Journal of Animal Husbandry and Dairy Science
Volume 5, Issue 1, 2021, PP 1-9
ISSN 2637-5354
DOI: https://doi.org/10.22259/2637-5354.0501001

Effects of Allium Sativum Powder on in Vitro Digestibility of Maize Stover in Cattle

Lemoufouet Jules[1*], Kana Jean Raphael[1], Taboumda Evariste[1], Mube Kuitche Hervé[1], Mekuiko Watsop Hippolyte[2], Miégoué Emile[1], Tendonkeng Fernand[1], Mouchili Mama[1], Matumuini Ndzani Essie Ference[3] et Pamo Tedonkeng Etienne[1]

[1]Department of Animal Science, Faculty of Agronomy and Agricultural Sciences, University of Dschang, Dschang, Cameroon

[2]Department of Animal Production, School of VeterinaryMedecine and Sciences, University of Ngaoundere, Ngaoundere, Cameroon

[3]Higher institute of Agronomy and Biotechnology (INSAB), University of Science and Technology of Masuku, Franceville, Gabon

***Corresponding Author:** Lemoufouet Jules, Department of Animal Science, Faculty of Agronomy and Agricultural Sciences, University of Dschang, Dschang, Cameroon.*

ABSTRACT

The study of the effects of Allium sativum powder on the in vitro digestibility of maize stover treated with 5% urea and enriched with 5% molasses was conducted in April 2019 in the Production Research Unit and Animal Nutrition (URPRONAN) of the University of Dschang. A source of ruminal fluid (Cattle), energy (maize stover enriched with 5% molasses), nitrogen (Urea 5%) and additive (powder of Alllium sativum with variable incorporation rate: 0; 2.5; 3.75 and 5 mg) were used for this study. A sample of each ration based on maize stover treated with urea and enriched with molasses associated with various proportions of Allium sativum powder (0; 0.5%; 0.75 and 1%) was taken in view of the determination of the chemical composition and to assess the in vitro digestibility. The results of this study showed that: gas production (11.19; 10.54; 11.11 and 9.26 ml / 200 mg DM and fermentation of nitrogenous matter by microorganisms (0.75; 0, 78; 0.62 and 0.98 NDF-N in% DM), decreased with the addition of various proportions of garlic powder. It also led to a decrease in IVDOM (29, 30; 28, 8; 29 and 27%), VFAs (0.21; 0.19; 0.21 and 0.16 mmol / 40 ml) and ME (4.22; 4.14; 4.22 and 3, 9 KJ / Kg DM). The highest value of the metabolizable energy was reached with an incorporation rate of 0.75% of the powder of Allium sativum. This study made it possible to show that the addition of Allium sativum powder helps reduce protein fermentations in the rumen, making them more available for intestinal enzymes. We can thus retain that, the addition of Allium sativum powder to corn stubble treated with 5 % urea and enriched with 5% molasses induced changes in in vitro digestibilities. Certain modifications could have beneficial effects on feeding efficiency in ruminants. Further studies are needed to complete the effects of Allium sativum on the microbial population.

Keywords: *Allium sativum, in vitro digestibility, maize stover, protozoa.*

INTRODUCTION

Food remains one of the main constraints to the development of livestock farming in Sub-Saharan Africa (Tendonkeng et *al*., 2011; Lemoufouet et *al*., 2014). According to Meyer et *al*. (2010), animals ingest food to meet their energy needs. In fact, the fodder is of good quality only at the start of the rainy season (Pamo et *al*., 2007). This represents the major handicap in livestock production as the deterioration of its fodder quality is accompanied by a decrease in digestibility. Indeed, the digestibility of forages is very dependent on their nutritional value. Animals that only consume fodder during the dry season have difficulty in externalizing their production potential (Lemoufouet et *al*., 2014). With a view to meeting the nutritional needs of ruminants and hoping for a minimum of production and productivity especially during the difficult period, it is almost inevitable to call on agricultural by-products to sustainably increase the production yield of livestock. This is how maize stover, whose Cameroonian production is estimated at over 1.6 million tons per year, could be valued.

