Volume 58, January-December 2024, ISSN: 2079-3480

ANIMAL SCIENCE

CUBAN JOURNAL OF AGRICULTURAL SCIENCE

Journal site: https://cjascience.com Cu-ID: https://cu-id.com/1996/v58e09

IN VITRO EFFECT OF TREES FORAGES INCLUSION FROM THE AMAZONIAN PIEDMONT IN SUPPLEMENTS FOR CATTLE

EFECTO *IN VITRO* DE LA INCLUSIÓN DE ARBÓREAS FORRAJERAS DEL PIE DE MONTE AMAZÓNICO EN SUPLEMENTOS PARA BOVINOS

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The *in vitro* effects of the inclusion of three native species and adapted to the Amazonian piedmont on supplements for cattle were evaluated. A total of seven treatments were constituted: T1) control (concentrate without trees), T2 and T3 (20 and 40 % of *Trichantera gigantea* inclusion, respectively), T4 and T5 (20 and 40 % of *Piptocoma discolor* inclusion, respectively), and T6 and T7 (20 and 40 % of *Hibiscus rosa-sinensis* inclusion, respectively). The *in vitro* technique of gas production was applied and the *in vitro* degradability of the dry matter and organic matter was determined. A completely randomized design with four repetitions per treatment was used. The higher gas production corresponded to the control (T1), although did not showed differences with respect to the treatment with 40 % *Piptocoma* (T5) (p=0.016). The lower values of accumulated gas production were obtained when including 20 % de *Piptocoma* (T4), which not differ of the treatments T2, T5, T6 and T7. The highest values of *in vitro* degradability of the dry matter corresponded to the control (T1) and the supplements with 20 % of *Trichantera* inclusion (T2) and 20 and 40 % of *hibiscus* (T6 and T7) (p<0.008). The lower degradability value was for T5. The highest figures of *in vitro* digestibility of organic matter corresponded to the control and to the supplement with 20 % of *Ttrichantera* (T2) and with *hibiscus* (T6 and T7) (p<0.0001). The rest of treatments had the fallowing performance: T4 > T3 > T5. It is concluded that the inclusion in the concentrate of 20 % of *T. gigantea* and 20 and 40 % of *H. rosa-sinensis* did not affected the DM and OM degradability of the supplement and improved the nutrition partition to higher deposition of the fermented matter as microbial biomass, when decreasing the gas production with respect to the concentrate without trees.

Key words: *fermentation, livestock, nutrition, Putumayo*

Se evaluaron los efectos *in vitro* de la inclusión de tres forrajeras nativas y adaptadas al pie de monte amazónico en suplementos para bovinos. Se conformaron siete tratamientos: T1) control (concentrado sin arbóreas), T2 y T3 (20 y 40 % de inclusión de *Trichantera gigantea*, respectivamente), T4 y T5 (20 y 40 % de inclusión de *Piptocoma discolor*, respectivamente) y T6 y T7 (20 y 40 % de inclusión de *Hibiscus rosa-sinensis*, respectivamente). Se aplicó la técnica *in vitro* de producción de gas y se determinó la degradabilidad *in vitro* de la materia seca y materia orgánica. Se utilizó diseño completamente aleatorizado con cuatro repeticiones por tratamiento. La mayor producción de gas correspondió al control (T1), aunque no mostró diferencias con respecto al tratamiento con 40 % de *Piptocoma* (T5) (p=0.016). Los menores valores de producción acumulada de gas se obtuvieron al incluir 20 % de *Piptocoma* (T4), lo que no difirió de los tratamientos T2, T5, T6 y T7. Los mayores valores de degradabilidad *in vitro* de la materia seca correspondieron al control (T1) y los suplementos con 20 % de inclusión de *Trichantera* (T2) y 20 y 40 % de *Hibiscus* (T6 y T7) (p<0.008). El menor valor de degradabilidad fue para el T5. Las mayores cifras de digestibilidad *in vitro* de la materia orgánica correspondieron al control y a los suplementos con 20 % de *Trichantera* (T2) y con *Hibiscus* (T6 y T7) (p<0.0001). El resto de los tratamientos tuvieron el siguiente comportamiento: T4 > T3 > T5. Se concluye que la inclusión en el concentrado de 20 % de *T. gigantea* y de 20 y 40 % de *H. rosa-sinensis* no afectó la degradabilidad de la MS y la MO del suplemento y mejoró la partición de nutrientes a mayor deposición de la materia fermentada como biomasa microbiana, al disminuir la producción de gas con respecto al concentrado sin arbóreas.

