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Vitamins for beef cattle

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INTRODUCTION

Vitamins are important metabolic catalysts used in livestock animal diets (Ball, 2006). As micronutrients, they are required in very small amounts in beef cattle diets. However, they are essential to meet the demands related to immunological processes (health), growth, and reproduction (McDowell, 2000). Moreover, vitamins are required for the metabolism of other nutrients, such as carbohydrates and proteins. Thus, vitamins must be present in diets in adequate amounts.

Vitamin supplementation, which is commonly incorporated into the mineral supplements, may contribute to a greater growth performance of beef cattle in intensive production systems, where the metabolic requirements may be increased. In addition, vitamins help to prevent diseases and to maintain animal health (Spears and Weiss, 2014).

Vitamins are mainly available in plant tissues as provitamins. In bovine tissues, they occur due their consumption or production by microorganisms in the digestive tract (Berchielli et al., 2006). Vitamins are classified according to their solubility in lipids and organic solvents as fat-soluble vitamins (A, D, E, and K), or in water as water-soluble vitamins (pantothenic acid, biotin, thiamine, niacin, riboflavin, and cyanocobalamin).

According to Acedo et al. (2018), only 69 articles studying vitamins A, D, and E for beef cattle were published in the Journal of Animal Science over the last 83 years. It represented only 0.16% of the total number of publications in this Journal. Moreover, the recommendations available in the literature regarding vitamin nutrition for beef cattle are mostly from studies developed between the 1960s and 1980s. Considering the genetic improvement and changes in energy and protein requirements over time, it may be necessary to adjust the current recommendations on vitamin supplementation for beef cattle. Thus, further studies on vitamin supplementation are still necessary.

Studies evaluating the effects of vitamin supplementation on beef cattle productive performance under Brazilian conditions are scarce. Over the last 27 years, only two articles were published in Brazil, evaluating vitamin supplementation for dairy cattle, in which the inclusion of individual vitamins was tested (Acedo et al., 2018). Hence, due the scarcity of data on vitamin supplementation for ruminants, the use of studies in non-ruminants and humans is inevitable. Therefore, the objective of this chapter was to discuss the main aspects regarding the supplementation of water and fat-soluble vitamins for beef cattle. Subsequently, two studies regarding the effects of vitamin supplementation on productive performance, physiology, and metabolism of feedlot cattle raised under Brazilian conditions are presented.

WATER-SOLUBLE VITAMINS

Water-soluble vitamins are mainly found in plant tissues. However, the ruminal microbiota can synthesize and provide water-soluble vitamins for ruminants. In addition, water-soluble vitamins are usually not stored in the animal body in large amounts and must be supplied daily. Nevertheless, water-soluble vitamin requirements have not been established for beef cattle. Failure to establish supplementation levels may be primary because adequate amounts of water-soluble vitamins are provided by ruminal bacterial synthesis in most dietary situations (NASEM, 2016). Furthermore, ruminants are able to produce their own vitamin C (NASEM, 2016).

Water-soluble vitamins, especially thiamine (vitamin B_1), niacin (vitamin B_3), and biotin (vitamin B_7) play relevant roles as coenzymes in the metabolism of carbohydrates, proteins, and lipids. They increase protein synthesis by ruminal

microorganisms (vitamin B_3); they are responsible for the enzymatic carboxylation of ketoacids in the tricarboxylic acid cycle (vitamin B_1), and play a very important role in glucose metabolism (vitamin B_7; NASEM, 2016). Besides, niacin acts on the hepatic conversion of ammonia to urea, as well as in the hepatic metabolism of ketones.

In general, B-complex vitamins have similar functional characteristics and act synergistically. The synthesis of B-complex vitamins in ruminants is influenced by the type of diet; however, considerable diet modifications can occur without causing any signs of deficiency (NASEM, 2016). The deficiency of B-complex vitamins in ruminants is usually limited to young animals that have not completed rumen development yet. Deficiency may also happen when vitamin antagonists are present in the rumen or when ruminal synthesis is limited by the absence of precursors. Next, a brief description of the main water-soluble vitamins for beef cattle will be presented. Additional information can be obtained from the studies listed in the references section at the end of this chapter.

Thiamine (Vitamin B_1)

Thiamine is widely distributed in plant and animal tissues. In animal tissues, it occurs as thiamine pyrophosphate (Ball, 2004), which contains a pyrimidine ring (2,5-dimethyl-6-aminopyrimidine) and a thiazole ring (4-methyl-5-hydroxy ethyl thiazole) linked by a methyl bridge (Aragón, 2018).

Deficiency of thiamine in ruminants is rare. However, it may cause weakness, retracted head, and cardiac arrhythmia, just like other water-soluble vitamins. Thus, water-soluble vitamins deficiencies can result in anorexia, diarrhea, and reduced animal growth (NASEM, 2016).

Thiamine plays a central role in the energy supply to the animal, especially in the metabolism of carbohydrates (Rodwell et al., 2015). Thiamine pyrophosphate is the coenzyme of three important enzyme complexes, which are involved in the main steps of decarboxylation in carbohydrate metabolism: pyruvate dehydrogenase (glycolysis), transketolase (pentose phosphate pathway), and α-ketoglutarate dehydrogenase (citric acid cycle pathways; Cunha et al., 2006). Therefore, the incorrect supply of thiamine may impair energy supply in all bovine tissues.

Although ruminants can usually synthesize vitamin B_1 in adequate amounts (Miller et al., 1986), there are reports about thiamine deficiency in sheep and cattle related to its increased degradation by thiaminase and decreased microbial thiamine synthesis due to the occurrence of ruminal acidosis (Brent, 1976; Pan et al., 2016). The production of thiamine analogues by thiaminase (thiaminase I, mainly) in grain-fed cattle can induce central nervous system disorders such as polioencephalomalacia (PEM), which is clinically a softening or degeneration of the gray matter of the brain (Brent et al., 1984).

In addition, some synthetic compounds (pyrithiamine and oxythiamine) are considered thiamine antagonists as they have a similar composition and act as inhibitors (competitive) of thiamine, interfering with different stages of animal metabolism (González and Silva, 2019).

Recent studies have demonstrated positive effects of thiamine supplementation on epithelial transport in the rumen and reduction of inflammation induced by high-grain diets in high producing dairy cows and finishing beef cattle (Karapinar et al., 2010; Pan et al., 2017; Aschenbach et al., 2019). In a study performed by Pan et al. (2016), thiamine supplementation for cattle fed high-grain diets improved ruminal pH and decreased ruminal lactate concentrations. In addition, the authors stated that thiamine supplementation was able to regulate the ruminal microbial population in cows with subacute rumen acidosis (SARA), by reducing the population of *Streptococcus bovis* and stimulating the growth of *Megasphaera elsdenii*.

In most cases, available studies have evaluated the supplementation of water-soluble vitamins combined as blends, composed mainly of thiamine (vitamin B_1) and/or niacin (vitamin B_3), and/or biotin (vitamin B_7; Zinn et al., 1987; Ebtehag et al., 2016; Aragón, 2018). Thus, the effects of water-soluble vitamin supplementation on the productive performance of ruminants will be discussed based on blends rather than individual supplementation in many sections of this chapter.

Improved average daily gain (ADG) and total body weight (BW) gain were observed for finishing buffaloes supplemented with vitamins B_3 and/or B_1 (Ebtehag et al., 2016). The authors of this study reported that animals supplemented with niacin (0.992 kg/d of ADG; total BW gain of 119 kg), thiamine (0.963 kg/d ADG; total BW gain of 115.6 kg), and both niacin and thiamine (1.01 kg/d ADG; total BW gain of 121.6 kg) showed greater ADG and total BW gain when compared to the control treatment (0.747 kg/d ADG; total BW gain of 89.6 kg). According to Aragón (2018), the improvements in the productive performance of animals supplemented with vitamins B_1 and/or B_3 may be related to their role on energy metabolism, resulting in a more efficient use of feed energy.

Riboflavin (Vitamin B_2)

Riboflavin, also known as vitamin B_2, exists in nature in three forms: free riboflavin and its coenzyme derivatives flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD). Riboflavin in its coenzyme form (FMN or FAD) is called flavoprotein and acts in intermediary metabolism, especially in oxidation-reduction reactions (McDowell, 2000). Vitamin B_2 is also required for the activation and metabolism of different B-complex vitamins, such as cyanocobalamin (vitamin B_{12}), folic acid (vitamin B_9) and pyridoxine (vitamin B_6; Wu et al., 2021). Additionally, riboflavin promotes the conversion of tryptophan to niacin and acts in the mobilization of iron (Ahmadian et al., 2021).

Zinn et al. (1987) observed that the synthesis and flow of riboflavin in the rumen are independent of the concentrate content in the diet fed to steers. However, Beaudet et al. (2016) suggested that ruminal synthesis of riboflavin was greater under dietary conditions that stimulate ruminal microbial growth (i.e., diets containing readily degradable carbohydrates sources and adequate supply of N). Nevertheless, there is a reasonable consensus that ruminal degradation of dietary riboflavin is almost complete (Zinn et al., 1987) and only approximately 1% of this vitamin could reach the small intestine and be absorbed (Santschi et al., 2005). Consequently, riboflavin supplementation in diets for adult ruminants would only be justified if technologies were used to protect it from ruminal degradation.