Maize cultivation is widely used and controlled by farmers in the western region of the country

with larger harvests between August and September, which results in the production of large quantities of stubble abandoned in fields at the start of the dry season. However, maize stover is a food of low nutritional value and, untreated, only represents 70% in energy value compared to late grass hay. In addition, the total nitrogen content of maize stover is very low (4.63% and 3 to 6% DM) according to Boukila et *al*. (2005) and Beaumont (2011), which is why their nitrogen value (PDIN) is only 40% of that of hay (Beaumont, 2011). In addition, the levels of soluble sugars, minerals and vitamins in the stover are low; they also constitute a bulky and hardly digestible fodder; but well supplemented, they can be used in the feed of ruminants with moderate needs (Devun et *al*., 2011; Lemoufouet et *al*., 2014). Thus, in order to allow optimal use of this poor forage by ruminants, it is often recommended to supplement with urea, ammonia or even molasses; but this improvement can also be achieved by supplementation with nitrogenous concentrates (oil cake), other agro-industrial by-products or even vitamin-enriched mineral foods (VEMF) enriched in trace elements and sulfur (Devun et *al*., 2011). It is from this perspective that the use of urea and molasses are of practical interest.

In addition, some authors (wanapat et *al*., 2008; Sahli et *al*., 2018) recommend the use of food additives (thyme, garlic, etc.) to boost the microbial degradation of fodder in the rumen. *Allium sativum* has been used in animal feed for its beneficial effects on rumen microbial populations (Harris et *al*., 2001). Works have been carried out with garlic clove powder on the digestibility of forages; this is the case with the work of Alamuyoe et *al*. (2016); Sahli et *al*. (2016). This powder has been used to control the microbial ecosystem of the gastrointestinal tract in small ruminants especially in the tropics with the aim of improving productivity. In addition, garlic cloves as well as the extractions obtained from the latter would allow the control of ruminal fermentation and improve the efficiency of nutrient use by ruminants (Wanapat et *al*., 2008; Santhosha et *al*., 2013). Garlic contains high levels of organosulphur compounds such as allicin, or s-allylcysteine, diallydisulphide, and s-methylcysteinesulfoxide (Dethier, 2009). Studies (Wanapat et *al*., 2008) have shown that garlic powder induces changes in rumen fermentation. Some of these modifications could have beneficial effects on the efficiency of the digestive use of rations in ruminants or even destructive activity in protozoa (Wanapat et *al*., 2008; Sahli et *al*., 2016). Thus, previous work (Wanapat et *al*., 2008) has shown that adding 1% of garlic powder to a ration could have beneficial effects on digestibility parameters in ruminants. However, no studies have been performed on the effects of garlic powder on the *in vitro* digestibility parameters of corn stubble treated with 5% urea and enriched with molasses. It is to remedy this shortcoming that this work was initiated with the general objective of contributing to the improvement of knowledge on the valorization of crop residues in ruminant feed.

Material and Methods

Study Area

The present study was conducted between the months of March and May 2019 in the Animal Production and Nutrition Research Unit (URPRONAN) of the Faculty of Agronomy and Agricultural Sciences (FASA) of the University of Dschang (Figure 1). Dschang is a locality located at 5 ° 26 'North latitude and 10 ° 26' East longitude and at an altitude of 1420 m. The climate is the equatorial, Cameronian type, modified by altitude and characterized by two seasons. The dry season goes from mid-November to mid-March and the rainy season from mid-March to mid-November corresponding to the period of crops. Rainfall varies between 1,500 and 2,000 mm of water per year. The average annual insolation is 1800 hours with temperatures varying between 10 ° C (July-August) to 25 ° C (February) and relative humidity varying between 40-97%. The original vegetation is a shrub savannah with in places gallery forests.

Material and Method

The fresh garlic cloves used in this study were purchased in the Dschang market, a locality located at 5 ° 26 'North latitude and 10 ° 26' East longitude and at an altitude of 1420 m and transported to the FAR for the cleaning, chopping and then in the nutrition laboratory for drying in an oven and grinding.

The ruminal contents used came from the municipal slaughterhouse of Dschang, collected very early in the morning after slaughter of an adult cattle and transported to the nutrition laboratory for the extraction of the juice and used for the *in vitro* digestibility of the different rations and the microscopic identification of the protozoa it contained.