Palabras clave: *fermentación, ganadería, nutrición, Putumayo*

Received: February 12, 2024
Accepted: May 20, 2024
Conflict of interest: The authors declare that there is not conflict among them.
CRediT authorship contribution statement: A.R. Riascos-Vallejos: **Conceptualization, Investigation, Data curation, Writing of the original draft.** R. Rodríguez Hernández: **Conceptualization, Writing of the original draft, Supervision.** Lina Y. Quintero: **Investigation, Data curation**. Diana P. Vargas: **Data curation, Writing of the original draft**

Introduction

In Colombia, 45 % of the milk is produce in extensive systems based on grasses with great fiber content, low nutritive value and low biomass production (Blanco-Wells and Günther 2019 and Ribeiro da Silva *et al.* 2021). The use of concentrates in the tropics is prohibitive for the most of farmers, due to their high prices. The incorporation of foliages from trees species to conventional concentrates could be a more economic option. It is know that the use of these forages with energy sources of easily fermentation improve the ruminal fermentation patterns and increase the dry matter and organic matter digestibility (Díaz Echevarria *et al.* 2023).

The technique of gas *in vitro* production is widely use in the evaluation of the nutritive value of new foods, supplements and additives, so it constitutes a less laborious and expensive procedure, require less time with respect to *in vivo* studies (Amanzougarene and Fondevila 2020) and it is combine with measurements of the degradability of the fermentation wastes which has high correlation with the *in vivo* degradability (Baffa *et al.* 2023). Therefore, the objective of this study was to evaluate the *in vitro* effects of the inclusion of three forages native and adapted to the Amazonian piedmont on supplements for cattle.

Materials and Methods

Preparation of the evaluated treatments. All the forages were obtained in areas from Villa Lucero farm, located at coordinates 0°35'25.6"N 76°32'05.3"W, Puerto Asís municipality, Santana Locality, Putumayo Department, at the southwest of Colombia Republic, at a latitude of 256m.os.l. The climatic conditions corresponds to the region of tropical humid forest, with average temperature of 25.3 ºC, relative humidity of 85 % and annual rainfall of 3 355 mm (Ruiz and Melo 2022).

Samples of *Trichantera gigantea* (Acanthaceae), *Piptocoma discolor* (Asteraceae) and *Hibiscus rosa-sinensis* (Malvaceae) were collected from a forage bank established in the farm with one year old plants, to which agronomical management of pruning, control of insects and weeds were carried out. An establishment cut was made and the plants regrowth at 60d was collected by manual cut. The cut material was dried in an air forced oven at 60 ºC until reaching constant weight. Later, the dried material was homogenized and milled with a hammer mill of 1.0mm sieve.

A total of seven supplements were formulated, taking into account the NRC (2001) recommendations. Each treatment consisted on the inclusion of 20 or 40 % of forage in the supplement and a commercial supplement as control. The fallowing treatments were evaluated:

1. Commercial supplement [T1 Control]
2. Commercial supplement with 20 % of *T. gigantean* inclusion [T2]
3. Commercial supplement with 40 % of *T. gigantea* inclusion [T3]
4. Commercial supplement with 20 % of *P. discolor* inclusion [T4]
5. Commercial supplement with 40 % of *P. discolor* inclusion [T5]
6. Commercial supplement with 20 % of *H. rosa-sinensis* inclusion [T6]
7. Commercial supplement with 40 % of *H. rosa-sinensis* inclusion [T7]

A total of 5 kg of each treatment to be evaluated were prepared. From each samples for the laboratory analysis and *in vitro* studies were taking. The treatments were prepared in the biotechnology laboratory from Centro Agroforestal and Acuícola Arapaima del SENA, Putumayo.

