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Garlic (*Allium sativum* L.) chemical composition, antioxidant and antimicrobial activities, and *in vitro* effect on digestion in sheep

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Abstract. An *in vitro* study also was conducted to evaluate the effect of garlic (*Allium Sativum* L.) powder (GaP) supplementation on rumen fermentation. Two successive 24 h-incubations were practiced. Growing doses of garlic powder were added to 500 mg of a diet composed (50% of rye-grass hay, 50% of concentrate on DM basis). pH, ammonia nitrogen (NH_3-N) and true organic matter degradability (TOMD) were measured and partitioning factor (PF) and microbial biomass (MBM) were estimated. Results showed that garlic powder was low in ash (3.5 % DM), relatively high in CP (16.6% DM) and very lowly lignified (ADL < 2.2 % DM). Colorimetric analysis revealed that total phenols, flavonoids and tannins contents were respectively 0.37 mg AG.eq, 0.11 mg EC and 30.2 mg EC /g DM. All aqueous extracts exhibited a high, concentration dependent, free radical-scavenging activity. It was found that all extracts exhibited antibacterial activity against Gram(–) (*Escherichia coli*) and Gram(+) bacteria (*Staphylococcus aureus* and *Bacillus subtilis*), but ethanol extract was proven to be more efficient against the Gram(–) bacteria. The *in vitro* trial results indicated that GaP (32 and 64 mg doses) increases significantly (P<0.001) gas production after 24 h. No effect on pH was observed. An increase (P<0.0001) in NH3-N concentration was recorded with 4 mg and 8 mg doses compared with control; whereas adding 32 and 64 mg resulted in a NH3-N concentration equivalent to control. Total VFA concentration noted with control (73.6 $mmolL^{-1}$) was lower (P<0.001), compared to that noted from adding GaP (averaged 99.28 $mmolL^{-1}$). The propionate (C3) increased with GaP, and the highest proportion was noted with the addition of 8 mg (*P* <0.001). The TOMD was similar for all the doses except for 64 mg GaP, where a slight but significant (*P* <0.001) increase was noted (77.7%). PF values were equivalent in all doses (averaged 3.2), while a significant (*P*<0.0001) increase (4.45) was noted with 64 mg. The same trend was observed in MBM (*P* <0.001). It was concluded that GaP present some advantages related to its antioxidant and antimicrobial actions and could be considered as a natural alternative to synthetic antioxidants. Also, it modified positively rumen fermentations. *In vivo* and performance and quality measurements are necessary to confirm the eventual positive benefits of garlic on ewes feeding.

Keywords. Garlic – Secondary compounds – Antioxidant – Antimicrobial activities – *In vitro* fermentation – Sheep.

L'ail* (Allium sativum *L.) dans la nutrition des ruminants: composition chimique, activités antioxydante et antimicrobienne et effet in vitro sur la digestion chez les ovins

