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# Effect of barley replacement by acorns (*Quercus coccifera* L.) as energy supplement on *in vitro* fermentation

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**SUMMARY** – The effect of barley replacement by acorns of kermes oak on *in vitro* gas production, using vetchoat hay basal diet, was studied. Control diet (D0) was composed of a mixture of oat-vetch hay and barley grains (2/3 and 1/3 respectively). Experimental diets included increased proportions of acorns (A) replacing barley (B) in the concentrate part of D0, on DM basis (D1: 25% A, 75% B; D2: 50% A, 50% B; D3: 75% A, 25% B; D4: 100% A). For all diets, kinetic of *in vitro* gas production was determined in 100 ml graduated glass syringes with inocula from fistulated goats. All diets were low in crude protein (6.5 to 8.3% DM, respectively in D3 and D0) and moderate in lignocellulose (ADF) (19.5 to 22.7% DM, respectively in D0 and D4). Acorns are lower in metabolisable energy (1767 kcal kg<sup>-1</sup> DM) than barley (2484 kcal kg<sup>-1</sup> DM). Diets 0, D1 and D2 exhibited similar total gas production (69.3 ml on average). Diet 3 and D4 produced similar amounts of gas (65.4 ml), significantly (P<0.01) lower than D0, D1 and D2. The rate of gas production values ranged from 0.057 (D4) to 0.062 h<sup>-1</sup> (D2). It was concluded that acorns could replace no more than 50% of the amount of barley grains in the concentrate used in this experiment otherwise a decrease of the *in vitro* gas production, thus of microbial activity is expected.

**Keywords:** Acorns, *Quercus coccifera*, barley, energy, *in vitro*, fermentation.

RESUME - "Effet du remplacement de l'orge par les glands (Quercus coccifera) comme supplément énergétique sur la fermentation in vitro". Nous avons étudié l'effet du remplacement de l'orge par les glands de chêne Kermès dans un régime à base de foin de vesce-avoine sur la fermentation in vitro. Le régime témoin (R0) est composé d'un mélange de foin (ration de base : 2/3) et d'orge (concentré : 1/3). Les régimes expérimentaux contiennent des proportions croissantes de glands (G) en remplacement de l'orge (O) sur la base de la MS (R1: 25% G, 75% O; R2: 50% G, 50% O; R3: 75% G, 25% O; R4: 100% O). Pour tous les régimes, nous avons mesuré la cinétique de production de gaz in vitro dans des seringues en verre à travers des incubations dans un inoculum provenant de caprins porteurs de canules ruminales. Les teneurs en MAT étaient faibles pour tous les régimes (de 6,5 à 8,3% MS, respectivement dans R3 et R0). Les teneurs en ADF étaient modérées (de 19,5 à 22,7% MS, respectivement dans D0 et R4). Les glands avaient une teneur en énergie métabolisable calculée en fonction de la production de gaz (1767kcal kg<sup>-1</sup> MS), plus faible que celle de l'orge (2484 kcal kg<sup>-1</sup> MS). Les régimes R0, R1 et R2 ont présenté des productions totales de qaz similaires (en moyenne 69.3 ml). Les régimes R3 et R4 ont produit des quantités équivalentes de gaz (en moyenne 65,4 ml) significativement inférieures à celles de R0, R1 et R2. La vitesse de production de gaz a varié de 0,057 (R4) à 0,062 h 1 (R2). Nous avons conclu que les glands ne peuvent remplacer l'orge à plus de 50% dans le concentré. Au-delà de ce niveau, nous avons constaté une diminution significative de la production de gaz, indiquant une diminution de l'activité microbienne.

Mots-clés: Glands, Quercus coccifera, orge, énergie, in vitro, fermentation.

# Introduction

In Tunisia, a growing interest is given to some forestry products such as shrubs leaves and fruits in small ruminants feeding. Acorns from oak trees, mainly Kermes oak (*Quercus coccifera*) and cork oak (*Quercus suber*) are abundant in Tunisian coastal-forestry regions (Ministry of Agriculture, 1995). Acorns are generally harvested by farmers in October to December and fed to goat, marketed or traditionally conserved (Kayouli and Buldgen, 2001). In a previous work, we studied the effect of maturity stage on the nutritive value of acorns from kermes oak (Moujahed *et al.*, 2005) in the region of Bizerte (North of Tunisia). The main results suggested that the period between the end of November and the end of December seemed to be the best time to harvest acorns. During this period, acorns are abundant and feature highest nutritional value, with highest *in sacco* degradation and *in vitro* fermentation. Acorns seemed to be a highly energetic resource for small ruminants (Kayouli and Buldjen, 2001) and often compared to barley (El Jassim *et al.*, 1998). This experiment aimed to study the effect of replacing partially or totally barley with acorns from Kermes oak in concentrate on *in vitro* fermentation of small ruminant diets.