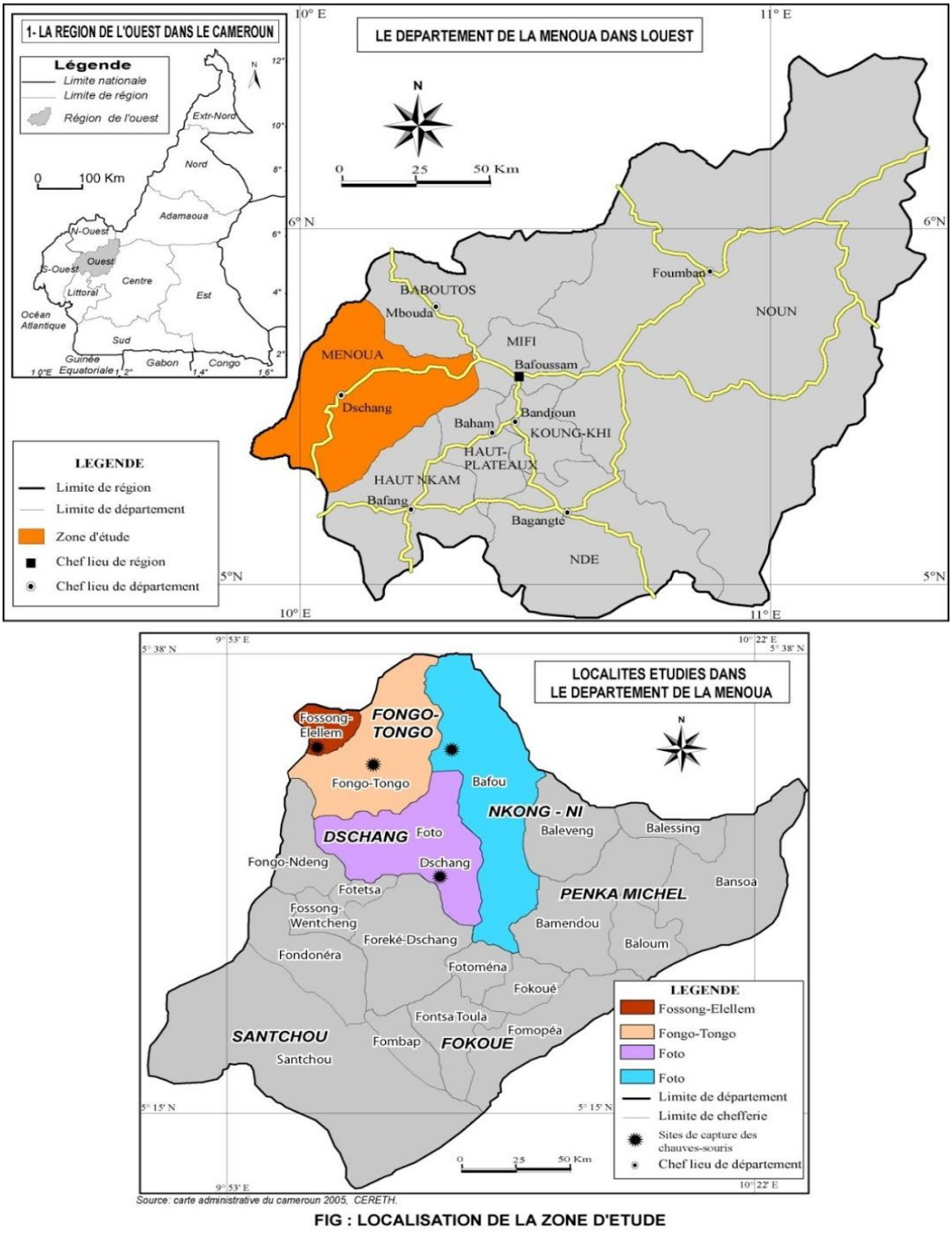

Figure1: *location of the study area*

Source: www.wikipedia.org/wiki/cameroun/ouest

Maize stubble was collected from IRAD plots one week after harvesting the maize stover and then transported to the FAR for chopping, urea treatment, then crushing and molasses treatment.

The harvested stubble was then chopped manually with a machete, to a size of about 2 to 3 cm. These stubbles were treated with urea according to the protocol described by FAO .(1994), namely 5 kg of urea dissolved in 100L of water per 100 kg of stubble and this treatment lasted three weeks. The stubble was then dried in the sun for two weeks, crushed using a hammer mill minus a 1mm mesh screen.

The molasses treatment was performed on maize stover powder previously treated with urea. This molasses treatment was carried out according to the method described by Chenost and Kayouli (1997) at the rate of 250 ml of water for 600 g of stover. From this could be formulated the ration based on stubble treated with 5% Urea and enriched with 5% molasses. After drying at 60 °C in a ventilated

Gallemkamp brand oven until a constant weight was obtained, the treated stover was stored in plastic bags. 500 g of sample from this ration was taken and stored for analyzes of chemical composition and *in vitro* digestibility.

