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EFFECT OF THE ESSENTIAL OIL OF RHIZOMES OF ZINGIBER OFFICINALE ON THE IN VITRO DIGESTIBILITY OF PENNISETUM CLANDESTINUM HAY IN SMALL RUMINANTS

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ABSTRACT

The study of the effect of essential oil (HE) of rhizomes of Zingiber officinale on the in vitro digestibility of Pennisetum clandestinum hay was undertaken between January 2016 and February 2017 at the Experimental Farm, and in the Laboratory of Animal Production and Nutrition of the University of Dschang. Two sources of rumen fluid (sheep and goat) were used for this study. The rhizomes of Zingiber officinale were harvested in parcel of culture in Santchou. Essential oil was extracted from the rhizomes of Zingiber officinale by the hydrodistillation technique. Ration included hay of Pennisetum clandestinum (90%) and concentrates (10%). Four doses (0, 100, 200 and 400mg/kg of dry matter) of this essential oil were used during the incubation of different rations. The results of this study showed that, no matter the ruminal liquid considered, gas production (GP) after 24h of incubation, volatile fatty acids (VFA), metabolizable energy (ME), as well as the in vitro digestibility of organic matter (IVOMD) significantly ($p<0.05$) increased with the addition of 100 or 200mg essential oil of rhizomes of Zingiber officinale with the rations-based of Pennisetum clandestinum hay. The highest values of these parameters (35.9ml/500mg; 0.79mmol/40ml; 8.11MJ/kg DM and 55.91% respectively) were obtained with the ration containing 200mg essential. The addition of 400mg of this oil significantly ($p<0.05$) lowered the concentration of these components. The microbial mass (MM) significantly ($p<0.05$) dropped with the addition of the essential oil of the rhizomes of Zingiber officinale in the rations. This study shows that the addition of the essential oil of rhizomes of Zingiber officinale in the rations-based of the Pennisetum clandestinum hay contributes to improve its digestibility in the small ruminants.

INTRODUCTION

The handling of the ruminal ecosystem to increase the capacity of digestion of poor fodder, to reduce methane emission and nitrogen excretion by ruminants are components of fundamental objectives that seek nutritionnists (Zhang *et al*., 2011). Microbial digestion in the rumen is a major phenomenon whose efficiency can be improved by the use of food additives (Noirot *et al*., 2007). By acting on the balance of the microbial population, these additives make it possible to control the place of digestion and/or an orientation of fermentations towards the formation of end products which must be used more effectively by animal (Macheboeuf *et al*., 2006). Many food additives were developed to improve the efficiency of the use of nutrients by decreasing the total quantity of methane or ammonia produced, thus increasing the quantity of energy or nitrogen available for the animal need. Among these additives, ionophores antibiotics such as monensin were employed successfully like food additives for decades to handle ruminal fermentations (Noirot *et al*., 2007). This, in order to improve the efficiency of feed in the systems of production of ruminants (Patra, 2011). However, the risk of the presence of antibiotic residues in milk, the meat and its effects on human health led to its prohibition for a use in animal feeds by the European Union (Aouadi and Ben, 2012). This prohibition caused a renewed interest for the natural substitutes which represent vegetable extracts (tannins, saponins, essential oils). Plant extracts contain secondary metabolites which showed important selective antimicrobial activities (Cowan, 1999; Benchaar *et al*., 2008).

Some of them (saponins, phenolic compounds and essential oils) are already recognized for their effects on microbial fermentations (Ryan *et al*.,1997; Evans and Martin, 2000; Cardozo *et al*., 2004; Busquet *et al*., 2005). Several essential oils and their active components have strong and selective antimicrobial activities against a broad range of micro-organisms, including bacteria, protozoa and mushrooms (Benchaar *et al*., 2008), and can be employed to control the competition between various microbial populations with the objective to improve the efficiency of energy utilization and protein in rumen (Calsamiglia *et al*., 2007). Several in vitro studies are already undertaken on the effect of essential oils on ruminal fermentations and the production of volatile fatty acids, yet the results are contradictory. The effect of essential oils depends on their structure, which results from the chemical composition and the type of functional group or the aromatic molecules which compose them (Bayourte *et al*., 2014). However, the data available on the effect of the essential oil of

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ginger on the digestibility of fodder in ruminants are limited. The objective of this study was to evaluate the effects of different doses of essential oil of ginger on in vitro digestibility of Pennisetum clandestinum hayin small ruminants.

