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Effect of white garlic powder (*Allium sativum*) on *in vitro* ruminal fermentation

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Abstract. The current study aimed to evaluate the effects of increasing doses (0, 4, 8, 16, 32 and 64 mg/50 ml of buffered rumen fluid from sheep) of garlic powder (GP) on *in vitro* fermentation characteristics. Doses of garlic powder were added to 500 mg of a diet composed of 50% of rye-grass hay and 50% of concentrate on dry matter (DM) basis. The medium of incubation consisted on ruminal liquid extracted from sheep, mixed in equal proportions with a buffer solution and introduced in 100 ml glass syringes (39°C). For each garlic dose and incubation, 2 syringes were reserved for sampling liquid to determine pH and ammonia nitrogen (NH3-N) and 3 for true organic matter degradability (TOMD) measurements (5 syringes in total). Two successive 24 h-incubations were practiced and at the end of each gas production was measured. Partitioning factor (PF) was estimated as the ratio between TOMD and the gas produced at 24 h of incubation. Microbial biomass (MBM) was estimated on the bases of truly degraded substrate and PF. Results showed that GP had no effect on gas production for doses 4, 8 and 16 mg compared with control. However, when 32 and 64 mg of GP were added, gas production increased (P<0.001) by about 13% for each dose. An increase (P<0.0001) in NH_3-N concentration was recorded with 4 mg and 8 mg of GP compared with control, Whereas adding 16 mg of GP resulted in a NH_3-N concentration equivalent to control. The doses of 32 and 64 mg of GP diminished the NH_3-N concentration by 20 % compared with control. Truly organic matter degradability was similar (72.2 % on average) for all doses except 64 mg of GP, where a slight but significant (P<0.001) increase was found (77.7%). Garlic powder did not affect PF values when adding 4, 8, 16 or 32 mg of GP compared with control. However, the dose of 64 mg increased significantly (P<0.0001) the PF value. Microbial biomass values obtained with doses 4, 8, 16 and 32 mg were equivalent to those obtained from control, while the value observed with 64 mg was significantly (P<0.001) higher. It was concluded that GP could induce changes in rumen fermentation as measured *in vitro*. Some of these modifications could have positive effect on feeding efficacy in ruminants. Further measurements are needed to investigate mainly the effect of garlic on microbial activities, such as effects on volatile fatty acid (VFA) production and composition and on animal performances.

Keywords. Garlic powder – *In vitro* Ruminal fermentation.

***Effet de l'apport de la poudre d'ail blanc* (Allium sativum) *sur les fermentations ruminales mesurées* in vitro**

Résumé. *La présente étude a pour objectif d'évaluer les effets de doses croissantes (0, 4, 8, 16, 32 et 64 mg / 50 ml de liquide de rumen de mouton tamponné) de la poudre d'ail (PA) sur les caractéristiques de fermentation* in vitro*. Les doses de poudre d'ail ont été ajoutées à 500 mg de matière sèche (MS) d'un régime composé de 50% de foin et de ray-grass et de 50% de concentré. Le milieu d'incubation se compose de liquide ruminal extrait de mouton, mélangé en proportions égales avec une solution tampon de salive artificielle et introduit dans des seringues calibrées de 100 ml en verre (39°C). Chaque incubation dure 24 heures, à l'issu de laquelle on mesure la production de gaz et une partie du liquide est recueillie pour le dosage de l'azote ammoniacal (NH_3-N sur 2 seringues). Trois seringues (5 au total par dose et incubation) ont été réservées pour déterminer la digestibilité réelle de la matière organique (DRMO). Le facteur de partition (FP) a été estimé comme étant le rapport entre la DRMO et les gaz produits à 24 h. La biomasse microbienne (BMM) a été estimée sur la base de substrat réellement dégradé et le FP. Les résultats ont montré que la poudre d'ail n'avait aucun effet sur la production de gaz pour les doses 4, 8 et 16 mg comparativement avec le témoin. Toutefois, lorsqu'on ajoute 32 et 64 mg de PA, la production de gaz augmente significativement (P <0,001) d'environ 13% pour chaque dose.*

Une augmentation (P <0,0001) de la concentration en NH_3-N a été enregistrée avec 4 et 8 mg de PA en comparaison avec le témoin. L'ajout de 16 mg de PA a entraîné une concentration en NH_3 -N équivalente au contrôle. Les doses de 32 et 64 mg de PAI ont diminué la concentration de NH_3-N de 20% comparativement au témoin. La valeur de la DRMO était similaire (en moyenne 72,2%) pour toutes les doses à l'exception de 64 mg, où une légère augmentation significative (P <0,001) a été observée (77,7%). La poudre d'ail n'a pas affecté les valeurs du FP lors de l'ajout de 4, 8, 16 ou 32 mg de PA comparativement avec le témoin. Cependant, nous avons enregistré une augmentation significative (P <0,0001) pour la dose 64 mg. La BMM était équivalente pour les doses 0, 4, 8, 16 et 32 mg, tandis que la valeur observée avec 64 mg était significativement (P <0,001) plus élevée. Il est à conclure que l'ail en poudre aurait induit des changements dans les fermentations du rumen mesurées in vitro. *Certaines de ces modifications pourraient avoir des effets positifs sur l'efficacité de l'alimentation chez les ruminants. D'autres mesures sont nécessaires pour compléter l'effet de l'ail sur l'activité de la population microbienne (production et composition des acides gras volatils) et sur les performances des animaux.*

Mots-clés. *Poudre d'ail – Fermentation ruminale* in vitro.

