LUIZ CARLOS OLIVEIRA DE SOUSA

DOES FREQUENCY OF SUPPLEMENTATION AFFECT THE PERFORMANCE OF CATTLE UNDER GRAZING IN TROPICAL PASTURES?

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

Adviser: Edenio Detmann

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Assent:

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Edenio Detmann
Adviser

I dedicate my master's degree to my grandfather ***Luiz*** *(in memoriam). I am grateful to God for all the moments I had the opportunity to spend with him. I treasure every word and every teaching of yours with great affection. I know that, wherever you are, you will be very happy with this achievement. You will always be in my heart.*

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“If there are many theories about something, it is because the unknown predominates”

(Katsumasa Hoshino)

ABSTRACT

SOUSA, Luiz Carlos Oliveira de, M.Sc., Universidade Federal de Viçosa, February, 2022. **Does frequency of supplementation affect the performance of cattle under grazing in tropical pastures?** Adviser: Edenio Detmann.

Our objective was to evaluate the effects of supplementation frequency on performance and voluntary intake of cattle under grazing in tropical pastures by using a meta-analytical approach. The dataset used to evaluate the voluntary intake was compiled from 18 experiments carried out in Brazil between 2009 and 2021, totaling 75 treatment means. In order to evaluate the animal performance, treatment means were collected from 19 experiments carried out in Brazil between 2003 and 2018, totaling 69 treatment means. The data were analyzed using meta-analysis techniques, considering the random effect of the experiments on the model parameters. There was no effect of supplementation ($P \geq 0.11$) or supplementation frequency ($P \geq 0.18$) on forage intake and dietary digested organic matter content (DOM). On the other hand, supplementation increased ($P<0.01$) total intake, dietary crude protein (CP) and CP-to-DOM dietary ratio. Supplementation enhanced ($P<0.01$) average daily gain (ADG), but without any influence of supplementation frequency ($P \geq 0.84$). The ADG increased linearly ($P<0.05$) as forage CP increased, but no difference was detected ($P>0.05$) among supplementation frequencies. The additional weight gain (AWG) caused by supplementation decreased as forage CP increased and became null at 150 g CP/kg dry matter. There was a quadratic pattern ($P<0.01$) of the AWG in response to variations in both supplement intake and supplemental CP intake, but no effect of different frequencies was detected on this variable ($P>0.05$). Maximal responses occur when 5.0 g of supplement and 1.14 g of supplemental CP per kg BW were provided. Reducing supplementation frequency down to thrice a week does not affect voluntary intake and performance of cattle under grazing in tropical pastures. There is a positive response to protein supplementation on animal performance even with medium to high-quality forages. However, that response decreases as forage crude protein increases and becomes null at 150 CP/kg dry matter.

Keywords: Average daily gain. N status. Protein supplementation.

RESUMO

SOUSA, Luiz Carlos Oliveira de, M.Sc., Universidade Federal de Viçosa, fevereiro de 2022. **A frequência de suplementação afeta o desempenho de bovinos sob pastejo em pastagens tropicais?** Orientador: Edenio Detmann.

Nosso objetivo foi avaliar os efeitos da frequência de suplementação no desempenho e consumo voluntário de bovinos sob pastejo em pastagens tropicais utilizando abordagem meta-analítica. O banco de dados utilizado para avaliar o consumo voluntário foi compilado a partir de 18 experimentos conduzidos no Brasil entre 2009 e 2021, totalizando 75 médias de tratamento. Para avaliar o desempenho animal, foram coletadas médias de tratamento de 19 experimentos conduzidos no Brasil entre 2003 e 2018, totalizando 69 médias de tratamento.

Os dados foram analisados utilizando técnicas de meta-análise, considerando o efeito aleatório de experimentos nos parâmetros do modelo. Não houve efeito da suplementação ($P \geq 0,11$) ou da frequência de suplementação ($P \geq 0,18$) no consumo de forragem e no conteúdo de matéria orgânica digerida da dieta (MOD). Por outro lado, a suplementação aumentou ($P<0,01$) o consumo total, a proteína bruta (PB) dietética e a razão entre os conteúdos de PB e MOD dietética. A suplementação melhorou ($P<0,05$) o ganho médio diário (GMD), mas sem qualquer influência da frequência de suplementação ($P \geq 0,84$). O GMD aumentou linearmente à medida que a PB da forragem aumentou, mas nenhuma diferença foi detectada ($P>0,05$) entre frequências de suplementação. O ganho de peso adicional (GPA) causado pela suplementação diminuiu à medida que a PB da forragem aumentou e tornou-se nulo a 150 g PB/kg de matéria seca. Houve padrão quadrático ($P<0,01$) do GPA em resposta a variações tanto no consumo de suplemento como no consumo de PB suplementar, mas nenhum efeito de diferentes frequências foi detectado nessa variável ($P>0,05$). Máximas respostas ocorrem quando 5,0 g de suplemento e 1,14 g de PB suplementar por kg de PC foram fornecidas. Reduzir a frequência de suplementação para três vezes por semana não afeta a ingestão voluntária e o desempenho de bovinos em pastejo em pastagens tropicais. Há resposta positiva à suplementação proteica no desempenho animal mesmo em forragens de alta qualidade. No entanto, essa resposta diminui à medida que a proteína bruta da forragem aumenta e torna-se nula a 150 PB/kg de matéria seca.

Palavras-chave: Ganho médio diário. Status de N. Suplementação proteica.

SUMMARY

1. INTRODUCTION

Tropical grasses are unlikely available as a balanced diet for grazing cattle as they may have multiple nutritional constraints throughout the year. As such, supplementation programs must be designed to overcome deficiencies in basal nutritional resources and, thereafter, provide nutrients directly to meet the animals' requirements according to the goals established in the system planning (Detmann et al., 2010). Nevertheless, supplementation strategies for grazing cattle includes costs related to acquisition of inputs, labor and equipment necessary for daily distribution of supplements. Thus, reducing frequency of supplementation has become an important issue in several studies carried out in tropical (Canesin et al., 2007; Morais et al., 2014; Moraes et al., 2017) and non-tropical regions (Beaty et al., 1994; Farmer et al., 2004; Drewnoski and Poore, 2012).

The main nutritional support for using infrequent supplementation for grazing cattle relies on nitrogen (N) recycling mechanisms (Farmer et al., 2004). From this, the increase in N status by supplementation event could supply the ruminal N demands on days when the animals are not supplemented (Detmann et al., 2014a). Besides N recycling, other mechanisms seem to be associated with dietary N conservation in infrequently supplemented animals. Several authors have found a decoupling between rumen ammonia and blood urea concentrations (Reis et al., 2020; Rufino et al., 2020; Silva, 2020). Even though the highest rumen ammonia concentrations are observed concomitantly with supplementation, the blood urea concentration peaks only 24 hours after supplementation. This pattern may be associated with a delay in the activity of the ornithine cycle enzymes (Cappellozza et al., 2015). In this case, N compounds that uptake ammonia in hepatocytes (e.g., glutamine) may act as a short-term N reserve, allowing to maintain an adequate N status in animal metabolism (Häussinger et al., 1992; Allen et al., 2009). Furthermore, infrequently supplemented animals may increase renal urea reabsorption on days without supplementation (Reis et al., 2020). Recently, Costa (2021) brought into evidence that some anabolic stimuli could aid in the dietary N conservation for infrequently supplemented animals. This was supported by an increased concentration of IGF-1 on the day after supplementation, which concurs with the blood urea peak.