Study Area

The study was conducted at the Experimental Farm of Dschang University in western Cameroon. The area is within the Sudano–Guinean zone of Central Africa (latitude 5–7°N, longitude 8–12°E; altitude 1400 m ASL). The annual temperature varies between 16 and 27°C with a relative humidity of 40–97%.

There are two main seasons: the rainy season (April–October) and the dry season (November–March), which is the main cropping season. The mean of the annual rainfall is about 2000 mm (Tendonkeng *et al*., 2014).

Animal material

One adult West African Dwarf goat and Djallonké sheep were used as donors of rumen fluid for the study of the digestibility of Pennisetum clandestinum hay associated with different level of Zingeber officinale essential oil. During the preliminary phase of the evaluation of the in vitro digestibility, which lasted a month, the animals were placed onstilts and were receiving Pennisetum clandestinum hay at libitum. A month before started the study, all animals were treated with oxytetracycline(20%), a preventive antibiotic. They were also dewormed with Ivermectin 1% (synthetic broad spectrumanthelmintic active against gastrointestinal nematodes and pulmonary adults and larvae).

Plant material

Two plant species (Zingiber officinale and Pennisetum clandestinum) were used in this study. The rhizomes of Zingiber officinale were collected in Santchou (West-Cameroun) in February 2016. These rhizomes were crushed in their fresh state for the extraction of essential oil. Meanwhile, the fresh leaves of Pennisetum clandestinum were cut 45 days after planting at the Experimental Farm of the University of Dschang and were dried aired and shade free at ambient temperature until obtention of hay whose chemical composition is presented in Table 1. The concentrate food used within this study consisted of: 50% maize, 30% wheat bran and 20% cotton seed cake.

Table 1 Chemical composition of Pennisetum clandestinum

Chemical composition	Quantity
Dry matter (%) (% DM)	96
Ash	15.19
Organic matter	84.80
Total nitrogenized matter	1 3.37
Lipids	2.09
Crude fibre	30.42
Neutral detergent fiber (NDF)	82
Total carbohydrate	64.82
dOM	32.91

dOM: digestibility of organic matter

Extraction of essential oil

The rhizomes of Zingiber officinale have been crushed into paste, for the extraction of essential oils. The extraction of oils was made in the laboratory of Physiology and Animal Health of the Faculty of Agronomy and Agricultural Science by hydrodistillation according to the method proposed by Wang and Waller (2006). This technique consists of placing the plant material in an alembic, then heated with water with 200°C. Intense heat causes the explosion of saccules which contain oil and these spread in the water vapor. They will thenbe channeled in a condenser and will be cooled to be liquified again. At the end, oil were separates to water and was dried using anhydrous sodium sulphate.

Four rations were used in this study:

1. Pennisetum clandestinum + concentrated + 0mg essential oil (FPc+HEZo0)/kg of DM: control;
2. Pennisetum clandestinum + concentrated + 100mg of essential oil of rhizomes of Zingiber officinale/ kg of DM (FPc+HEZo100);
3. Pennisetum clandestinum + concentrated + 200mg of essential oil of rhizomes of Zingiber officinale/ kg of DM(FPc+HEZo200);
4. Pennisetum clandestinum + concentrated + 400mg of essential oil of rhizomes of Zingiber officinale/ kg of DM(FPc+HEZo400).

In vitro digestibility

Preparation of inoculum and method used. The evaluation of the quantity of gas produced and preparation of reagent were made according to the method and the procedure described by Menke *et al*., (1979). The day before the test, the samples (weighed in triplicate and introduce into syringes) and two (one for goat ruminal liquid and one for sheep ruminal liquid) freshly prepared reagent (Menke *et al*., 1979) were placed in an incubator at 39°C overnight. Similarly, a water bath was turned on and the temperature was maintained by two thermostats set at 39°C. The morning before the collection of rumen fluid of goat and sheep, the reagent in which continually arrived a stream of gas (CO2) with a moderately pressure (4 Bars) was placed in a water bath at 39°C. Then, the sodium sulfide (417 mg) and 6N NaOH (0.444 ml) were added to this reagent. The ruminal liquid collected respectively from goat and sheep was immediately filtered under a stream of CO2 and 700 ml of each ruminal liquid were collected and introduced into the each reagent still under CO2 stream. This mixture (inoculum) was homogenized for 10 minutes using a magnetic rod and 40 ml of each ration with goat inoculum and 40 ml of each ration with sheep inoculum was put into the syringe using a precision dispenser and then placed in the water bath for incubation.

