Strategies to manipulate rumen fermentation for better utilizing feedstuffs in goats

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Strategies to manipulate rumen fermentation for better utilizing feedstuffs in goats

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Abstract. The development and implementation of strategies aiming to optimize rumen fermentation is key to improve feedstuffs utilization and then to optimize ruminant production. Of special importance are strategies that may be applied to unconventional feedstuffs. The main challenges nowadays of ruminant production are to reduce feeding costs, improve products quality and diminish the impact of production on environment. Strategies have to be developed to achieve these objectives. Strategies based on the use of unconventional feedstuffs like by-products and wastes may contribute to both decreased feeding cost and environmental impact of ruminant production thought both recycling by-products and decreasing methane emissions and, improved products quality (i.e. fatty acid profile in milk). Microbial protein synthesis, methanogenesis and biohydrogenation are key processes in ruminal fermentation. The development of efficient strategies is, in a great extent, based on the knowledge of those processes and rumen microorganisms involved. Some strategies based on both the use of feed blocks including by-products and wastes or additives are considered and their effects on microbial protein synthesis, methane emissions and products quality in goats described. Feed blocks including olive cake or waste fruits from greenhouse horticulture have been used to partially replace cereals-based concentrates in goats diet and the effects on feeding cost, ruminal fermentation, microbial protein synthesis, methanogenesis and milk yield and composition were studied. Changes in the abundances of total bacteria and methanogenics archaea were studied as well. A wide range of plant extracts and secondary compounds have been used as additives to manipulate the fermentation in the rumen to both reduce protein degradability and minimize methane emissions. The main challenge is to confirm in vivo the potential that in vitro trials have shown.

Keywords. Dietary strategies – Feedstuffs utilization – Rumen fermentation – Microbiota – Methane – Milk-Goat.

Stratégies pour manipuler les fermentations du rumen vers une utilisation optimale des aliments

Resumé. *Le développement de stratégies pour optimiser la fermentation du rumen est essentiel pour améliorer l'utilisation des aliments et pour optimiser la production des ruminants. Les stratégies appliquées aux aliments non conventionnels comme les sous-produits et les déchets sont particulièrement importantes. Les principaux objectifs de la production des ruminants sont de réduire le coût d'alimentation, d'améliorer la qualité des produits et de diminuer l'impact de la production sur l'environnement. Les aliments non conventionnels peuvent contribuer à diminuer le coût d'alimentation et l'impact environnemental et améliorer la qualité des produits (profil d'acides gras dans le lait par exemple). La synthèse des protéines microbiennes, la méthanogénèse et la biohydrogénation sont des facteurs clés des fermentations ruminales. Des stratégies pour maximiser la protéine microbienne, minimiser la production de méthane et optimiser la biohydrogénation sont présentées. Le développement de stratégies efficientes est basé en grande partie sur la connaissance du macrobiote ruminal. Certaines stratégies basées sur l'utilisation de blocs multinutriments, y compris les sous-produits et déchets agro-industriels, ou additifs ainsi que leurs effets sur la protéine microbienne, la production de méthane et la qualité du lait chez les chèvres seront décrites. Des blocs incluant des grignons d'olive ou déchets des fruits de l'horticulture sous serre ont été utilisés pour remplacer partiellement des concentrés à base de céréales dans les régimes des chèvres en lactation. Leurs effets sur le coût du régime, la fermentation ruminale, la synthèse de protéine microbienne, les productions de méthane et de lait ainsi que la composition du lait ont été étudiés. Les changements de l'abondance des bactéries totales et d'archaea méthanogènes ont été aussi étudiés. Une large gamme d'extraits de plantes et de composés secondaires ont été utilisés comme additifs pour manipuler la fermentation dans le rumen et la dégradabilité des protéines et à la fois réduire et minimiser les émissions de méthane. Le principal défi est de confirmer* in vivo *le potentiel que les essais* in vitro *ont montré.*

Mots-clés. *Stratégies nutritionnelles – Utilisation des aliments – Fermentations du rumen – Microbiote – Méthane – Lait – Chèvre.*

I – Introduction

The development and implementation of strategies aiming to optimize rumen fermentation is key to improve feedstuffs utilization and then to optimize ruminant production. Of special importance are strategies that may be applied to unconventional feedstuffs. The main challenges nowadays in ruminant production are to reduce feeding costs, improve products quality and diminish the impact of production on environment. The use of unconventional feedstuffs may contribute to decrease feeding cost and environmental impact through reduced methane emissions. In addition, improving products quality (i.e. fatty acid profile in milk) has been also shown potentially achievable by using such ingredients in the diet (Vasta *et al.*, 2008). Microbial protein synthesis, methanogenesis and biohydrogenation are key factors in ruminal fermentation. A better knowledge of those processes, driving factors and microorganisms involved and their mechanisms of action may help to develop efficient strategies.

In this work we present some results obtained in our group on the effects of using feed blocks for optimizing the use of agricultural by-products/wastes and also additives (i.e. essential oils, organosulphur compounds and other antimethanogenic chemicals) on rumen function, nutrient utilization and milk yield and composition in goats. Nowadays there is an increased interest on the development of dietary strategies that could reduce methane emissions by ruminants (Martín *et al.*, 2010). Methane emission from the enteric fermentation is of concern worldwide due to its potential as greenhouse gas (Wright and Klieve, 2011). Additionally methane represents an energy loss of around 2 to 12% of the gross energy supply for the animal (Johnson and Johnson, 1995; Hook *et al.*, 2010). Research has been focused on feed additives or diet ingredients that could reduce the formation of saturated FA and increase the concentration of unsaturated FA in ruminant products. As far as we know, few studies have investigated the effect of strategies based on the use of by-products on milk FA composition (Molina-Alcaide *et al.*, 2010; Modaresi *et al*., 2011) and methane emissions by ruminants at the same time.