Despite advances in understanding the mechanisms associated with infrequent supplementation, the effects of supplementation frequency on performance and voluntary intake remain unclear. In the tropics, some studies have indicated either a decreased (Silva-

Marques et al., 2018; Rufino et al., 2020; Silva, 2020) or an unaltered (Canesin et al., 2007; Morais et al., 2014; Moraes et al., 2017) performance and voluntary intake compared to daily supplemented animals. To the best of our knowledge, those differences are likely associated with variations in the supplement amount and composition, as well as the different supplementation frequencies adopted in each study. Hence, a meta-analytical approach may uncover a general pattern of animal response to a decreased supplementation frequency, allowing inferences on a wide range of productive conditions and considering the by-experiment random variation on the parameters that express animal performance.

Therefore, our objective was to evaluate the effects of the supplementation frequency on performance and voluntary intake of cattle under grazing in tropical pastures using a meta-analytical approach.

2. MATERIAL AND METHODS

This study used data from previously published papers and did not require an approval from the Animal Care and Use Committee of the Universidade Federal de Viçosa to be performed.

2.1. Data acquisition

The dataset used to evaluate voluntary intake was compiled from 18 experiments carried out in Brazil between 2009 and 2021, totaling 75 treatment means (Appendix). The literature search was performed in the Web of Science, Scopus, and Scielo databases. The following keywords were used: "supplementation frequency" or "infrequent supplementation", and "intake".

The experiments were carried out with cattle either under grazing (Urochloa sp. and Megathyrsus sp.) or fed tropical forages (Cynodon sp., Megathyrsus sp., and Urochloa sp.) and supplemented with different frequencies. Thus, during the process of contemplation and graphic analysis of the data, the following supplementation frequencies were found: 7X, 6X, 5X, 3X, and 1X (meaning that animals were supplemented seven, six, five, three, and one day a week, respectively). However, only the 7X, 5X, and 3X frequencies were considered for analysis, due to the low number of observations in relation to the other supplementation frequencies. Data from control (without supplementation) were also recorded when available. It is noteworthy that, in some experiments, treatments were evaluated with supplementation every three days. In these cases, we considered that supplementation was performed thrice a week (3X).

Treatment means of the following variables were recorded in the dataset: forage crude protein (CP), forage intake, dry matter intake, digested organic matter content in the diet (dietary DOM), dietary CP-to-DOM ratio (CP:DOM), supplement intake, supplement CP content, and supplemental CP intake. In some experiments, the organic matter digestibility was not available and, therefore, it was not possible to calculate the dietary DOM and CP:DOM, which caused a small heterogeneity for these variables in the dataset regarding the number of treatment means.

For experiments with grazing cattle, fecal output was estimated using external markers, namely: chromic oxide (Cr2O3), titanium dioxide (TiO2), or hydroxyphenyl propanol (LIPE®). Forage intake was estimated using indigestible neutral detergent fiber, indigestible acid detergent fiber or, indigestible dry matter. Forage sampling was performed by the hand-plucking method.

Additionally, for each selected study, other information was added to the dataset, such as: genetic group (Zebu, European, or crossbred), animal category (bulls, steers, and heifers), stocking method (rotational or continuous stocking), and season of the year (dry season, rainy season, and their transitions). However, due the possibility of cross-classification, categorical variables were not taken into account in the formal analysis, with exception for season of the year, which was indirectly incorporated in the dataset by using forage quality characteristics.

The dataset for the animal performance evaluation was formed from 19 experiments carried out in Brazil between 2003 and 2018, totaling 69 treatment means (Appendix). Publications were selected through literature searches in the Web of Science, Scopus, and Scielo databases. The following keywords were used: “supplementation frequency” or “infrequent supplementation”, and “performance” or “weight gain”.

All experiments were carried out with growing and finishing cattle under grazing (Urochloa sp. and Megathyrsus sp.) and supplemented with different frequencies. It must be emphasized that only the 7X, 5X, and 3X frequencies were considered for analysis, due to the low number of observations regarding the other supplementation frequencies. Data from control (without supplementation) were also recorded when available.

Treatment means of the following variables were recorded in the dataset: forage CP, forage availability, initial body weight (BW), average daily gain (ADG), supplement intake, supplement CP content and supplemental CP intake. Furthermore, several other characteristics were recorded in the dataset, as follow: experiment time, animals’ genetic group (Zebu, European, or crossbreds), stocking method (rotational and continuous stocking), and season of the year (dry season, rainy season, and their transitions). However, as previously described, the categorical variables were not taken into account in the formal analysis. On the other hand, it should be noted that experiments with beef cows were not considered in the current meta-analysis.

The additional weight gain (AWG, kg/d) was calculated as the difference between the ADG of supplemented animals and non-supplemented animals. Additionally, the variables related to supplement intake were calculated as a function of animal BW, as described by Detmann et al. (2014a):

$$ABW = iBW + \left(\frac{DIE}{2} \times ADG\right) \quad (1),$$

$$SUP = \frac{SUPM}{ABW} \times 1000 \quad (2),$$

$$SCPI = \frac{SUPM \times SCP}{ABW} \quad (3),$$

where ABW is the average BW of the animal (kg), iBW is the initial BW of the animal (kg), DIE is the number the days in the experiment, SUP is the supplement intake (g/kg BW), SUPM is the absolute mass of the supplement consumed by the animal (kg/d), SCPI is the supplemental CP intake (g/kg BW) and SCP is the supplement CP (g/kg DM).

2.2. Statistical analyses

Data were analyzed by meta-analysis techniques (St-Pierre, 2001), considering the random effect of different experiments on the model parameters. The choice of the best (co)variance structure was based on the lowest value of the Akaike's information criterion with correction. All variance components were estimated by the restricted maximum likelihood method and the degrees of freedom were estimated by the Kenward-Roger method.

Overall, comparisons between supplementation forms were performed by decomposing its sum of squares into orthogonal effects as follow: 1) control (without supplementation) versus supplemented animals. 2) evaluation of the linear and quadratic effect of reducing supplementation frequency. When pertinent, comparisons were performed using the forage CP content as a concomitant variable, which allowed adjusting for differences among seasons.

Linear regression models were also fitted to assess the effect of both supplementation and forage CP (an indicator of variation in forage quality) on animal performance. The models used to describe AWG were parameterized to pass through the origin (i.e., intercept = 0) (Detmann et al., 2014a). When pertinent, categorical variables were included in the models through dummy variables. The coefficients of determination (r^2/R^2) of the adjusted models were calculated as the square of the correlation between predicted and observed values. Influential data on model parameters were identified and removed from the dataset based on Cook's distance pattern.