I – Introduction

The use of antibiotics as additives has been proved to be an efficient tool to reduce energy and nitrogen losses from the diet (McGuffey *et al*., 2001). However, using antibiotics as feed additives was banned in the European Union since January 2006 (Russell and Houlihan, 2003, Regulation 1831/2003 / EC) because they contribute to antimicrobial resistance both in animals and humans (Chesson, 2006). This situation represents serious risks to the humans (Anassori *et al*., 2011), and for this reason scientists are interested in evaluating the potential use of natural antimicrobials resources such as plant extracts (Busquet *et al*., 2005; Castillejos *et al*., 2006). Since a long time, garlic (*Allium sativum*) has been used by humans as a source of antimicrobial agents for gastrointestinal infections (Kongmun *et al*., 2010), and it is recognized as a medicinal plant. Some research suggests garlic as a growth promoter in livestock (Yan and al., 2011). This study aimed to evaluate the potential of garlic to modify rumen fermentation and to investigate the eventual beneficial effects on digestion in sheep mainly in Tunisian conditions, using a local verity of garlic.

II – Material and methods

White garlic *(Allium sativum)* was collected from the region of Beja (Northern-west of Tunisia, humid). Samples were dried at 40°C during 48h, and then ground to pass through a 1 mm screen and stored for chemical analysis and *in vitro* measurements. For inoculum preparation, rumen content was collected from four adult Barbarine sheep (average age and live weight: 12 months and 30 kg respectively), freshly sacrified at the municipal slaughterhouse of Ariana (Tunis). The data about diets were checked from the owners of the animals and those receiving generally oat hay based diets supplemented with barley grains were chosen in order to standardize the rumen fluid. The rumen contents of the 4 slaughtered sheep (1L per animal) was collected immediately after evisceration and transferred in pre-warmed thermos flasks rapidly to the laboratory where the contents were mixed and filtered through 4 layers of surgical gauze.

A composed diet (D: 50% of ray-grass hay and 50% of commercial concentrate on DM basis) was used to determine the effect of growing doses (0, 4, 8, 16, 32 and 64 mg) of white garlic powder on *in vitro* rumen fermentation parameters. Samples (0.5 g DM) of D were incubated in 100 ml glass syringes according to the technique of Menke and Steingass (1988). The incubation medium (50 ml) was a mixture of rumen fluid and buffer solution (1:1). Gas production was measured at 1, 2, 4, 6, 12 and 24 h of incubation. Diets were incubated in 5 replications (syringes) and in two successive batches. Each incubation lasted 24 h, then gas production and pH were measured and fluid samples were taken for NH_3-N analysis. Truly degraded organic matter (TDOM) was measured in 3 syringes. The partitioning factor (PF) was calculated as the ratio of substrate TOMD (mg) to the volume of gas (ml) produced (Blümmel *et al*., 1997). The microbial biomass (MBM) yield was calculated using the TOMD, the gas volume and a stoichiometric factor (Blümmel *et al*., 1997) as follows: MBM (mg) = Substrate truly degraded - (gas volume × stoichiometric factor), where the stoichiometric factor used was equal to 2.20 (value used for roughages).

Feeds were analyzed for dry matter (DM), ash and crude protein (CP) contents (AOAC, 1984). Cell wall composition (NDF, ADF and ADL) in feeds were analyzed as described by Van Soest *et al*. (1991). Ammonia-N was analyzed according to the method of Conway (1962).

The General Linear Model procedure (GLM) of SAS (2009) with the option of LS MEANS multiple ranges was used to analyze data. The model included effects of GP dose, incubation batch and interaction. The control syringes (T: containing buffered solution with inoculum) were used as co-variable in order to control rumen liquid variation.

III – Results and discussion

Chemical composition of feeds is presented in Table 1. Dry matter content of garlic was around 43.3%. Garlic is relatively high in CP (18.8% DM). This value is very close to that reported by Kongmun *et al.* (2010) and Klevenhusen *et al.* (2011). The total cell wall content (NDF) of garlic was low (7.9 %DM). This value is higher than that found by Manasri et *al.* (2012) and Kongmun et *al.* (2011). The ADF fraction (6.5% DM) was also higher than the values reported by Kongmun *et al*. (2011) and Manasri *et al*. (2012). Garlic cell wall was very lowly lignified and the obtained value of ADL did not exceed 2% DM. The differences observed in cell wall contents comparatively with literature may be related to the differences in remaining teguments in the analyzed substrate.

Table 1. Chemical composition of feeds (% of DM)

Feed	DM (%)	Ash	CP	NDF	ADF	ADL
White garlic	43.3	3.6	18.8	7.9	6.5	1.9
Ray grass hay	95.1	14.4	16	51.1	29.3	9.3
Concentrate	89	7.6	15.5	27.3	7.3	7.1

DM: Dry matter, CP: Crude protein, NDF: Neutral detergent fiber, ADF: acid detergent fiber, ADL: Acid detergent lignin.