# **Material and methods**

### Plant material

Acorns (*Quercus coccifera*) were harvested in the forestry region of Bizerte (North of Tunisia, humid). Samples from 4 trees were harvested at maturity stages 3 and 4 (Moujahed *et al.*, 2005; respectively November and December 2002) and mixed to make a composite sample. Dry matter (DM) was determined at 105°C in a forced-air oven and the samples were dried at 40°C during 48h, ground to pass through 1 mm screen and stored for chemical analysis and *in vitro* determinations.

# Diets, animals and measurements

Five diets using oat-vetch hay as roughage and barley and/or acorns as concentrate were studied. Control diet (D0) was composed of a mixture (2/3 and 1/3 respectively) of hay (H) and barley(B). Experimental diets included increased proportions of acorns (A) replacing barley (B) in the concentrate part of D0, on DM basis (D1: 25% A, 75% B; D2: 50% A, 50% B; D3: 75% A, 25% B; D4: 100% A). Diets mixtures were made of ground feeds using a mixer.

Four adult local goats with rumen cannula (average liveweight: 44.5 kg) were used for *in vitro* determinations. They were housed in individual pens and received 70 g kg<sup>-1</sup>LW<sup>0.75</sup> of a ration composed of 70% of oat-vetch hay and 30% of commercial concentrate on dry matter (DM) basis twice per day.

Samples of diets D1-D4 (300 mg DM) were incubated in triplicate in 100 ml glass syringes according to the technique of Menke and Steingass (1988). Gas production was recorded after incubation for 1, 2, 4, 6, 12, 24, 36, 48, 72 and 96 h. Two runs were performed for each diet.

Metabolisable energy (ME) and degradable organic matter (dOM) of feeds were calculated as described by Menke and Steingass (1988). Gas productions were fitted using the model of Ørskov and McDonald (1979): p=a+b(1-e<sup>-ct</sup>), where: (i) p is the gas production at time t; (ii) 'a' is the immediate gas production; (iii) 'b' is the slowly fraction or gas production; (iv) 'c' is the rate of gas production; and (vi) 'a+b' is the total gas production. Parameters were calculated using the Non Linear procedure (SAS, 1985). The General Linear Model procedure (GLM) with the option of Duncan multiple ranges was used for statistical analysis of data (SAS, 1985).

# Laboratory analysis

Feeds and acorn samples, ground to pass through a 1 mm screen sieve, were analysed for DM, ash and crude protein (CP) according to AOAC (1984). Neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) were analysed as described by Goering and Van Soest (1970). Acorns were analysed for total tannins (Makkar and Goodchild, 1996).

# Results and discussion

The chemical composition, *in vitro* organic matter degradability and metabolisable energy of feeds and diets are presented in Table 1. Acorns were low in CP and ash (3.9 and 2.2% DM respectively). All diets were low in CP (6.5 to 8.3% DM, respectively in D3 and D0). Irrespective of there highest barley contents, D0 and D1 presented the highest levels of nitrogen. ADF was moderate in diets (19.5 to 22.7% DM, respectively in D0 and D4). Overall, nutrient contents are in line with those reported in the literature dealing with acorns of Vallenea and Kermes oaks (El Jassim *et al.*, 1998), and cork oak (Kayouli and Buldgen, 2001). Calculated *in vitro* organic matter digestibility (dMO) was highest in barley (64.2%) when compared to hay and acorns. Acorns are lower in metabolisable energy estimated on the basis of gas production technique (1767.4 kcal kg<sup>-1</sup> DM) than barley (2484 kcal kg<sup>-1</sup> DM). Total tannins content in acorns was low (4% DM).

Table 1. Chemical composition (% DM), *in vitro* organic matter degradability (dMO: %) and metabolisable energy (ME: kcal kgDM<sup>-1</sup>) of feeds and diets

	Ash	СР	NDF	ADF	ADL	dMO	ME
Hay	8.6	6.8	40.6	25.8	4	48.7	1578
Barley	2.5	11.4	28.2	6.8	1.6	64.2	2484
Acorns	2.2	3.9	36.25	14.3	5	47.6	1767
D0	6.6	8.3	36.5	19.5	3.2	56.3	1881
D1	6.6	7.7	37.1	20.3	3.6	55.3	1842
D2	6.5	7.1	37.7	21.1	4	56	1867
D3	6.5	6.5	38.4	21.9	4.4	54	1797
D4	6.5	5.8	39	22.7	4.8	52.6	1739

D0: H + 100% B; D1: H + 25% A + 75% B; D2: H + 50% A + 50% B;

D3: H + 75% A + 25% B; D4: H + 100% A.

