Comparative study of biotin supplementation on weight gain and occurrence of digital diseases in cattle (Bos taurus x Bos indicus)*

Estudo comparativo da suplementação com biotina sobre o ganho de peso e ocorrência de enfermidades digitais em bovinos (Bos taurus x Bos indicus)

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Abstract

This study aimed to assess the weight gain and the incidence of foot diseases in male, crossbred (Bos taurus x Bos indicus) bovines that were supplemented with biotin. An amount of 240 animals, supplemented or not with biotin, allocated in 12 groups of 20 animals, was assessed for a period of six months. The study was conducted during three years and the groups were divided according to the forage available, corn, corn residue and sorghum silage, and initial weights between 100 and 200 kg and between 200 and 300 kg. The statistical analyses used were the Tukey's Test, in triple factorial scheme (type of silage x use of biotin x initial body weight) and the Fisher's Exact Test, both at 5% significance level. The biotin supplementation in bovines did not influence weight gain and the incidence of foot diseases, however, when comparing only the type of forage, corn and sorghum silage provided higher weight gain than silage made of corn residue.

Keywords: Body weight, hoof, ruminants, vitamins

Resumo

Esse estudo teve como objetivo avaliar o ganho em peso e a ocorrência de enfermidades digitais em bovinos do sexo masculino, mestiços (*Bos taurus x Bos indicus*) que foram suplementados com biotina. Avaliaram-se, por um período de seis meses, 240 bovinos, suplementados ou não com biotina, alocados em 12 grupos de 20 animais. O estudo foi realizado durante três anos e os grupos foram divididos de acordo com o volumoso disponibilizado, silagem de milho, de resíduo de milho e de sorgo, e pesos iniciais entre 100 e 200 kg e entre 200 e 300 kg. As análises estatísticas empregadas foram o Teste de Tukey, em esquema de fatorial triplo (tipo de silagem x uso da biotina x peso corporal inicial) e o Teste Exato de Fisher, ambas em nível de significância de 5%. A suplementação com biotina nos bovinos não exerceu influência sobre o ganho em peso e a ocorrência de enfermidades digitais, mas, quando se comparou apenas o tipo de volumoso, a silagem de milho e sorgo, pode se observar um maior ganho em peso que a silagem confeccionada de resíduo de milho.

Palavras-chave: Casco, peso corporal, ruminantes, vitaminas

Introduction

Over the years, the Brazilian beef cattle farming has undergone intense changes, participating significantly in the local economy and reaching, according to data from the Instituto Brasileiro de Geografia e Estatística (IBGE, 2011), two hundred and twelve million cattle units of different breeds and exploited in three breeding systems: extensive, semi-extensive and intensive methods. Intensive farming features some advantages, such as reduced slaughter age, higher production flexibility and the use of a limited territory, favoring the release of foraging are as for other categories during the confinement period (QUADROS, 2014), in addition to controlled diet offering.

Despite intensive farming contributes positively to improve the bovine productive performance (COSTA et al., 2002), the type of nutrition used, the nutritional conditions and the overcrowding can interfere with the weight gain and health of animals. In intensive system, complications such as laminitis, sole ulcer, white line disease, foot dermatitis and septic pododermatitis are common, in addition to bloat, pneumonia and ammonia poisoning, considered multifactorial problems and difficult to control (GREENOUGH, 2007). In an attempt to solve those problems, biotin vitamin has

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been adopted in bovine supplementation (SILVA et al., 2010). Biotin is a cofactor of pyruvate carboxylase and propionyl-coenzyme A carboxylase, actively participating in body energetic metabolism, such as carbon dioxide fixation in aluconeogenesis. The use of biotin supplements can enhanced amino acid metabolism and increases dry matter intake (WEI et al., 2018). In the synthesis of keratinized tissues, biotin participates in the differentiation of keratinocytes and the production of the intercellular cementing substance (MULLING et al., 1999), thus being an important vitamin for the health of bovine hooves. The supplement aims to prevent and help the cure of hoof diseases (LISCHER et al., 2002), once it strengthens the hoof wall and helps the locomotion of animals when searching for food, thus minimizing weight losses (FITZGERALD et al., 2000).