II – Strategies based on the inclusion of by-products and wastes in feed blocks

Livestock production in Mediterranean countries is constrained by the scarcity of pastures and high prices of feedstuffs such as cereals, the base of most of the concentrates. Feed blocks (FB) based on local resources and by-products (Ben Salem and Nefzaoui, 2003) might be used to overcome this situation. Because olive trees and the derived industries are of great importance in Mediterranean countries, their by-products are of economic and environmental interest. The most important by-product is the crude 2-stage olive cake (CTSOC), composed of olive pulp, skin, and stones as well as water (Hermoso *et al.,* 1995). Production of CTSOC accounts for 2,000,000 t/yr (Molina-Alcaide and Yáñez-Ruiz, 2008) with high pollutant potential, in part because of its high moisture content. Greenhouse horticulture account for 15% of world horticulture production in the Mediterranean area, Spain being the main producer (MARM, 2009). This intensive agriculture generates large amounts of fruit wastes, mainly tomato and cucumber, which have to be stored with economic and environmental troubles. Increased in cereal prices (43% from 2008 to 2011; FAO, 2011) has driven the attention of ruminant nutritionists toward local alternative feedstuffs (Ben Salem, 2010; Molina-Alcaide *et al.,* 2010) in order to reduce production cost. To our knowledge, the use of tomato by-products for ruminant feeding has been explored (Ben Salem and Znaidi, 2008) but no information is available

regarding the inclusion of cucumber in ruminant diets. Either 2-stage crude olive cake (COC) and wastes of tomato (T) and cucumber (C) fruits have been included in feed blocks to state their respective potential to replace concentrate in diets for both unproductive and lactating goats.

1. In vivo trials with unproductive goats

A. Feed blocks based on COC

Six dry non-pregnant, rumen-fistulated Granadina goats (49 ± 2.2 kg of BW) were used to study the effect of three experimental diets on ruminal fermentation and microbial N flow by following a 3 x 3 Latin square design with repetition. Diets were composed (fresh matter basis) of 600 g alfalfa hay and 400 g of cereals-based concentrate (diet AC), 600 g alfalfa hay, 200 g concentrate, and 180 ± 80 g feed block including 120 g/kg of crude olive cake (diet ABCI) and, 600 g alfalfa hay, 200 g concentrate, and 291 ± 61 g feed block including 100 g/kg of crude olive cake (diet ACBII). No effect ($P \geq 0.18$) of diet was found for pH and VFA concentration (Table 1) indicating that partial replacement of concentrate with FB did not compromise the ruminal fermentation of carbohydrates. On the contrary, both microbial N flow and efficiency of microbial protein synthesis in the rumen decreased ($P \leq 0.001$) in animals fed diets including feed blocks compared with the control one. A better supply of available carbohydrates and N utilization in diet AC, compared to those including feed blocks, could occur as previously suggested by Lee *et al.* (2003) and Merry *et al*. (2006). Although the most important factors influencing microbial growth are rumen outflow and energy available to microbes, growth may also be influenced by the substrate and type of microbes growing in the rumen (Van Soest *et al.*, 1988).

Table 1. Effect of the partial replacement of concentrate with feed blocks including crude olive cake on the average values of pH, volatile fatty acid (VFA), and microbial growth and efficiency in the rumen of unproductive goats

Item	Diets†			*SEM*	*P-value*
	AC	ACBI	ACBII		
pH	6.35	6.45	6.51	0.030	0.10
VFA mmol/L	80.5	88.8	95.7	4.3	0.35
Acetate	59.1	64.1	69.1	3.2	0.45
Propionate	13.3	16.3	15.5	0.73	0.24
Isobutyrate	0.38	0.62	0.53	0.052	0.18
Butyrate	7.03	6.89	9.24	0.61	0.23
Isovalerate	0.60	0.54	0.67	0.063	0.68
Valerate	0.74	0.89	1.05	0.071	0.23
Acetate/propionate	4.44	3.93	4.46	0.23	0.38
Microbial N flow, g/d	17.0^{b}	9.82^{a}	11.3^{a}	1.05	0.001
g microbial N/kg DOM	31.4^{c}	18.2^{a}	18.0^{a}	1.4	<0.001

†AC: control diet (alfalfa hay:concentrate, 1:1); ACBI and ACBII: alfalfa hay and concentrate (1:0.5) and feed blocks including 120 and 100 g of crude olive cake/kg feed block fresh matter basis, respectively.

B. Feed blocks based on greenhouse waste fruits

Four adult dry non-pregnant rumen-fistulated Granadina goats (32 ± 5.5 kg BW) were fed four experimental diets formulated with alfalfa hay and concentrate in a 1:1 ratio (diet AC), alfalfa hay, concentrate and feed blocks (1:0.5:ad libitum) including wastes of tomato (diet ACT), cucumber (diet ACC) or barley (diet ACB). The effect of diet on ruminal fermentation, microbial N flow and efficiency of synthesis, methane emission, nutrients utilization and abundances of

