microbial populations in diets containing high concentrate levels. Also, steers fed with a high concentrate diet had lower ruminal thiamine concentrations than steers fed with a low concentrate diet (Miller et al., 1986a).

Fat-soluble vitamins requirements in beef cattle increase with high-concentrate diets (Quarterman, 1966; Topps et al. 1966;). Rode et al. (1990) showed that the disappearance of vitamin A during *in vitro* ruminal fermentation was approximately 80% when donor steers were fed with a high-concentrate diet. Also, significant amounts of vitamin E were destroyed in the rumen when the concentrate in the diet increased (Alderson et al., 1971). In addition, ruminants have higher vitamin D requirements since their metabolism begins before absorption, as rumen microorganisms are capable of degrading vitamin D into inactive metabolites (Sommerfeldt et al., 1983).

Previous experiments, *in vivo* (Pan et al., 2016; Wei et al., 2016; Hausmann et al., 2017), and *in vitro* (Riddell et al., 1981; Cruywagen and Bunge, 2004; Tagliapietra et al., 2013) have shown that B-vitamins and fat-soluble vitamin supplementation improves some ruminal parameters and digestibility when utilized individually. Nevertheless, it is not yet clear elucidate how the individual or combined use of vitamin affects ruminal microorganisms. Besides that, studies using B vitamin blend (biotin, niacin, and thiamine), fat-soluble vitamin blend (A, D, and E), or combination with these two blends in beef cattle were not found in the literature.

Due to the need to reduce costs and time, in addition to meeting the growing demand of ethics committees to reduce animals' use in researches, the *in situ* method has been used as an alternative to estimate digestibility *in vivo* of complete diets (Silva et al., 2020; Benedeti et al.,2020). However, limited studies were done evaluating the effect of dietary vitamin

supplementation on ruminal *in situ* degradation (Doreau and Ottou, 1996; Shaver and Bal, 2000; Paiva Ferreira et al., 2020).

We hypothesize that the vitamin blend supply would improve ruminal fermentation, *in situ* degradability, vitamin D status, ingestive and digestive parameters of Nellore bulls fed with a high concentrate diet. The objective of the present study was to evaluate the effect of B-vitamin blend (biotin, niacin, and thiamine), fat-soluble vitamin blend (ADE), or a combination with these two blends supplementation on ingestive, digestive, ruminal parameters, serum concentrations of 25-hydroxyvitamin D [25(OH)D] and *in situ* degradability of complete diets in Nellore bulls receiving diets with high concentrate.

2. MATERIALS AND METHODS

This study was conducted at the Experimental Feedlot of the Animal Science Department at the Universidade Federal de Viçosa (UFV), Viçosa, Minas Gerais, Brazil. All procedures were previously approved by the Animal Ethics and Welfare Committee of the Universidade Federal de Viçosa (CEUAP-UFV), according to protocol number 037/2018.

2.1 Animals, diets, and management

Four rumen cannulated Nellore bulls (body weight = 289 ± 11.2 kg; age = 8 ± 1.0 mo) were used in a 4 × 4 Latin square design. The following diets were evaluated: Diet without vitamin supplementation (**CTL**), diet with B vitamin blend (biotin, niacin, and thiamine) supplementation (**Vit B blend)**, diet with fat-soluble vitamin blend (**Vit ADE**) supplementation and combination with these two blends (**Vit B blend+ADE**) supplementation.

Prior to the start of the experiment, bulls underwent a 30-d adaptation period to experimental facilities and conditions. The animals were identified, weighed, treated against endo and ectoparasites (Dectomax®, Zoetis Indústria de Produtos Veterinários Ltda., Brazil). Each experimental period lasted 25 days with 14 days of adaptation to the diets (Machado et

al., 2016), and 11 days for data collection. The total mixed rations were provided twice a day, at 0700 and 1600 h. Feed delivery was adjusted daily to maintain minimum refusals (5%) the next day and ad libitum intake.

The roughage:concentrate ratio was 30:70 (DM basis) and diets were formulated to meet animals' requirements according to the BR-CORTE system (Valadares Filho et al., 2016) to support an average daily gain of 1.2 kg/day. The levels of vitamin supplementation were determined according to Optimum Vitamin Nutrition (OVN® - DSM, 2012). Vitamins blends were premixed into the concentrate, and the levels of each vitamin per kg of DM were: 3.3 mg of biotin (D-biotin; DSM), 111.1 mg of niacin (niacin; DSM), 28.9 mg of thiamine (thiamine hydrochloride; DSM), 6666.7 IU of vitamin A (retinyl acetate; DSM), 5111.1 IU of vitamin D (13% D_3-cholecalciferol and 87% 25-Hydroxyvitamin D_3 - Hy-D®; DSM), and 70 IU of vitamin E (DL- alfa-tocopheryl acetate; DSM). Chemical composition and amount of feed in diets are shown in Table 1.

2.2 Intake, total digestibility trial, and nitrogen balance

Feeds offered and orts from each animal were weighed daily, sampled and frozen from days 15 to 25. Furthermore, feeds and orts samples from the ten days were pooled for each animal per experimental period. These samples were pre-dried in a forced-circulation oven for 72 hours at 55 ° C. The total dry matter (DM) was evaluated using a drying oven at 105 °C for 16 h. Samples of each one of the concentrate ingredients were collected directly at the feed mill, and corn silage samples were collected daily and stored in a freezer at -20ºC.

Dry matter intake was daily measured by weighing offered and refused feedstuffs. To estimate nutrients digestibility, total feces were collected from all animals for 5 consecutive days, from days 15 to 19 of each experimental period. After 24 h of collection, the total feces were weighed, homogenized and approximately 250 g was oven-dried (55ºC for 72 h). After each collection period, a proportional sample was composed for each animal using samples

and data from the 5 d of fecal collection, based on fecal DM production of each day. Then, feces, orts and feed samples were ground using a mill (Willye, model TE-680, TECNAL, Brazil) with a 1 mm sieves and stored for further chemical analyses.