All statistical analyzes were performed using the MIXED procedure of SAS 9.4 considering 0.05 as the critical level of probability for the occurrence of type I error.

3. RESULTS

3.1. Voluntary intake

The dataset used to evaluate the voluntary intake is summarized in Table 1. The dataset included animals fed low- to medium-quality forage (forage CP and CP:DOM ranging from 36 to 93 g/kg and 75 to 179 g/kg, respectively), receiving supplements that provided diets with either deficit or excess of dietary N (dietary CP and CP:DOM ranging from 71 to 193 g/kg and 118 to 371 g/kg, respectively).

There was no effect of supplementation ($P \geq 0.11$) or supplementation frequency ($P \geq 0.18$) on forage intake and dietary DOM (Table 2). On the other hand, supplementation enhanced ($P<0.01$) total intake, dietary CP, and CP:DOM when compared to control. On average, supplementation increased CP:DOM from 119 g/kg to 180 g/kg. Among supplemented animals, a quadratic effect ($P<0.02$) of the supplementation frequency was found on dietary CP and CP:DOM. At first glance, this pattern indicated that animals supplemented five days a week had higher and lower values of dietary CP and CP: DOM, respectively. However, the 5X presented a lower number of treatments means than the other supplementations schemes, which probably led to a slightly overestimated supplement intake. The effect of 5X supplementation on dietary CP was almost negligible compared to 3X and 7X (+2.5 g CP/kg DM on average). Moreover, the lower CP:DOM reflects the higher supplement intake observed for 5X, which increased the DOM intake from supplement and diluted dietary CP. Thus, the observed quadratic effect does not represent a true effect of the supplementation scheme itself.

3.2. Animal performance

The dataset used to assess the performance is summarized in Table 3. The dataset encompassed animals fed low- to high-quality forage (here indicated by both forage CP and ADG, which ranged from 32 to 156 g/kg and -0.160 to 0.940 kg/d, respectively). Also, range variation of both supplement intake (ranged from 0.9 to 9.6 g/kg BW) and ADG (ranged from 0.130 to 1.17 kg/d) indicates that the data used in this meta-analysis comprised systems of low- to high-technological levels.

Supplementation enhanced ($P<0.01$) ADG when compared to control (Figure 1). On average, ADG was increased from 0.413 kg/d to 0.656 kg/d for control and supplemented animals, respectively. Nevertheless, supplementation frequency did not affect ($P \geq 0.84$) the animal performance.

The ADG increased linearly ($P<0.05$) as forage CP increased (Figure 2A). However, no difference ($P>0.05$) was detected among supplementation frequencies. Thus, the different frequencies were clustered in a single category called supplemented animals. In this sense, different patterns of response to forage CP were observed for control and supplemented animals ($P<0.01$). Overall, control's ADG increased by 0.0077 kg/d in response to one unit of forage CP. On the other hand, for each forage CP unit, the supplemented animals' ADG increased by 0.0047 kg/d (see model details in Figure 2A). It must be emphasized that adjusted model showed no lack of fit ($P>0.85$) and adequately explained the ADG pattern (Figure 2B). When evaluating the adjusted model of ADG as a function of forage CP by both control and supplemented animals, the break-even point (i.e., control ADG equals supplemented ADG) would be achieved when forage CP approaches 150 g/kg DM (Figure 2A).

The overall effects of the amount and composition of supplements were interpreted using the AWG. Firstly, it must be highlighted that no effect of different frequencies was detected on this variable ($P>0.05$), so the supplemented animals were interpreted as a single and homogeneous group. Secondly, the amount and composition of supplements could not be used in a same model, a probable effect of collinearity between supplement amount and CP content. Thus, the models were adjusted separately for the amounts of supplement and supplemental CP.

There was a quadratic pattern ($P<0.01$) of AWG in response to variations in both supplement intake (Figure 3A) and supplemental CP intake (Figure 4A). Overall, both adjusted models showed no lack of fit ($P \geq 0.74$, Figures 3B and 4B). From the adjusted models, maximal responses of AWG were estimated at 5.0 g of supplement and 1.14 g of supplemental CP per kg BW. In this case, the maximum response in terms of AWG would be achieved by providing supplements close to 228 g CP/kg DM.

We did not find any effect ($P>0.05$) of forage availability on animal performance.

4. DISCUSSION

Overall, we did not observe any effect of supplementation on forage intake. In the tropics, improvements in forage intake have been associated with both supply of N compounds to rumen fibrolytic microorganisms and the metabolic adequacy of absorbed nutrients (Detmann et al., 2014a). Whereas supplementation was able to increase dietary CP above the minimum required to maximize microbial growth and fiber degradation (80-100 g CP/kg DM; Lazzarini et al., 2009; Detmann et al., 2014a), an increase in forage intake would be expected, but this was not observed.

On the other hand, it seems logical to state that supplementation was able to increase N availability in animal metabolism. Such observation can be supported by evaluating the CP:DOM. On average, the supplementation increased the CP:DOM from 119 to 180 g/kg. This ratio has been established as an indicator of intake adequacy in the tropics, as it reflects both ruminal and metabolic events (Poppi and McLennan, 1995; Detmann et al., 2014a). According to Reis et al. (2016), forage intake would be optimized when CP:DOM is raised up to 220 g/kg. Hence, although the increase of CP:DOM has been lower than recommended to optimize intake, positive responses on forage intake could be expected, which was not observed in our study. Despite of the lack of effect on forage intake, supplementation increased ADG on average by 243 g/d. It seems to indicate that, at least partially, metabolic or post-digestive effects of supplements were more prominent in the animals' response than any change in intake and digestive characteristics.

Several studies in the tropics have reported that protein supplementation increases performance and, or N balance in cattle fed either low-quality (Franco et al., 2017; Almeida et al., 2018; Rufino et al., 2020) or high-quality (Batista et al., 2016; Figueiras et al., 2016; Reis et al., 2020) forages; with concurrent either small or null effects on fiber degradation, microbial growth, and forage intake. However, those results have been always associated with an increased N status in animal metabolism.

Theoretically, the term "N status" defines the quantitative and qualitative availability of N compounds for different physiological functions in animal metabolism, including functions associated with the metabolism of other compounds (e.g., energy) (Detmann et al., 2014a). In this sense, the N usage by animal metabolism would follow an order of priority, according to the importance of each function, as follow: survival, maintenance, and production (e.g., tissue synthesis and deposition). Hence, accretion of N as tissues would occur only after the highest priority functions have been supplied (e.g., N recycling to the rumen) (Detmann et al., 2014a).

In the tropics, evidences in this regard have been obtained from the evaluation of ruminal nitrogen balance (RNB). Several studies have observed negative RNB regardless of forage quality (Batista et al., 2016; Oliveira et al., 2020; Reis et al., 2020). This pattern means that the flow of nitrogen from the rumen to the abomasum is greater than the N intake. Under this condition, a greater proportion of dietary N will be directed towards N recycling, which increases the participation of microbial N from recycled N (Batista et al., 2016; 2017; Oliveira et al., 2020). Thus, there will be less N available for other functions (e.g., tissue synthesis and deposition), which results in lower N retention and utilization efficiency (Detmann et al., 2017).