total bacteria and methanogens were studied following a 4 x 4 Latin square design. The feed blocks intakes were 203 ± 73, 179 ± 40 and 144 ± 68 g/animal/d for those including wastes of tomato, cucumber and barley grain, respectively. Diets including tomato and cucumber wastes promoted (Table 2) decreased ($P < 0.001$) methane emissions (37 and 13%, respectively) in comparison with diets control and the one including barley-based FB, which showed similar ($P>0.05$) methane emissions. Average pH and ammonia concentration values in the rumen were similar ($P \geq 0.108$) independently of diet supplied to the animals. The VFA content was higher ($P = 0.001$) in goats fed diets ACT and ACC compared to AC and ACB diets. The acetate:propionate ratio decreased ($P < 0.001$) with diets including tomato wastes and barley grain the first promoting the higher decreasing. Both microbial N flow and efficiency of synthesis were modified ($P = 0.047$ and 0.044, respectively) decreasing with diet ACT and increasing with ACC diet. The partial replacement of concentrate with feed blocks modified digestibility of DM ($P = 0.022$), OM ($P = 0.021$) and fat ($P = 0.049$). Total bacteria abundance in the rumen was not affected ($P = 0.072$) by diet while methanogens abundance increased ($P = 0.015$) in animals fed diets including wastes-based FB in comparison with diet AC and ACB. Diet including tomato based feed blocks decreased MNF without affecting the efficiency of synthesis but it decreased methane emission by 33% compared to the other studied diets. This dietary approach involves the use of agricultural by-products and reduced methane emissions, which represents advantages against approaches using antibiotics or other chemicals potentially harmful to the animal or the environment. Feeding cost decreased by 10% with diets including feed blocks compared with control diet. Further research is needed to understand the mechanisms involved in the antimethanogenic effect of greenhouse waste fruits-based feed blocks and in order to improve ruminal protein yield and energy utilization, which may limit the use of this type of blocks in non-productive ruminants practical feeding (Romero-Huelva and Molina-Alcaide, 2013)

Table 2. Effects of the partial replacement of concentrate with feed blocks including wastes of tomato or cucumber and barley grain on average values for methane emissions, pH, concentrations of NH_3-N and VFA, acetate:propionate ratio, microbial N flow and efficiency of synthesis, nutrients apparent digestibility and total bacteria and methanogens abundances in unproductive goats

Item	Diet†				*SEM*	*P-value*
	AC	ACT	ACC	ACB		
CH_4 L / kg DMI	29.3^{c}	18.5^{a}	25.4^{b}	28.2^{c}	0.330	< 0.001
pH	6.94	7.05	7.09	7.09	0.024	0.108
NH_3-N, mg / 100 mL	16.4	15.6	16.3	16.0	0.483	0.865
Total VFA, mmol / L	66.9^{a}	80.3^{b}	82.2^{b}	67.2^{a}	2.011	0.001
Acetate:propionate	5.03^{c}	3.89^{a}	4.58^{bc}	4.12^{b}	0.036	< 0.001
Microbial N flow, g/d	16.2^{b}	13.1^{a}	18.9^{c}	15.4^{b}	1.00	0.047
EMNS, g/kg of OMAFR	24.2^{b}	21.1^{a}	30.8^{c}	24.9^{b}	1.03	0.044
Apparent digestibility (g/g)						
Dry matter	0.68^{b}	0.62^{a}	0.64^{a}	0.67^{ab}	0.003	0.022
Organic matter	0.71^{b}	0.66^{a}	0.67^{a}	0.70^{ab}	0.003	0.021
Fat	0.68^{b}	0.58^{a}	0.63^{ab}	0.55^{a}	0.014	0.049
CP	0.72	0.70	0.72	0.73	0.005	0.802
NDF	0.59	0.60	0.57	0.59	0.006	0.939
ADF	0.56	0.55	0.57	0.58	0.007	0.923
log gen copies / g fresh matter						
Bacteria	11.2	11.4	11.4	11.2	0.058	0.072
Methanogens	8.48^{a}	8.86^{b}	8.85^{b}	8.49^{a}	0.050	0.015

†AC: control diet (alfalfa hay:concentrate, 1:1); ACT and ACC and ACB: alfalfa hay and concentrate (1:0.5) and feeding blocks including tomato and cucumber fruit wastes and barley grain, respectively.

2. *In vivo* trials with lactating goats

Dairy goat farming is of relevant economic, environmental, and sociological importance in the Mediterranean basin (Rancourt *et al.*, 2006) with an increasing demand for gourmet cheeses, yogurt, and milk from sheep and goats (Haenlein, 2001). Milk and dairy products from goats are important foods for man, especially for people with food allergies or for those living in dry areas where cow's milk is scarce (Park, 2006; Sanz Ceballos *et al.*, 2009). The influence of diet on milk production may depend more on ruminal fermentation balance and end products than on the digestible or metabolizable energy content (Sanz- Sampelayo *et al.,* 1998). It is well documented that diet composition affects ruminal fermentation, and hence milk composition (Morand-Fehr *et al.,* 1991). Lipid composition is one of the most important factors determining the technological, nutritional, and health quality of goat's milk (Chilliard *et al.*, 2003).