No daily requirements for riboflavin have been established for beef cattle, as ruminal microorganisms synthesize abundant amounts of this vitamin, representing approximately 1.5 times the estimated metabolic requirements of riboflavin (NASEM, 2016). Hence, riboflavin deficiencies are unlikely to occur in adult ruminants, and it has only been observed in young pre-ruminants (Casals and Calsamiglia, 2012). Zinn (1992) calculated, using data collected from pigs (NRC, 1979), that the requirements for feedlot cattle were 0.32 mg/kg $BW^{0.75}$. Recent evidence has shown that increasing levels of dietary riboflavin supplementation from 600 to 900 mg/d had no influence on dry matter (DM) intake but tended to increase ADG in bulls (Wu et al., 2021). Nonetheless, riboflavin supplementation should only be considered in animals under stress and with a low feed intake when the ruminal microbial population may be impaired (Zinn, 1992).

Niacin (Vitamin B_3)

Ruminants can be supplied with Niacin (vitamin B_3) from three sources: 1) dietary niacin; 2) conversion of tryptophan to niacin; and/or 3) synthesis of ruminal microorganism (NASEM, 2016). Niacin is part of the coenzymes NAD and NADP, being important in all the animal metabolism. Moreover, niacin is required for ammonia detoxification to urea and ketone metabolism in the liver in ruminants.

Niacin supplementation of niacin is usually not required due to its microbial synthesis in the rumen. However, studies have shown a variation in niacin concentration in the rumen according to the type of feed provided and roughage/concentrate ratio in the diet (forage: concentrate; Niehoff et al., 2009; Schwab *et al.,* 2006). However, it is unclear which diet component most influences the ruminal concentration of niacin. Nevertheless, of the total of niacin supplemented, only a small part reaches the duodenum (Niehoff et al., 2009), which may be due to ruminal degradation or absorption in the abomasum.

According to Zinn et al., (1987), ruminal starch degradation stimulates the synthesis of all B-complex vitamins. Results by Schwab et al. (2006) corroborate the previous findings of Zinn et al. (1987), who observed a positive correlation between non-fibrous carbohydrates (NFC) and starch concentrations in the diet, as well as their intake and ruminal degradation, and niacin synthesis in dairy cattle. However, no effect of diet forage (35 or 60% on a DM basis) or NFC (30 or 40% on a DM basis) ratio was observed on the production of water-soluble vitamins.

The effects of vitamin B_3 supplementation on the productive performance of beef cattle have been variable. Byers (1981) summarized 14 studies on the effects of vitamin B_3 supplementation for beef cattle in a meta-analysis. The authors concluded that niacin supplementation ranging from 50 to 250 mg/kg DM during diet adaptation (first 21 to 38 d) improved growth rate and feed efficiency of beef cattle by 9.7 and 10.9 %, respectively. Luo et al. (2019) evaluated the effects of vitamin B_3 supplementation at levels of 320, 480 and 640 mg/kg DM on the productive performance of finishing *Jinjiang* cattle. The results showed that cattle fed 640 mg/kg DM of supplemental niacin presented ADG 43.75% greater than those in the control group during the initial period (1 to 28 d). However, dietary doses of 480 and 640 mg/kg DM of supplemental niacin reduced feed efficiency by 26.07% and 31.61%, respectively, compared to the control group. According to Zinn et al. (1987), the absence of beneficial effects of vitamin B_3 supplementation at low doses (100-400 ppm) on the growth performance of cattle may be related to the degradation of this vitamin by the ruminal microbiota.

Nevertheless, previous research has suggested that dietary supplementation with niacin may improve rumen fermentation and microbial protein synthesis in beef cattle (Flachowsky, 1993; Girard, 1998). In addition, an increase in nutrient digestibility and productive performance was observed when vitamin B_3 was supplemented at a dose of 6g/cow/d and 1g/bull/d (Flachowsky, 1993).

Pantothenic acid (Vitamin B_5)

Pantothenic acid (vitamin B_5) is a component of two key enzymes in carbohydrate, lipid, and protein metabolism: coenzyme A (CoA) and the acyl group carrier protein (ACP; NASEM, 2016). This vitamin is composed of β-alanine and pantoic acid (Bässler et al., 2002) and it is found in almost all feed and living cells, associated with CoA, ACP, or as free pantothenic acid (Finlayson e Seeley, 1983; Ragaller et al., 2011).

The biological activity of vitamin B_5 is directly related to CoA and ACP. When associated with the CoA, vitamin B_5 is involved in energy-releasing reactions from carbohydrates, fatty acids, and amino acids, whereas it is involved in fatty acid biosynthesis when associated with ACP (Ball, 2006). In carbohydrate metabolism, acetyl-CoA provides acetyl groups for the tricarboxylic acid cycle. In addition, acetyl-CoA plays a role in the acetylation of amino sugars and choline to form mucopolysaccharides.

The quantification of vitamin B_5 in the rumen is complex since it is subjected to the processes of synthesis, degradation, and passage through the digestive tract, and probably absorption, simultaneously (Kon and Porter, 1954). Furthermore, variations in pantothenic acid concentrations in the rumen fluid of cattle fed concentrate diets tend to be greater than for those fed roughage diets (Hayes et al., 1966). Zinn et al. (1987) reported a negative apparent synthesis of B_5 vitamin (-13.5 mg/d) in growing male crossbred calves (116 kg) under transport stress fed a corn-based diet, indicating that there was not a net synthesis of B_5 vitamin. Authors stated that it may be related to the lower feed intake of growth when compared to older animals and to the characteristics of the diet, since the synthesis of vitamin B_5 varies according to the diet composition.

There are cases in the literature suggesting that vitamin B_5 is necessary for the proper growth of some ruminal bacteria, such as *Lactobacillus* sp., *Streptococcus bovis*, and *Megasphaera elsdenii* (Ford et al., 1958; Wolin et al., 1997). A beneficial influence on the *in vitro* protozoa count has also been reported (Völker et al., 2011). It is estimated that the amount of vitamin B_5 synthesized in the rumen is 20 to 30 times greater than the amount present in diets commonly offered to beef cattle (NASEM, 2016) and, because of that, nutritional requirements for vitamin B_5 have not been established yet.

About 80% of supplemental vitamin B_5 is degraded in the rumen (Zinn et al., 1987) and the use of its protected form (pantothenate) may be a viable alternative. According to Liu et al. (2018), increased ruminal concentrations of total volatile fatty acids (VFA), the degradability of neutral detergent fiber (NDF) and crude protein (CP), bacterial abundance, microbial enzyme activity, and microbial protein synthesis were observed when pantothenate was supplemented to Blonde d'Aquitaine × Simmental cattle. In a previous study, Li et al. (2017) observed better nutrient utilization and ADG for Blonde d'Aquitaine × Simmental calves supplemented with pantothenate.

Pyridoxine (Vitamin B_6)

Pyridoxine is also known as vitamin B_6 and represents a group of three compounds: pyridoxol, pyridoxal, and pyridoxamine. The latter two forms are predominantly found in animal products (McDowell, 2000). The vitamin activities of these three compounds are similar when offered to animals but are not equivalent when given to microorganisms (Morris, 2001). Pyridoxal phosphate and pyridoxamine phosphate are other chemical forms associated with this vitamin. Pyridoxal phosphate is a cofactor in over 60 enzymatic reactions associated with carbohydrate, protein, and lipid metabolisms (McCormick, 2006). However, it is best known for its important role in most reactions associated with amino acid metabolism (mainly methionine and tryptophan, Mohammed et al., 2004). Therefore, vitamin B_6 requirements should be established according to protein intake (Casals and Calsamiglia, 2012). This means that the use of higher levels of methionine and tryptophan in feedlots diets may increase the pyridoxine requirement in bovines.

Modifications in diet composition may affect the ruminal microbial population and probably the vitamin B_6 metabolism. Although not much is known about the factors that affect ruminal synthesis of this vitamin, it appears to be increased when high concentrate diets are fed (Schwab et al., 2006) and to be reduced in cattle fed high-forage diets (Castagnino et al., 2016). Recently, Clemmons et al. (2020) indicated that metabolism of the vitamin B_6 byproduct was higher in the rumen of more feed-efficient animals, and that this may have allowed a more efficient protein use and muscle gain.

Most published data show that pyridoxine is abundantly synthesized, and very low amounts are degraded in the rumen. Hence, the dietary supply of vitamin B_6 is sufficient to prevent deficiency symptoms and allow normal cattle growth and production. Zinn (1992), extrapolating data collected from studies with pigs, recommended the supply of 0.14 mg/kg $BW^{0.75}$ for feedlot cattle. However, vitamin B_6 requirements have not been set for beef cattle.