In the current study, the average dietary CP of the control was 58 g/kg DM. Thus, it can be considered that animals were under dietary N deficiency. On the other hand, supplementation was able to increase dietary CP to 112 g/kg DM, on average. According to Detmann et al. (2014a), supplementation should improve dietary CP up to 124 g/kg DM to achieve an equilibrium between N intake and flow into the abomasum (i.e., RNB = 0), which would improve N availability for tissue synthesis and deposition. Hence, it can be assumed that supplementation was able to overcome, at least partially, the deficiencies of N compounds in animal metabolism, which explains the increased ADG for supplemented animals compared to control.

Our initial concern in the current investigation was to quantify the effect of supplementation frequency on voluntary intake. In this sense, we did not observe any effect of reducing supplementation frequency on forage intake. Several studies have reported reduced forage intake in infrequently supplemented animals, especially on the day that all animals are supplemented (Drewnoski and Poore, 2012; Rufino et al., 2020; Silva, 2020). Overall, these effects have been attributed to either an excessive amount of supplemental N (Rufino et al., 2020) or high amounts of supplement given at once (Drewnoski and Poore, 2012), or still associated with very long intervals between supplementation events (Silva, 2020).

To our knowledge, there are three possible reasons for the lack of effects of decreased supplementation frequency on forage intake. First, the supplemental CP intake for infrequently supplemented animals was, on average, 1.19 g/kg BW. The additional CP provided by the supplementation led to diets with CP and CP:DOM of 111 g/kg DM and 193 g/kg respectively. In the tropics, the negative effects of supplementation on forage intake become more prominent when dietary CP and CP:DOM are greater than 145 g/kg DM and 220 g/kg, respectively (Detmann et al., 2014a; Reis et al. al., 2016). This explains, at least partially, the lack of effect on forage intake for daily and infrequently supplemented animals. Second, supplement intake for infrequently supplemented animals was, on average, 3.2 g/kg BW. Given this and considering that the supplements were essentially proteinaceous (on average, 391 g CP/kg DM), any negative effect of excessive energy on forage intake would be unlikely. Finally, this study only covers animals supplemented down to thrice a week. Thus, changing supplementation frequency down to thrice a week seems to be a safe limit since as the interval among supplementation exceeds three days the amount of supplement per supplementation event increases, which in turn amplifies the negative effects of excessive protein or energy on forage intake (Drewnoski and Poore, 2012; Silva, 2020).

Such statements regarding forage intake are supported by evaluating animals' performance. Generally, negative effects of reduced supplementation frequency on animal performance have been associated with both a decrease in forage intake (as previously discussed) and an increase in urinary N losses, which in turn leads to a decrease in N retention and utilization efficiency (Farmer et al., 2004; Silva-Marques et al., 2018; Silva, 2020). However, such statements do not agree with the performance results obtained herein.

When evaluating the overall effects of supplementation frequency on animal performance, we did not detect any difference on the of the animals' ADG. Subsequently, we evaluated the effect of supplementation frequency taking into account for forage quality, here represented by forage CP. The ADG increased linearly as forage CP increased in the same way for both daily and infrequently supplemented animals. Thus, regardless of forage quality, reducing supplementation frequency did not affect animal performance, which corroborates several studies carried out in the tropics (Canesin et al., 2009; Miorin et al., 2016; Moraes et al., 2017). This seems to indicate that regardless of forage quality, the same mechanisms are involved in the conservation of dietary N in animal metabolism when infrequent supplementation is provided to animals.

On the other hand, from the adjusted model, it was possible to observe a different pattern between control and supplemented animals. The model pointed out that non-supplemented animals respond better to an improvement in forage quality compared to supplemented animals. In terms of management, it allows inferring that, whenever possible, any investment in forage quality must be a priority, as forage production costs are lower than any investment in concentrate feeds and feeding. The difference between the two slopes

(Figure 2A) represents the additional weight gain provided by supplementation. Thus, the main inference is that the response to supplements decreases as forage quality increases, as stated by Detmann et al. (2014b). It should be emphasized that the estimated AWG with supplementation for a forage with 90 g CP/kg DM approaches 200 g/d. Our findings align with established concept based on previous studies performed in Australia (Poppi and McLennan, 1995) and Brazil (Paulino et al., 2008), which have shown that, even with high-quality forage, there is a potential AWG of 200-300 g/d to be explored by using supplementation.

Nevertheless, the adjusted model showed that a positive AWG with supplementation occurs when forage CP raises up to 150 g/kg DM. We should highlight that our dataset covers only information regarding weight gain on an animal basis and no information concerning weight gain per unit of area was taken into account. Thus, the model indicates that any investment in terms of supplementation would not result in positive responses on an animal basis when forage CP exceeds 150 g/kg DM. From this point on, the expected economical return of supplementation must be calculated taking into account the impacts on systemic production indexes (e.g., gain per area, stocking rate).

However, there are three additional issues here. First, grazed forages with more than 150 g CP/kg DM are not a reality for most livestock production systems in the tropics (Detmann et al., 2014b). Such forage quality would only be observed in intensive grazing systems with high N fertilization and rotational stocking. Thus, as reported by Detmann et al. (2014a), responses to protein supplementation can occur throughout the year, even with high-quality tropical forages. Second, as previously discussed, it would be necessary to increase the dietary CP to values close to 124 g CP/kg DM in order to ensure an adequate N availability for the various physiological functions (e.g., tissue synthesis and deposition) and decrease the relative participation of N recycling to supply ruminal demands. Finally, negative metabolic effects of supplemental CP are observed when dietary CP exceeds 145 g CP/kg DM (Detmann et al.,2014a). The combination of these two last arguments seems to explain the positive response to supplementation even with high-quality forages (i.e., up to 150 g CP/kg DM), as observed in this study.

We also evaluated the effects of supplementation frequency taking into account both the amount of supplement and supplemental CP on AWG. Overall, both daily and infrequently animals presented the same quadratic pattern in response to increases in supplement intake and supplemental CP intake. This means that, regardless of the amount of supplement and supplemental CP, reducing supplementation frequency does not affect animals' response. From a theoretical point of view, it could be expected that infrequently supplemented animals will achieve maximum response at smaller amounts of supplement intake and supplemental CP, since the negative effects of excessive energy or protein are amplified as the interval between supplementation increases (Drewnoski and Poore, 2012; Silva, 2020). However, this pattern was not observed in our study. To the best of our knowledge, it seems reasonable to assume that, besides any possible mechanisms of adaptation to supplementation schemes, the animals were not subjected to conditions in which the negative effects of supplementation frequency on animal performance could be intensified (e.g., excessive energy or protein), as supplement and supplemental CP intakes ranged from 1.0 to 7.3 and 0.3 to 1.5 g/kg BW, respectively.