Incubation

After 24 hours of incubation, the gas products and corrected by the gas produced by the inoculum in control tubes were used to determine the organic matter digestibility (OMD) using the regression equation proposed by Menke and Steingass (1988):

OMD (%) = 14.88 + 0.889GP + 0.45CP + 0.0651Ash, where:

GP = Amount of gas produced after 24 hours of incubation,
CP = Crude protein of the initial sample, Meanwhile, the content of metabolizable energy (ME), partitioning factor (PF) which is the amount of organic matter which is degraded to produce 1 ml of gas, the microbial mass (MM), the volatile fatty acids (VFA) were calculated using the equations proposed by Makkar (2002):

ME (MJ/kg.DM) = 2.20 + 0.136GP + 0.057CP;

$$PF\ (mg/ml) = \frac{OMD}{GP}$$

VFA (mmol/ml) = 0.0239GP – 0.0601;
MM (mg) = OMD – (GP x SF);
OMD (mg) = Amount of organic matter degraded.
GP (ml) = Amount of gas produced after 24hours of incubation,
SF = stoichiometric factor (2.20 for fodder).

After 24 hours of incubation, the contents of each syringe was transferred into a 600 ml beaker and the syringe washed twice with two portions of 15 ml Neutral detergent solution (NDS) (Van Soest *et al*.,1991) and emptied into the beaker. The samples were boiled gently for one hour and filtered intopre-tared filter crucibles. These crucibles were dried at 103°C overnight and weighed (Van Soest *et al*.,1991). The in vitro dry matter digestibility (IVDMD) was calculated as the difference between the weight of the substrate and the weight of the incubated residue after NDS treatment, at the end of the incubation. The residues obtained after treatment with NDS were used to determine the residual nitrogen (NDF-N) by Kjeldahl method.

Statistical Analysis

The data on in vitro digestibility were subjected to one way analysis of variance following General Linear Models. When differences exist between treatments, the means were separated by the Waller Duncan test at 5% significance level (Steel and Torrie, 1980). The t-test of Student was used for comparisons between the digestibility parameters depending on the source of rumen fluid.

RESULTS

Effect of sheep rumen fluid on the in vitro digestibility of Pennisetum clandestinum hay associated with different levels of essential oil of rhizomes of Zingiber officinale

The parameters of the in vitro digestibility of the various rations incubated with the sheep rumen fluid are presented in Table 2. It can be observed from this table that the incorporation of the essential oil of the rhizomes of Zingiber officinale significantly ($p<0.05$) improved the production of gases of rations FPc+HEZo100 and FPc+HEZo200. Indeed, volumes of gas of these rations were significantly ($p<0.05$) higher than those of the rations FPc+HEZo0 and FPc+HEZo400 which were comparable between them. The same observation was made for the ME, the VFA, the IVDMD and the IVOMD. The PF and the residual nitrogen (NDF-N) ($p>0.05$) were not significantly influenced by the addition of essential oil in the ration. The microbial mass in addition significantly ($p>0.05$) dropped with the addition of essential oil in the ration.