Before the digestibility test, the different rations were weighed in triplicate with the variable proportions (0; 2.5; 3.75; 5 mg / kg) of garlic powder and analyzed for the determination of the Dry matter (DM), organic matter (OM), crude fiber (CF), lipids and total nitrogenous matter (TNM) contents of the different rations were carried out according to the methods described by AOAC (2002). The cell wall content (NDF) was determined according to the method proposed by Van Soest et *al.* (1991).

On the eve of the test, the stock solution prepared according to the method and procedure described by Menke et *al*. (1979) as well as 0.5 g of each ration weighed in triplicate and deposited at the bottom of the syringes previously embalmed with petroleum jelly were placed in a Memmert brand incubator set at 39 ° C. overnight. Likewise, the water bath was started up and the temperature controlled by two LAUDA E300 brand thermostats set to 39 ° C, the water was heated to 100 ° C then stored in the thermos which should be used to collect the water ruminal contents in order to keep it warm at the time of collection of the contents and at a temperature of survival of microorganisms (39-41 ° C).

The morning before the *in vitro* digestibility test, the stock solution was placed in the water bath set at 39 ° C; in this solution came a continuous flow of CO_2 from a gas cylinder with a pressure set at 4 bars. Sodium sulfide (417 mg) and 6N NaOH (0.444 ml) were added to the stock solution. The thermos filled with hot water was transported to the slaughterhouse, immediately after slaughter and evisceration of an adult bovine, this thermos was emptied of its contents and filled with ruminal contents then transported to the nutrition laboratory where was to be perform the *in vitro* digestibility test. Once in the lab, this ruminal content was immediately squeezed out and its liquid filtered under a stream of CO_2 that came continuously from a gas cylinder.

A quantity of liquid was withdrawn and treated with the solution of blue methyl formaldehyde saline (MFS) prepared according to the protocol described by Ogimoto and Imai. (1981).

The formaldehyde in this solution allows microbial fixation while methylene blue stains cell nuclei blue and observation and identification under the microscope at objective 40X is then possible according to the principle:

Principle: The protozoa are treated with an MBFS (methylene blue-formalin saline) solution which allows the cells to be fixed by formaldehyde and the nuclei to be stained with methyl blue.

The enumeration and identification of protozoa was carried out on a Malassez cell, which is a thick glass slide in which is hollowed a counting chamber consisting of 100 rectangles, of which 25 are subdivided into 20 small squares to facilitate counting. The total volume of the cell is equal to 1 µl, or 0.01 µl per rectangle. This allows a quantitative and qualitative assessment of protozoa (Ogimoto and Imai, 1981). This same operation is repeated at the end of digestibility.

For the preparation of 2100 ml of inoculum, 700 ml of the liquid were taken and introduced into the stock solution still under the flow of CO_2. This mixture (inoculum) was homogenized for 10 minutes using a magnetic rod. 40 ml of this inoculum was taken and injected into each syringe using a Fortuna Optifix brand precision dispenser, then all placed in a water bath for incubation. The incubation lasted 24 hours and the volumes of gas produced were recorded at 0, 3, 6, 9, 12, 18 and 24 H. At the end of the incubation, the contents of the syringes were emptied into beakers of 600 ml. These syringes were rinsed twice in succession with two 15 ml portions of Neutral Detergent Solution Dual (NDS) and emptied into these beakers. The samples were brought to a boil over low heat for one hour and filtered in pre-tared filter crucibles. These crucibles were dried at 103 ° C overnight and then weighed. This operation had made it possible to remove more or less undegraded substrates and microorganisms which, when they died, are generally reused in the digestive tract of ruminants.

Gas production was calculated and corrected using the formula proposed by Menke and Steingass. (1988).

$$GP\ \ (ml/500mg\ DM) = \frac{(V_{24} - V_o - GP_o) \times 500mg \times GP_h}{m \times DM}$$

V24 = Volume of gas read after 24 hours of incubation;

V0 = Volume of inoculum in the syringe at the start of incubation;

GP0 = Volume of gas produced by the blank after 24 hours of incubation;

GPh = Volume of gas produced by the standard after 24 h of incubation;

DM = degraded dry matter and m = mass of the sample.