A. Feed blocks based on COC

Eighteen Granadina goats (39.6 ± 1.89 kg of BW) in the middle of the third lactation were fed four diets made (fresh matter basis) of alfalfa hay and concentrate (1:1), alfalfa hay (diet AC), concentrate and feed block (1:0.5: ad libitum) including 120 or 100 g/kg of crude olive cake (diets ACBI and ACBII, respectively). The effect of diet on nutrients utilization, microbial growth and milk yield and composition was studied by following an experimental design 3 x 3 Latin square with 6 replications. Blocks intake resulted in 209 ± 32 and 346 ± 56 g/animal/d for diets ACBI and ACBII, respectively. The feeding cost was 36% lower with diets including feed blocks compared to the control one. Body weight of animals fed the different diets (Table 3) was similar ($P \geq 0.626$). The N intake was similar ($P = 0.201$) but milk total and protein N and the milk protein N/N balance were lower ($P = 0.012$, 0.023 and 0.050, respectively) for goats fed diets including feed blocks compared with control diet. On the contrary, retained N was higher ($P = 0.050$) for animals fed diets including FB than control diet. Regarding energy the intake and urinary energy were lower ($P = 0.016$ and 0.043, respectively) for diet ACBI in comparison with diets AC and ACBII. Milk energy was lower ($P = 0.049$) for diets including FB compared to control diet. Both microbial N flow and efficiency of synthesis were lower ($P = 0.045$ and 0.036, respectively) in goats fed diet ACBI than in those receiving diets control and ACBII. The presence of soybean meal in the concentrate, with high CP and lysine content (De Blas *et al.*, 1994) could explain the results obtained in the present work.

Milk yield (Table 4) decreased ($P = 0.016$) by 22 and 18%, respectively, with diets ACBI and ACBII, respectively, in comparison to diet AC. The milk contents in casein, whey protein, fat, lactose, total solid and gross energy did not vary ($P \geq 0.144$) with diet. However changes in milk fatty acids profile were promoted by the partial replacement of concentrate with FB: saturated fatty acids decreased ($P = 0.046$) by 3 and 7% in milk from goats fed diets ACBI and ACBII, respectively, in comparison with milk from goats receiving diet AC; mono and polyunsaturated fatty acids content in milk was not ($P \geq 0.164$) modified by diet; medium chain fatty acids content decreased ($P = 0.043$) by 12% in milk from goats fed diet ACBII in comparison with diets AC and ACBI; vaccenic acid content increased ($P = 0.050$) 1.5 and 3.5 folds in milk from goats fed diets ACBI and ACBII, respectively, compared to milk from animals fed diet AC; rumenic acid content was 19% and 35% higher ($P = 0.020$) in milk from goats fed diets ACBI and ACBII, respectively than in milk from those fed diet AC; the C18:3 (6, 9, 12) acid content was 41.5% higher ($P = 0.033$) in average in milk from goats fed diets ACBI and ACBII than from goats fed diet AC. Because CLA is considered healthy for consumers (Pariza, 2004) the inclusion of FB containing crude olive cake in goats feeding could be an option to improve the nutritional and healthy quality of milk. The partial replacement of concentrate with FB promoted decreased feeding cost by 36%. The decrease of milk yield with diet ACBII could be tolerated when considering the healthier quality of milk obtained with this diet compared with diets AC and ACBI, the lower cost of feeding diets including FB, and the environmental advantage of recycling by-products.

Table 3. Effect of the partial replacement of concentrate with feed blocks including crude olive cake on the average values of body weight, N and energy intake and utilization, microbial N flow and efficiency of microbial N synthesis in dairy goats

Item	Diets†			*SEM*	*P – value*
	AC	ACBI	ACBII		
BW, kg	38.3	39.0	39.5	0.523	0.626
	g/kg $BW^{0.75}$				
N intake	2.46	2.20	2.30	0.082	0.201
Faecal N	0.773	0.636	0.657	0.060	0.322
Urine N	0.886	0.819	0.901	0.036	0.455
Milk total N	0.497[b]	0.343[a]	0.343[a]	0.036	0.012
Milk protein N	0.405[b]	0.317[a]	0.317[a]	0.012	0.023
Digestible N	1.68	1.56	1.65	0.069	0.583
N balance	0.798	0.741	0.745	0.065	0.929
Retained N	0.301[a]	0.397[b]	0.402[b]	0.067	0.050
	%				
Digestible N/intake N	69.2	71.4	71.5	1.96	0.452
N balance/digestible N	46.8	46.7	43.1	2.47	0.792
Milk protein N/digestible N	25.1	20.6	19.8	1.77	0.239
Milk protein N/N balance	55.6[b]	48.2[a]	53.1[b]	6.21	0.050
Milk protein N/milk total N	88.6	93.1	92.2	0.433	0.491
Milk protein N/intake N	16.9	14.5	14.0	1.06	0.289
	MJ/kg $BW^{0.75}$				
Energy intake	1.51[b]	1.37[a]	1.52[b]	0.490	0.016
Faecal energy	0.488	0.448	0.449	0.031	0.502
Urine energy	0.056[b]	0.043[a]	0.062[b]	0.003	0.043
Milk energy	0.258[b]	0.218[a]	0.219[a]	0.017	0.049
Digestible energy	1.03	0.92	1.07	0.04	0.116
Methane energy	0.106	0.095	0.111	0.04	0.180
ME	0.863	0.781	0.899	0.035	0.173
	%				
Digestible energy/energy intake	68.3	67.5	70.3	1.61	0.418
Milk energy/digestible energy	25.1	24.0	21.0	1.75	0.136
Milk energy/ME	29.8	28.4	25.2	2.12	0.194
ME/energy intake	57.5	57.3	58.9	1.47	0.676
ME/digestible energy	84.3	84.9	83.7	0.312	0.052
Microbial N flow, g/d	27.6[b]	17.3[a]	23.4[b]	1.26	0.045
EMNS, g/ kg OMAFR	49.1[b]	38.8[a]	44.4[ab]	2.75	0.036

†AC: control diet (alfalfa hay:concentrate, 1:1); ACBI and ACBII: alfalfa hay and concentrate (1:0.5) and feeding blocks including 120 and 100 g/kg of crude olive cake, respectively.