Table 2 Effect of sheep rumen fluid on the in vitro digestibility of Pennisetum clandestinum hay associated with different levels of essential oil of rhizomes of Zingiber officinale

Rations	24h GP (ml/500mg)	ME(MJ/kgMS)	MM (Mg)	PF (mg/ml)	VFA(mmol/40ml)	IVDMD (%)	IVOMD (%)	NDF-N
FPc+HEZo0	30.59c	7.38c	98a	2.7a	0.67c	41.82b	51.1a	1.77a
FPc+HEZo100	33.42b	7.76b	84.79b	2.9a	0.73b	48.97a	53.63b	1.5a
FPc+HEZo200	35.9a	8.11a	78.49c	2.8a	0.79a	51.4a	55.91a	1.49a
FPc+HEZo400	30.6c	7.36c	83.24a	2.81a	0.66c	41.86b	50.99c	1.3a
SEM	0.15	0.02	0.37	0.02	0.00	0.39	0.13	0.08
P	0.00	0.00	0.00	0.15	0.00	0.00	0.00	0.48

a,b,c: the mean bearing the same letter in the same column are not significantly different. FPc+HEZo0 = P. clandestinum hay nonassociated with essential oil; FPc+HEZo100 = P. clandestinum hay+100mg essential oil of the rhizomes of Z. officinale; FPc+HEZo200 = P. clandestinum hay + 200mg essential oil of the rhizomes of Z. officinale; FPc+HEZo400 = P. clandestinum hay + 400mg essential oil of the rhizomes of Z. officinale; SEM = standard Error of mean; P = Probability. GP = gas production; ME = metabolisable energy; MM = microbial mass; PF = partitioning factor; VFA = volatile fatty acid; IVDMD = in vitro dry matter digestibility; IVOMD = invitro organic matter digestibility; NDF-N = residual nitrogen.

Effect of goat rumen fluid on the in vitro digestibility of Pennisetum clandestinum hay associated with different levels of essential oil of rhizomes of Zingiber officinale

Table 3 presents the parameters of the in vitro digestibility of the various rations incubated with the goat rumen fluid. It is noticed from this table that the level of inclusion of the essential oil of rhizomes of Zingiber officinale influenced the in vitro studied parameters of digestibility. Indeed, the production of gas, the ME, the VFA and the IVOMD of ration FPHEZo200 were significantly ($p<0.05$) higher than those of rations FPHEZo0 and FPHEZo100 which were comparable between them. In addition, the lowest values of these parameters were obtained with the ration FPHEZo400. The MM significantly ($p<0.05$) dropped with the incorporation of essential oil in the various rations. The PF decreased with the level of incorporation of the essential oil of Zingiber officinale in the ration, without any significant difference ($p>0.05$) being observed.

Table 3 Effect of goat rumen fluid on the in vitro digestibility of Pennisetum clandestinum hay associated with different levels of essential oil of rhizomes of Zingiber officinale

Rations	24h GP (ml/500mg)	ME(MJ/kgMS)	MM (Mg)	PF (mg/ml)	VFA(mmol/40ml)	IVDMD (%)	IVOMD (%)	NDF-N
FPc+HEZO0	31.67b	7.52b	97.1a	3.7a	0.69b	60.00a	52.07b	1.5a
FPc+HEZO100	31.31b	7.47b	80.33c	3.40a	0.68b	53.32b	51.76b	1.4a
FPc+HEZo 200	35.1a	8.00a	93.72b	3.07a	0.7a	52.35b	55.17a	1.5a
FPc+HEZo400	26.4c	6.8c	89.07c	3.19a	0.57c	42.23c	47.39c	1.4a
ESM	0.11	0.01	0.85	0.06	0.00	0.77	0.09	0.05
P	0.00	0.00	0.00	0.18	0.00	0.00	0.00	0.74

a,b,c: the mean bearing the same letter in the same column are not significantly different. FPc+HEZo0 = P. clandestinum hay nonassociated with essential oil; FPc+HEZo100 = P. clandestinum hay+100mg essential oil of the rhizomes of Z. officinale; FPc+HEZo200 = P. clandestinum hay + 200mg essential oil of the rhizomes of Z. officinale; FPc+HEZo400 = P. clandestinum hay + 400mg essential oil of the rhizomes of Z. officinale; SEM = standard Error of mean; P = Probability. GP = gas production; ME = metabolisable energy; MM = microbial mass; PF = partitioning factor; VFA = volatile fatty acid; IVDMD = in vitro digestibility of dry matter; IVOMD = in vitro digestibility of the organic matter; NDF-N = residual nitrogen. Comparative effect of rumen fluid source on the in vitro digestibility of Pennisetum clandestinum hay associated with different levels of essential oil of rhizomes of Zingiber officinale