Résumé. *Une étude* in vitro *a été menée pour évaluer l'effet de la supplémentation avec de la poudre d'ail (PA) sur la fermentation dans le rumen. Deux incubations successives de 24 h ont été pratiquées. Des doses croissantes de poudre d'ail ont été ajoutées à 500 mg d'un régime composé de 50% de foin de ray-grass et de 50% de concentré. Le pH, l'azote ammoniacal (NH3-N) et la dégradabilité réelle des matières organiques (TDMO) ont été mesurés, el le facteur de partition (PF) et la biomasse microbienne (MBM) ont été estimés. La PA était faible en cendres (3,5% MS), relativement élevée en MAT (16,6% MS) et très faiblement lignifiée (teneur en ADL <2,2% MS). L'analyse colorimétrique a révélé que les teneurs totales en phénols, en flavonoïdes et en tanins étaient respectivement de 0,37 mg d'AG.eq, 0,11 mg EC et 30,2 mg EC / g de MS. Tous les extraits aqueux présentaient une activité de piégeage des radicaux libres élevée. Les extraits présentaient une activité antibactérienne contre presque toutes les bactéries (Escherichia coli, Staphylococcus aureus et Bacillus subtilis) mais l'extrait d'éthanol était plus efficace contre les bactéries Gram(–). L'essai in vitro a indiqué que la PA (en doses de 32 et 64 mg) augmente (P <0,001) la production de gaz pour après 24 h. L'ail n'a eu aucun effet sur le pH. Une augmentation (P <0,0001) de la concentration en NH3-N a été enregistrée avec les* *doses 4 mg et 8 mg, alors que l'ajout de 32 et 64 mg a entraîné une concentration équivalente au contrôle (moyenne de 39,25 mg / 100 ml). L'ajout de PA a entraîné une augmentation équivalente des concentrations en AGVs totaux pour toutes les doses (moyenne de 99,28 mmolL^{-1}, par rapport aux 73.6 mmL^{-1} du contrôle). Le propionate (C3) augmentait avec les doses et la proportion la plus élevée était observée avec 8 mg de PA (P <0,001). La TDMO était similaire pour toutes les doses, à l'exception de 64 mg de PA, où une augmentation légère mais significative (P <0,001) a été observée (77,7%). La TDMO ne différaient pas entre les doses de PA, à l'exception de la dose de 64 mg, pour laquelle la valeur observée (77,7%) était significativement plus élevée (P <0,001) que toutes les autres (en moyenne73,1%). Les valeurs de PF étaient équivalentes pour toutes les doses (en moyenne 3,2), sauf pour 64 mg (P <0,0001). La même tendance a été observée pour MBM (p <0,001). Il a été conclu que la poudre d'ail pouvait présenter des avantages liés à ses actions antioxydante et antimicrobienne et pourrait être considérée comme une alternative naturelle aux antioxydants synthétiques. En outre, il s'est avéré modifier positivement les fermentations dans le rumen. Des mesures in vivo et des performances sont nécessaires pour confirmer les éventuels avantages positifs et pour étudier les effets de l'ail sur la digestion, les performances des animaux et la qualité du produit.*

Mots-clés. *Ail – Composés secondaires – Antioxydant – Activités antimicrobiennes – Fermentation* in vitro *– Moutons.*

I – Introduction

Recently, the administration of antibiotics in animal feed as growing promoters is going to be increasingly alarming because of the emergence of potential bacterial resistance among animal food products consumers (Anassori *et al*., 2011).The utilization of antibiotics could result in the appearance of residues in the final animal products. This situation has prompted scientists to find other naturals alternatives. In this connection, researches have shown that many plants and their extracts have a very strong antibacterial activity and can be exploited to develop antibiotics activities for the treatment of humans and animals (Busquet *et al*., 2005; Castillejos *et al*., 2006). Aromatic plants are able to naturally produce biologically active metabolites (Khadri *et al*., 2010). Indeed, in addition to the primary metabolites, aromatic plants accumulate secondary metabolites. Among these plants, garlic *(Allium sativum* L.) is beginning to have much interest as a potential source of natural active bio-molecules in animal nutrition. Some research suggests garlic as a growth promoter in livestock (Yan and et al., 2011). Anassori *et al*. (2011) demonstrated that raw garlic and garlic oil resulted in a range of beneficial effects on rumen fermentation. This study was carried in order to evaluate the chemical composition, the secondary compounds contents and the antioxidant and the antimicrobial activity of a local Tunisian variety. Also, effect of garlic powder on *in vitro* rumen fermentation characteristics was investigated.

II – Materials and methods

Garlic *(Allium sativum)* was harvested from the region of Beja (Northern-west of Tunisia, humid). Samples were dried at 50°C during 48h, and then ground to pass through a 1 mm screen and stored for chemical analysis and other determinations.

Garlic powder was analyzed for dry matter (DM), ash and crude protein (CP) contents (AOAC, 1984). Cell wall composition in feeds (NDF, ADF and ADL) was analyzed as described by Van Soest *et al.* (1991).The total phenols content was determined using Folin-Ciocalteu reagent and gallic acid was used as standard (Lister and Wilson, 2001). Total flavonoids were measured by a colorimetric assay according to Dewanto *et al*. (2002) using te method based on the formation of flavonoid-aluminum complex.Tannins contents were determined as described by Joslyn (1970) and absorbance was measured at 760 nm after addition of Folin-Denis reagent and Na_2CO_3. Each assay was repeated in triplicate.