In vitro experimental procedure. The gas *in vitro* technique described by Theodorou *et al.* (1994) was used. A total of 1.0 g of DM of each treatment was incubated in 110 mL amber bottles in culture media and a microbial inoculum (in 85:10 proportions). The ruminal content of three Holstein crossbreed heifers was used as inoculum, obtaining during the slaughter process in a beneficial center, according to the certificated standards of animal welfare. The animals were feed with a diet of tropical grasses and had free access to water and minerals salts. The ruminal content of each animal was collected and stored in closed vacuum flasks until go to the laboratory, where was filtered and mixture the inoculums in same proportions.

The bottles were sealed and incubated in water bath at controlled temperature (39 ºC). That moment was taking as the zero hour of incubation. A total of four bottles per treatment and four bottles without substrate as control were incubated.

The gas production was measured until 72 h of incubation by a pressure transducer (Autonics, PSA-1 model) with digital scanner. When each scanner finished, the bottles were manually shake. When the incubation ends, they were opened and their content were filter through nylon bags (45µm of porosity) previously tare in analytical scale (OHAUS, ax224/e, ± 0.0001). The bags with the fermentation wastes were dried until reach constant weight. A forced air oven with regulated temperature to 60 ºC during 72 h was used. The *in vitro* degradability of the dry matter (IVDDM) and the *in vitro* degradability of the organic matter (IVDOM) of the evaluated supplements were determined by gravimetry, understood as the difference of both indicators. This is: the difference between the incubated substrate and the fermentation waste expressed as the incubated substrate proportion and multiply by 100 (%), respectively.

Chemical analysis. The dry matter (DM), organic matter (OM), ether extract (EE) and crude protein (CP) was determined according to AOAC (2016). The neutral detergent fiber (NDF) and acid detergent fiber (ADF) were obtained using the procedure described by van Soest *et al.* (1991).

Statistical analysis. To determine the effect of the forage species inclusion on the treatments, a complete randomized design with four repetitions was used, which corresponded to each bottle or bag by supplement. The results were analyzed by ANOVA through the statistical package InfoStat (Di Rienzo *et al.* 2012). When there were differences (P<0.05), the means of treatments were compared by the Duncan (1955) multiple ranges test.

Results and Discussion

Table 1 shows the chemical composition of the evaluated supplements. It was formulated on the base of making isoenergetic treatments with CP variation, which not exceeded 3 % between the supplements with low and high protein content. The relation protein/ME was between 9.8 and 11.6 g MJkg DM^{-1}, aspect to be consider because a change in the protein contribution can influence on the cattle productive yield, when modifying the general level of nutrition.

Figure 1 shows the gas *in vitro* accumulated production at 72 h of fermentation. The higher production corresponded to the control (T1), although did not showed differences with respect to the treatment with 40 % of *Piptocoma* (T5) (p=0.016). The lower values of gas accumulated production were obtained when including 20 % of *Piptocoma* (T4). These ones did not differ of the treatment with 40 % of this plant (T5) neither of the supplements with 20 % of *Trichantera* (T2) and with *Hibiscus* (T6 and T7).

The IVDDM and IVDOM are show in figures 2 and 3, respectively. The higher values of IDDM corresponded to the control (T1) and the supplements with 20 % of *Trichantera* inclusion (T2) and 20 and 40 % of *hibiscus* (T6 and T7) (p<0.008).The lower degradability value was observed when including 40 % of *Piptocoma* (T5). The supplement with 40 % of *Trichantera* did not showed differences regarding the supplements with *Piptocoma* (T4 and T5).