B. Feed blocks based on greenhouse waste fruits

Eight Granadina goats (39.4 ± 5.39 kg BW) in the middle of the third lactation were fed four experimental diets formulated with 1kg alfalfa hay plus 1 kg concentrate (diet AC), and alfalfa hay, concentrate and feed blocks containing greenhouse wastes of tomato (diet ACT) and cucumber (diet ACC) fruits or barley (diet ACB) which replaced 22% of the concentrate in the control diet. Although this substantial reduction in feeding cost, wastes transportation and FB

manufacturing should be considered in each situation in order to estimate the profit margins. The effect of diet on ruminal fermentation, microbial N flow and efficiency, methane emissions, nutrients utilization, milk yield and composition and microbial abundances was studied by following a 4 x 4 Latin square experimental design with repetitions. The feed blocks intakes were 231 ± 69, 238 ± 59 and 223 ± 88 g/animal/d for tomato, cucumber and barley-based feed blocks. Methane emission (Table 5) was reduced ($P < 0.001$) by diets including blocks with tomato and cucumber wastes (38.5% in average) and barley grain (30%) compared to diet AC. Therefore, FB including tomato or cucumber fruit wastes may also have an added value derived from the presence of plant secondary compounds which could act as natural safe antimethanogenics additives, alternative to the chemical ones as suggested by Patra and Saxena (2010). Diets ACT and ACC showed similar antimethanogenic effect, but different fermentation patterns, which could support previous speculations concerning the association between the antimethanogenic effect of plant secondary compounds and their molecular structure and weight together with chemical composition of diets (Guo *et al.,* 2008). Similar values for methane emissions (L / kg DMI) using respiration chambers were also found in goats treated with bromochloromethane with proved antimethanogenic activity (Abecia *et al.,* 2011). An additional advantage of the strategy involving the replacement of 35% of concentrate with feed blocks including tomato or cucumber fruits, rely to the lack of effect on DMI that has been shown to decreased with some antimethanogenic strategies (Beauchemin *et al.*, 2008). In addition, the abundances of total bacteria and methanogens were not affected by diet, suggesting the absence of any relationship between the reduction in methane emissions and abundances of methanogens (Machmüller *et al.,* 2003). It has been hypothesized that rather than the number is the species composition of archaea community what drives the synthesis of methane in the rumen (Morgavi *et al*., 2010) but it still remains unknown which genera or species of archaea are more involved in ruminal methane production.

Table 4. Effect of the partial replacement of concentrate with feed blocks including crude olive cake on the average values of milk yield and composition in dairy goats

Item	Diets†			*SEM*	*P-values*
	AC	ACBI	ACBII		
Milk, g/d	1255[b]	973[a]	1029[a]	81.4	0.016
g/kg milk Protein	31.8	33.8	31.1	1.20	0.657
Casein	25.3	26.7	23.7	1.12	0.581
Whey protein	6.60	7.12	7.48	0.327	0.294
Fat	45.9	49.7	42.7	2.24	0.547
Lactose	47.6	44.9	43.3	2.11	0.882
Total solid	131	139	127	3.12	0.144
Gross energy, MJ/d	3.25	3.51	3.36	0.113	0.533
FA profile, g/100 g total identified FA					
C18:1 (trans 11)	0.122[a]	0.187[a]	0.432[b]	0.040	0.050
C18:2 (cis 9, trans 11)	0.436[a]	0.522[b]	0.590[b]	0.040	0.020
C18:3 (cis 6, 9, 12)	0.475[a]	0.671[b]	0.676[b]	0.042	0.033
Medium chain	37.5[b]	37.2[b]	32.8[a]	0.913	0.043
Monounsaturated fatty acids	18.6	20.4	23.3	0.835	0.164
Polyunsaturated fatty acids	3.34	3.71	3.68	0.185	0.241
Saturated fatty acids	78.1[b]	75.9[b]	73.1[a]	0.773	0.046

†AC: control diet (alfalfa hay:concentrate, 1:1); ACBI and ACBII: alfalfa hay and concentrate (1:0.5) and feeding blocks including 120 and 100 g/kg of crude olive cake, respectively.

Table 5. Effects of the partial replacement of concentrate with feed blocks including wastes of tomato or cucumber and barley grain on average values for methane emissions, pH, concentrations of ammonia N an VFA, acetate:propionate ratio, abundances (log gene copies/g fresh matter) of total bacteria and methanogens, microbial N flow and efficiency and N and energy balances in dairy goats

Item	Diet†				*SEM*	*P-value*
	AC	ACT	ACC	ACB		
CH_4 L/kg DMI	28.2[c]	17.4[a]	17.2[a]	19.7[b]	0.558	< 0.001
pH	6.94	7.00	6.93	6.91	0.036	0.391
NH_3-N, mg/100 mL	28.1[b]	18.2[a]	18.5[a]	25.3[b]	0.497	0.003
Total VFA, mmol/L	149[c]	109[b]	161[c]	85.6[a]	2.62	< 0.001
Acetate:propionate	5.35	5.01	4.48	5.44	0.152	0.269
Bacteria††	11.5	11.4	11.5	11.4	0.092	0.423
Methanogens††	9.04	8.83	8.91	8.86	0.095	0.441
Microbial N flow, g/d	16.2[b]	13.1[a]	18.9[c]	15.4[b]	1.00	0.047
EMNS, g/kg of OMAFR	24.2[b]	21.1[a]	30.8[c]	24.9[b]	1.03	0.044
Body weight (BW), kg	38.6	38.7	40.3	40.2	1.79	0.411
g/kg of $BW^{0.75}$						
N intake	3.25	3.12	3.01	3.10	0.13	0.384
Fecal N	0.730	0.760	0.737	0.704	0.066	0.922
Urine N	1.51[b]	1.31[a]	1.32[a]	1.40[ab]	0.064	0.037
Milk total N	0.398	0.346	0.386	0.373	0.036	0.728
MJ/kg of $BW^{0.75}$						
Energy intake	1.93[b]	1.80[a]	1.77[a]	1.79[a]	0.060	0.029
Fecal energy	0.567	0.569	0.543	0.532	0.038	0.517
Urine energy	0.080[b]	0.070[a]	0.074[a]	0.075[a]	0.004	0.008
Milk energy	0.251	0.229	0.243	0.233	0.019	0.970
Methane energy	0.110[b]	0.072[a]	0.067[a]	0.080[a]	0.004	< 0.001
ME	1.18	1.09	1.09	1.10	0.033	0.575