From the adjusted model, maximal responses to supplementation were estimated at 5.0 g of supplement and 1.14 g of supplemental CP per kg BW. From this, the additional gain would be maximized with a supplement CP close to 228 g CP/kg DM. At first glance, these values would be recommended for both daily and infrequent animals supplemented. In fact, Simioni et al. (2009) did not observe any negative effect in reducing the frequency of supplementation by cattle grazing low-quality forage supplemented with protein supplements up to 6 g/kg BW. Nevertheless, we should highlight that the values found in our study are a general recommendation, as we did not find any relationship with forage CP, which seems unlikely. Therefore, both supplement amount and supplemental CP must be adjusted according to variations in forage quality.

Concerning to overall effects of the amount and composition of supplements on animal performance, the decreasing in additional gain above the aforementioned limits may be due to the deleterious effects of excessive N on forage intake, which in turn can decrease animal performance. In fact, some authors have found a decrease in weight gain due to the excess of N provided by supplementation (Detmann et al., 2004; Barros et al., 2015).

5. CONCLUSIONS

Reducing supplementation frequency down to thrice a week does not affect the voluntary intake and performance of cattle under grazing in tropical pastures. There is a positive response of protein supplementation on animal performance even with medium to high-quality forages. However, that response decreases as forage crude protein increases and becomes null at 150 g CP/kg DM.

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Table 1. Overall description of the dataset used to evaluate voluntary intake characteristics.

Item[a]	Statistics				
	Mean	Minimum	Maximum	s	n
Only forages					
Forage CP (g/kg DM)	59	36	81	17.2	11
Forage intake (g/kg BW)	15.6	9.3	24.6	3.99	11
Dietary CP (g/kg DM)	58	37	80	17.1	11
Dietary DOM (g/kg DM)	498	266	622	104.9	9
CP:DOM (g CP/kg DOM)	119	75	179	38.2	9
Supplemented					
Forage CP (g/kg DM)	66	36	93	19.4	64
Forage intake (g/kg BW)	15.8	9.30	26.3	3.86	64
Total intake (g/kg BW)	18.9	12.1	29.8	3.95	64
Supplement intake (g/kg BW)	3.09	0.200	7.60	2.152	64
Supplement CP (g/kg DM)	460	139	1140	317.1	64
Supplemental CP intake (g/kg BW)	1.12	0.220	3.50	0.777	64
Dietary CP (g/kg DM)	114	71	193	29.3	64
Dietary DOM (g/kg DM)	549	272	786	119.7	41
CP:DOM (g CP/kg DOM)	194	118	371	50.1	41

[a] Dietary DOM, digested organic matter content in diet; CP:DOM, ratio between dietary CP and dietary DOM.

Table 2. Voluntary intake and dietary characteristics in cattle under grazing in tropical pastures supplemented at different frequencies.

Item[b]	Supplementation[a]				P value[c]		
	Control	3X	5X	7X	C vs. S	L	Q
Forage intake (g/kg BW)[d]	16.2±1.09	16.1±0.86	19.4±1.51	16.3±0.86	0.41	0.83	0.18
Total intake (g/ kg BW)[d]	16.2±1.12	19.2±0.88	23.4±1.54	19.3±0.88	0.001	0.92	0.62
Supplement intake (g/kg BW)	-	3.12±0.425	4.05±0.729	2.96±0.425	-	-	-
Supplemental CP intake (g/kg BW)	-	1.19±0.186	1.62±0.316	1.08±0.186	-	-	-
Dietary CP (g/kg DM)	58±7.9	111±6.2	114±11.2	112±6.2	<0.001	0.94	0.008
n	11	30	6	28			
Dietary DOM (g/kg DM)	498±39.8	555±35.7	631±69.6	548±35.7	0.11	0.88	0.86
CP:DOM (g/kg)	119±15.8	193±13.8	156±28.4	191±13.7	0.004	0.91	0.014
n	9	19	3	19			

[a] Control, without supplementation; 3X, 5X, and 7X, supplemented three, five, or seven days a week.

[b] Dietary DOM, digested organic matter content in diet; CP:DOM, ratio between dietary CP and dietary DOM.

[c] C vs S, control versus supplementation; L, Q, linear and quadratic effects of supplementation frequency.

[d] Comparisons performed using the forage CP content as a concomitant variable.

Table 3. Overall description of the dataset used to evaluate animal performance

Item[a]	Statistics				
	Mean	Minimum	Maximum	s	n
Only forages					
Forage CP (g/kg DM)	73	32	147	34.7	11
Forage availability (ton DM/ha)	6.13	2.00	11.9	3.909	11
ADG (kg/d)	0.445	-0.160	0.940	0.3967	11
Supplemented					
Forage CP	78	32	156	33.1	58
Forage availability (ton DM/ha)	6.40	2.00	19.6	3.801	58
Supplement intake (g/kg BW)	4.34	0.900	9.60	2.184	58
Supplement CP (g/kg DM)	279	133	585	106.4	58
Supplemental CP intake (g/kg BW)	1.12	0.300	2.39	0.549	58
AWG (kg/d)	0.255	0.050	0.540	0.1479	31
ADG (kg/d)	0.637	0.130	1.170	0.2696	58

[a] ADG, average daily gain; AWG, additional weight gain in relation to animals without supplementation (control).