Table 1. Proportion of ingredients and nutrient composition of the experimental diets

Item	Experimental Diets[1]			
	CTL	VitB blend	VitADE	VitB blend+ADE
Ingredients, g/kg				
Corn Silage	300.8	300.8	300.8	300.8
Ground corn	637.6	637.6	637.6	637.6
Soybean meal	38.5	38.5	38.5	38.5
Urea	9.9	9.9	9.9	9.9
Mineral premix [2]	13.2	-	-	-
Vit. B mineral premix[2]	-	13.2	-	-
Vit. ADE mineral premix[2]	-	-	13.2	-
Vit. B+ADE mineral premix[2]	-	-	-	13.2
Chemical composition (g/kg)				
Dry matter	527.0	527.0	527.0	527.0
Organic matter	961.0	961.0	960.8	960.7
Neutral detergent fiber [3]	206.8	206.8	206.8	206.8
Indigestible neutral detergent fiber	56.8	56.8	56.8	56.8
Crude protein	117.1	117.1	117.1	117.1
Ether extract	37.0	37.0	37.0	37.0
Starch	494.9	494.9	494.9	494.9
Non-fiber carbohydrates	614.8	614.8	614.8	614.8

[1]CTL= diet without vitamin supplementation, Vit B blend= diet with biotin, niacin, and thiamine supplementation, Vit ADE= diet with vitamins A, D and E supplementation, Vit B blend+ADE= diet with vitamin B blend, A, D and E supplementation.[2]Ingredients per kg mineral: 172.5 g Ca; 20.8 g P; 31.25 g S; 20.8 g Mg; 31.25 g K; 68.75 g Na; 10.4 mg Co; 679 mg Cu; 8.35 mg Cr; 34.5 mg I; 1333 mg Mn; 8.35 mg Se; 2500 mg Zn and 208 mg F. [3]Corrected for residual ash and residual nitrogenous compounds.

To assess the nitrogen compound balance, a total collection of urine from each animal was made in each experimental period, during the same 5 consecutive days as the total feces collection. Collector funnels were used coupled to hoses, which conducted the urine to plastic

containers with 200 mL H_2SO_4 at 20%, to conserve the nitrogenous compounds. At the end of each collection day (24 h), the daily urine volume was quantified, and a sample was stored proportional to the daily volume excreted by each animal. Urine samples were composed per period for each animal. The composite samples were stored at −20 °C for further analysis.

2.3 *In situ* ruminal degradation procedures

The *in situ* ruminal incubation as described by Silva et al. (2020) was used to estimate the degradation parameters and *in vivo* digestibility of the evaluated diets.

From day 10 to 14 of each experimental period, the *in situ* degradability of each total mixed ration was performed to evaluate the influence of vitamin supplementation on the DM and OM degradability of these diets. All ingredients of the diets were collected daily and stored at −20°C between day 1 and 7 of each experimental period, dried in a forced-air oven at 55°C for 72 h, were ground using a mill (Willye, model TE-680, TECNAL, Brazil) with a 2 mm sieve. To compose the diet, corn silage and concentrate were weighed separately, maintaining the same roughage:concentrate ratio on a DM basis. And, 6 g of dried sample was individually weighed into nylon bags (Sefar Nitex, Switzerland; 50-µm porosity, 400 cm2 surface area) and incubated in each animal receiving the corresponding treatment in the *in vivo* procedure. Incubation was performed to allow the following ruminal degradation times: 0, 2, 4, 6, 12, 24, 48, 72 and 96 h.

The number of bags varied as a function of the incubation time to guarantee enough residual samples after incubation, where more bags per sample were incubated for the longer incubation times relative to the shorter incubation times. Samples were incubated into the rumen in reverse order of incubation hours so that all bags were removed at the same time, washed in running water, oven-dried, ground, and stored for further analysis.

2.4 Omasal and ruminal sampling

The determination of omasal flow was performed using the double marker system technique, being cobalt and iNDF as markers, as described by Rotta et al. (2014). A continuous infusion of 5 g/d of Co-EDTA (0.51 g cobalt /d) from 17 to day 22 of each experimental period using a peristaltic pump (model BP-600.4, Colombo, Paraná, Brazil). Omasal digesta collections were performed from the 20th to the 22th day of each experimental period. A total of 8 samples per animal were collected from the omasum at a 9-h interval for 3 d; sampling times were: day 1 at 8:00 and 17:00, day 2 at 2:00, 11:00 and 20:00, day 3 at 5:00, 14:00 and 23:00, simulating 24 h of collections with 3-h intervals.

The technique, that was developed by Huhtanen et al. (1997) and adapted by Leão et al. (2004) was used for sampling of the omasal digesta, where approximately 500 mL of omasal digesta was collected, where 250 mL for a bacterial isolate, 200 ml to estimate the flow and ruminal digestibilities, and 50 ml to NH_3-N analyses. The samples for NH_3-N were immediately stored in plastic containers and kept at -80° C for further analysis. The samples for bacterial isolate were stored in plastic containers and kept at 4° C. Furthermore, 1L of omasal digesta from four omasum collection times were composed for each animal for bacterial isolate: isolate particle-associated bacteria (PAB) and fluid-associated bacteria (FAB). The processing of these samples was carried out according to Reynal et al. (2005) and with adaptations suggested by Krizsan et al. (2010). On the 18th day of each experimental period, the same procedure was repeated resulting in two centrifugations per period. All samples were stored in plastic containers and kept at -80° C for further lyophilization (LP510; Liobras, São Paulo, Brazil). After these samples were lyophilized, PAB from each centrifugation were macerated and grouped for each animal per experimental period and stored in plastic bags for further analyses. The same procedure was done for FAB.

A 200 mL of digesta was also collected, filtered using a 100 μm nylon filter with an open area of 44% (Sefar Nytex 100/44; Sefar, Thal, Switzerland), thereby obtaining two phases: liquid added to small particles and large particles. The two phases were stored separately in plastic containers and kept at -80° C for further lyophilization (LP510; Liobras, São Paulo, Brazil). After these samples were lyophilized, large particles samples were milled at 1 mm sieves mills (Willye, model TE-048, TECNAL, Brazil) and liquid added to small particles were macerated. Then, liquid added to small particles samples from the eight collection times were composed for each animal per experimental period and stored in plastic containers for further laboratory analyses. The same was done to large particles, thus obtaining two samples of omasum (liquid and particles) per animal per period for the flow and ruminal digestibilities estimation.

Samples of ruminal content were manually collected from the cranial, ventral and caudal areas of the rumen, during the same times as omasal sampling, resulting in eight samples. Approximately 250 ml of ruminal content were manually collected at the liquid-solid interface of the rumen, filtered through a 100 μm nylon filter (Sefar Nitex; Sefar, Thal, Switzerland; porosity of 100 μm), and were subjected to pH measurement by using a digital potentiometer (pH-221, Lutron Electronics, Taiwan). Then, 50 ml samples of ruminal fluid from each time of omasal collection were used for VFA and NH_3-N. All samples were immediately stored in Eppendorf tubes and kept at -80° C for further analysis.