Biotin (Vitamin B_7)

Biotin is commonly known as vitamin B_7, vitamin H, or coenzyme R. This vitamin is present in nature in both protein-bound and free forms. Nevertheless, most of the ingredients used in feedlots contain biotin bound to the protein (covalent bonds), which hinders its digestion and bioavailability for the animal (Habeeb and Gad, 2019). Proteases in the digestive system degrade this bound form, releasing biocytin (vitamin linked to lysine; McDowell, 2000). The bound formed between lysine and biotin can only be broken under the action of a specific enzyme (biotinidase; McMahon, 2002), which is present in the pancreas, intestinal brush cells, and some bacterial species, releasing biotin in its free form to be absorbed (Santschi et al., 2005). Therefore, higher protein feed usually contains more biotin than feed with low protein concentrations (e.g., soybean meal = 0.30 mg/kg vs. barley = 0.08 mg/kg; Ammerman et al., 1995).

Biotin is a cofactor for many enzymes involved in many metabolic pathways. Biotin is required as a coenzyme in amino acid metabolism, cellular respiration, gluconeogenesis, and lipogenesis (McDowell, 2000). Besides, this vitamin is necessary for the normal functioning of the thyroid and adrenal glands and the reproductive and nervous systems (Habeeb and Gad, 2019). Specifically, biotin is a cofactor for four carboxylase enzymes: pyruvate carboxylase and propionyl-CoA- carboxylase, which are crucial for gluconeogenesis; acetyl-CoA-carboxylase, the first and rate-limiting step in

de novo fatty acid synthesis; and methylcrotonyl-CoA carboxylase (Weiss and Zimmerly, 2000). Moreover, biotin is necessary for many processes that contribute to the mechanical properties of the hoof and horn. These processes include keratinization, the formation of intracellular cementing substances, and the production of other lipids required to maintain epidermal permeability and integrity (Lischer et al., 1996; Mulling et al., 1999). It has been shown that biotin supplementation is recommended to improve the hardness of the hoof and horn in dairy cattle (Higuchi et al., 2003; Chen et al., 2012).

Although feeds commonly used in feedlots contain varying concentrations of biotin, the primary source of this vitamin for ruminants is the one synthesized by ruminal microorganisms (NRC, 2001; Enjalbert et al., 2008). Biotin is also required for some ruminal bacteria, especially cellulolytic bacteria for their growth (Baldwin and Allison, 1983). Furthermore, this vitamin is also needed for the ruminal microbiota to produce propionate because biotin is a cofactor in the microbial enzyme methylmalonyl-CoA-carboxytransferase, which catalyzes a step in the synthesis of propionic acid (Zempleni and Mock, 1999).

Ruminal bacteria synthesize large quantities of biotin depending on the energy availability (0.79 mg/kg of digested organic matter [DOM]; Briggs et al., 1964, cited by Casals and Calsamiglia, 2012). However, biotin synthesis may be reduced with high-energy diets. High-concentrate diets, usually fed in feedlots, can create an acidic rumen or change the site of starch fermentation. Thereby, the ruminal and intestinal flora may be altered and, in turn, affect the synthesis or degradation of microbial biotin. Thus, biotin supplementation may be required for ruminants fed high- concentrate diets.

Decreased biotin synthesis has been reported *in vitro* for diets containing more than 50% concentrate (Da Costa Gomez et al., 1998). In addition, Abel et al. (2001) observed that ruminal biotin synthesis decreased by 50% as the proportion of barley to hay increased from 50:50 to 83:17 in an *in vitro* continuous culture study, likely as a result of an imbalance among ruminal microorganisms species caused by pH decrease. Furthermore, the proportion and type of grain in the diet influenced the ruminal and intestinal apparent syntheses and absorption of biotin in duodenally cannulated beef steers (Miller et al., 1986).

However, Rosendo et al. (2004) suggested that the reduction of cellulolytic microbial growth caused by low ruminal pH (5.3) may decrease utilization rather than synthesis of ruminal biotin. Nonetheless, it has been reported that biotin is not extensively degraded in the rumen (NRC, 2001). Schwab and Shaver (2005) observed a significant increase in the concentration of biotin in the milk and plasma of dairy cattle, suggesting that this vitamin supplemented in an unprotected way against ruminal degradation was not completely degraded or used by ruminal microorganisms.

A meta-analysis using 9 studies indicated that supplementation with unprotected biotin positively affected milk yield in dairy cows (Chen et al., 2011), suggesting that biotin does not require ruminal protection. Nevertheless, a limited number of studies have been conducted evaluating biotin supplementation in beef cattle. De Silva et al. (2018) found that biotin supplementation (20 mg/d) to crossbred cattle did not alter their growth rates. Moreover, previous work indicated that dietary biotin largely escaped the rumen and supplementation at high levels with vitamins blend for feedlot cattle did not affect animal performance but tended to decrease morbidity (Zinn et al., 1987). Results from Campbell et al. (2000) indicated that biotin supplementation (10 mg/d) improved hoof health in Hereford cows. The combination of biotin synthesized by ruminal microorganisms and its supply in the basal diet seems to be sufficient to meet requirements of biotin for beef cattle.

The NASEM (2016) did not establish biotin daily requirements for beef cattle. Besides that, Zinn (1992) estimated the requirements of biotin for feedlot and/or finishing calves at 0.13 mg/kg of $BW^{0.75}$. In contrast, OVN (2016) recommends 10 to 20 mg/d for optimum hoof health and optimum meat marbling.

Choline (Vitamin B8)

Choline is often not considered a vitamin because, unlike classical vitamins, its

endogenous synthesis is possible. Therefore, it has been suggested that this compound may be an essential nutrient for ruminants, regardless of whether it is classified as a true vitamin (Pinotti et al., 2020). Accordingly, choline is required in relatively large amounts (grams and not in milligrams), usually more than other vitamins (NASEM, 2016).

Although a small amount of free choline may exist in some plant materials, phosphatidylcholine is the major natural form of choline in feed. Choline is essential for the synthesis of vital molecules in the body (e.g., phosphatidylcholine and acetylcholine); thus, it plays an essential role in energy, protein, and lipid metabolism (Berdanier, 2008). In addition, it is an important donor of methyl groups, contributing to the biosynthesis of other methylated compounds in animal metabolism (McDowell, 2006). Therefore, one of the advantages of supplementing choline is to increase the availability of methionine for protein synthesis, as biologically active methyl groups may also be obtained from this essential amino acid (NRC, 2001).

Choline requirements for beef cattle are not well established because of several reasons. For example, feed ingredients contain different concentrations of choline. The choline content in corn is approximately 80.04 mg/kg, while in soybean meal this concentration is 1686.54 mg/kg (Chen et al., 2019). Additionally, the combination of different levels and composition of protein, fat, and carbohydrates in the diet as well as the age, category, energy intake, and growth rate of animals have an influence on the lipotropic action of choline, and thus, the requirement of this vitamin (Mookerjea, 1971).

Furthermore, dietary choline is only available to adult cattle if it escapes ruminal degradation. One of the few reports available in the literature regarding the effects of unprotected choline supplementation for beef cattle observed no improvement in feedlot performance, carcass measurements, acidosis, or ruminal fermentation products in choline-steers supplemented with choline fed an all-concentrate diet (Morris, 2001). Dietary choline and synthetic supplements are rapidly and extensively degraded by the rumen microbiota (Baldi and Pinotti, 2006). Ruminal choline degradation values vary between 80 and 99%, depending on the source (Sharma and Erdman, 1989). Thus, even choline-rich feed can only contribute marginally to the post-ruminal supply of choline. Therefore, an effective method to increase choline availability to ruminants is to feed it in a way that is protected from ruminal degradation.

In this regard, rumen-protected choline, containing choline chloride covered by a protective layer of fatty acids, has received interest for research and production applications (Jayaprakash et al., 2016). Some studies have reported improvement in growth performance (Bindel et al., 2000; Pinotti et al., 2009) and carcass traits (Drouillard et al., 1998) in beef cattle supplemented with rumen-protected choline. Due to the limited number of available research and the inconsistency of the results, no practical recommendation for daily unprotected or rumen protected choline supplementation has been made in this chapter. However, it has been suggested that milk-fed calves should be supplemented of 0.26% choline in milk replacers (NASEM, 2016).

Folic acid (Vitamin B_9)

Folic acid, also known as vitamin B_9, folacin, or folate, is involved in the transfer of one-carbon units, playing a crucial role in the biosynthesis of purine and pyrimidines, synthesis of DNA, and amino acid metabolism (McDowell, 2000). Folic acid acts synergically with other vitamins, minerals, amino acids, and metabolites to maintain a functional one-carbon metabolic pool (e.g., choline, methionine, glycine, cobalt, and others; NASEM, 2016).

Ruminal microorganisms synthesize a great amount of folic acid, which depends on energy availability (NRC, 2001). Zinn (1992) estimated that folic acid synthesis in the rumen was 0.42 mg/kg of DOM. Thus, animals with functional rumen must have an adequate supply of folic acid (McDowell, 2000). Moreover, it is estimated that approximately 97% of dietary supplementary folic acid is degraded by ruminal microorganisms (Zinn et al., 1992; Wang et al., 2017) and only 3% of dietary folic acid could reach the small intestine to be absorbed (Santschi et al., 2005). Thus, folic acid supplementation in adult animals would only be justified with the use of

protection technology from ruminal degradation.