Therefore, when considering the positive aspects of biotin supplementation and inconstant findings related to weight gain and foot diseases in intensively farmed bovines, it is evident that there are still unanswered questions about those matters. One of the most important questions on the subject, the increase of this vitamin in weight gain and the influence in reducing foot diseases in bovines confined and receiving separate diet including distinct forages still need to be investigated.

This study aimed to assess the weight gain and the incidence of foot diseases in male, crossbred (*Bos taurus* x *Bos indicus*) bovines, farmed intensively and supplemented with biotin.

Material and methods

The research was developed at Fazenda Tomé Pinto, in the Municipality of São Francisco de Goiás, State of Goiás, Brazil, owned by the Escola de Veterinária e Zootecnia da Universidade Federal de Goiás (EVZ/UFG), located at Rodovia BR 153, with permission of the Ethics Committee of the UFG, under protocol No. 150/2010.

The assessments were conducted between April and September, in the years 2010, 2011 and 2012. The study was divided into three experimental stages, according to the type of forage and period of year assessed, as specified below:

Stage I: Year 2010, with four experimental groups.

In stage I, 80 male crossbred (*Bos taurus x Bos indicus*), not castrated, clinically healthy (GREENOUGH, 2007; ROSEMBERGER, 1988) bovines between 6 and 20 months of age were used, allocated in four groups with 20 animals each (GI, GII, GIII and GIV) and corn silage was used as forage (Table 1). Concerning body weight, animals initial weighing between 100 and 200 kg were considered light units and those between 200 and 300 kg were considered heavy units. The farming adopted for all groups was intensive, conducted in 200 sqm pens, with a 10 sqm area per animal. The forage was provided at ease free at covered feeders, respecting a minimum space of 50 cm for every animal, and water was available in Australian-type fountains with capacity for 2,000 liters.

Table 1: Distribution of male crossbred (Bos taurus x Bos indicus)
intensively farmed bovines according to the group, body
weight, assessment period, forage used in the diet and biotin
supplementation, in the year 2010, at Fazenda Tomé Pinto
of the Escola de Veterinária e Zootecnia da Universidade
Federal de Goiás, in the Municipality of São Francisco de
Goiás, State of Goiás, Brazil

Group	Nr. of Animals	Body Weight (kg)	Weight Division	Year	Forage (Silage)	Biotin*
I	20	100 to 200	Light	2010	Corn	+
П	20	100 to 200	Light	2010	Corn	-
Ш	20	200 to 300	Heavy	2010	Corn	+
IV	20	200 to 300	Heavy	2010	Corn	-

*The symbol "+" was used for animals that received biotin addition. The symbol "-" was used for animals that received no biotin

Stage II: Year 2011, with four experimental groups.

In stage II, 80 male crossbred (*Bos taurus x Bos indicus*), not castrated, clinically healthy (GREENOUGH, 2007; ROSEMBERGER, 1988) bovines between 6 and 20 months of age were used, allocated in four groups with 20 animals (GV, GVI, GVII and GVIII) and corn stover residue was used as forage (Table 2). Housing conditions, diet offering, concentrate composition, clinical assessment moments and weighing of animals were identical to those instage I.

Table 2: Distribution of male crossbred (Bos taurus x Bos indicus)
intensively farmed bovines according to the group, body
weight, assessment period, forage used in the diet and biotin
supplementation, in the year 2011, at Fazenda Tomé Pinto
of the Escola de Veterinária e Zootecnia da Universidade
Federal de Goiás, in the Municipality of São Francisco de
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Group	Nr. of	Body	Weight	Year	Forage	Biotin*
	Animals	Weight (kg)	Division		(Silage)	
V	20	100 to 200	Light	2011	Residue	+
VI	20	100 to 200	Light	2011	Residue	-
VII	20	200 to 300	Heavy	2011	Residue	+
VIII	20	200 to 300	Heavy	2011	Residue	-

*The symbol "+" was used for animals that received biotin addition. The symbol "-" was used for animals that received no biotin

Stage III: Year 2012, with four experimental groups.

In this stage, 80 male crossbred (*Bos taurus* x *Bos indicus*), not castrated, clinically healthy (GREENOUGH, 2007; ROSEMBERGER, 1988) bovines between 6 and 20 months of age were used, allocated in four groups with 20 animals (GIX, GX, GXI and GVXII) and sorghum silage was used as forage (Table 3). Housing conditions, diet offering, concentrate composition, clinical assessment moments and weighing of animals were identical to those instages I and II.