On the day 23 of each experimental period, total rumen emptying was carried out 4 h after feeding to estimate the rate of passage and digestion (Allen e Linton, 2007). After removal of all ruminal contents, the digesta was weighed and filtered in a double layer of cheesecloth for separation of solid and liquid fractions, which were then weighed and sampled. After sampling, the digesta was reconstituted and returned to the rumen of the respective animals. On the day 25, the entire rumen emptying procedure was repeated before

morning feeding. The collected samples were lyophilized and ground in a knife mill with 1 mm sieve (Willye, model TE-680, TECNAL, Brazil). Furthermore, solid- and liquid- fractions samples from the two ruminal emptying procedure were composed for each animal per experimental period (dry weight basis) and stored in plastic bags for further laboratory analyses.

2.5 Blood Sample

Blood samples were drawn from the jugular vein to measured serum 25-hydroxyvitamin D [25(OH)D] concentrations, on the 25th day of each experimental period before feeding. Blood was sampled using vacuum tubes with a clot activator and gel for serum separation (BD Vacutainer® SST® II Advance®, São Paulo, Brazil). After collection, samples were centrifuged at 3,600 × g for 15 min for serum separation. Serum samples were transferred into microtubes and stored frozen at −20°C.

The levels 25(OH)D were measured using a chemiluminescent immunoassay (Atellica Solution; Siemens Healthineers, São Paulo, Brazil).

2.6 Laboratory analysis and calculations

Samples of orts, omasal digesta, feces, feed, FAB, PAB and ruminal emptying were analyzed for DM (AOAC, 2012; method 934.01), organic matter (OM; AOAC, 2012; method 930.05) and CP (AOAC, 2012; 981.10 method). The ether extract analysis was performed according to AOAC (2005) method 2003.05 after acid hydrolysis with HCl in samples of orts, omasal digesta, feces and feed. The analysis of neutral detergent fiber (NDF) in orts, feed, omasal digesta, fecal samples, and rumen emptying samples was performed according to the techniques described by Mertens (2002), without the addition of sodium sulphite, but with the addition of thermostable alpha-amylase to the detergent. The NDF content was corrected for residual ash and protein (apNDF). Indigestible NDF (iNDF) was estimated according with Casali et al. (2008), in triplicate for orts, feed, omasal digesta (particle phase) and fecal

samples. The starch content of orts, feed, omasal digesta, fecal samples, and rumen emptying samples was quantified according to Silva et al. (2019). Non-fiber carbohydrates (NFC) were calculated according to Detmann and Valadares Filho (2010):

NFC (% DM) = 100 - [%CP - (%CP derived from urea +% urea) + %apNDF +% EE + %ash],

The total-tract apparent digestibility was calculated as: Digestibility (%) = {(x – y)/x} × 100, where x (kg/d) and y (kg/d) are the intake and the output in feces of each component, respectively.

Ruminal digestibility coefficients were estimated by measuring the difference between the nutrient intake and the flow of the nutrients through the rumen. The calculation of intestinal digestibility was estimated by measuring the difference between the flow of the nutrients through the rumen and the quantities of these in feces.

For the *in situ* evaluations, the DM and OM degradation profiles were estimated by using the first-order asymptotic model proposed by Ørskov and McDonald (1979), as formulated below: $Yt = a + b \times (1 - e^{(-kd.t)})$, where Yt = degraded fraction of DM or OM in time 't', g/kg; a = readily soluble fraction, g/kg; b = potentially degradable fraction in the rumen, g/kg; kd = rate constant for degradation of b, per h and t = time, h.

Estimated times for the *in situ* incubations to assess the *in vivo* digestibility of DM and OM were defined as the time in which *in situ* degradation equalled *in vivo* digestibility. This can be obtained using the equation: $T = -(\ln(1 - ((\ in\ vivo \text{ digestibility} - a)/b)))/kd$, where T = estimated time; a = readily soluble fraction, g/kg; b = potentially degradable fraction in the rumen, g/kg and kd = rate constant for degradation of b, per h.

To estimate the digesta flow, the omasal digesta samples were quantified for Co concentration by an atomic absorption spectrophotometer (Spctr AA-800; Varian spectrometer, Harbor City, CA) after digestion with nitropercloric acid. The flow of the omasal digesta was estimated through the reconstitution of digesta technique (Faichney, 1975)

using the double marker system. Cobalt was used as the liquid added to small particles marker and the iNDF as the large particles phase marker. The reconstitution factor was calculated based on the concentrations of the markers during the different phases of the digesta (France and Siddons, 1986). The nutrient flow (g/d) was obtained by multiplying its concentration in the digesta (g/kg DM) by the DM flow.

The composite samples of omasal digesta, FAB and PAB samples were also analyzed for N-RNA concentration, as suggested by Zinn and Owens (1980) and modified by Ushida et al. (1985).

For VFA analysis (lactate, acetate, butyrate, and propionate), rumen fluid samples were centrifuged (12,000 × g, 10 min, 4°C), and supernatants were treated as described by Siegfried et al. (1984). Ruminal VFA were analyzed by HPLC by HPLC in a Dionex Ultimate 3000 Dual detector HPLC (Dionex Corporation, Sunnyvale, CA, USA) coupled to a refractive index (RI) Shodex RI-101 maintained at 40°C using a ion exchange column Phenomenex Rezex ROA, 300 × 7.8 mm maintained at 45°C. Mobile phase was prepared with 5 mmol/l sulfuric acid (H_2SO_4) and the flow was 0.7 ml/min. The concentration of NH_3-N in rumen fluid and omasal liquid was obtained according to the technique described by Okuda et al. (1965).

Urine samples were analysed for N content using the same methods described for the CP analysis of feed samples The amount of absorbed N was obtained by the difference between N-intake and N-fecal, while the N-retained was obtained by subtracting the N-urinary and N-fecal from the N-intake.

The rates (%/h) of ingestion (k_i), passage (k_p), and digestion (k_d) were calculated using the pool-and-flux method by Allen and Linton (2007), according to the following models:

$$k_i = (\text{intake/rumen pool}) \times 100,$$

$$k_p = (\text{rumen flow/rumen pool}) \times 100,$$

$$k_d = k_i - k_p ,$$

Microbial efficiency was expressed in g MCP / kg TDN and in g MCP / kg of digestible organic matter (DOM). DOM was calculated by the difference between the OM ingested and the amount of organic matter passing through the feces. The microbial efficiencies calculated between the production of microbial N (MN) and the amount of rumen-degraded organic matter (RDOM) were also obtained. The RDOM was calculated by the difference between the OM ingested and the amount of organic matter passing through omasum.