†AC: control diet (alfalfa hay:concentrate, 1:1); ACT and ACC and ACB: alfalfa hay and concentrate (1:0.65) and feeding blocks including tomato and cucumber fruit wastes and barley grain, respectively.
††Log gen copies/g wet weight

The pH values were similar (P = 0.391) for all the diets while VFA concentration was affected (P < 0.001) by diet and not (0.269) the acetate to propionate ratio. The lack of correlation between pH values and VFA concentration agrees with observation of other authors (Busquet *et al.*, 2005a; Cantalapiedra *et al.*, 2009) and may be due to the contamination of rumen samples with saliva. The type of carbohydrates present in the concentrate (Ørskov and Fraser, 1975) and the buffer properties attributed to alfalfa (Dixon and Stockdale, 1999) could contribute to the lack of variations in rumen pH with dietary treatments. Ammonia concentration decreased (P = 0.003) with diets including blocks containing tomato and cucumber wastes. Either bacteria or methanogens abundances were not (P = 0.423 and 0.441, respectively) modified by diet. Both microbial N flow (P = 0047) and efficiency (P = 0.044) decreased and increased, respectively, with diet including tomato and cucumber wastes. Body weight was not affected by diet (P = 0.411) and regarding N balance only N in urine was affected by diet (P = 0.037) with lower values for diets including wastes which may have importance from the environmental point of view. Energy intake, energy in urine and in methane decreased (P ≤ 0.029) with diets including feed blocks compared to the control one.

Milk yield (Table 6) was not affected (P = 0.826) by diet. With the exception of lactose (P = 0.037) no effect (P ≥ 0.037) was detected in milk composition. Regarding milk fatty acids profile

no effect of diet was observed both on total saturated (P = 0.578) and monounsaturated (P = 0.762). On the contrary total PUFA increased (P = 0.034) by with diets including FB (15, 14 and 9%, respectively for tomato, cucumber and barley-based FB compared to control diet) and rumenic acid content increased (0.043) with diet including FB containing tomato wastes (9% in comparison to control diet). Feeding cost was reduced by 22% in diets including FB.

Table 6. Effects of the partial replacement of concentrate with feed blocks including wastes of tomato or cucumber and barley grain on average values for milk yield, composition and fatty acids profile

Item	Diet†				*SEM*	*P-value*
	AC	ACT	ACC	ACB		
Milk, g/d	1019	944	1041	1000	83.8	0.826
g/kg milk						
Protein	34.7	33.9	33.9	33.9	1.68	0.935
Fat	55.1	55.7	50.8	54.4	2.00	0.067
Lactose	52.6^{b}	46.0^{a}	55.2^{b}	59.5^{c}	2.55	0.037
Total solid	148	141	145	153	4.94	0.069
Gross energy, MJ/kg	3.76	3.77	3.67	3.72	0.149	0.744
g/100 g of identified FA						
Total SFA	74.7	73.4	73.4	74.4	0.458	0.580
Total MUFA	19.7	20.3	20.4	19.6	0.498	0.762
Total PUFA	3.34^{a}	3.83^{b}	3.80^{b}	3.65^{b}	0.108	0.034
cis-9, trans-11 CLA	0.57^{ab}	0.62^{b}	0.55^{ab}	0.50^{a}	0.048	0.043

†AC: control diet (alfalfa hay:concentrate, 1:1); ACT and ACC and ACB: alfalfa hay and concentrate (1:0.65) and feeding blocks including tomato and cucumber fruit wastes and barley grain, respectively

In addition to FA supply, other factors such as energy supply, proportion of fiber or concentrate and the presence of plant secondary compounds should be considered when the effects of dietary treatments on milk FA profile are assessed (Leiber *et al.,* 2005). Therefore, the higher accumulation of LNA in the milk of goats receiving FB could be associated with changes in the ruminal ecosystem due to energy shortage or specific secondary plant metabolites presence in the diet (Leiber *et al.*, 2005). Moreover, the synchronous and fractionated supply of nutrients allowed when using FB in ruminants feeding (Ben Salem and Nefzaoui, 2003) may have been associated with a better FA absorption in the small intestine (Romero-Huelva *et al.,* 2012).

III – Use of additives to manipulate rumen fermentation

An enormous variety of secondary compounds are produced by plants to provide protection against microbial and insect attack (Levin, 1976, Cowan, 1999, Isman, 2000 and Iason, 2005). Some are toxic to animals, but others may not be, and indeed many have been used to manipulate gut function in both ruminant and non-ruminant animals (Greathead, 2003). Broadly, these compounds fall into four categories: essential oils, organosulphurs, polyphenols and saponins. We will consider the effect of essential oils and organosulphur compounds on rumen fermentation in goats.