IVDDM was obtained by the difference between the weight of the incubated substrate and the weight of the undegraded residue after the treatment with NDS at the end of the incubation from the following formula (Van Soest and Robertson, 1985):

$$\text{IVDDM (\%)} = \frac{Pe - R}{Pe} \times 100$$, where:

Pe = weight of the incubated sample;

R = weight of the sample after incubation.

After 24 h of incubation, the gases produced and corrected by the gases from the control tube were used to calculate the *in vitro* digestibility of organic matter (IVDOM) using the regression equation of Menke and Steingass. (1988). As for the metabolizable energy (ME), it was calculated according to the equation proposed by Makkar. (2002):

IVDOM (%) = 14.88+ 0.889 GP + 0.0651 Ash

ME (MJ / Kg MS) = 2, 20 +0.136 GP + 0.057 CP

RESULTS

The chemical composition and the nutrient content of the different rations (Table 1) show that the contents of dry matter, TNC, crude fiber, lipids, dOM and NDF of the different rations have increased.

Table1: *Chemical composition and the nutrient content of the different rations*

Chemical Composition	Rations			
	R0	R1	R2	R3
DM (%)	91.10	94.30	94.50	95.00
(%DM)				
Ash	7.75	8.16	7.92	7.76
OM	92.20	91.8	92.10	92.20
TNM	8.80	8.88	8.96	8.98
NDF	79.70	80.80	81.30	81.40
Crude cellulose	37.90	37.50	37.40	40.40
Lipids	1.71	1.71	1.72	1.74
Nutritive value				
dOM	17.10	18.10	17.60	12.00

R0 (control ration) = Treated maize stover + 0% garlic powder; R1 = Treated maize stover + 0.5% Allium sativum powder; R2 = Treated maize stover + 0.75% Allium sativum powder; R3 = Treated maize stover + 1% Allium sativum powder; DM = Dry matter; OM = organic matter; TNM = Total nitrogenous matter; NDF: Neutral detergent fiber and dOM = Digestibility of organic matter.

The effect of the powder level of *Allium sativum* on the evolution of gas production from maize stover treated with 5% urea and enriched with 5% molasses then incubated with bovine ruminal fluid (Figure 2) shows that the gas production has been increasing for all rations. Gas production was comparable (p> 0.05) for the R0, R1 and R2 rations and significantly (p <0.05) higher than that of the R3 ration (CM5U5 + PA 1).

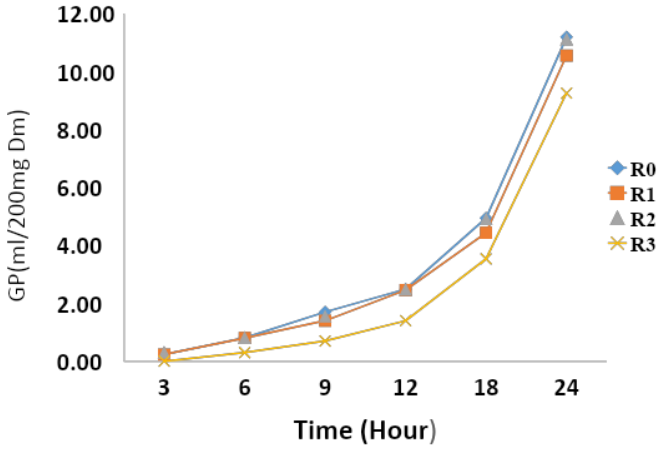

Figure2: *Evolution of gas production from treated maize stover according to the Level of Allium sativium powder incubated with bovine ruminal fluid*

R0 (control ration) = Treated maize stover + 0% garlic powder; R1 = Treated maize stover + 0.5% garlic powder; R2 = Treated maize stover + 0.75% garlic powder; R3 = Treated maize stover + 1% garlic powder and GP = Gas production.

In addition, the downward trend of the regression curve indicates a decrease in gas production with the level of incorporation of garlic powder into the ration. The relationship between the two parameters is average, with a coefficient of determination (R2 = 0.59) associated with the line. Reflecting the fact that 59% of the variation in gas production could be related to garlic powder.