2.7 Statistical analysis

The experiment was analyzed according to a 4 × 4 Latin square design including the fixed effects of treatments and the random effects of animal and experimental period.

The *in situ* parameters *a*, *b* and *kd* of the described models were fitted using the NLIN procedure of SAS® (version 9.4, SAS Institute Inc., Cary, NC), from the Marquardt algorithm, to obtain the parameters of the non-linear regression for each animal and period. A confidence interval was adopted to *in vivo* digestibility instead of a single average value of *in vivo* digestibility. The asymptotic confidence intervals for the *in vivo* digestibility of DM and OM ($1 - \alpha = 0.95$).

Data of intake, total and partial digestibility, the analysis of the *in situ* degradation parameters, rates (intake, passage and degradation), VFA, N balance, microbial efficiency, and levels of serum 25(OH)D were evaluated using the PROC MIXED of SAS, version 9.4 (SAS Institute Inc., Cary, NC), following model:

$$Yijk = \mu + Ti + aj + pk + \varepsilon ijk,$$

where Y_{ijk} = dependent variable μ is the overall mean, Ti is the fixed effect of treatments (i= 1 to 4), aj is the random effect of animal (j= 1 to 4), pk is the random effect of period (k= 1 to 4), and εijk is the random element of variation.

Data of ruminal pH and NH_3-N were analyzed as repeated measures. The best structure of the (co)variance matrix was chosen based on Akaike's information criterion with correction.

The averages were compared using the Tukey test, where differences were considered significant at $P \leq 0.05$.

RESULTS

The results of intake, ruminal, intestinal and apparent total-tract digestibility are shown in Table 2. There were no effects ($P > 0.41$) of vitamin supplementation on DM and nutrients intake. Ruminal digestibility of DM, OM, apNDF, CP, EE, starch and NFC were not affected ($P > 0.14$) by vitamins. Also, no effect ($P > 0.10$) of vitamin was observed for intestinal digestibility. The added vitamin in diets did not affect the apparent total-tract digestibility of DM, OM, CP, apNDF, and NFC, however, tended to increase ($P < 0.10$) the apparent total-tract digestibility of EE and starch.

Table 2. Intake, ruminal, intestinal and apparent total-tract digestibility of Nellore bulls fed diets with or without vitamin supplementation.

Item	Experimental Diets[1]				SEM[2]	*P*-value
	CTL	Vit B blend	Vit ADE	Vit B blend+ADE		
Intake, kg/d						
DM	7.3	7.2	7.3	6.7	0.54	0.601
OM	7.1	6.9	7.0	6.5	0.53	0.615
apNDF	1.5	1.5	1.5	1.4	0.12	0.669
CP	0.9	0.9	0.9	0.8	0.06	0.612
EE	0.3	0.3	0.3	0.3	0.02	0.415
NFC	4.5	4.4	4.5	4.1	0.35	0.604
Starch	3.7	3.6	3.6	3.3	0.29	0.581
Intake, g/kg BW						
DM	22.1	21.2	21.9	20.0	0.44	0.870
Ruminal, g/kg						
DM	418.8	401.7	398.9	433.4	27.23	0.394
OM	494.7	477.2	475.4	510.4	23.35	0.339
apNDF	313.2	301.4	278.4	364.6	27.12	0.144
CP	-8.7	-65.4	-63.9	-22.1	55.90	0.649
EE	-34.8	-38.3	14.4	-3.1	65.44	0.737
NFC	700.4	675.0	690.8	709.5	22.58	0.384
Starch	842.0	852.4	841.1	862.3	16.26	0.648
Intestinal, g/kg						
DM	272.0	304.3	294.5	292.0	32.71	0.814
OM	212.8	243.4	232.0	232.8	28.11	0.819
apNDF	198.8	198.9	219.1	186.5	33.77	0.758
CP	730.0	786.6	776.3	740.4	56.39	0.769
EE	816.5	838.3	773.1	773.3	62.90	0.596
NFC	73.5	112.6	89.7	111.2	28.92	0.694
Starch	34.4	44.9	66.7	56.7	19.93	0.698
Apparent total-tract, g/kg						
DM	690.8	706.1	693.4	725.4	15.42	0.175
OM	707.6	720.5	707.4	742.2	15.04	0.145
apNDF	512.0	500.3	497.5	551.1	24.70	0.223
CP	721.3	721.2	712.5	718.3	9.79	0.894
EE	781.8	812.3	787.4	774.3	11.66	0.072
NFC	773.9	796.3	780.4	820.7	16.89	0.160
Starch	876.4	897.3	907.8	918.9	12.30	0.083

[1]CTL= diet without vitamin supplementation, Vit B blend= diet with biotin, niacin, and thiamine supplementation, Vit ADE= diet with vitamins A, D and E supplementation, Vit B blend+ADE= diet with vitamin B blend, A, D and E supplementation. [2]Standard error mean.

There was no difference ($P > 0.43$) in readily soluble fraction (*a)*, potentially degradable fraction in the rumen (*b*) or in the rate constant for degradation of *b* (*kd)* of DM and OM between the experimental diets (Table 3). The *in situ* incubation time to access *in vivo* degradability of DM and OM ranged between 16 and 24 h (Table 4, Fig. 1).

Table 3. Ruminal degradation parameters estimate of DM and OM of diets with or without vitamin supplementation in Nellore bulls.

	Experimental Diets[2]					
Parameter[1]	CTL	Vit B blend	Vit ADE	Vit B blend+ADE	SEM[3]	*P*-value
Dry Matter						
a	337	337	339	341	6.07	0.920
b	521	530	530	512	9.00	0.470
kd	0.06	0.06	0.06	0.07	0.006	0.827
Organic Matter						
a	323	322	326	326	6.18	0.902
b	540	547	547	530	8.94	0.434
kd	0.06	0.06	0.07	0.07	0.005	0.800

[1]*a,* readily soluble fraction (g/kg); *b*, potentially degradable fraction in the rumen (g/kg); *kd,* rate constant for degradation of *b* (per h); *T*, estimated time for *in situ* incubations to access *in vivo* digestibility, h. [2]CTL= diet without vitamin supplementation, Vit B blend= diet with biotin, niacin, and thiamine supplementation, Vit ADE= diet with vitamins A, D and E supplementation, Vit B blend+ADE= diet with vitamin B blend, A, D and E supplementation. [3]Standard error mean.