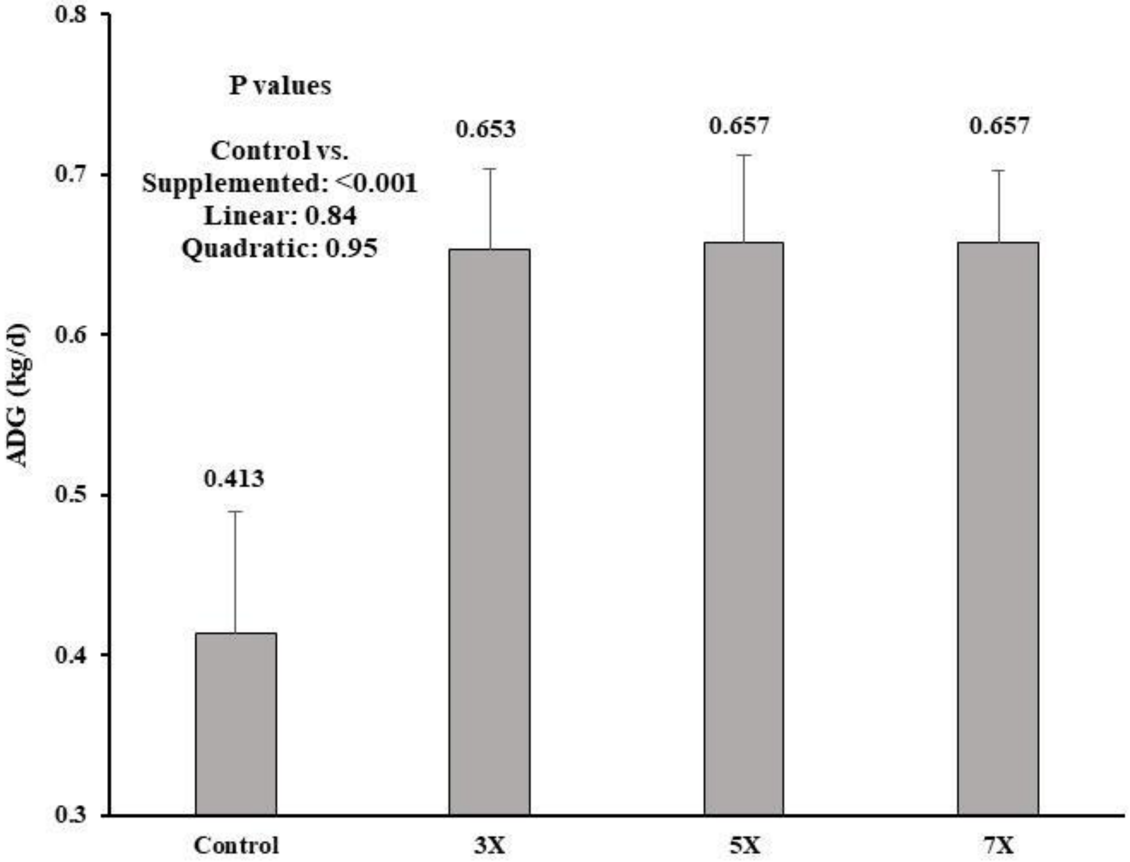
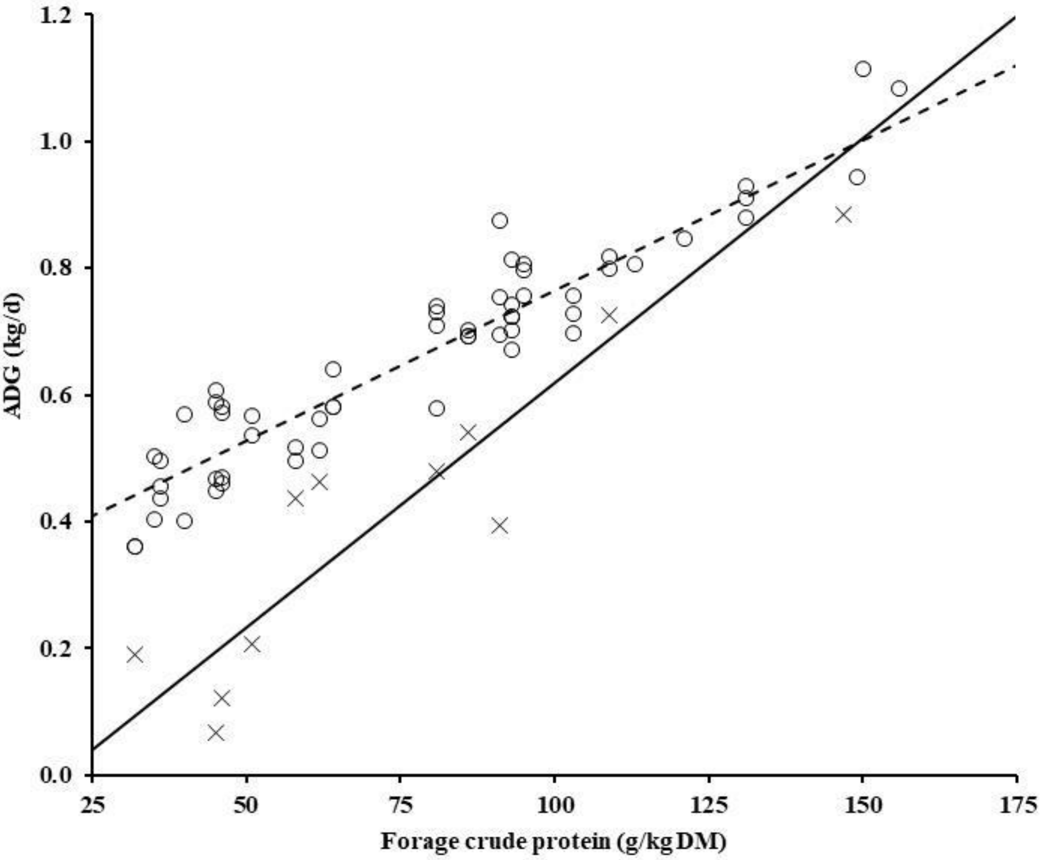
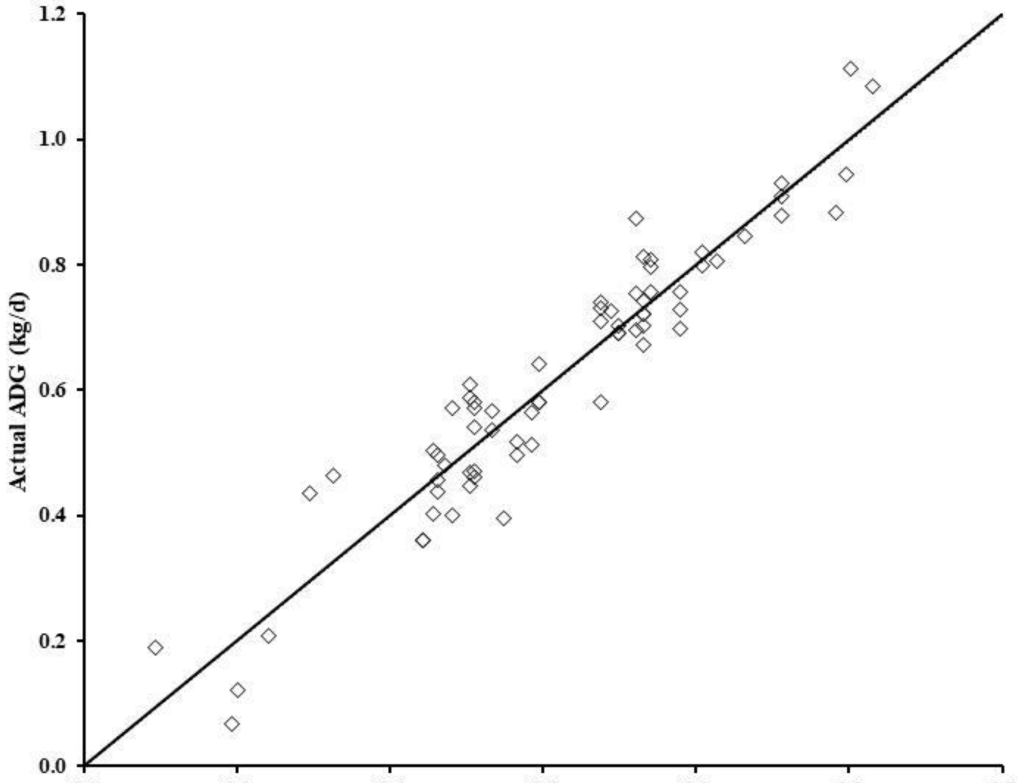


Figure 1. Least square means of average daily gain (ADG) of cattle under grazing in tropical pastures without supplementation (control) or supplemented with different frequencies (3X, 5X, and 7X: supplemented three, five or seven days a week). Comparisons performed using forage crude protein content as a concomitant variable.