In this context, recent studies have found that rumen-protected folic acid supplementation to steers increased total ruminal VFA concentration, nutrient degradability, microbial protein synthesis, cellulolytic bacterial abundance, enzyme activity, ADG, and feed efficiency (Liu et al., 2020; Wang et al., 2016, 2017). Zinn (1992) extrapolating data collected from pigs (NRC, 1998), concluded that the requirements of folic acid were 0.75 mg/kg $BW^{0.75}$ for feedlot cattle. However, international nutrient requirements systems (e.g., NASEM, 2016; OVN, 2016) and this current publication have not established the requirements of vitamin B_9 for beef cattle.

Cobalamin (Vitamin B_{12})

Vitamin B_{12} is a generic name used to refer to a group of compounds that have vitamin B_{12} activity, such as cyano, hydroxy, methyl, or deoxyadenosylcobalamin. Cobalamin, the biologically active form of vitamin B_{12}, is involved on the metabolism of nucleic acids, protein, carbohydrates, and lipids (González-Montaña et al., 2020). Meeting vitamin B_{12} requirements is of a special interest in ruminants than in non-ruminant animals, as it is required for proper propionate metabolism. Methylmalonyl-CoA mutase is a vitamin B_{12}-dependent enzyme that is essential for the conversion of propionate to succinate, which by gluconeogenesis is later converted into glucose, influencing energy production in animals (Mansur, 2009; Rowley and Kendall, 2019).

Unlike other B vitamins, vitamin B_{12} is not present in plants and is only produced by bacteria if cobalt supply is adequate, which is usually used as an indicator of the B_{12} status in the animal organism (González-Montaña et al., 2020). The rumen microbiota can synthesize vitamin B_{12} if concentrations of free cobalt in the ruminal fluid are greater than 0.5 mg/mL (Underwood and Suttle, 2002). Thus, ruminants obtain the preformed vitamin B_{12} from their natural bacterial flora or by eating animal products.

Physiologically, the selective uptake of active vitamin B_{12} requires interaction between pre-carrier proteins, haptocorrin (HP), intrinsic factor (IF), transcobalamin (TC), and different receptors. Haptocorrin is part of many biological fluids, including gastric juice, and helps to break down vitamin B_{12} analogues. Gastric (humans) or pancreatic (dogs) IF selectively binds to B_{12} and enters intestinal cells via receptor-mediated endocytosis. Subsequently, the absorbed vitamin B_{12} is transmitted to the TC and delivered to the tissues via bloodstream. It is noted, therefore, that the complexity of the transport system allows efficiency in the absorption process of vitamin B_{12}. However, any failure at any point in this process can lead to the deficiency of this vitamin, which would lead to irreversible neurological damage, anemia, and even death (Fedosov, 2012; Kather *et al.,* 2020).

In a study comparing the pharmacokinetics of two forms of cobalamin (OHB12 and CNB12) associated with trace minerals in Holstein steers, Gonzalez-Rivas et al. (2021) found that animals that received the OHB12 form presented an increase of 11% in ADG when compared to those treated with CNB12 (1.17 and. 1.06 kg/d for steers receiving OHB12 and CNB12 forms of cobalamin, respectively). Furthermore, the authors stated that a single injection of OHB12 was effective in increasing the blood level of cobalamin in the first 24 hours and its liver storage, which was sustained for at least 28 days in a situation of deficiency. In contrast, supplementing cobalamin with the CNB12 form did not increase cobalamin in the liver (Gonzalez-Rivas et al., 2021).

FAT-SOLUBLE VITAMINS

Fat-soluble vitamins are found in association with the lipid constituents of plants and animals and are characterized by their long-term storage capacity in the animal organism (McDowell, 2000). Fat-soluble vitamins A, D, E, and K are absorbed concomitantly with the absorption of lipids and depend on the presence of bile and micelle formation in the gut (McDowell, 2000).

Fat soluble vitamins have several essential functions in the body of ruminants. For example, vitamin A is involved in the formation, regeneration, and protection of the ectoderm and mucous membranes (*Toledo et al.,* 2006). Vitamin E improves antibody formation and humoral immune system. It is also necessary for cell metabolism (cellular respiration and nucleic acid metabolism), cell division, and protein synthesis. Also, vitamin E acts as an antioxidant

compound for unsaturated fatty acids and vitamin A, contributing to improve meat quality (Leda *et al., 2018;* Toledo *et al.,* 2006).

On the other hand, vitamin D regulates calcium (Ca) and phosphorus (P) homeostasis, increasing intestinal uptake and bone resorption. In addition, it increases the activity of Ca-dependent proteolytic enzymes, which can lead to improved meat quality (Montgomery *et al.,* 2000; Toledo *et al.,* 2006). Vitamin K is required for the synthesis of several plasma clotting factors (factors II, VII, IX, and X) and may also participate in Ca homeostasis (Dores et al., 2001).

Vitamin A

Although all vitamins are equally important in maintaining ruminant life, vitamin A is the most relevant vitamin for beef cattle production, being essential for healthy growth and development (NRC, 2001). Vitamin A performs many functions in the animal's body, and the most important of which is associated with vision (vision pigment), embryo development, immunity, the maintenance of homeostasis, and maintenance of skeletal and epithelial tissue (Bendich, 1993; McDowell, 2006). Vitamin A and β-carotene are required to optimize the reproductive performance of Nellore cows (Gouvêa et al., 2018; Vasconcellos et al., 2018ab) and embryonic quality (Amaral et al., 2004). Furthermore, this vitamin has a positive role in the spermatogenesis of Nellore bulls (Silva et al., 2020).

Vitamin A is a family of molecules grouped under the name of retinol, which is nearly colorless, long-chain, and unsaturated compound. As a fat-soluble compound, normal processes of fat digestion, and absorption, and adequate dietary fat content are necessary to allow its absorption. The liver and adipose tissues are the local in the body where absorbed retinol can be stored. Several studies have confirmed that the liver (usually contains around 90% of total-body vitamin A) can store enough vitamin A to preserve animals from a deficiency caused by long periods of low vitamin A intake (McDowell, 2000).

The basic structure of vitamin A is all-trans retinol, which can originate several isomers. Nevertheless, the common forms of vitamin A supplementation used in commercial feeds are the all-trans retinyl acetate and all-trans retinyl palmitate. These are normally produced as hardened or cross-linked gelatin beadlets to improve stability. Thus, vitamin A requirements for ruminant nutrition are reported in either International Units (IU) or Retinol Equivalents (RE; NASEM, 2016). The definitions and relationships between IU and RE are shown in Table 15.1.

Table 15.1 - Definitions and relationships between International Units (IU) and Retinol Equivalents (RE) related to vitamin A

1 IU	0.3 μg of all-*trans* retinol
1 IU	0.344 μg of retinyl acetate
1 IU	0.55 μg of retinyl palmitate
400 IU	1 mg β-carotene
1 RE	1 μg of all-*trans* retinol, 2 μg of synthetic supplemented all-*trans* β-carotene, 6 μg of dietary all-*trans* β-carotene, or 12 μg of other dietary provitamin A carotenoids

*Adapted from NASEM (2016) and Casals and Calsamiglia (2012).

Retinol is not present in plant-based ingredients commonly used in ruminant diets, but its precursors (carotenes or carotenoids) occur in different forms. However, β-carotene activity is substantially higher than other carotenoids. β-carotene (or pro-vitamin A) is converted to vitamin A (retinol) by enzymes located in the intestinal mucosal cells. Besides, other carotenoids (e.g., α-carotene and cryptoxanthin) can also be converted to vitamin A. However, the efficiency of conversion of carotenoids to retinol appears to be lower in beef cattle than in non ruminants animals (Ullrey, 1972). The reduced conversion rate of carotene to retinol may be due to ruminal degradation of vitamin A (McDowell, 2000).

Ruminal degradation of vitamin A may be affected by the type of diet fed to beef cattle. Rode et al. (1990) reported that vitamin A

disappearance during *in vitro* ruminal fermentation increased from 20% to approximately 80% when donor steers were fed a high-concentrate diet in relation to those fed a high-forage diet. In this regard, new technologies have been commercially developed to protect vitamin A from ruminal degradation. In this case, commercial products are composed of vitamin A and other compounds, such as carbohydrates and antioxidants, aiming its stabilization (Alosilla et al., 2007).

Several factors can influence the carotenoid composition of a feedstuff, such as production conditions (e.g., temperature and humidity), maturity at harvest, and postharvest storage conditions and processing (Pickworth *et al.,* 2012). Retinol and carotenoids are highly sensitive to oxidation, exposure to UV light and the presence of acids, humidity, and microminerals (NRC, 2001). Prolonged storage in premixes containing trace minerals (particularly copper), elevated moisture, pelleting, extrusion processing, or the presence of rancid fats reduces the stability of vitamin A (Olson, 1991; Coelho, 2002).