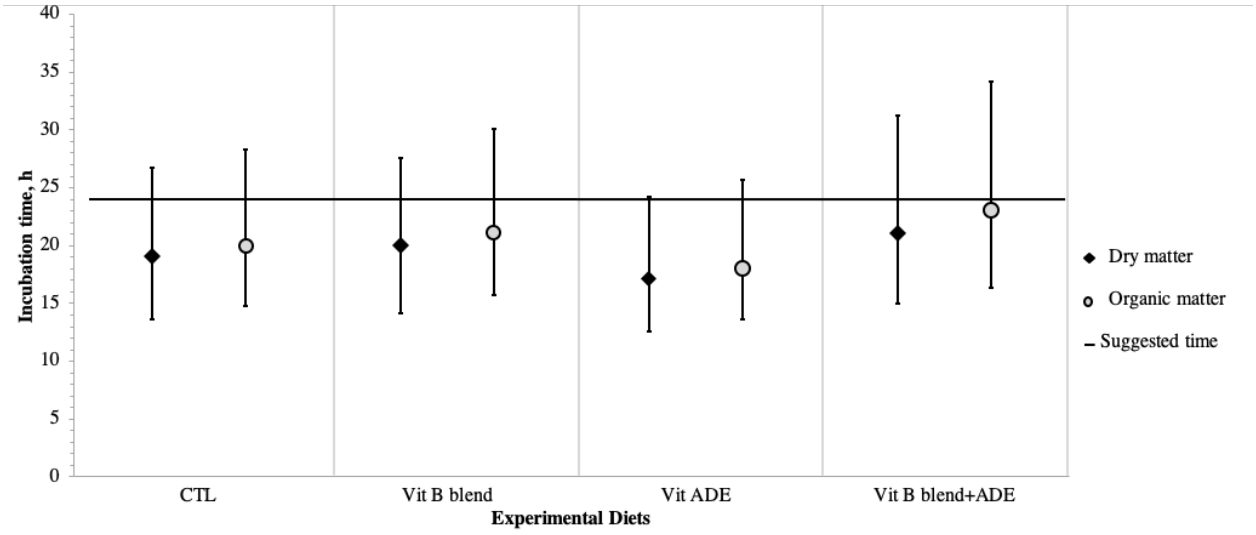

Fig. 1. Suggested incubation times for *in situ* procedures and incubation time intervals in which the *in situ* degradability predicts the *in vivo* digestibility of DM and OM for the evaluated diets.

Table 4. Means and confidence intervals ($1 - \alpha = 0.95$) of the *in vivo* digestibility coefficients and *in situ* degradability coefficients with 24 h of incubation for the DM and OM of diets with or without vitamin supplementation in Nellore bulls.

	Experimental Diets[1]			
Item	CTL	Vit B blend	Vit ADE	Vit B blend+ADE
DM *in vivo* digestibility				
Mean	0.69	0.71	0.69	0.73
Lower limit	0.65	0.67	0.66	0.69
Upper limit	0.73	0.74	0.73	0.76
24-h *in situ*[2]	0.73	0.74	0.75	0.75
OM *in vivo* digestibility				
Mean	0.71	0.72	0.71	0.74
Lower limit	0.67	0.68	0.67	0.71
Upper limit	0.74	0.76	0.74	0.78
24-h *in situ*[2]	0.74	0.74	0.76	0.75

[1]CTL= diet without vitamin supplementation, Vit B blend= diet with biotin, niacin, and thiamine supplementation, Vit ADE= diet with vitamins A, D and E supplementation, Vit B blend+ADE= diet with vitamin B blend, A, D and E supplementation. [2] Estimated by Eqn (1), degradation parameters from Table 3 and 24 h of incubation time.

As shown in Table 5, the addition of vitamins in the diets did not affect ($P > 0.16$) ruminal kinetics, k_i, k_p, and k_d, values for DM, OM, apNDF and starch. The means of DM k_i, k_p, and k_d were 9.62, 5.61, and 4.01% h^{-1}, respectively. The diets did not affect ($P = 0.83$) the ruminal pool of starch, however, tended to increase ($P < 0.10$) ruminal pool of DM, OM and apNDF.

The results in Table 6 show that supplementing vitamins did not affect ($P > 0.58$) intake of TDN, DOM, RDOM and N. Also, the treatments did not affect ($P > 0.23$) the omasal flow (g/d) of total N, NH_3-N, Non ammonia N (NAN), microbial N, and Non ammonia non microbial N (NANMN). The efficiency of microbial protein synthesis based in total digestible nutrients (MCP/kg TDN) and digestible organic matter (g MCP/kg DOM) was not affected ($P > 0.19$) by vitamin supplementation, thus obtaining mean values 128.5 g MCP/kg and 138.7 g MCP/kg, respectively. Furthermore, no effect ($P > 0.51$) of vitamin was observed for efficiency of microbial N synthesis based in rumen-degraded organic matter (RDOM, g MN/kg).

Table 5. Digestion kinetics for Nellore bulls fed diets with or without vitamin supplementation.

Item		Experimental Diets[1]				SEM[2]	*P*-value
		CTL	Vit B blend	Vit ADE	Vit B blend+ADE		
Rates, % h^{-1}							
DM	k_i	10.2	9.6	9.4	9.3	0.74	0.773
	k_p	5.9	5.7	5.5	5.3	0.37	0.533
	k_d	4.3	3.9	3.8	4.0	0.48	0.833
OM	k_i	10.7	10.0	9.8	9.7	0.79	0.814
	k_p	5.4	5.2	5.1	4.8	0.35	0.543
	k_d	5.3	4.8	4.8	5.0	0.53	0.837
apNDF	k_i	3.7	3.4	3.3	3.5	0.28	0.674
	k_p	2.5	2.4	2.4	2.2	0.22	0.611
	k_d	1.2	1.0	0.9	1.3	0.13	0.164
Starch	k_i	52.0	53.6	71.5	58.6	10.04	0.548
	k_p	7.9	7.9	11.1	8.0	1.58	0.229
	k_d	44.1	45.7	60.4	50.6	8.77	0.586
Rumen pool sizes, kg							
DM		3.0	3.2	3.4	3.0	0.37	0.084
OM		2.8	3.0	3.1	2.8	0.35	0.082
apNDF		1.7	1.8	2.0	1.7	0.24	0.094
Starch		0.3	0.3	0.3	0.2	0.05	0.830

[1]CTL= diet without vitamin supplementation, Vit B blend= diet with biotin, niacin, and thiamine supplementation, Vit ADE= diet with vitamins A, D and E supplementation, Vit B blend+ADE= diet with vitamin B blend, A, D and E supplementation. [2]Standard error mean.