Previous studies reported that essential oils and garlic extracts in certain amounts can enhance rumen fermentation (Cardozo *et al.,* 2006; Castillejos *et al.*, 2008; Kamel *et al.*, 2008). However, effects reported in the literature are variable and contradictory, which may be due to the different concentrations, plant and basal diets used (Hart *et al.,* 2008). Among the garlic derived compounds, some thiosulfate compounds have been shown to exhibit methane reduction

potential (Kamel *et al.*, 2008; Benchaar and Greathead, 2011; Soliva *et al.*, 2011). Furthermore, some of the recently developed thiosulfates with high antimicrobial activity (Ruiz *et al*., 2010) have not been fully tested in ruminants and offer room for further screenings (Martin-Garcia *et al.,* 2011).

In our group several in vitro trials have been performed to screen the effects of some essential oils and organosulphur compounds on rumen fermentation and methane emissions using rumen fluid from goats. Some of the compounds tested in vitro have been further tested in goats fed at maintenance level. Furthermore, an in vivo trial was conducted to evaluate the effect of reducing methane emissions in dairy goats on milk production and milk composition by using bromochloromethane, a synthetic compound with a well known antimethanogenic effect.

1. *In vitro–In vivo* assays

Different experiments have been conducted in our group to investigate the effects of some essential oils and organosulphur compounds on rumen fermentation and methane emissions. Table 7 summarizes the effects observed on some parameters by the addition of carvacrol (CAR), cinnamaldehyde (CIN), propyl-propane-thiosulfinate (PTS), propyl-propane-thiosulfonate (PTSO), diallyl disulfide (DDS) and bromochloromethane (BCM) as antimethanogenic reference (Martinez et al., submitted). We have used two different substrates as diet, which are based on a alfafa hay:concenrrate mix (1:1), in which the concentrate included sources of starch and protein with high (I: barley and faba beans) or low (II: maize and sunflower cake) degradability.

As shown in Table 7, the asymptotic gas production was affected (P<0.001) by substrate with all compounds with the exception of PTS (P=0.386). The gas production rate only increased (P<0.001) with CAR and DDS at the highest dose (320 µl/L) while PTS and PTSO reduced it (P<0.001). The highest doses of BCM and CIN did not have any effect (P>0.100) on gas production rate. The gas produced per gram of digested DM (Table 7) was not affected (P=0.225) by BCM, while it was reduced (P≤0.047) by the rest of compounds at different doses. Substrate had statistical significant effect (P≤0.002) with all compounds, decreasing gas produced per gram DM digested by substrate II compare with substrate I. Truly digested DM after 72 h of incubation (Table 6) was affected (P≤0.015) by all doses of CAR and PTS, while only the highest dose (320 µl/L) of DDS decreased it (P=0.022). Finally, BCM tended (P=0.099) to decrease truly digested DM while CIN and PTSO showed no effect (P>0.170). Methane produced in vitro from 12 to 24 h (Table 6) was reduced by all doses of PTS and DDS (P<0.001). The PTS exhibited a methane reduction (P<0.001) up to 88% at the highest dose (320 µl/L), while DDS reduced methane (P<0.001) with both doses up to 55% compared with the control. Likewise, the addition of both doses of BCM decreased (P< 0.001) methane concentration by 95% as compared to the control.

As outlined above, the addition of CAR did not affect (P >0.05) methane concentration in vitro suing rumen liquor from goats, which is in contrast to Macheboeuf *et al.* (2008), who reported a linear decrease in CH_4 production with CAR at 225, 300, 450 and 750 mg/L in batch cultures, with a reduction in acetate, propionate and total VFA concentrations. Methane concentration was not affected either by CIN, which disagrees with other authors (Macheboeuf *et al.*, 2008), that reported a reduction of methane production with similar doses to those used in the present work. This difference might have been due to the different origin of the inoculum (ovine vs. caprine), the basal diet (25:75 vs. 50:50 forage:concentrate) used or even the extraction process of the essential oil. PTS and DDS reduced methane emission up to 90% and 60 %, respectively, which are comparable to those reported in other in vitro studies. Busquet *et al.* (2005b) reported a decrease of about 70% in methane emission after 17 h of incubation in batch cultures with a similar dose (300 mg/L) of garlic oil and DDS. Likewise, Soliva *et al.* (2011) showed 90% inhibition of methane production with garlic oil (300 mg/L) in an experiment carried out with Rusitec fermenters. However, Kamel *et al.* (2008) did not find such effect of DDS on methane emission in 24 h incubations using batch cultures, although doses were lower

than in other experiments. The antimicrobial effect of garlic derived compounds has been suggested to be realted to its reaction with thiol groups of some enzymes as acetyl-CoA (Focke *et al.*, 1990) or to the inhibition of HMG-CoA reductase (Busquet *et al*., 2005a; 2005b). PTSO did not affect methane production, although its chemical structure is very similar to that of PTS. Whereas PTS is more active against enterobacteria. PTSO has higher activity on lactobacilli, bifidobacteria, bacteroides and clostridia (Ruiz *et al.,* 2010). This differential inhibition showed by PTS and PTSO may be due to different composition of microbial membranes and their permeability to these compounds as suggested by Miron *et al.* (2000).