Table 3: Distribution of male crossbred (Bos taurus x Bos indicus)
intensively farmed bovines according to the group, body
weight, assessment period, forage used in the diet and biotin
supplementation, in the year 2012, at Fazenda Tomé Pinto
of the Escola de Veterinária e Zootecnia da Universidade
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Group	Nr. of	Body Weight	ody Weight Weight		Forage	Biotin*
	Animals	(kg)	Division		(Silage)	
IX	20	100 to 200	Light	2012	Sorghum	+
Х	20	100 to 200	Light	2012	Sorghum	-
XI	20	200 to 300	Heavy	2012	Sorghum	+
XII	20	200 to 300	Heavy	2012	Sorghum	-

* The symbol "+" was used for animals that received biotin addition. The symbol "-" was used for animals that received no biotin

The concentrate supplied to all groups was composed of corn bran, soy bran and mineral mix (Performance®, Boi Forte Produtos Agropecuários, City of Goiânia, State of Goiás), representing 1.5% of body weight and amount adjusted according to the weight gain of animals. The total amount of the diet and the concentrate/forage concentration were adjusted through observations conducted on the occasion of diet replenishment, and significant left overs were not reported, which assured that the concentrate offered to the batches were consumed according to the adaptation recommended in the study. The vitamin was associated with the concentrate, calculated in order that every animal received daily 20 mg/animal/day (BERGSTEN et al., 2003) for the assessment of the biotin effect. The vitamin was provided to groups I, III, V, VII, IX and XI. Regardless of the group to which the animal belonged, the diet was offered twice a day, being the first in the morning, between 6 and 8 a.m., and the second in the afternoon, between the 2 and 4 pm. In 2010, corn silage was used as forage, in the subsequent year the silage was made of corn stover residue (Quero®, City of Nerópolis, State of Goiás) and lastly the sorghum silage.

The offering of concentrate before forage was adopted as nutrition farming conduct to assure that the concentrates, along with biotin, were entirely consumed, and such offering was supplied to animals, regardless of the group and the stage of the study, only after the total consumption of the concentrate offered.

Nutrition chemical assessments were conducted at the Departamento de Produção Animal¹(DPA) of the Universidade Federal de Goiás to determine the dry matter (DM), crude protein (CP), mineral matter (MM) and pH of silages offered (Table 4).

Table 4: Nutritional analyses of corn, corn residue
and sorghum silage, being 71.73% of total
digestible nutrients, 5.5% of NDF and 18%
protein, usedin the diet of crossbred (*Bos*
taurus x *Bos indicus*) intensively farmed
bovines, between 2010 and 2012, at Fazenda
Tomé Pinto of the Escola de Veterinária
e Zootecnia da Universidade Federal de
Goiás, in the Municipality of São Francisco
de Goiás, State of Goiás, Brazil

Demonsterne	Corn Corn Residu		ue Sorghum	
Parameters	Silage	Silage	Silage	
Dry Matter	26.6%	23.07%	25.47%	
Mineral Matter	4.01%	2.8%	3.79%	
Crude Protein	6.53%	5.5%	6.12%	
рН	4.12	3.35	3.7	

After15 days of adaptation, six monthly assessments were conducted, called Moments, to determine the body weight gain. Those assessments occurred at the initial Moment

(M0), at 30 days (M1), 60 days (M2), 90 days (M3), 120 days (M4), 150 days (M5) and 180 days (M6). All assessments were conducted after 16 hours of complete fasting. At the same time, the incidence of foot diseases, encompassing 15 types of diseases, was also assessed. Such diseases were subclinical and clinical laminitis, septic pododermatitis, inter-foot vegetative pododermatitis, foot dermatitis, sole ulcer, claw ulcer, stress line, heel growth, heel erosion, sole erosion, claw growth, white line disease, cracks in the hoof and interdigital abscess. It was considered that the 20 animals from each group could suffer from all those diseases and that every animal could suffer froman association of the different illnesses.