Incorporation of *Allium sativum* powder into treated maize stover did not significantly ($p < 0.05$) affect some digestibility parameters (Table 2). A significant drop in gas production is observed for ration R3 ($p < 0.05$). Indeed, the volume of gas produced by this ration was significantly ($p < 0.05$) low and lower than those of the other rations, which seem to be comparable with each other ($p > 0.05$). This same observation is made with ME, VFA, IVDOM; parameters that registered a significant change. However, no significant change ($p < 0.05$) was observed with the incorporation of *Allium sativum* powder into maize stover treated with 5% Urea and enriched with 5% molasses on MM, CF and IVDM which recorded comparable values between them ($p > 0.05$). On the other hand, the residual nitrogen of the different rations increased significantly ($p < 0.05$) with the incorporation of the powder of *Allium sativum*

Table2: *Effect of Allium sativum powder on the in vitro digestibility parameters of the different rations incubated with bovine ruminal fluid*

	GP after 24 H	ME	MBM	CF	VFA	IVDDM	IVDOM	NDF-N
Rations	(ml/200mgDM)	(KJ/KgD M)	(mg)	(mg/ml)	(mmol /40ml)	(%)	(%)	(%)
R0	11.2^{a}	4.22^{a}	136a	4.26^{a}	0.21a	55.8^{a}	29.3^{a}	0.75^{b}
R1	10.6^{a}	4.14^{a}	146^{a}	4.48^{a}	0.19^{a}	57.2^{a}	28.8^{a}	0.78^{b}
R2	11.1^{a}	4.22^{a}	136^{a}	4.39^{a}	0.21^{a}	54.6^{a}	29.3^{a}	0.620^{c}
R3	9.26^{b}	3.97^{b}	153^{a}	4.77^{a}	0.16^{b}	56.7^{a}	27.7^{b}	0.970^{a}
ESM	0.249	0.033	4.69	0.100	0.006	0.748	0.217	0.038
P	0.001	0.001	0.571	0.369	0.001	0.706	0.001	0.00

VFA = volatile fatty acids; IVDOM = In vitro digestibility of organic matter; IVDDM = In vitro digestibility of dry matter; NDF-N = Residual nitrogen; CF=Crude fiber; ME= metabolizable energy; MBM= Microbial Biomass; GP= Gas production.

Observation of ruminal fluid under a light microscope prior to *in vitro* digestibility testing revealed the presence of *Balantidium coli* and *Isospora beli* after staining with methylformalin blue saline solution. However, after the *in vitro* digestibility test, neither of these two protozoa was found in the various incubation residues of the rations.

Moreover, mineralized dry matter and the level of incorporation of garlic powder in the ration is very low, with a coefficient of determination (R2 = 0.03) associated with the right. Indicating the fact that only 3% of the variation in mineralized dry matter could be related to the dose of this spice.

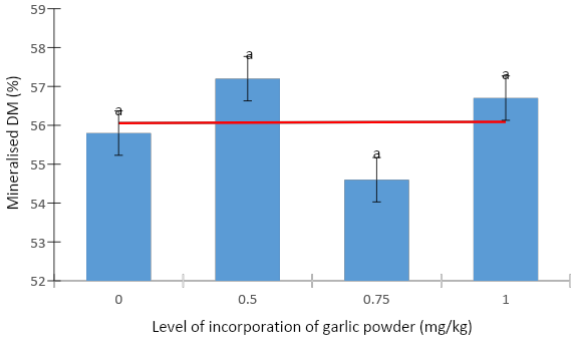

Figure3: *Effect of garlic powder on the mineralized dry matter content of maize stover*

0 (control ration) = Treated maize stover + 0% garlic powder; 0.5 = maize stover treated + 0.5% Allium sativum powder; 0.75 = Treated maize stover + 0.75% Allium sativum powder; 1 = Treated maize stover + 1% Allium sativum powder.

DISCUSSION

The DM content obtained with the CM5U5 + PA0 ration in this study was lower than those obtained by Matumuini. (2014), namely 96.3% DM; Lemoufouet et *al*. (2014) notably (94.8 and 95% DM) for culms treated with 5% urea and enriched with 5% molasses (Lemoufouet et *al*., 2016) and for culms treated with 28% hen droppings enriched with 5% molasses respectively; but higher than that obtained by Tchoffo.(2018), notably 89.87% DM with stover treated with 5% molasses. The organic constituents varied very little between the different rations. The organic matter content (92.25% DM) for maize stover treated with 5% urea enriched with 5% molasses was similar to that of Tchoffo.(2018) namely 92.75% DM with treated stover. 5% molasses; but lower than that of Matumuini et *al*. (2014) which was 96% DM and higher than that of Lemoufouet et *al*. (2014) namely 84.3% DM and 67% DM respectively for culms treated with 5% urea and enriched with 5% molasses and for culms treated with 28% hen droppings and enriched with 5% molasses.