Vitamin supplementation did not affect ($P > 0.22$) the fecal and urine nitrogen compounds excretion as shown in Table 7. The nitrogen balance was also not affected by diets, thereby presenting with an average nitrogen balance of 36.27 g/d.

Table 6. Omasal flow, microbial protein synthesis and efficiency in Nellore bulls fed diets with or without vitamin supplementation.

Item	Experimental Diets[1]				SEM[2]	*P*-value
	CTL	Vit B blend	Vit ADE	Vit B blend+ADE		
Intake, kg/d						
TDN[3]	5.4	5.4	5.4	5.1	0.45	0.803
DOM[4]	5.0	5.0	5.0	4.8	0.42	0.889
RDOM[5]	3.5	3.3	3.4	3.3	0.26	0.904
N intake, g/d	139.2	136.9	139.9	127.9	9.90	0.589
Omasal flow, g/d						
Total N	140.4	145.6	148.5	131.5	13.04	0.342
NH_3-N	2.9	2.7	2.7	2.3	0.49	0.345
NAN[6]	137.5	143.0	145.9	129.2	12.66	0.344
Microbial N	107.6	105.3	118.5	99.7	9.68	0.355
NANMN[7]	29.9	35.5	27.4	29.5	9.10	0.331
NAN, % of total N	98.0	98.2	98.3	98.3	0.23	0.234
Microbial efficiency						
gMCP[8] /kg TDN	124.9	128.4	139.3	121.6	11.01	0.224
gMCP/kg DOM	135.0	139.0	150.7	130.1	12.04	0.197
gMN/kg RDOM	31.6	33.2	36.2	30.2	3.27	0.511

[1]CTL= diet without vitamin supplementation, Vit B blend= diet with biotin, niacin, and thiamine supplementation, Vit ADE= diet with vitamins A, D and E supplementation, Vit B blend+ADE= diet with vitamin B blend, A, D and E supplementation.[2]Standard error mean. [3]Total digestible nutrients. [4]Digestible organic matter. [5]Rumen-degraded organic matter. [6]Non ammonia N.[7]Non ammonia non microbial N. [8]Microbial crude protein.

Table 7. N balance in Nellore bulls fed diets with or without vitamin supplementation.

Item	Experimental Diets[1]				SEM[2]	*P*-value
	CTL	Vit B blend	Vit ADE	Vit B blend+ADE		
N balance, g/d						
N intake	139.2	136.9	139.9	127.9	9.90	0.589
Fecal N	38.8	38.2	40.1	36.2	3.02	0.782
Urinary N	64.5	60.5	67.2	56.5	5.93	0.228
N retention	36.0	38.2	35.7	35.2	5.40	0.434

[1]CTL= diet without vitamin supplementation, Vit B blend= diet with biotin, niacin, and thiamine supplementation, Vit ADE= diet with vitamins A, D and E supplementation, Vit B blend+ADE= diet with vitamin B blend, A, D and E supplementation. [2]Standard error mean.

The effects of vitamins blend on pH, ammonia-N, acetate, propionate, butyrate, total VFA, and acetate/propionate ratio are presented in Table 8. The NH_3-N concentration expressed in mg/dL, and rumen pH were similar ($P > 0.13$) among treatments. Results did not show a significant effect ($P > 0.48$) in total VFA concentration due to vitamin blend supplementation with the mean total VFA values of 94.72, 97.8, 92.87 and 88.81 mmol/L, respectively in CTL, Vit B blend, Vit ADE and Vit B blend+ADE. Individual VFA concentration (mol/100mol) and acetate:propionate ratio did not differ ($P > 0.21$) across treatments.

Serum 25-hydroxyvitamin D [25(OH)D] concentrations increased ($P < 0.01$) in Nellore bulls fed diets with vitamin supplementation with the mean values of 38.7 and 31.5 (ng/mL), respectively in Vit ADE and Vit B blend+ADE (Fig. 2).

Table 8. Rumen pH, NH_3-N and VFA of Nellore bulls fed diets with or without vitamin supplementation.

Item	Experimental Diets[1]				SEM[2]	*P*-value
	CTL	Vit B blend	Vit ADE	Vit B blend+ADE		
Rumen pH	6.3	6.1	6.4	6.2	0.10	0.297
Rumen NH_3-N, mg/dL	11.8	9.3	9.7	10.0	1.38	0.133
Total VFA, mmol/L	94.7	97.8	92.9	88.8	6.75	0.484
Individual, mmol/100mmol						
Lactate	4.8	4.5	4.2	4.2	0.40	0.742
Acetate	63.6	63.2	64.5	66.5	1.51	0.251
Propionate	21.5	23.6	22.0	19.9	1.28	0.196
Butyrate	10.2	8.7	9.3	9.4	0.50	0.269
Ratio A:P	3.0	2.8	3.0	3.5	0.26	0.213

[1]CTL= diet without vitamin supplementation, Vit B blend= diet with biotin, niacin, and thiamine supplementation, Vit ADE= diet with vitamins A, D and E supplementation, Vit B blend+ADE= diet with vitamin B blend, A, D and E supplementation. [2]Standard error mean.