Antioxidant activity was evaluated as free radical scavenging capacity by measuring the scavenging activity of garlic extract on DPPH (Sánchez-González *et al*., 2011).

The ethanol and the methanol extract of garlic were individually tested for their antibacterial activity against Gram negative bacteria *(Escherichia coli* (E.coli)) and Gram positive bacteria *(Staphylococcus aureus* and *Bacillus subtulis*). Petri dishes were prepared and the discs with extract were applied. Bacterial strains were grown in trypto-caseine soja agar (TSA) and incubated at 37°C for 24 h. Antimicrobial activity was assessed as described by Teixeira *et al*. (2012). All these measurements were carried out in triplicates.

Rumen content was collected from 4 adult Barbarine sheep (average 12 months, 30 kg live weight) slaughtered at the municipal slaughterhouse of Ariana (Tunis). Animals were chosen in order to standardize the rumen fluid. The rumen contents of the 4 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 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 (50ml) 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. At the end of incubation, two syringes from each dose were reserved for NH_3-N and VFA analysis using gas chromatography.Three syringes were used to determine DMO. Cumulative gas production data were fitted according to the model of Orskov and McDonald (1979). The partitioning factor (PF) was calculated as the ratio between TODM (mg) and the gas produced at 24 hours of incubation of substrate truly degraded (Blümmel *et al*., 1997). The microbial biomass (MBM) yield was calculated using the TODM (mg) and gas volume (24 h) and stoichiometric factor (Blümmel *et al.,* 1997) as follows: MBM (mg) = substrate truly degraded (gas volume × stoichiometric factor), where the stoichiometric factor was equal to 2.25 (value used for roughages).

For the *in vitro* trial, 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 garlic powder is presented in Table 1. Garlic is relatively high in CP (16.6% DM). Our results are 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 (58 %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 (ADL: 3.5% DM). Garlic contains various secondary compounds at different levels, such as phenols, flavonoids and tannins. Results of the quantitative determination of total phenol, flavonoids and total tannins are also reported in Table 1.

The obtained results of the antioxidant activity shows that the percentage inhibition of garlic was 155.16 µg/ml. Both ethanolic and methanolic extracts of garlic showed variable antibacterial activity against Gram (+) and Gram (–) strains. The ethanolic extract affected more the Gram (+) bacteria than the Gram (–) ones. Our results are in agreement with Gulfraz *et al.* (2011) who showed that the ethanolic extracts of garlic show an inhibitory activity against all bacteria tested Gram (+)

and Gram (–) with zones of inhibition between 10.3 (+/– 0.5 mm) and 14.3 (+/– 0.5 mm).The ethanolic extract has the strongest antibacterial activity against the different bacteria tested. This activity was more important against Gram (+) bacteria essentially against Staphylococcus aureus with a diameter of inhibition: 18 mm (ZouariChekki *et al*., 2014).

Table 1. Chemical composition of garlic used in the experiment (% of dry matter)

DM_F (%)	DM Powder (%)	Ash %DM	MO %DM	CP %DM	NDF %DM	ADF %DM	ADL %DM	Total polyphenols (mg/g eq.AG)	Flavonoids (mg EQ/g MS)	Tanins (mg EQ/g MS)
35.5	87.7	3.4	96.5	16.6	5.8	3.2	–	0.37	0.11	30.2

DM: dry matter, CP: crude protein, NDF: neutral detergent fiber, ADF: acid detergent fiber, ADL: acid detergent lignin.