The parameters of in vitro digestibility of the various rations were influenced by the source of the rumen fluid, except residual nitrogen (NDF-N) (Table 4). The higher amounts of gas production after 24 hours of incubation P. clandestinum hay, VFA, ME, IVOMD and IVDMD both with sheep and goat rumen fluid were obtained with FPc+HEZo200 while the lower amount sheep were obtained with hay incubated with 400mg essential oil/ kg of dry matter. The production of gas, of VFA, the ME, the IVOMD, the MM and the residual nitrogen (NDF-N) of the control ration (FPc+HEZo0) were not influenced ($p>0.05$) by the source of theruminal liquid. The same observation was made for the MM and the IVDMD of P. clandestinum hay associated of 100 or 200mg essential oil of the rhizomes of Zingiber officinale. However, with the rations FPc+HEZo100, FPc+HEZo200 and FPc+HEZo400, production of gas, VFA, the ME, and the IVDOM obtained with the sheeprumen fluidwas significantly higher ($p<0.05$) than that recorded with the goat rumen fluid. The microbial mass and PF of P. clandestinum incubated with 400mg essential oil/ kg of DM were significantly ($p<0.05$) higher with goat rumen fluid. The source of rumen fluid did not significantly ($p>0.05$) affect residual nitrogen (NDF-N) of the P. clandestinum incubated with or without essential oil of rhizomes of Zingiber officinale.

Table 4 Comparative effect of rumen fluid source on the in vitro digestibility of Pennisetum clandestinum hay associated with different levels of essential oil of rhizomes of Zingiber officinale

Rations	Rumen fluid	GP after 24h (ml/500 mg)	ME(MJ/kgMS)	MM (Mg)	PF (mg/ml)	VFA(mmol/40 ml)	IVDMD (%)	IVOMD (%)	NDF-N
FPc+HEZo 0	Sheep	30.59a	7.3a	98a	2.73 b	0.67a	41.82 b	51.11 a	1.77a
	Goat	31.67a	7.52 a	97.1a	3.78 a	0.69 a	60.00 a	52.07 a	1.5a
FPc+HEZo 100	Sheep	33.42a	7.76a	84.79a	2.93a	0.73a	48.97a	53.63a	1.5a
	Goat	31.31 b	7.47 b	80.3a	3.40a	0.68b	53.32a	51.76b	1.4a
FPc+HEZo 200	Sheep	35.9a	8.11a	78.49a	2.8a	0.79a	51.4a	55.91a	1.49a
	Goat	35.15b	8.00b	93.7a	3.07a	0.78b	52.3a	55.17b	1.5a
FPc+HEZo 400	Sheep	30.61a	7.36a	83.24b	2.81b	0.66 a	41.86 a	50.99a	1.3a
	Goat	26.41b	6.81b	89.07a	3.19 a	0.57 b	42.23 a	47.39b	1.4a

a,b: the mean bearing the same letter in the same column for the same sample are not significantly different. FPc+HEZo0 = P. clandestinum hay nonassociated with essential oil; FPc+HEZo100 = P. clandestinum hay+100mg essential oil of the rhizomes of Z. officinale; FPc+HEZo200 = P. clandestinum hay + 200mg essential oil of the rhizomes of Z. officinale; FPc+HEZo400 = P. clandestinum hay + 400mg essential oil of the rhizomes of Z. officinale; SEM = standard Error of mean; P = Probability. GP = gas production; ME = metabolisable energy; MM = microbial mass; PF = partitioning factor; VFA = volatile fatty acid; IVDMD = invitro digestibility of dry matter; IVOMD = in vitro digestibility of the organic matter; NDF-N = residual nitrogen.

DISCUSSION

The in vitro studied parameters of digestibility were influenced when the rations were incubated with the essential oil of rhizomes of Zingiber officinale no mather the rumen fluid considered, except the partitioning factor (PF) and residual nitrogen (NDF-N).