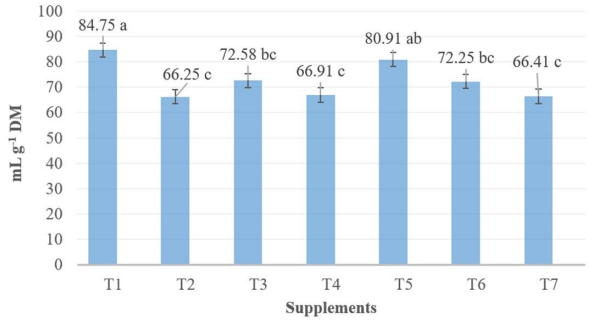

Figure 1. Effect of the supplement on the gas *in vitro* accumulated production at 72 h of fermentation (SE= ±3.71 and p=0.0158)

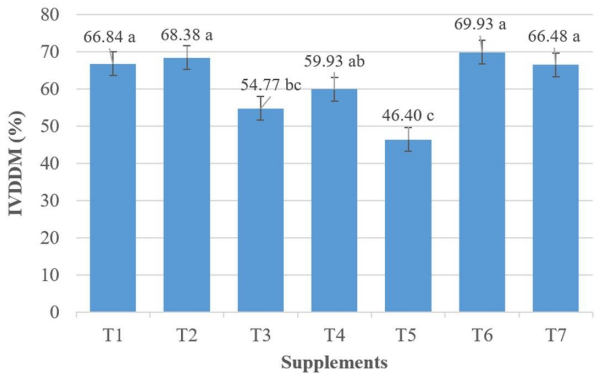

Figure 2. Effect of the supplement on the IVDDM at 72h of incubation (SE=±3.18 and p=0.0076)

With respect to the IVDOM, the higher degradabilities were for the control and the supplements with 20 % of *Trichantera* (T2) and with *Hibiscus* (T6 and T7) (p<0.0001). The rest of the treatments had the fallowing performance: T4 >T3 >T5.

Often, the manipulation of the composition of the diet nutrients is propose as strategy to improve feeding to more competitive costs and the ruminal fermentation, as well as to increase energy efficiency or reduce the methane production (Baffa *et al.* 2023). According to the scrubs characterization in the Amazonian piedmont, the species *P. discolor, T. gigantea* and *H. rosa-sinensis* has adequate

Table 1. Chemical composition of the evaluated supplements

Treatments	T1	T2	T3	T4	T5	T6	T7
DM, %	88.70	89.08	90.22	89.81	91.57	88.88	90.66
OM, %	96.87	96.30	94.04	96.20	95.00	97.14	95.82
Ether extract, %	4.96	6.44	9.32	6.98	7.98	2.72	5.65
Crude protein, %	11.53	11.50	12.49	11.78	13.95	11.50	11.54
NDF	49.03	50.56	52.57	45.83	52.12	53.99	53.98
ADF	28.32	26.81	28.06	28.27	29.27	28.90	31.76
ME (MJkg^{-1} DM)	11.79	11.50	11.50	11.76	11.98	11.51	11.83
Relation CP/ME (g MJkg^{-1} DM)	9.80	10.00	10.90	10.00	11.60	10.00	9.80

content of proteins, energy and degradability (Riascos *et al.* 2020).That is why, their inclusion in the supplements could reduce costs, without affecting their quality and even, to contribute moderate contents of secondary metabolites that will improved the ruminal fermentation and increased the partition of the degraded nutrients to high microbial synthesis and lower gas production per unit of fermented organic matter.

When jointly analyzing the three measured indicator, the control (T1) always showed the higher values of gas production, IVDDM and IVDOM, as it would be when including in the rest of the supplements a fibrous source (20 and 40 %). The supplements with 20 % of *Trichantera* (T2) and 40 % of *Hibiscus* (T7) showed high DM and OM degradabilities, but low gas productions regarding the control. A similar performance had the treatment with 20 % of *Hibiscus* inclusion (T6), so these ones and the previous should be considered to future studies under *in vivo* conditions.