Among the diseases, animals that were reluctant to move due to pain, were stepping on the ground mainly on heels, showed mechanical unbalance and after 6 to 8 weeks of the process onset showed lesion son the sole and on the surface of the hoof such as hematomas, bleeding or ulcers and stress lines (LISCHER et al., 2002) were considered as suffering from clinical laminitis. Cases marked by an asymptomatic period, followed by secondary lesions characterized by changes such as bleeding and sole ulcer, lesions in the white line (VERMUNT, 2007) and claw ulcer (OSSENT & LISCHER, 1998; LISCHER et al.,2002) were considered as subclinical laminitis.

The data were subjected to analysis of variance and Tukey's Test, in triple factorial scheme, involving the type of silage x use of biotin x initial body weight. The joint analysis of the three years of assessment was considered to assess the weight gain of bovines. The rate of positive results was computed in the assessment of incidence of digital lesions, and every animal could show 15 types of lesions. For that, the Fisher's Exact Test was applied individually. In all situations, a 0.05 significance level was adopted and software R (R Development Core Team 2012) was used to conduct the statistical analyses.

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Results and discussion

In table 5, the average weight gain, concerning the six assessment months, of the different groups of bovines examined can be observed. The effects of the use of biotin vitamin, the type of silage adopted and the initial weight of groups on the weight gain are demonstrated. In 2010, the bovines fed with corn silage, belonging to groups I, II, III and IV showed, respectively, an average weight gain of 125 kg, 111.65 kg, 123.6 kg and 133.4 kg. In 2011, the bovines fed with corn residue silage allocated in groups V, VI, VII and VIII obtained, sequentially, an average weight gain of 109.25 kg, 103.4 kg, 103.65 kg and 105.6 kg. The average weight gain, in 2012, in groups IX, X, XI and XII fed with sorghum silage was, consecutively, of 120.35 kg, 119.4 kg, 149.05 kg and 133.7 kg.

The biotin used in the oral supplementation of animals provided no statistically difference regarding weight gain. The use of the vitamin provided an average weight gain of 122.44 kg. Bovines not supplemented gained 118.54 kg. However, it was possible to detect a favorable gain of 3.9 kg for the groups treated with biotin, regardless of the forage provided. The average weight gain of 130.62 kg obtained for the sorghum silage showed no difference when compared to the 125.25 kg found for corn silage. However, comparing the average weight gain of 105.6 kg obtained for the corn residue silage, difference was noticed among the other types of silage, indicating that the type of forage influenced the weight gain, regardless of the biotin supplementation.

There was significant statistical difference when comparing the initial weight of the bovines allocated in the 12 groups. The groups whose initial weight was considered heavy obtained higher average of weight gain, with value of 126.04 kg. On the other hand, in the group with initial weight assessed as light, the average weight gain was 114.94 kg. This result had no relation with the forage offered to the animals either with the use of biotin supplemented to bovines. Table 6 shows the effect of type of silage when compared to the initial weight.

The type of silage offered to animals showed relation with the initial weight. Regardless of the biotin supplementation, the groups, with the initial body weight considered heavy, fed with corn or sorghum silage, showed higher average weight gain, while the bovines fed with corn residue silage showed lower average weight gain differing statistically.

The probability of each animal suffer from the 15 lesions resulted in a total of 300 podal lesions, due to the existence of 20 animals in every group. Thus, and being aware of the association of different hoof changes diagnosed in the same animal, the 12 groups of animals that were supplemented with biotin (Groups I, III, V, VII, IX and XI) showed no statistical difference compared to those that did not receive the vitamin in the diet (Groups II, IV, VI, VIII, X and XII) with regard to podal lesions. In addition, there was no statistical difference when analyzing the ratio of incidence of foot diseases individually with the type of silage adopted and the initial body weight. The quantification of the presence incidence of foot disease in the animals may be observed in table 7.

Factor	Levels	[•] Weight Gain (kg)	**p Value	
Distin	Biotin addition	122.44ª	0.245	
Biotin	No biotin addition	118.54ª		
	Corn silage	125.25ª		
Silage	Corn residue silage	105.6 ^b	< 0.001	
	Sorghum silage	130.62ª		
Maight	Light	114.94 ^b	0.001	
Weight	Heavy	126.04ª	0.001	

'Means followed by the same letter, within the same factor, are statistically equal, by the Tukey's test, at 5% of significance. "Probability values of F-test for the isolated factors, of the variance analysis