Vitamin A deficiency in beef cattle raised in tropical conditions can occur during the winter, as forage mass availability and quality (e.g., low β-carotene concentration) are low. Vitamin A deficiency may also occur in high concentrate diets in feedlot systems, and when the feed ingredients have been exposed to sunlight and/or high temperatures or stored for a long period (Akhtar et al. 2011). Considering that fresh forage (e.g., pasture) has relatively higher concentrations of β-carotene than conserved forages (e.g., silage), the amount of supplemental vitamin A required when fresh forage is fed would be less. Although toxicity due to an excess of this vitamin in the diet is uncommon in ruminants due to its high rate of ruminal degradability, it also limits the amount of vitamin A available to the animal (Casals and Calsamiglia, 2012).

Some *in vivo* studies have documented that cattle fed 2,134 - 2,300 IU/kg DM of vitamin A supplement had greater gains and feed efficiency than those consuming approximately 6,274-11,000 IU/kg DM (Zinn et al., 1996). Furthermore, Gorocica-Buenfil et al. (2007bc; 2008) conducted three trials evaluating the supplementation of vitamin A at levels of 2,200 or 2,700 IU/kg DM for finishing beef cattle. Compared with the non-supplemented group, vitamin A-supplemented group improved gains in one trial (Gorocica-Buenfil et al., 2007b) and feed efficiency in the other two trials (Gorocica-Buenfil et al., 2007c; 2008). Bryant et al. (2010) evaluated the effects of vitamin A supplementation (0; 1,103; 2,205; 4,410; or 8,820 IU/kg DM) on the growth performance and carcass quality of steers fed a high-grain diet. The authors did not find differences in the final BW, feed efficiency, ADG, and daily DM intake (DMI) among treatments within each 28-days or the overall feedlot period. Thus, it was suggested that vitamin A supplementation for finishing cattle should be carefully evaluated.

According to these authors, typical feedlot diets (approximately 90% of concentrate in the total diet) have an average of 5,215 IU vitamin A/kg DM. The vitamin A requirement for finishing cattle fed with similar basal diets is ≤ 2,205 IU/kg DM (NASEM, 2016). Thus, diets with values above 2,205 IU/kg DM of vitamin A can overload the ability of the lower gastrointestinal tract to absorb other nutrients, not necessarily benefiting animal health and performance.

Recently, Wellmann et al. (2020) evaluated the supplementation of vitamin A at the estimated requirement (2,200 IU/kg DM) or 5 times the estimated requirement (11,000 IU/kg DM; NASEM 2016) for Nellore steers and did not observe differences across treatments for ADG, feed efficiency, hot carcass weight, rib eye area, or backfat thickness. Therefore, the current NASEM (2016) recommendations of 2,200 IU vitamin A/kg DM seem to be adequate for restoring the vitamin A status of feedlot steers.

Previous studies have suggested that vitamin A feeding strategy plays a crucial role in intramuscular fat deposition in beef cattle (Oka, 1996; Gorocica-Buenfil et al., 2007a; Bryant et al., 2010; Knutson et al., 2020; Peng et al., 2020). In contrast, a study showed that vitamin A restriction during the fattening period increased intramuscular fat in cattle's carcasses by adipocyte hypertrophy (Peng et al., 2021). Nonetheless, the general applicability of vitamin A restriction in beef

cattle production is still ambiguous and deserves further investigation.

Vitamin A requirements can be expressed as IU per kg of body weight (IU/kg BW), per head per day (IU/d), or per kg of diet DM (IU/kg DM). In this chapter, the requirements have been expressed as IU/kg DM when based on NASEM (2016) and as IU/d when based on OVN (2016) recommendations in Table 15.6.

Vitamin D

The main sources of vitamin D are cholecalciferol (vitamin D_3) and ergocalciferol (vitamin D_2). They are preformed sources of vitamin D and differ by the presence of an additional double bond and a methyl group in the plant sterol. Vitamin D_3 is known as the natural form found in animals and can be synthesized under the skin by the action of ultraviolet rays exclusively from 7-dehydrocholesterol (pro-vitamin D_3), which is derived from cholesterol or squalene molecules present in large amounts in the skin, intestinal wall, and other tissues. Vitamin D_2 is the vitamin D present in plants.

The synthesis of vitamin D_3 consists of several steps involving the photochemical modification of 7-dehydrocholesterol followed by non-enzymatic isomerization (Castro, 2011). Thus, the amount of cholecalciferol formed will depend on the frequency and intensity of the ultraviolet rays that reach the animal's skin. Hence, vitamin D requirements will depend on the degree of sunlight exposure. Consequently, the conversion of the provitamin form to active vitamin will be more intense in the tropics than in temperate and arctic zones, at high altitudes. Also, this conversion will be more intense in summer when compared to winter, and at noon when compared to the morning period.

Large amounts (approximately 70%) of vitamin D intake is degraded in the rumen (Ballet et al., 2000). In addition, ruminal degradation of vitamin D can generate metabolites with antivitamin D activity, which protect ruminants from vitamin D toxicity when it is ingested in excessive amounts (Ballet et al., 2000). The remaining vitamin D can be absorbed in the small intestine.

Vitamin D and its metabolites have functions other than acting on Ca metabolism and regulating bone mineralization. These well-known effects of vitamin D are related to biochemical changes that occur in the intestine, bone and kidneys. Vitamin D also has hormone-like functions. Like steroid hormones, 1,25–$(OH)_2D_3$ (Calcitriol) regulates gene expression through interaction with specific receptor proteins in the nucleus. Moreover, 1,25–$(OH)_2D_3$ synthesis is involved in the regulation of growth and differentiation of a variety of cell types, including hematopoietic and immune system cells (Lemire, 1992).

Steroid hormones, prolactin, growth hormone (GH), and insulin-like growth factor 1 (IGF-1) stimulate renal synthesis of 1,25-$(OH)_2D_3$ to regulate Ca absorption and meet higher Ca requirements during pregnancy, lactation, and growth in ruminants (Holick, 2007). When these hormones do not act efficiently, animals may present insufficient productive performance.

Young animals may have rickets caused by a decrease in the concentration of Ca, P or both, and a significant increase in acid phosphatase. In adult animals, vitamin D deficiency can lead to "milk fever" in lactating cows, mainly in multiparous ones. This is because older animals usually have a lower response to dietary Ca due to the decreased production of 1,25-$(OH)_2D_2$ and lower target tissue response to 1,25-$(OH)_2D_3$, which may result in metabolic problems. The NASEM (2016) still uses the NRC (1984) guidelines for vitamin D supplementation for beef cattle, which recommends a supplementation of 275 IU/kg DM or approximately 5.7 IU/kg BW.

Vitamin E

Vitamin E is mainly derived from compounds known as tocopherols, which are defined as fat-soluble biological antioxidants in animal tissues. Thus, this vitamin is important for the metabolism of essential fatty acids (Andriguetto and Perly, 1994). Moreover, it prevents the formation of peroxides resulting from *in vivo* oxidation of certain fatty acids (McDowell, 2000).

Among the different types of tocopherols, α-tocopherol is the most biologically active (100% of biological activity relative to vitamin E), being the predominant preformed vitamin E (McDowell, 2000) and the most used in commercial supplements (Scherf *et al.*, 1996). α-tocopherol is an excellent natural antioxidant that protects carotene and other oxidizable compounds in the feed and body (Buckley et al.,

1995), improving ADG (Pehrson *et al.,* 1991) and feed efficiency in beef cattle (Mir *et al.,* 2003).

The concentration of α-tocopherol (vitamin E) in the feed is variable. In forages, the concentration varies according to the vegetative stage of the plant, ranging from 80 to 200 IU/kg DM or from 80 to 200 mg of α-tocopherol acetate (1 UI is equivalent to 1mg of α-tocopheryl acetate; Insani et al., 2008; Acedo et al., 2018). In addition, concentrations of α-tocopherol decrease rapidly after the plant is harvested (Acedo et al., 2018). Therefore, silage and hay used in ruminant diets may contain from 20% to 80% less α-tocopherol than the plant before the conservation process. Concentrates contain reduced concentrations of vitamin E.

Vitamin E requirements can be met through exogenous dietary supplementation, which may improve animal performance. Requirements of vitamin E for ruminants can be expressed in IU, μmol, and α-tocopherol (mg/IU; NASEM, 2016). Definitions and relationships among units for expressing vitamin E are shown in Table 15.2.