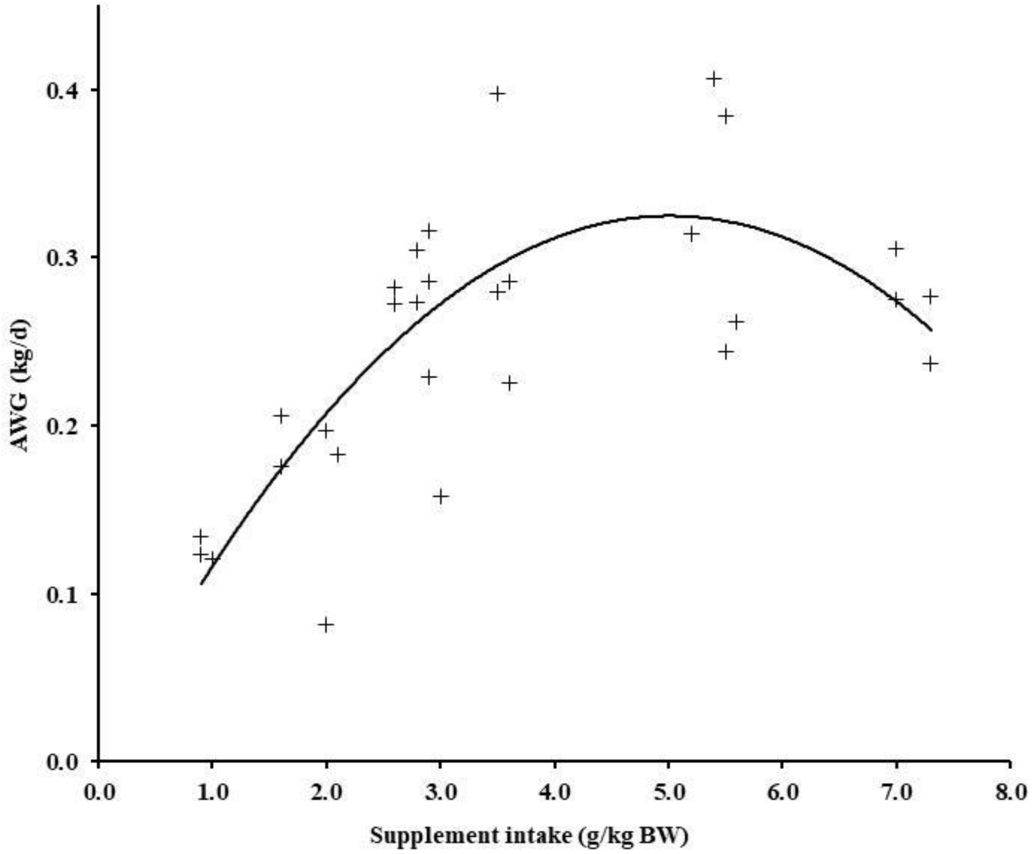

ADG (kg/d)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
25
50
75
100
125
150
175
Forage crude protein (g/kg DM)

(A)

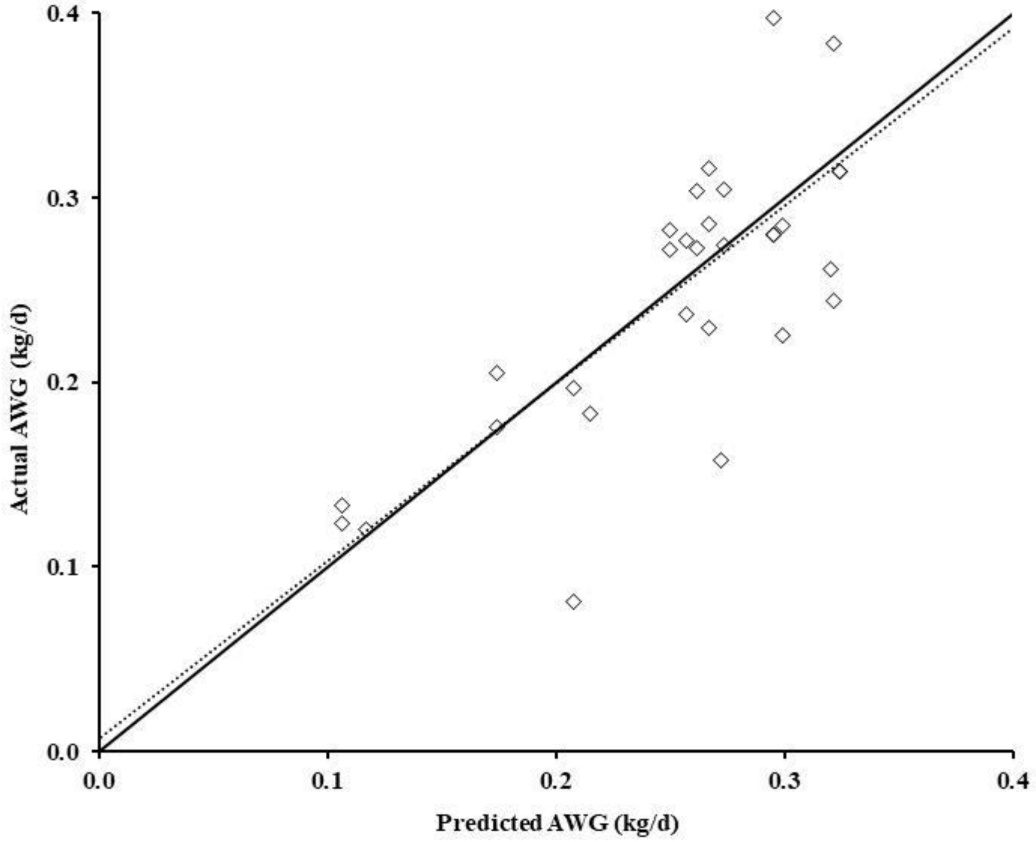

Actual ADG (kg/d)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0.0
0.2
0.4
0.6
0.8
1.0
1.2
Predicted ADG (kg/d)

(B)

Figure 2. Relationship between average daily gain (ADG) and forage crude protein (FCP) content in animals under grazing in tropical pastures receiving or not supplements [A – ADG according to FCP: $\hat{Y} = -0.1529 + 0.4449 \times D + 0.007722 \times FCP - 0.00299 \times FCP \times D$, where D=0 for control and D=1 for supplemented, $s_{XY} = 0.18$, $R^2 = 0.953$, × and solid line represent control, ○ and dashed line represent the supplemented. The data points were adjusted for the random effect of experiments; B = relationship between predicted and actual ADG: $\hat{Y} = -0.0096 + 1.0157 \times X$, P ($\beta_0 = 0$ and $\beta_1 = 1$): 0.85, the solid and dashed lines represent the equality ($Y = X$) and least square straight line, respectively).