Table 6: Analysis of the interaction between the factors of initial weight and type of silage offered to bovines, regarding the variable weight gain (kg), between 2010 and 2012, at Fazenda Tomé Pinto of the Escola de Veterinária e Zootecnia da Universidade Federal de Goiás, in the Municipality of São Francisco de Goiás, State of Goiás, Brazil

"Maight		**p Value		
*Weight	Corn	Corn Residues	Sorghum	-
Light	118.375 ^{Aa}	106.575 kg ^{Aa}	119.875 kg ^{aA}	0.016
Heavy	132.125 kg ^{aB}	104.625 kg ^{Ba}	141.375 kg ^{aB}	

'Means followed by the same letter, in the line, and the same capital letter, in the column, are statistically equal, by the Tukey's test, at 5% of significance. "Probability value of F-test for the interaction between the initial weight and the type of silage, of the analysis of variance

Table 7: Absolute frequencies and percentages of lesions in animals supplemented and not supplemented with biotin, in groups with different initial weights and different types of silage, between 2010 and 2012, at Fazenda Tomé Pinto of the Escola de Veterinária e Zootecnia da Universidade Federal de Goiás, in the Municipality of São Francisco de Goiás, State of Goiás, Brazil

Weight	Silage -	Biotin				*p Values
weight		With		With	out	p values
		Ν	(%)	Ν	(%)	
Light	CS	10/300	3,33	12/300	4	0.3149
Heavy	CS	9/300	3	11/300	3.66	0.2929
Light	CR	18/300	6	23/300	7.66	0.3819
Heavy	CR	18/300	6	22/300	7.33	0.3984
Light	SS	9/300	3	11/300	3.66	0.2929
Heavy	SS	15/300	5	22/300	7.33	0.3141

Probability values of the Fisher's Exact Test. CS: Corn Silage; CR: Corn Residue Silage; SS: Sorghum Silage; N: Individual probability of showing 300 podal lesions

The ruminal pH measured in the early hours after the diet offering showed average values of 5.1 for animals fed with corn residue silage, 5.9 for corn silage-based forage and 5.7 for bovines fed with sorghum silage, indicating that this parameter was below the average values considered normal for the specie.

Initially, by analyzing the negative results and the possible factors responsible for the failure of the biotin supplementation in crossbred (*Bos taurus x Bos indicus*) bovines, it was verified that some aspects involving weight gain and this vitamin still require further clarifications. According to SILVA et al. (2009), biotin promotes higher growth of hoof and assists in the prevention and recovery of foot disease in bovines, but they did not mentioned the possibility of increasing weight gain. In the present study, it is possible to observe that the influence of biotin on this parameter is related to the pH, the quality of forages and the rate of concentrated ration ingested by animals, objects also considered in the assessment of weight gain in animals.

Considering the isolated analysis of category, age and breed of the animals used in the study, it is likely that those parameters have not influenced negatively the parameters analyzed, including the weight gain. However, when associated with intensive farming and the type of forage offered, they may have limited the maximum expression of the benefits originated from the biotin supplementation. Thus, following this reasoning, it is possible to infer that the effect of the forage used in animal nutrition seemed to be more important than the vitamin, indicating the complexity of the subject. Nevertheless, studies by LEAN & RABIEE (2011) reported favorable results of this vitamin on the production and level of milk protein and fat. SILVA et al (2009), studying the action of biotin, verified that the vitamin provides higher growth of the hoof compared to its wear in young bovines (Bos taurus x Bos indicus) farmed extensively. LEAN & RABIEE (2011) stated that biotin may be related with the slightest onset of podal problems.

Still considering the key factors that may have limited the effect of biotin, the low pH and the lower quality of corn residue silage, when compared to the other forages assessed, could have also minimized the beneficial effects of this vitamin. It is possibly because both the pH and the quality of the forage may have triggered immediate consequences on the decline of the ruminal pH, influencing in the absorption of this vitamin. According to ABEL et al. (2001), the use of biotin by ruminal microbiota is pH dependent, if there is a decrease in the amount of infusoria and failure in the absorption of this vitamin, in cases of acidification of the ruminal fluid. SATO et al. (2012) stated that shortly after the food intake, the ruminal pH decreases dramatically, and it may result in cases of transitory subacute ruminal acidosis.