Table 15.2 - Factors for converting international units (IU) of Vitamins E to α-tocopherol in milligrams (mg)[a]

Form of Vitamin E	USP Conversion Factors[b]		Molar Conversion Factors [c] μmol/IU	α-Tocopherol Conversion Factors[d] mg/IU
	IU/mg	mg/IU		
Synthetic Vitamin E and Esters				
DL-α-Tocopherol acetate	1.00	1.00	2.12	0.45
DL-α-Tocopherol succinate	0.89	1.12	2.12	0.45
DL-α-Tocopherol[e]	1.10	0.91	2.12	0.45
Natural Vitamin E and Esters				
DL-α-Tocopherol acetate	1.36	0.74	1.56	0.67
DL-α-Tocopherol succinate	1.21	0.83	1.56	0.67
DL-α-Tocopherol[e]	1.49	0.67	1.56	0.67

[a] Adapted from IOM (2000).
[b] Official United States Pharmacopeia (USP) conversions where 1 IU is defined as 1 mg of all-rac-a-tocopheryl acetate (USP, 1979, 1999).
[c] to convert mg to umol divide the mg by the molecular weight of the vitamin E compound (c-tocop eryl acetate=472; a-tocopheryl succinate = 530; a-tocopherol = 430) and multiply by 1,000. Because the amount of free and succinate compounds is adjusted for their different molecular weights relative to a-tocopheryl acetate, these forms have the same conversion factors as the corresponding tocopherol compound.
[d] to convert the umol of the vitamin E compound to mg of a-tocopherol, multiply the umol by the mc ecular weight of a-tocopherol (430) and divide by 1,000. The activities of the three synthetic a-tocopherol compounds have been divided by 2 because the 2 is ereoisomers contained in synthetic a-tocopherol are not maintained in the blood.
[e] DL-c-Tocopherol = all-rac-(racemic) a-tocopherol = synthetic vitamin E; all rac-a-tocopherol = RK R- RRS-, RSR-, RSS-, SSS-, SRS-, SSR-, and SRR a-tocopherol isomers.
F D-α-Tocopherol = RRR-a-tocopherol = natural vitamin E.

Roeber et al. (2001) evaluated the levels of α-tocopherol acetate in round steaks, T-bones steaks (loin and tenderloin), and ground beef from finishing cattle that were supplemented with 500 and 1000 IU/d of α-tocopherol acetate during commercial shelf display. Dietary supplementation of α-tocopherol acetate at 1000 IU/d ensured a better shelf life for sirloin steaks compared to supplementation at 500 IU/d. In addition, cuts classified as containing a high concentration of α-tocopherol presented greater shelf life (increase of 10.7 and 4.0 h for steaks and ground beef, respectively). Thus, these results suggested a relationship between the concentrations of α-tocopherol in the shelf life of ground beef and cuts. In contrast, Pereira (2002) did not observe effects of supplementation with α-tocopheryl acetate (1,000 mg/d) on the chemical and physicochemical characteristics of the meat of finishing Nellore steers. Nevertheless, shelf life was not determined in this study. According to Acedo et al. (2018), the beneficial effects of vitamin E supplementation on shelf life are related to the reduction of myoglobin oxidation and its conversion to metmyoglobin, responsible for giving the brown/dark red color to the meat; and the

reduction in lipid oxidation (antioxidant effect).

Secrist et al. (1997) carried out an extensive literature review about the effects of vitamin E supplementation on the productive performance of beef cattle. Authors summarized 21 studies and concluded that vitamin E supplementation improved weight gain rate and final body weight of feedlot cattle. Moreover, this study showed that vitamin E supplementation to beef cattle during the first days of feedlot (stressful period due to the transport and adaptation to a new environment) tended to improve feed efficiency. Furthermore, authors reported that vitamin E supplementation positively affected the immune system of cattle, increasing the immune response to diseases and pathogens, as well as to other disorders that arise at the beginning of the feedlot period. Consequently, these results suggested that vitamin E may usually be at a suboptimal level in feedlot diets, as its supplementation promoted a general improvement in growth performance of feedlot cattle (Secrist *et al.*, 1997). Therefore, when an adequate amount of vitamin E is not supplied in the diet formulation, animal performance may be impaired.

Vitamin K

Vitamin K is a generic term used to characterize a homologous group of fat-soluble vitamins, consisting of a 2-methyl-1,4-naphthoquinone, with various isomers that differ in nature and side-chain length, and have anti- hemorrhagic effects (NASEM, 2016). In addition, there are some cases in the literature that describe a relationship between vitamin K and some vitamin K-dependent proteins (proteins C, S, Z, and M), suggesting other functions in addition to its clotting action. Proteins C and S play an anticoagulant rather than a procoagulant role in normal hemostasis (Stenflo, 1999), and are also involved in the regulation of bone turnover (Binkley e Suttie, 1995). The functions of proteins M and Z are still unknown.

The forms of vitamin K can be grouped as: phylloquinone (K_1), menaquinones (K_2), and menadiones (K). Phylloquinone and menaquinones are natural forms of vitamin K. Phylloquinone is extracted from plants (chloroplasts) and presents a phytyl sidechain composed of four isoprene units. Menaquinone is synthesized by bacteria from compounds containing active vitamin K through fermentation. This vitamin K form presents double bonds that succeed each other periodically. The synthetic menadione (K) has an active nucleus (2-methyl-1,4-naphthoquinone) and does not have sidechain. It is usually used as a vitamin K supplement (Dôres et al., 2001).

Phylloquinone and menaquinone have similar biological activity in the blood clotting process. However, the activity of menadiones and their derivatives depends on the degree of stability of the synthetic product; and they are usually less effective than phylloquinone on antagonist substances (sulfaquinoxaline, dicumarol, warfarin, or actinomycin D; McDowell, 2000).

Vitamin K_2 is synthesized in large amounts by rumen bacteria (Berchielli et al., 2006). Thus, vitamin K requirements of ruminants are met by a combination of their intake and ruminal and intestinal microbial biosynthesis, which may involve intestinal microorganisms such as *Escherichia coli*. Moreover, ruminants can absorb considerable amounts of vitamin K synthesized in the rumen and small intestine through active transport (Mendonça Júnior *et al.*, 2010). According to McDowell (2000), males are more susceptible to vitamin K deficiency in the diet than female animals, apparently due to estrogen stimulation of phylloquinone absorption.

PRACTICAL EVALUATION OF VITAMINS SUPPLEMENTATION IN BEEF CATTLE DIETS

Two studies were carried out concurrently to evaluate the effects of vitamin supplementation on the metabolism (Study 1) and productive performance (Study 2) of beef cattle under Brazilian conditions. Therefore, feed intake, nutrient digestibility, and ruminal parameters of Nellore cattle fed diets containing different vitamin

supplements were evaluated in Study 1 (Silva et al., 2022). In study 2, the effects of different vitamin supplements on feed intake, ADG, and feed efficiency of Nellore young bulls were evaluated (Andrade, 2022).

In both studies, animals were fed a basal diet composed of 30% corn silage and 70% concentrate (DM basis) with a 2 × 2 factorial scheme. The factors consisted of two fat-soluble vitamin blend (A, D, and E) supplementation levels (ADE− or ADE+) and two B vitamin blend (biotin, niacin, and thiamine) supplementation levels (B-blend− or B-blend+). Thus, the treatments consisted of no vitamin supplementation (ADE− B-blend−), supplementation with a B vitamin blend (ADE− B-blend+), supplementation with a fat-soluble vitamin blend (ADE+ B-blend−), or supplementation with a combination of these two blends (ADE+ B-blend+). Blends were included in the concentrate and the levels of vitamins supplementations in the diet were: 3.3 mg/kg DM of biotin (D-biotin), 111.1 mg/kg DM of niacin (niacin), 28.9 mg/kg DM of thiamine (thiamine hydrochloride), 6666.7 IU/kg DM of vitamin A (retinyl acetate), 5111.1 IU/kg DM of vitamin D (13% D3-Cholecalciferol and 87% 25-Hydroxyvitamin D3 - Hy-D®), and 70 IU/kg DM of vitamin E (DL-alpha tocopherol acetate).

Study 1

In this study, four rumen-cannulated Nellore bulls (age = 8 ± 1.0 months; initial BW = 289 ± 11.2 kg) were used in a 4 × 4 Latin square design, with a 2 × 2 factorial scheme. The experiment lasted 100 d, with four periods of 25 d each. Each period consisted of 14 d for dietary adaptation, 5 d for feces and urine collection, 3 d for omasal and ruminal digesta collection, and 3 d for ruminal emptying. The main results are described below. More details can be obtained in Silva et al. (2022).

Dry matter and nutrient intake, as well as ruminal digestibility were not affected ($P \geq 0.072$) by any of the vitamin supplementation (Table 15.3). The apparent total-tract digestibility of DM, organic matter (OM), and neutral detergent fiber corrected for ash and protein (apNDF) were not influenced by the experimental diets ($P > 0.114$). However, an increase in starch total-tract digestibility was verified with ADE supplementation ($P = 0.031$).

Individual and total VFA concentrations (mol/100mol), as well as lactate concentrations were not affected ($P \geq 0.133$) by vitamin supplementations (Table 15.4). Bulls that consumed the combination of ADE and B-blend supplementation had lower ruminal NH_3-N concentrations ($P=0.008$). The B-blend vitamin supplementation decreased ruminal pH ($P = 0.003$). Besides that, serum 25-hydroxyvitamin D (25(OH)D) concentrations increased ($P <0.01$), with mean values of 38.7 and 31.5 (ng/mL), respectively, for diets with ADE and ADE+B-blend supplementation (Figure 15.1).