The ash obtained with the CM5U5 + PA0 ration in this study was similar to that of Tchoffo .(2018), namely 7.25% DM, but higher than that of Matumuini et *al*. (2014) namely 3.6% DM and lower than those of Lemoufouet et *al*. (2014) were 10.5 and 14% DM, respectively.

The TNC varied very little between the different rations, but its value obtained with the CM5U5 + PA0 ration in this study was close to those of Lemoufouet et *al*. (2014) namely 9.19 and 9.1% DM respectively, but higher than those obtained by Matumuini et *al*. (2014) namely 4% DM and Tchoffo.(2018) namely 5.88% DM for stubble enriched with 5% molasses and associated with *Thitonia diversifolia* and stubble enriched with molasses associated with pods of *Acacia albida* respectively.

The variations observed in the chemical composition of the different rations would surely be due to the period, the level of fertilization and the stage of harvesting of the stover on the one hand and the sources of nitrogen and energy (chicken droppings, *Acacia* pods *albida* and *Thitonia diversifolia* as well as the level of incorporation.

In this work, the values of some *in vitro* digestibility parameters decreased or increased with the incorporation of increasing levels of garlic powder. Thus gas production has decreased with the incorporation of increasing rates of garlic powder. The highest value was obtained with the CM5U5 + PA0 ration and the lowest with the rate of garlic powder of 1%; this is the reverse of the observations made by Sahli et *al*. (2018) who had instead recorded an increase in gas production with 64 mg of garlic powder incubated with a ration containing 50% concentrate and 50% forage.

The VFA values obtained in this work decreased with the incorporation of increasing levels of garlic powder, which corresponds to the observations of Wanapat et *al*. (2008) who found a decrease in VFAs with increasing rates of garlic; but for these, this decrease would be in favor of an increase in the propionate fraction.

In this study, an increase in nitrogen residue was observed with the incorporation of increasing levels of garlic powder; this corresponds to the observations made by Wanapat et *al*. (2008); Sahli et *al*. (2018) in different studies. According to these authors, garlic induces a decrease in the fermentation of nitrogenous matter in the rumen; thus increasing the retention and absorption of nitrogenous matter.

The absence of protozoa in the various digestibility residues *in vitro* seen in the CM5U5 + PA0 ration would be due to the presence of large quantities of lignin in the maize stover which, because of its encrustation, hindered the fixation of the protozoa on plant particles in order to benefit from the nutrients therein.

In addition, garlic did not promote the development of protozoa in rations where its powder was added. Garlic powder (especially its active component: *alliin*) would have had a destructive effect on the populations of protozoa by causing a destabilization of the structures of the latter, thus pushing them to death (wanapat et *al*., 2008).

CONCLUSION

At the end of this work, which focused on the effect of *Allium sativum* powder on the *in vitro* digestibility of maize stover treated with 5% urea and enriched with 5% molasses incubated with bovine ruminal fluid, the following observations can be retained: The inclusion of the powder of *Allium sativum* at increasing rates led to a very small variation in the chemical composition of the different rations even if the dry matter, cell walls and the crude fiber were the most affected. The incorporation of *Allium sativum* powder into the rations has led to considerable changes in certain digestibility

parameters *in vitro*, in particular: a decrease in gas production, metabolizable energy, partitioning factors, volatile fatty acids, the *in vitro* digestibility of organic matter and the degradation of nitrogenous matter and an increase in the *in vitro* digestibility of dry matter, a decrease in the use of nitrogenous matter by microorganisms.

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Citation: *Lemoufouet Jules, Kana Jean Raphael, Taboumda Evariste, Mube Kuitche Hervé, Mekuiko Watsop Hippolyte, Miégoué Emile, Tendonkeng Fernand, Mouchili Mama, Matumuini Ndzani Essie Ference et Pamo Tedonkeng Etienne, "Effects of Allium Sativum Powder on in Vitro Digestibility of Maize Stover in Cattle", Journal of Animal Husbandry and Dairy Science, 5(1), 2021, pp. 1-9.DOI: https://doi.org/10.22259/2637-5354.0501001*