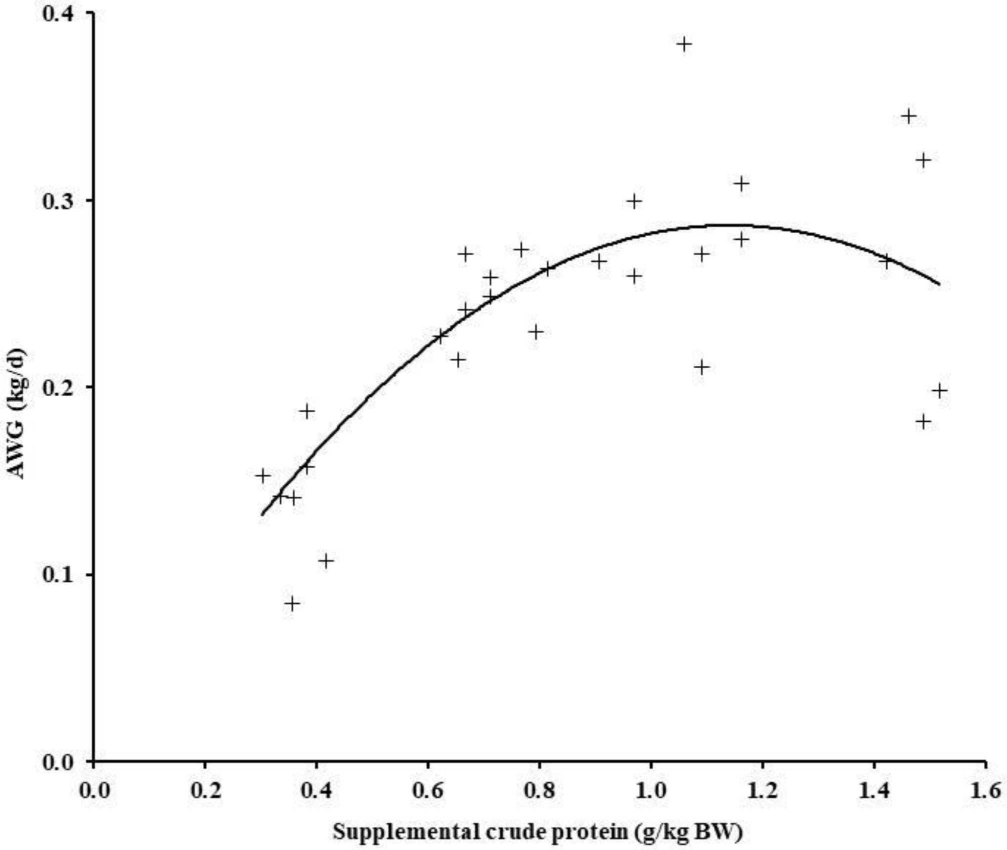

AWG (kg/d)
0.0
0.1
0.2
0.3
0.4
0.0
1.0
2.0
3.0
4.0
5.0
6.0
7.0
8.0
Supplement intake (g/kg BW)

(A)

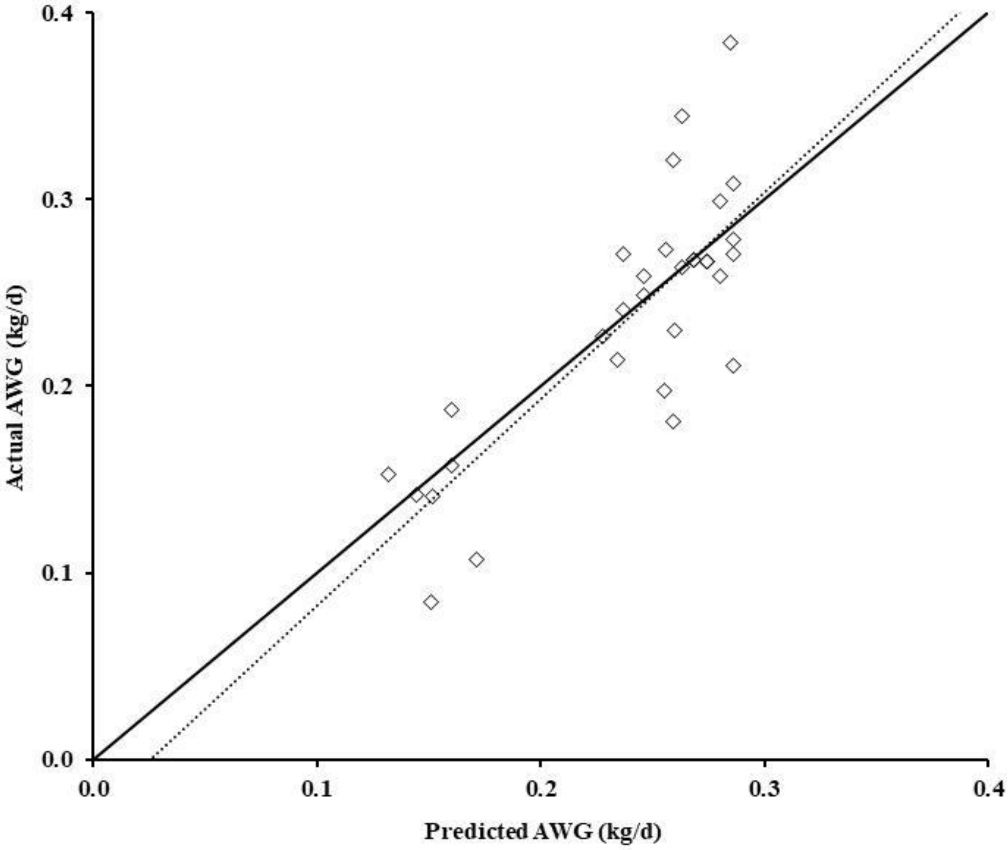

Actual AWG (kg/d)
0.0
0.1
0.2
0.3
0.4
0.0
0.1
0.2
0.3
0.4
Predicted AWG (kg/d)

(B)

Figure 3. Relationship between additional weight gain (AWG) in relation to animals without supplementation and supplement intake (SUP) in animals under grazing in tropical pastures with supplementation [A – AWG according to SUP: $\hat{Y} = 0.1296 \times SUP - 0.01293 \times SUP2$, sXY = 0.13, $R^2 = 0.881$]. The data points were adjusted for the random effect of experiments; B = relationship between predicted and actual AWG: $\hat{Y} = -0.0177 + 1.0431 \times X$, P ($\beta0 = 0$ and $\beta1 = 1$): 0.86, the solid and dashed lines represent the equality (Y = X) and least square straight line, respectively).


0.4
0.3
0.2
0.1
0.0
AWG (kg/d)
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
Supplemental crude protein (g/kg BW)

(A)


0.4
0.3
0.2
0.1
0.0
Actual AWG (kg/d)
0.0
0.1
0.2
0.3
0.4
Predicted AWG (kg/d)

(B)

Figure 4. Relationship between additional weight gain (AWG) in relation to animals without supplementation and supplemental CP intake (SCPI) in animals under grazing in tropical pastures with supplementation [A – AWG according to SCPI: $\hat{Y} = 0.5023 \times SCPI - 0.2202 \times SCPI2$, $sXY = 0.11$, $R^2 = 0.926$; The data points were adjusted for the random effect of experiments; B = relationship between predicted and actual AWG: $\hat{Y} = -0.0133 + 1.0431 \times X$, P ($\beta 0 = 0$ and $\beta 1 = 1$): 0.74, the solid and dashed lines represent the equality (Y = X) and least square straight line, respectively).

APPENDIX

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