Table 15.3 - Dry matter and nutrient intake, ruminal, and apparent total-tract digestibility of Nellore bulls receiving diets with or without vitamin blend supplementation

Item	Experimental Diets[1]				SEM[2]	*P*-value		
	ADE$^-$		ADE$^+$			B-blend	ADE	B-blend × ADE
	B-blend$^-$	B-blend$^+$	B- blend$^-$	B-blend$^+$				
Intake (kg/d)								
Dry Matter	7.3	7.2	7.3	6.7	0.54	0.312	0.545	0.558
Organic Matter	7.1	6.9	7.0	6.5	0.53	0.326	0.539	0.570
apNDF[3]	1.5	1.5	1.5	1.4	0.12	0.301	0.630	0.765
Starch	3.7	3.6	3.6	3.3	0.29	0.331	0.465	0.549
Intake (g/kg BW)								
DM	22.1	21.2	21.9	20.0	0.94	0.175	0.448	0.578
Ruminal (g/kg)								
Dry Matter	418.8	401.7	398.9	433.4	27.23	0.577	0.705	0.132
Organic Matter	494.7	477.2	475.4	510.4	23.35	0.557	0.637	0.111
apNDF	313.2	301.4	278.4	364.6	27.12	0.149	0.549	0.071
Starch	842.0	852.4	841.1	862.3	16.26	0.273	0.744	0.694
Apparent total-tract (g/kg)								
Dry Matter	690.8	706.1	693.4	725.4	15.42	0.062	0.331	0.450
Organic Matter	707.6	720.5	707.4	742.2	15.04	0.056	0.327	0.322
apNDF	512.0	500.3	497.5	551.1	24.70	0.281	0.345	0.114
Starch	876.4	897.3	907.8	918.9	12.30	0.141	0.031	0.625

[a–b] mean values in the same line with different letters are statistically different. [1] ADE$^-$ = No vitamin ADE supplementation; ADE$^+$= diet with fat-soluble vitamin blend (ADE) supplementation, B-blend$^-$ = No B vitamin blend (biotin, niacin, and thiamine) supplementation, B-blend$^+$= diet with B vitamin blend (biotin, niacin, and thiamine) supplementation.[2]Standard error mean. [3]Neutral detergent fiber corrected for residual ash and protein.

Table 15.4 - Effects of vitamin supplementation on ruminal pH, NH_3-N, VFA and lactate concentrations in Nellore bulls

Item	Experimental Diets [1]				SEM[2]	*P*-value		
	ADE$^-$		ADE$^+$			B-blend	ADE	B-blend × ADE
	B-blend $^-$	B-blend $^+$	B-blend $^-$	B-blend $^+$				
Rumen pH	6.1	5.8	6.0	5.9	0.27	0.003	0.679	0.24
Rumen NH_3-N, mg/dL	11.8 [a]	9.3 [b]	9.7 [b]	10.0 [b]	1.38	0.058	0.205	0.008
Total VFA, mmol/L	94.7	97.8	92.9	88.8	6.75	0.907	0.215	0.396
Individual, mmol/100mmol								
Lactate	4.8	4.5	4.2	4.2	0.40	0.768	0.341	0.749
Acetate	63.6	63.2	64.5	66.5	1.50	0.485	0.106	0.318
Propionate	21.5	23.6	22.0	19.9	1.27	0.972	0.174	0.091
Butyrate	10.2	8.7	9.3	9.4	0.50	0.198	0.917	0.137
Ratio A:P	3.1	2.8	3.2	4.1	0.52	0.423	0.136	0.180

[a–b] mean values in the same line with different letters are statistically different.
[1]ADE$^-$ = No vitamin ADE supplementation; ADE$^+$= diet with fat-soluble vitamin blend (ADE) supplementation, B-blend$^-$ = No B vitamin blend (biotin, niacin, and thiamine) supplementation, B-blend$^+$= diet with B vitamin blend (biotin, niacin, and thiamine) supplementation. [2]Standard error mean.

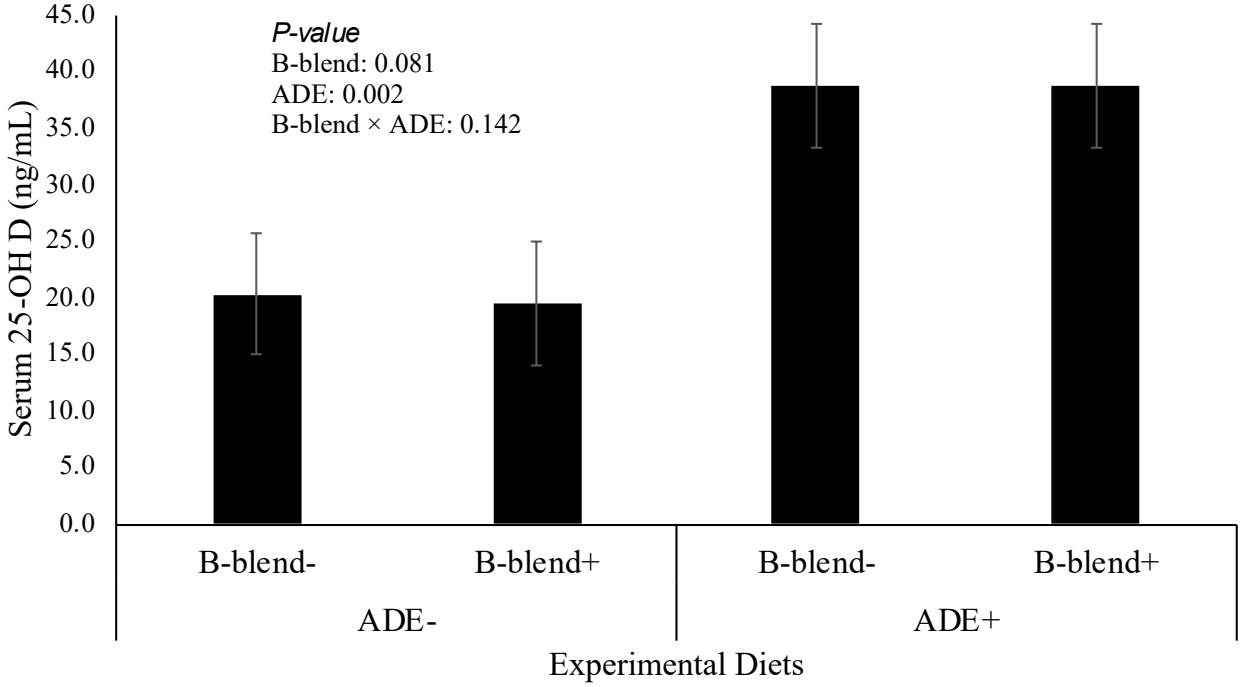

Figure 15.1 - Serum 25-hydroxyvitamin D [25(OH)D] concentrations in Nellore bulls fed diets with or without vitamin blend supplementation. ADE^- = No vitamin ADE supplementation; ADE^+= diet with fat-soluble vitamin blend (ADE) supplementation, B-$blend^-$ = No B vitamin blend (biotin, niacin, and thiamine) supplementation, B-$blend^+$= diet with B vitamin blend (biotin, niacin, and thiamine) supplementation.

Thus, this study showed that supplementing Nellore bulls with fat-soluble vitamin blend (ADE) in high concentrate-diets under Brazilian conditions increased starch total-tract digestibility.

Furthermore, supplementing Nellore bulls with a vitamin blend containing 25(OH)D_3 was a successful strategy for increasing circulating concentrations of 25(OH)D.

Study 2

In this study, 45 Nellore bulls (age = 8 ± 1.0 months; initial body weight = 261 ± 27.3 kg) were used. Five animals were slaughtered at the beginning of the experiment (REF) to determine the initial empty body weight (EBW) of the animals that remained in the experiment. The remaining 40 animals were randomly distributed into 4 groups of 10 animals each. The four treatments previously described were randomly assigned to each group. The experiment lasted 140 d. Dry matter ($P>0.05$) and OM (OMI) intakes were not influenced ($P>0.05$) by the experimental diets (Figure 15.2; A and B).