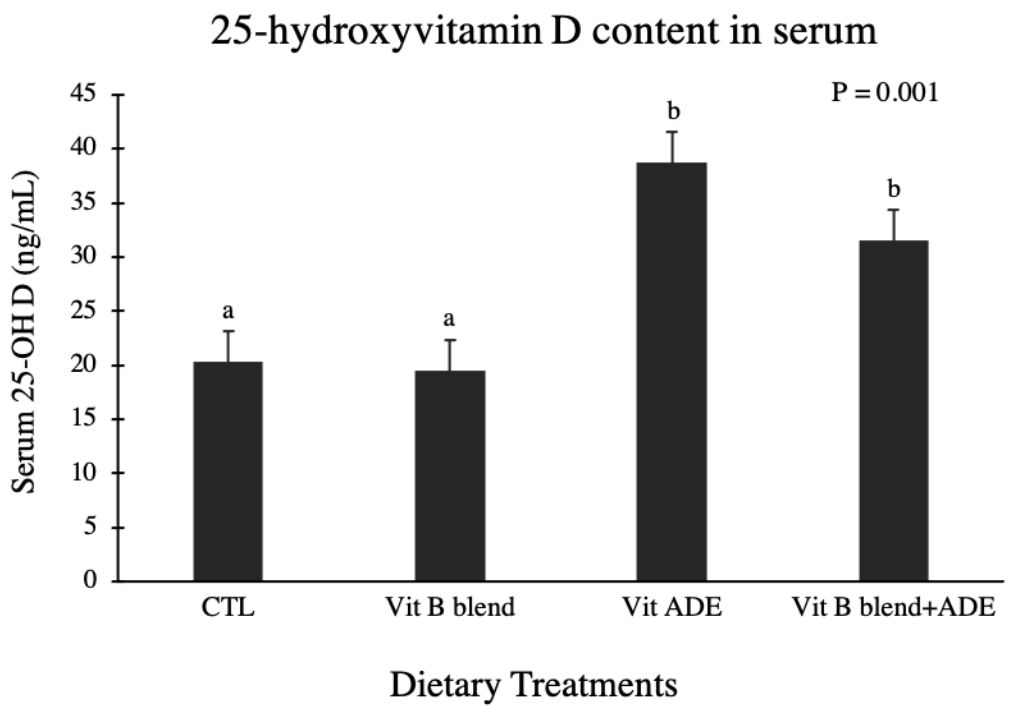

Fig. 2. Serum 25-hydroxyvitamin D [25(OH)D] concentrations in Nellore bulls fed diets with or without vitamin supplementation. Note: Different letters on the same line indicate significant differences between groups using the Tukey test. Dietary treatments: CTL= diet without vitamin supplementation, Vit B blend= diet with biotin, niacin, and thiamine supplementation, Vit ADE= diet with vitamins A, D and E supplementation, Vit B blend+ADE= diet with vitamin B blend, A, D and E supplementation.

DISCUSSION

There is a lack of information about the effects of vitamin supplementation on intake, digestibility, and ruminal fermentation in Nellore cattle. To our knowledge this present study was the first attempt to investigate the effect of B vitamin blend (biotin, niacin, and thiamine), fat-soluble vitamin blend (A, D, and E), or combination with these two blends. Proceeding from the positive health and production effects that had been suggested for each vitamin in previous trials, this study aimed to investigate whether a combination of these vitamins could be beneficial to enhance the digestibility and ruminal fermentation in bulls. Several previous studies had investigated the isolated effects of each vitamin on the ruminant metabolism, however, most of these studies were on dairy cows. Half of the studies showing the effects of

vitamins on ruminal fermentation were performed *in vitro*, while few showed these effects *in vivo* using beef cattle.

Earlier researches evaluating the effects of each vitamin supplementation did not exert a significant effect on DM (DMI) and nutrients intake, for example supplementing with vitamin E (Khodamoradi et al. 2012; Paiva Ferreira et al., 2020), niacin (Campbell et al., 1994; Doreau and Ottou, 1996; Luo et al., 2019) and biotin (Zimmerly and Weiss, 2001;Suksombat et al., 2011). In the present study, treatment differences also were not significant.

Luo et al. (2019) showed that nutrient digestibility was increased in a dose-dependent manner with niacin supplementation, when they supplemented with 320 mg of niacin/kg DM there were no significant differences in the apparent digestibility of all nutrients, with 420 mg of niacin/kg DM exhibited a significant difference in the apparent digestibility of CP, but with supplementation of 640 mg of niacin/kg DM the digestibility of DM, OM, CP, apNDF and ADF increased. In addition, researches indicated an increase of cellulolytic bacteria (Bryant and Robinson, 1961; Cruywagen and Bunge, 2004) with various B vitamin supplementation and *in vitro* experiments showed that biotin supplementation to donor cows improved cellulose digestion (Milligan et al.,1967; Cruywagen and Bunge, 2004). However, the results in the present trial showed that supplementing vitamin blend did not affect the nutrient digestibility of beef cattle. Kandathil and Bandla (2019) also showed that supplementing an optimized vitamin mixture orally also did not change nutrient digestibility. And, it has been previously reported that there were no significant differences in nutrient digestibility in cattle fed diets supplemented with niacin (Kumar and Dass 2006; Doreau and Otto 1996; Campbell et al., 1994), and vitamin E (Khodamoradi et al. 2012; Wei et al., 2016). The reason could be that the vitamins contents of the basal ration in the present study met the vitamins requirements of the bulls, the supplementation level was not high enough, these vitamins have a dose-

dependent or these vitamins present a combined effect requirement to influence the nutrient digestibility.

In the present trial, *in situ* DM and OM degradation of complete diets was also unaffected by dietary vitamin blend supplementation. Limited studies were done evaluating the effect of dietary vitamin supplementation on ruminal *in situ* degradation. For example, Shaver and Bal (2000), did not observe any effect on *in situ* DM degradation of alfalfa silage in supplemented cows with 300 mg/d of thiamine. Also, Doreau and Otto (1996) did not find an effect on *in situ* DM degradation of ensiled ear corn when cows were supplemented with 6g of niacin/d. Besides that, the *in situ* incubation for 24h was sufficient to access *in vivo* degradability of DM and OM, similarly to that found by Silva et al. (2020). However, most of the studies using *in situ* methods on the determination of feed digestibility in ruminants have evaluated individual ingredients rather than complete diets.

Niehoff et al. (2013) observed increased duodenal flows of total N and NAN, and an increase in the amount of microbial protein and effectiveness of microbial protein synthesis in animals treated with niacin (6 g/d; 60:40), but the current results with the omasal flow do not confirm this finding. In the present trial, we use 111.11 mg of niacin/kg DM (DMI=7.13; total niacin= 792.22 mg/d), the total consumed per day was lower than that dose used by Niehoff et al. (2013), this can explain the different results. However, Campbell et al. (1994) supplemented cows with the same 6g/d of niacin and did not find significant effects on the duodenal flow of total N and in the amount of N microbial. In general, niacin plays an important role in the energy metabolism because it is incorporated in the two electron-carrying coenzymes NAD(H) and NADP(H), so it has a significant impact on microbial fermentative activity. Nevertheless, the effect of niacin on the proteolytic activity of microbial is unknown.