Data on in vitro gas production parameters are presented in Table 2. The immediate gas production (a) was low among the five studied diets. This parameter ranged from -1.3 (D4) to 0.85 ml (D0). The negative values observed in D2, D3 and D4 may indicate a lag time at the beginning of fermentation. This phenomenon is less pronounced in D0 and D1 (P<0.001), probably due to differences in diets composition (higher content of barley in D0 and D1). Diets D0, D1 and D2 exhibited similar total gas production (69.3 ml on average). Diet 3 and D4 produced similar amounts of gas (65.4 ml on average), significantly (P<0.01) lower than D0, D1 and D2. This may indicate that increasing the level of acorns to replace barley in the diets reduces their fermentation pattern when it exceeds 50% of concentrate. This result could be explained by the relatively higher content of fibre, mainly ADF and ADL in D2, D3 and D4 when compared to D0 and D1. In addition, it is possible to attribute this result to the higher content of nitrogen in D1 and D2, since availability of degradable nitrogen enhances fermentation and increase digestibility of the diet (Chenost and Dulphy, 1987) through the better utilization of ammonia-nitrogen sourcing from proteolysis (Faverdin et al., 2003), mainly in presence of energy from barley and/or acorns. The rate of gas production values (c) ranged from 0.057 (D4) to 0.062 h<sup>-1</sup> (D2). In spite of the significant effect (P<0.01) observed for "c", it seems that the intensity of fermentation activity is similar among diets (0.059 h<sup>-1</sup> on average).

Table 2. Effects of barely replacement of acorns on in vitro gas production parameters

	Diets					
Parameters	D0	D1	D2	D3	D4	SEM
a (%)***	0.85 <sup>a</sup>	0.24 <sup>a</sup>	-0.5 <sup>b</sup>	-1 <sup>bc</sup>	-1.3 <sup>c</sup>	0.22
b (%)*	68.7 <sup>ab</sup>	68.4 <sup>ab</sup>	70.2 <sup>a</sup>	67.2 <sup>bc</sup>	65.8 <sup>c</sup>	0.75
c (h <sup>-1</sup> )**	0.06 <sup>abc</sup>	0.059 <sup>bc</sup>	0.062 <sup>a</sup>	0.061 <sup>ab</sup>	0.057 <sup>c</sup>	0.0008
a+b(%)**	69.6 <sup>a</sup>	68.6 <sup>a</sup>	69.8 <sup>a</sup>	66.2 <sup>b</sup>	64.5 <sup>b</sup>	0.76

<sup>&</sup>lt;sup>a, b, c</sup> row-wise values with the same letter do not differ significantly, \* P<0.05, \*\* P<0.01, \*\*\*P<0.001.

Our results are in line with those of El Jassim *et al.* (1998) who studied the efficiency of utilization of concentrate diets containing acorn (*Quercus aegilops* and *Quercus coccifera*) and urea by growing lambs. The authors reported that the substitution of barley with acorns at the rates of 50 and 100% in concentrate decreased digestibility coefficient of dietary constituents (DM, OM, CP, NDF and ADF). They suggested that these results may be due to the presence of antinutritive factors in acorns, such as tannins. These secondary compounds may inhibit cellulolysis by forming tannins-cellulase (Griffiths and Jones, 1977) and tannin-protein complexes (Van Soest, 1982). In our experiment, in spite of the possibility of inhibition of microbial fermentation and *in vitro* gas production (Khazaal *et al.*,

1993) due to acorns tannins, such explanation could not be clamed since total tannins content was low (4% DM). This trend could be explained by the decrease of the levels of nitrogen and energy in diets as consequence of increased substitution level of barley with acorns. Moreover, the low tannin intake by small ruminants, could involve positive effects mainly on N metabolism. Indeed, condensed tannins, at low levels, may complex proteins and protect them from microbial enzymes (Reed *et al.*, 1995) and protect soluble proteins from microbial degradation (Wang *et al.*, 1994). This may result in increased supply of dietary amino acids in the small intestine. El Jassim *et al.* (1998) concluded that acorns could replace barely in concentrate until the rate of 50%.

### **Conclusions**

It seems that acorns from Kermes oak could not replace barley over than 50% of concentrate in a vetch-oat hay basal diet. Beyond this level, there is a significant decrease of *in vitro* gas production which may indicate a decrease in microbial activity. However, this effect could be counterbalanced positively by an eventual beneficial effect of tannins, mainly in nitrogen metabolism. Such hypothesis needs to be confirmed *in vivo*.

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