Whatever is the rumen considered fluid, the production of gases (GP) after 24h of incubation, of volatile fatty acids (VFA), metabolizable energy (ME) as well as the in vitro digestibility of the organic matter (IVDOM) increased significantly with the addition of 100 or 200mg oil essential of rhizomes of Zingiber officinale with the ration-based of the of Pennisetum clandestinum hay. On the other hand, the addition of 400mg of this oil significantly lowered the concentration of these components. These results are in agreement with those obtained by Zhang *et al*. (2011) with the officinal powder of Zingiber in sheep. This effect of the essential oil of Zingiber officinale of the two species at the dose 400mg could be due to the antimicrobial activity more pronounced with the high amounts of active compounds of this oil against the bacteria gram-positive and gram-negative as reported by Mascolo *et al*. (1989). Busquet *et al*. (2006); Castillejos *et al*. (2006); Santoso *et al*. (2007); Patra (2011) and Cobellis *et al*. (2015) also showed that several extracts and secondary metabolites of plants at higher doses in the ration of ruminants will cause a reduction in the parameters of digestibility because of their antimicrobial effects. These results can be reinforced by the assumption that the use of essential oil at higher doses would inhibit all ruminal fermentation, which suggests the passage of the selective effect towards a more general effect which brings about the inhibition of the majority of micro-organisms (Binder *et al*., 2008). This indicates that the intensity of the antimicrobial activity of essential oil is strongly related to the concentration used (Binder *et al*., 2008).

This evolution of the production of gases after 24h of incubation, of volatile fatty acid, metabolizable energy, the in vitro digestibility of dry matter and the in vitro digestibility of the organic matter could also be related to the variations of pH as observed by the work of Benchaar *et al*. (2007) on compound essential oil. So, it's utilization at higher doses might increase in the pH thus creating a microbiote incompatible with the fermented activity of the micro-organisms.

The microbial mass (MM) was significantly lowered with the ovine ruminal liquid no matter the type or the level of essential oil considered. This fall of the MM would be due to the strong production of gas, as stated by Menke *et al*. (1979), Who observed that the rations with a high gas, production have a low microbial mass.

The values of total VFA produced after 24h of incubation are close to those obtained by Hundal *et al*. (2016) with the pure compounds (cinnamaldehyde, carcacrol, carvone and limonene) of essential oil, and for substrate straw of corn, but inferior to those obtained by Gabriella *et al*. (2016) with a mixture of essential oil of cinnamon bark, leaves of eucalyptus and origan in bovine. This can be explained by the amount and the chemical composition of the essential oil used in these studies, since the bactericidal capacity of essential oil in the rumen is related to the chemical structure of the aromatic molecules which constitute them (Bayourthe *et al*., 2014). This latter varying with conditions like the nature of the ground, the geographical origin, the climate, altitude (Dudareva *et al*., 2004), the physiological state of the plant, as well as the body of the plant used to extract essential oil (Delaquis *et al*., 2002). The hypothesis of a natural effect of the ration on the answer obtained with essential oil cannot be isolated as seems to show the study of Noirot and Bayourthe (2008). The varied effects of

essential oil on the concentration of the total volatile fatty acids can also depend on other factors, such as the type of the substrate as well as the conditions of medium (Gabriella *et al*., 2016). The parameters of the in vitro digestibility of the various rations incubated with the goat rumen fluid or the sheep rumen fluid in this study are very close. These observations are similar to those observed by Matumuini *et al*. (2014) who obtained comparable results ofin vitro digestibility with the rumen fluid of the goat and sheep incubated with the same ration.

CONCLUSION

At the end of this study, on the effect of essential oils of rhizomes of Zingiber officinale on the in vitro digestibility of of Pennisetum clandestinum hay in small ruminants, it arises that the parameters of in vitro digestibility evolved in a variable way independently of the source of the rumen fluid. Indeed, the production of gas and VFA, the ME and the IVDOM increased with the incorporation of 200mg essential oil in the ration. The PF was not influenced by the addition of essential oil in the different rations. The addition of 400mg of essential oil in the ration lowered ($p<0.05$) in a general way the parameters of in vitro digestibility. Although the results of this study are satisfactory with 100 or 200mg essential oil of rhizomes of Zingiber officinale, it would be desirable to carry out a test of in vivo digestibility to appreciate the effect of the addition of this essential oil on voluntary ingestion and the digestive utilization of hay of Pennisetum clandestinum in small ruminants.

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