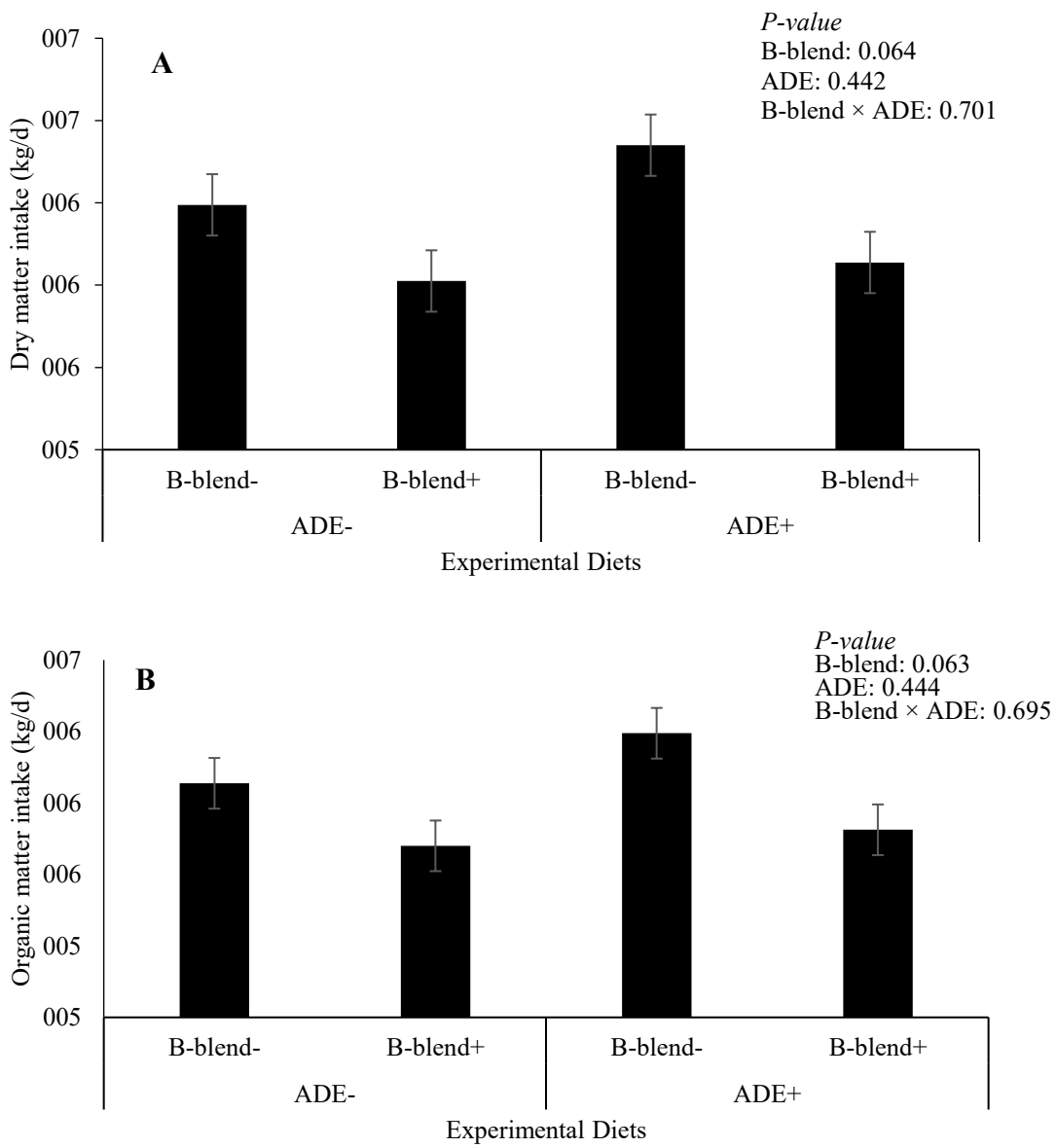

Figure 15.2 - Effect of fat- and/or water-soluble vitamins blends on intakes of dry matter (A) and organic matter (B) of young Nellore bulls. Treatments: ADE-/B-blend- = no vitamin supplementation; ADE-/ B-blend+ = supplemented with a water-soluble blend of vitamins biotin, niacin, and thiamine; ADE+/B-blend- = supplemented with a fat-soluble blend of vitamins A, D, and E; ADE+/B-blend+ = supplemented with both water and fat-soluble vitamin blends

No effects (P>0.05) of vitamin supplementation were observed on final body weight (fBW), final EBW (fEBW), ADG, empty body gain (EBG), hot carcass gain (HCG), cold carcass weight (CCW), hot carcass weight (HCW), backfat thickness (BFT), carcass length (CL), rib eye area (REA) and hot carcass yield (HCY; Table 15.5). Thus, no benefits were observed when supplementing water- (B-blend) and/or fat-soluble (ADE-blend) vitamins blends on DMI, OMI, productive performance, and carcass traits of young Nellore bulls receiving high grain diets under Brazilian feedlot.

Further research is needed regarding vitamin supplementation to feedlot diets. Thus, the committee of BR-CORTE current edition suggests the use of values recommended by NASEM (2016) and OVN (2016) for requirements of vitamins for beef cattle (Table 15.6.)

Table 15.5 - Effect of vitamin supplementation on animal performance and carcass traits of young Nellore bulls

Item[1]	Diets[2]					SEM[3]	P-value		
	REF	ADE-		ADE+			B-blend	ADE	B-blend × ADE
		B-blend-	B-blend+	B-blend-	B-blend+				
Number of animals	5	9	10	9	10	-	-	-	-
fBW (kg)	261	445	422	446	427	12.3	0.104	0.800	0.875
fEBW (kg)	234	408	386	410	390	11.7	0.089	0.811	0.962
ADG (kg/d)	-	1.25	1.14	1.26	1.19	0.054	0.120	0.608	0.693
EBG (kg/d)	-	1.19	1.08	1.20	1.12	0.050	0.075	0.624	0.834
HCG (kg/d)	-	0.77	0.72	0.75	0.74	0.032	0.268	0.946	0.594
CCW (kg)	-	258	244	259	247	8.1	0.131	0.823	0.882
HCW (kg)	155	268	256	266	258	7.9	0.211	0.995	0.824
BFT (mm)	-	7.16	6.58	6.33	6.34	0.768	0.717	0.502	0.711
CL (cm)	-	127	126	126	125	1.8	0.523	0.564	0.978
REA (cm^2)	55.9	75.8	73.2	77.1	75.8	2.44	0.449	0.441	0.792
HCY (%)	-	60.3	60.7	59.8	60.4	0.52	0.336	0.437	0.842

[1]fBW = Mean final body weight; fEBW = Final empty body weight; ADG = Average Daily Earnings; EBWG = Empty body weight gain; HDCG = Hot carcass gain; CCW = Cold carcass weight; HCW = Hot carcass weight; BFT = backfat thickness; CL = Carcass Length; REA = Loin eye area; HCY = Hot carcass yield.

[2]REF = reference group, reference animals were used to estimate the initial empty body weight, carcass, non-carcass and body composition of the other experimental animals. They were not included in the statistical analysis; (ADE-/B-blend-) = mineral supplementation without vitamins; (ADE-/B-blend+) = mineral supplementation with B vitamins (Thiamine = 28.9 mg/kg DM, Niacin = 111.1 mg/kg DM and Biotin = 3.3 mg/kg DM); (ADE+/B-blend-) = mineral supplementation with fat-soluble vitamins (A = 6,666.7 IU/Kg DM, D = 5,111.1 IU/Kg DM (13% D3 and 87% Hy-D®) and E = 70 IU/Kg DM); (ADE+/B-blend+) = mineral supplementation with B vitamins (Thiamine = 28.9 mg/kg DM, Niacin = 111.1 mg/kg DM and Biotin = 3.3 mg/kg DM) and fat-soluble vitamins (A = 6,666.7 IU/Kg DM, D = 5,111.1 IU/Kg DM (13% D3 and 87% Hy-D®) and E = 70 IU/Kg DM).

[3]The standard error of the mean for the treatments (ADE-/B-blend-) and (ADE+/B-blend+) are: [1]fBW = 13.0; fEBW = 12.3; ADG = 0.057; EBWG = 0.052; HDCG = 0.034; CCW = 8.5; HCW = 8.3; SFT = 0.809; CL = 1.8; REA = 2.57; HCY = 0.54.

TABLES OF VITAMIN REQUIREMENTS (NASEM, 2016 and OVN, 2016)

Table 15.6 - Requirement of fat-soluble and water-soluble vitamins for beef cattle according to NASEM (2016) and OVN (2016)

		Category/Phase					
Vitamins	Reference Table	[1]Calves, milk replacer (0-3 months)	Growing[2]	Fattening & finishing[2]	Pregnant Cows and Heifers[2]	Lactating Cows and Heifers[2]	Breeding bulls[2]
A[3]	OVN-DSM 2016 (IU/d)	20000-32000	25000-50000	40000-80000	40000-70000	40000-70000	50000-80000
	NASEM- 2016 (IU/ kg DM)	-	2200	2200	2800	3900	3900
B-carotene	OVN-DSM 2016 (mg/d)	100	-	-	300-500	300-500	-
D[3]	OVN-DSM 2016 (IU/d)	1400-1800	6000-90000	5000-70000	5000-10000	5000-10000	5000-10000
	NASEM- 2016 (IU/ kg DM)	-	275	275	-	-	-
E	OVN-DSM 2016 (mg/d)	100-150	200-300	500-2000[4]	300-500	300-500	300-500
	NASEM- 2016 (IU/ kg DM)	15-60	25-35[5]	25-35[5]	-	-	-
Tiamin	OVN-DSM 2016 (mg/d)	2.5-5	60-250[6]	60-250[6]	-	-	-
Biotin	OVN-DSM 2016(mg/d)	0.05-0.10	10-20[7]	10-20[7]	20	20	20

[1]Added per kg of powdered substitute; [2]Supplementary amount per animal per day; [3]Supplementary amount per animal per day; [4]Top level for longer color life, 100 to 120 days before slaughter; [5]400-500 IU/d for stressed calves newly received in confinement; [6]Higher level for cattle on high concentrate diets; [7]For optimal hoof health and meat marbling.

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