In vitro results are reported in Table 2. Garlic powder increases significantly (P<0.001) gas production for 32 (104,32 ml) and 64 mg (108,64 ml). An increase (P<0.0001) in NH3-N concentration was recorded with doses 4 and 8 mg, compared with control; whereas adding 32 and 64 mg resulted in a NH3-N concentration equivalent to control (averaged 39.25 mg/100 ml). The total VFA concentration noted with control (73.6 $mmolL^{-1}$) was the lowest value (P<0.001). Adding GaP resulted in increased, but equivalent VFA concentrations for all the other doses (averaged 99,28 $mmolL^{-1}$). The propionate (C3) increased with garlic adding and a higher proportion was noted with all the supplemented samples (averaged 19,08%, *P* <0.001). Wanapat *et al.* (2008), reported that increased supplementation of garlic powder in diets resulted in increased C3 and C4 proportions. The authors reported that the increased proportion of C3 was simultaneous with a reduction of the proportion of C2.

Table 2. Effect of increasing doses of garlic powder on rumen fermentation

Dose (mg)	0	4	8	16	32	64	SEM
Gas 24 (ml)	86.31^{c}	87.07^{c}	88.08^{c}	91.53^{bc}	104.32^{ab}	108.64^{a}	1.39
pH	6	5.97	5.99	6.0	5.97	5.95	0.0259
NH_3-N (mg/100ml)	35.49^{c}	40.95^{a}	40.8^{a}	39.17^{b}	38.15^{bc}	40.25^{ab}	0.572
Total VFA ($mmolL^{-1}$)	73.6^{b}	133.5^{a}	142.2^{a}	133.69^{a}	122.96^{a}	136.35^{a}	15.1
$\%C_2$	62.81^{a}	57.86^{b}	58.86^{b}	58.53^{b}	57.87^{b}	59.86^{b}	0.93
$\%C_3$	17.54^{b}	19.01^{a}	19.04^{a}	19.2^{a}	18.95^{a}	19.19^{a}	0.32
$\%C_4$	11.5^{b}	15.14^{a}	15.23^{a}	15.23^{a}	15.72^{a}	14.95^{a}	0.83
C_2/C_3	3.55^{a}	2.92^{b}	3.09^{b}	3.06^{b}	3.01^{b}	3.12^{b}	0.07

$^{a, b, c, d}$. Values with different letters in the same line are statistically different, *** P<0.001, **** P<0.0001, SEM: Standard error of the mean.

Microbial activity results are presented in Table 3. The TOMD was similar for all the doses except for 64 mg GaP, where a slight but significant (*P* <0.001) increase was noted (77.7%; vs an average of 73.1% for all the others. PF values were equivalent in all doses (averaged 3.2), while a significant (*P* <0.0001) increase was noted with 64 mg. The same trend was observed in MBM (*P* <0.001). The current results with the dose of 64 mg may be related to the small amounts of nutriment supplied from garlic, and are in line with the increase in gas production registered at this dose (Anassori *et al.,* 2011).

Table 3. Effects of increasing doses of garlic powder on DMO, PF and MBM

Dose (mg)	0	4	8	16	32	64	SEM
TOMD (%)	64.58^{a}	64.22^{a}	67.04^{a}	69.74^{a}	68.34^{a}	77.7^{b}	0.009
PF	3.14^{cd}	3.09^{b}	3.39^{bcd}	3.48^{bc}	3.64^{bc}	4.45^{a}	0.1
MBM (mg)	99.09^{b}	115.93^{b}	133.27^{b}	158.62^{b}	187.72^{ab}	281.66^{a}	7.19

$^{a, b, c, d}$. Values with different letters in the same line are statistically different, *** P<0.001, **** P<0.0001, SEM: Standard error of the mean.

IV – Conclusions

Garlic powder was relatively high in CP. Extracts had different contents of secondary compounds. For the anti-microbial activity, methanol extract was more active against Gram (–) bacteria than ethanol extract. Garlic was found to modify positively rumen fermentations. Red garlic powder could present some feeding advantages related to its antioxidant and antimicrobial actions and could be considered as a natural alternative to synthetic antioxidants. Some interesting modifications in rumen fermentation trends were observed. The effects of garlic on animal performances and product quality, considering the corresponding doses are to be investigated.

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