The effect of increasing doses of GP on fermentation parameters is reported in Table 2. Compared to control (0 mg of GP), gas production increased significantly ($P<0.0001$) for doses 16, 32 and 64 mg. The highest gas production was observed for the highest dose (64 mg) and was significantly ($P <0.001$) higher than the value recorded at 32 mg. This confirmed the results of Anassori *et al*. (2011), who found that garlic increased the gas production and thus the digestibility of the substrate. In the same sense, Kongmun et *al*. (2010) showed that garlic powder used in *in vitro* assays, increased the density of the population of cellulolytic bacteria. Actually, increasing doses of garlic may increase gas production because it represents an organic matter and protein supply. These nutrients are generally associated to establish adequate environment for microflora (Chen *et al*., 2008). No effect was noted on pH values when doses of GP were increased, and values remained suitable for ruminal fermentation. This result is in agreement with the results found by Kongmun et *al.* (2011) and Manasri *et* al. (2012), which showed that supplementation with garlic powder and garlic oil did not affect rumen pH. An increase ($P<0.0001$) in NH_3-N concentration was recorded with 4 and 8 mg of GP comparatively with control (+ 9%). However, adding 16 mg of GP or more resulted in a NH_3-N concentration equivalent to control. Results mentioned in literature are somewhat controversed compared to ours. Indeed, Kongmun et *al*. (2010) showed that in a diet containing coconut, supplemented with different doses of garlic powder, NH_3-N concentrations decreased mainly by the dose of 16 mg compared to dose 32 and 64 mg. Also, the *in vitro* findings of Cardozo et *al*. (2014) mentioned that at 7.5 mg of GP, there is a decrease in the rate of NH_3-N by about 25%. These differences in results may be related to the differences in garlic verities and the wide variations of their chemical compositions and in experimental conditions.

Table 2. Effect of increasing doses (mg/incubation bottle) of garlic powder on fermentation parameters

	0	4	8	16	32	64	SEM
Gas 24 (ml)****	95.1^{d}	95.8^{dc}	96.5^{dc}	98.8^{c}	105.3^{b}	109^{a}	0.083
pH	6.58	6.60	6.58	6.59	6.54	6.50	0.0261
NH3-N (mg/100ml)***	39,4^{b}	43^{a}	43^{a}	40,4^{ab}	39,1^{b}	39,4^{b}	0.658

$^{a, b, c, d}$ Values with different letters in the same line are statistically different.

*** P<0.001, **** P<0.0001,SEM: Standard error of the mean.

Table 3 reports the results of TOMD, PF and MBM. The TOMD values did not differ among GP doses except for dose 64 were the observed value was significantly (P<0.001) higher. This higher value may be related to the nutriment supply from garlic, manly nitrogen, which may be associated to an increase in the activity of cellulolytic bacteria (Kongmun *et al*., 2010), and is in concordance with the increase in gas production registered at this dose (Anassori *et al*., 2011). Our results are in contrast (only for dose 64) with those found *in vivo* by Manasri *et al.* (2012), who reported that supplementation with garlic powder did not affect the OM digestibility in cattle. As for TOMD, the PF values (P<0.0001) and the MBM (P<0.001) only increased at a dose of 64 mg GP. Indeed, the 64 mg dose provided larger proportions of degradable organic matter, mainly protein that could be captured for the microbial synthesis in the rumen. This would have led to an improvement of the efficiency of the microbial activity, with an increase in digestibility (Krishnamoorthy and Robinson, 2010). On the other hand, our results did not agree with those reported by Kumar *et al*. (2012) who found that MBM values decreased with increasing doses of GP. The observed results for dose 64 mg may be much more related to the supply of digestible OM and N from garlic, rather than to a specific effect of bioactivity of garlic components (Chen *et al*., 2008).

Table 3. Effects of increasing doses (mg/incubation bottle) of garlic powder on true organic matter degradability (TOMD), partitioning factor (PF) and microbial biomass yield (MBM)

	0	4	8	16	32	64	SEM
TOMD (%) ***	72.1^{b}	72.6^{b}	72.9^{b}	72.9^{b}	73.9^{b}	77.7^{a}	0.0048
PF (mg/ml) ****	3.23^{b}	3.19^{b}	3.19^{b}	3.22^{b}	3.25^{b}	3.75^{a}	0.0429
MBM (mg) ***	153.7^{b}	147.5^{b}	149.5^{b}	152.3^{b}	165.7^{b}	228^{a}	5.48

$^{a, b, c, d}$ Value with different letters in the same line are significantly different.

*** P<0.001; ****: p<0.0001; SEM: Standard error of the mean.

IV – Conclusion

It was concluded that GP could induce changes in *in vitro* fermentation. Some of these modifications could have a positive effect on feeding efficacy in ruminants. Further measurements are needed to investigate mainly the effect of garlic on microbial activities, such as effects on volatile fatty acid (VFA) production and composition and on animal performances.

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