In an *in vivo* trial, Schäfers et al.(2018) reported that supplementing 138.6 IU of vitamin E/kg DM to the rations of Holstein cows did not affect the flow of microbial N and

NAN(g/d), microbial protein synthesis and efficiency. Further, supplementing approximately 153.38 IU of vitamin E/ kg DM (1500 IU vitamin E/d) to the rations of Tabapuã bulls did not affect the efficiency of microbial protein synthesis (Paiva Ferreira et al., 2020). Besides, in the present trial Nellore bulls were supplemented with 70 IU vitamin E/kg DM and did not influence all variables related to nitrogen flow, microbial protein synthesis, and efficiency. However, according to Chikunya et al.(2004) when supplementing 100 or 500 IU vitamin E /kg DM to the rations of sheep with 50 g fatty acids/kg DM, the higher dose of vitamin E increased rumen microbial N flow to the proximal duodenum. Also, supplementing vitamin E at 80.4 IU/kg DM improved the utilization efficiency of dietary N, decreasing the urinary N excretion and increasing the N retention of beef cattle (Wei et al., 2016). The reason for those results could be that vitamin E supplementation raises the growth of rumen protozoa and bacteria (Hino et al., 1993; Naziroğlu et al., 2002) because it is an antioxidant and maintaining the integrity of cell membranes from oxidation (Burton et al., 1990). For this reason, we assume to this trial that due to the potentially high ruminal degradation of vitamin E, higher or a specific dose of this vitamin might be necessary to elicit an effect on ruminal microorganisms in beef cattle.

Some researchers showed that individual vitamin supplementation, for example, biotin (Suksombat et al., 2011), niacin (Doreau and Ottou, 1996) or vitamin E (Paiva Ferreira et al., 2020), did not affect ruminal pH. Though, it is well established that thiamine, in the form of thiamine pyrophosphatase (TPP), is the coenzyme of pyruvate dehydrogenase (PDH) and α-ketoneglutaric acid dehydrogenase (α-KGDHC) in carbohydrate metabolism (Bubber et al., 2004). Moreover, the deficiency of this vitamin inhibits pyruvate decarboxylation and causes the accumulation of pyruvate; consequently, enhance lactate dehydrogenase activity, increasing lactate concentrations, and decreased ruminal pH (Wang et al., 2015).

Also, high concentrate diets decrease ruminal pH, as a result, promotes the growth of thiaminase-producing bacteria, increasing thiaminase production, and thereby increasing thiamine ruminal degradation (Miller et al., 1986b). Thus, the supplementation of thiamin is very important in this kind of diet to avoid the deficiency and improve the growth of lactate-consuming bacteria (M. elsdenii) and suppressing that of lactate-producing bacteria (S. bovis). Recent researches revealed that supplementing 180 mg of thiamine/kg DM in high concentrate diets increased ruminal pH and decreased lactate concentration. However, differences were not observed in the present trial, although a lower supplementation level of thiamine was used (28.9 mg of thiamine/kg DM), and this level could be insufficient to induce an effect on ruminal microorganisms in beef cattle fed with high concentrate diets.

The bacterial enzyme, methylmalonyl-CoA decarboxylase, requires biotin and is involved with the production of propionate in the rumen (Dakshinamurti and Chauhan, 1988). And, the conversion of this VFA to glucose requires the enzyme propionyl-CoA carboxylase (Aschenbach et al., 2010), which is biotin-dependent (Waldrop et al. 2012). Earlier results showed that biotin supplementation did not affect VFA and NH_3-N concentration (Zimmerly and Weiss, 2001; Suksombat et al., 2011), consistent with the present results. Therefore, these findings might have been a result of insufficient levels of biotin supplementation, or this vitamin content in the basal feed attend microorganism requirements.

Supplementation of vitamin blend did not lead to significant changes in total VFA and individual molar proportions of major VFA (acetic, propionic and butyric acid) in the present trial, which is in accordance with other studies when supplemented with niacin (Riddell et al., 1980), and vitamin E (Schäfers et al., 2018). However, supplementation with 7,500 IU vit E/kg DM (Naziroğlu et al.,2002) or 800 mg of niacin/kg DM (Ouyang et al., 2014), affected VFA molar proportions in the rumen, so it might be concluded that in the present investigation vitamin supplementation was too low to elicit an effect on ruminal VFA concentrations.

Ruminal NH_3-N concentration was also unaffected by vitamin supplementation in this study. A similar result was also reported when supplemental dietary biotin (Suksombat et al., 2011), niacin (Doreau and Ottou, 1996), and vitamin E (Nogueira et al., 2020). However, Niehoff et al. (2013) observed an increase in ruminal ammonia concentration when the same dose of niacin (6g/d) than Doreau and Ottou (1996) was used. Another experiment evaluating the effect of vitamin E on ruminal fermentation *in vitro* (Naziroglu et al., 2002), showed that the NH_3-N level was higher in the supplemented groups compared with the control. Thus showing that the data about the effects of vitamins on ruminal ammonia are varied, and one explanation for higher NH_3-N concentration could be an effect on rumen protozoa, because it is often assumed that niacin and vitamin E is beneficial for ruminal protozoa, and they help to enhance the ammonia nitrogen levels in the rumen fluid. Nevertheless, the mechanism by which vitamin might increase the ruminal protozoa concentration has not been elucidated yet.

This experiment showed that the fat-soluble blend did not affect ruminal parameters in Nellore cattle. Similarly, Kandathil and Bandla (2019), concluded that 100 IU of vitamin A/kg DM or 6000 IU of vitamin D/kg DM did not affect *in vitro* ruminal fermentation. However, the vitamin A dose (6666.7 IU vitamin A/kg DM) used in the present study was higher than those used on *in vitro* trial, but no differences were also found. Furthermore, Hymøller and Jensen (2010) reported that vitamin D did not disappear in the rumen of dairy cows, and hence they concluded that vitamin D was not essential for optimum ruminal microorganisms' metabolism. Therefore, this information confirms the concept that both vitamin A and D do not affect ruminal metabolism, although more research needs to be done to better explain those parameters.

The concentration of 25(OH)D circulating is the best indicator of vitamin D status (Hollis and Horst, 2007). It is readily converted from vitamin D by 25-hydroxylases in the liver and serves as the precursor for the active vitamin D hormone, 1,25-dihydroxyvitamin D

(1,25(OH)2D). In the present trial, supplementing Nellore bulls with 25(OH)D_3+ Vitamina D_3 increased serum concentrations of 25(OH)D, with the mean values of 38.7 and 31.5 (ng/mL), respectively in Vit ADE and Vit B blend+ADE. According to Hollis (2005), those values (> 20 ng/mL) have generally been considered adequate for cattle.

CONCLUSION

Supplementing Nellore bulls with 25(OH)D_3 (Hy-D®) was a successful strategy for increasing circulating concentrations of 25(OH)D. However, supplementation of a B-vitamin blend, fat-soluble vitamin blend, or a combination with these two blends in high concentrate-diets did not improve ruminal fermentation, *in situ* degradability of complete diets, ingestive and digestive parameters in Nellore bulls.

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