The fact that corn residue silage is an industrial by-product is another negative aspect to be analyzed. In this case, it is not possible to ignore that the forage shows industrial contaminants, impairing the nutritional value of the ensiled material. Therefore, even if there has been intake of 1.5% of concentrated ration by the animals, weight gain was not significant, possibly because the by-product used as forage intensified, in part, the reduction of the ruminal pH, reducing the production and absorption of biotin and resulting in less weight gain of bovines. MOREIRA et al. (2009) pointed out the existence of a tendency of decrease in the forage intake by bovines when the amount of concentrate is increased in the diet. This reinforces the possibility that the biotin intake and weight gain of animals have been impaired. On the other hand, SCHWAB et al. (2006) assured that high-production bovines, due to being commonly fed with high amounts of concentrate and corn silage, when supplemented with biotin, may obtain better performance. However, they did not mention if the corn residue-based forage would have the same effect.

Considering the possibility of the intensive farming, the average amount of fiber and the increased volume of concentrate ingested by animals may influence the synthesis of biotin, another aspect to be analyzed is the age of the bovines. On average, the age of the animals allocated in groups I, II, V, VII, IX and X was seven months and in the other groups (III, IV, VI, VIII, XI and XII) 16 months. The first ones were younger, recently weaned and had just changed extensive by intensive farming. Therefore, at the beginning of the study, the rumen of those bovines was adjusting to a new full forage-based diet situation. However, as in intensive farming animals began to receive 1.5% of the concentrate related to body weight, it is natural that the forage intake tends to be lower, circumstance which may also have influenced the synthesis and value of biotin. The studies by GOMEZ et al. (18) have warned that the synthesis of biotin may show 80% reduction when the amount of forage ingested is reduced from 80% to 50%, because, under such circumstances, the ruminal microbiota becomes less able to produce this vitamin. Nevertheless, in this study, considering that the groups of bovines supplemented with this vitamin gained 3.9 kg more than those not supplemented, it is possible to infer that supplementation was not completely innocuous.

Findings contradicting the expectations showed that the biotin had no interference in the incidence of foot diseases. However, it is mentioned that many foot diseases in bovines are related to multifactorial causes and, under those circumstances, even if the biotin has a preventive action on the incidence of such diseases, the environment, the compact floor and the excess of moisture at the beginning of the rainy season may have masked the effect of supplementation with this vitamin. Still, even considering the influence of the environmental factors and the type of silage used in the nutrition of the animals, it is observed that the amount of cases of foot diseases showed a reduction tendency when the bovines were supplemented with biotin. LISCHER et al. (2002) studied the effect of oral biotin supplementation on healing sole ulcers in lactating cows. The researchers obtained optimistic results, since the study showed that the histological nature of the newly formed epidermis, to the extent that the repair of the sole ulcers evolved, was significantly better after the 50 days of treatment. SILVA et al. (2009) analyzed the effects of biotin supplementation administered orally on the growth and structure of the hoof of young Girolando breed animals, concluding that the average growth of the hoof of the animals supplemented with biotin was higher than those in the control group.

As the supplementation with biotin presented no significant results for the prevention of foot diseases in the bovines of this study, the results found contradict the assertions of LEAN et al. (2013). Although it is difficult to find a convincing explanation for those findings, a possibility for the failure of treatment, even having showed good results in other research, may be related to feeding in collective feeders. Under those circumstances, some bovines may have not ingested the minimum amount of vitamin and other more dominant animals may have ingested higher values. This situation should be considered when planning

the use of biotin in the supplementation of bovines, in addition to the need of conducting experimental studies to ensure the intake of the daily dose of 20 mg/kg of body weight of the vitamin established by BERGSTEN et al. (2003). The authors observed lower incidence of sole bleeding in animals supplemented with that dose of biotin. However, even being necessary to define criteria to supplement bovines with biotin, authors such as SILVA et al. (2010), LISCHER et al. (2002) and HIGUCHI et al. (2004) stated that this vitamin maybe recommended for the prevention of such diseases. HIGUCHI et al. (2014) assured that

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the supplementation with biotin increases rigidity and decreases the moisture rate of the hoof, indicating promising results for preventing foot diseases in bovines.

Conclusion

The conclusion of this study points out that the supplementation with biotin in young male crossbred (*Bos taurus x Bos indicus*) bovines, intensively farmed and receiving different forages had no influence on weight gain and incidence of foot diseases.

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