When these supplements had similar degradabilities with respect to the control, the performance of the gas production showed that these two treatments promote higher synthesis of microbial biomass, which is a desirable thing when using this type of food. Makkar (2000) stated that the foods should be selected by having true high degradability and low gas production per unit of degraded substrate.

Otherwise, the supplement with 20 % of *Piptocoma* showed lower values of IVDDM, IVDOM and gas production. This performance show that this inclusion level of the tropical forage affects the nutritive value of this treatment, so it was rejected as feed option. The same happen with the treatment with 40 % of inclusion of this plant. This performance could be due, probably; to the presence of secondary metabolites that affects the ruminal fermentation. It is know that this species has secondary metabolites that could affects the ruminal fermentation, like polyphenols, alkaloids and, in less amount saponins (Riascos *et al.* 2020). Likewise, the inclusion of *P. discolor* granules in ruminal activators showed that the blocks with high levels of this trees had lower IVDDM (Riascos *et al.* 2024).

The tropical forages species show variation in their nutrients and secondary metabolites content according to their phenological state, age or exposition to biotic and abiotic stress. These changes influence on the fermentation dynamic and the nutrient digestibility (Pérez-Can *et al.* 2020). Future studies should deep on these aspects, in a way that can obtain supplements that, in addition to contribute nutrients with low production costs, improve the ruminal fermentation of the basic grass.

It has been proved that the supplementation with protein foliages with a fast availability source energy make more efficient the nitrogen metabolism in the rumen (Arjona-Alcocer *et al.* 2020). Luna (2021) report under *in vitro*

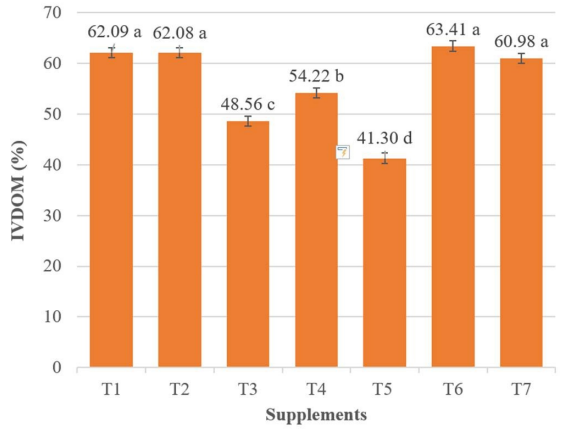

Figure 3. Effect of the supplement on the IVDOM at 72h of incubation (SE= ±1.02 and p<0.0001)

conditions improvement in the nitrogen availability and in the energy contribution in diets with *T. diversifolia,* due to high protein solubility and to the increase in the concentration of non structural carbohydrates in the diet. In this study, the supplements showed high energy content and CP values that exceeded 11.5 %, which guaranteed protein availability by energy unit higher than 9.8 g, although it has been to evaluate in the future the effect of the trees inclusion on the solubility of these proteins in the rumen and their availability at intestinal level.

In addition, the inclusion at 20 and 40 % of trees in the supplements did not caused tannins concentrations that exceeded the 50 g kg^{-1} DM, level which is consider lower to those that should be correspond to these metabolites has beneficial effects, and also affects the growing of microorganisms in the rumen, the ruminal metabolism and the diet digestibility (Díaz Echevarría *et al.* 2023). Therefore, in the case of *piptocoma*, these metabolites could be the cause of the recorded effects on the degradability of the supplements. These could be due to the particular reactivation of these phenolic compounds that, despite their moderate contents, can have an effect higher than other tannins to same concentrations.

Conclusions

The inclusion of 20 % de *T. gigantea* and 20 and 40 % de *H. rosa-sinensis* in the concentrate did not affect the DM and OM degradability of the supplement and improved the nutrition partition as to high deposition of the fermented matter as microbial biomass, when decreasing the gas production with respect to the concentrate without trees.

Acknowledgments

Thanks to the support offered by Servicio Nacional de Aprendizaje SENA and the biotechnology laboratory, SENNOVA project, Putumayo regional, Colombia.

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