Table 7. Effects of the substrate and additives dose on gas production (GP, gas mL/g digested DM), kinetics gas parameters (A: potential gas volume at steady state, mL; c: gas production rate, h-1), truly digested dry matter (tDDM, g/g) after 72 h incubation and on methane (mL/ mL total gas) production over 24 h of incubation in batch cultures

Compound†		Substrate		Dose††			*SEM*	*P-value*		
		I	II	0	I	II		Substrate	Dose	SxDo†††
CAR	GP	254	215	265^{a}	252^{a}	188^{b}	3.40	<0.001	<0.001	0.825
	A	103	87	111^{a}	100^{b}	73^{c}	1.14	<0.001	<0.001	0.998
	c	0.108	0.109	0.089^{b}	0.099^{b}	0.136^{a}	0.00	0.985	<0.001	0.984
	tDDM††††	0.86	0.85	0.89^{a}	0.85^{b}	0.83^{b}	0.01	0.730	0.015	0.781
	CH_4	0.127	0.118	0.130	0.127	0.110	0.00	0.312	0.168	0.977
CIN	GP	275	248	265^{ab}	272^{a}	250^{b}	3.37	0.002	0.047	0.685
	A	118	103	111^{ab}	113^{a}	108^{b}	0.82	<0.001	0.048	0.836
	c	0.084	0.084	0.089	0.085	0.077	0.00	0.957	0.139	0.934
	tDDM††††	0.89	0.88	0.89	0.88	0.89	0.00	0.067	0.405	0.884
	CH_4	0.127	0.122	0.130	0.126	0.117	0.00	0.503	0.307	0.794
PTS	GP	234	201	265^{a}	246^{b}	142^{c}	3.10	<0.001	<0.001	0.654
	A	102	95	111^{a}	98^{a}	87^{b}	4.54	0.386	0.127	0.648
	c	0.063	0.060	0.089^{a}	0.073^{a}	0.019^{b}	0.00	0.674	<0.001	0.847
	tDDM††††	0.86	0.85	0.89^{a}	0.84^{b}	0.83^{b}	0.01	0.231	0.006	0.930
	CH_4	0.086	0.082	0.130^{a}	0.108^{b}	0.014^{c}	0.00	0.451	<0.001	0.697
PTSO	GP	279	251	265^{ab}	274^{a}	256^{b}	2.36	<0.001	0.032	0.603
	A	116	102	111^{a}	112^{a}	105^{b}	0.80	<0.001	0.015	0.467
	C	0.083	0.083	0.089^{a}	0.090^{a}	0.069^{b}	0.00	0.879	0.002	0.906
	tDDM††††	0.89	0.87	0.89	0.87	0.87	0.00	0.088	0.171	0.976
	CH_4	0.124	0.116	0.130	0.119	0.111	0.00	0.210	0.096	0.947
DDS	GP	243	213	265^{a}	229^{b}	190^{c}	2.50	<0.001	<0.001	0.782
	A	100	87	111^{a}	94^{b}	76^{c}	0.88	<0.001	<0.001	0.419
	C	0.109	0.111	0.089^{b}	0.113^{a}	0.130^{a}	0.00	0.755	<0.001	0.991
	tDDM††††	0.88	0.87	0.89^{a}	0.87^{a}	0.86^{b}	0.01	0.540	0.065	0.360
	CH_4	0.077	0.076	0.130^{a}	0.056^{b}	0.044^{b}	0.00	0.912	<0.001	0.533
BCM	GP	280	238	265	252	260	2.76	<0.001	0.225	0.441
	A	117	98	111^{a}	103^{b}	107^{a}	0.85	<0.001	0.010	0.244
	C	0.091	0.099	0.089	0.097	0.098	0.00	0.095	0.160	0.501
	tDDM††††	0.89	0.87	0.89	0.87	0.88	0.00	0.015	0.099	0.935
	CH_4	0.048	0.045	0.130^{a}	0.005^{b}	0.004^{b}	0.00	0.636	<0.001	0.619

†Interaction between Substrate and Dose. ††Dose I for CAR, CIN, PTS and BCM was 160 µl/L, for PTSO was 40 µl/L and for DDS was 80 µl/L; Dose II for CAR, CIN, PTS, DDS and BCM was 320 µl/L and for PTSO was 160 µl/L. †††Compounds: CAR (carvacrol), CIN (cinnamaldehyde), PTS (propyl propane thiosulfinate), PTSO (propyl propane thiosulfonate), DDS (diallyl disulfide) and BCM (Bromochloromethane). ††††Calculated as proposed by van Soest *et al*. (1966): true digested DM = (DM input – FND output)/DM input, FND output being that analyzed in the residue after 72 h incubation. [a-c] Within a row doses means without a common superscript letter differ, P < 0.05. (Multiple comparisons LSD test).

Table 8. Effects of PTS and BCM on body weight (BW), daily dry matter intake (DMI) and methane (CH_4) emissions by goats

Compound†		Doses††				*SEM*	*P-value*	Contrast†††	
		0	I	II	III			L	Q
PTS	BW, kg††††	33.3	34.4	32.5	32.5	1.47	0.968	n.d.	n.d.
	DMI, g/day	601	584	628	478	64.10	0.654	0.426	0.463
	CH_4, L/day	21.8^{a}	19.9^{ab}	18.5ab	11.0^{b}	2.53	0.191	0.050	0.443
	CH_4, L/Kg DMI	34.5^{a}	29.8^{a}	30.4^{a}	23.0^{b}	1.14	0.002	<0.001	0.407
BCM	BW, kg††††	32.3	32.1	34.1	36.2	1.43	0.720	n.d.	n.d.
	DMI, g/day	440	434	580	458	52.85	0.470	0.545	0.440
	CH_4, L/day	17.4^{a}	12.1^{a}	13.4^{a}	6.35^{b}	1.37	0.010	0.003	0.627
	CH_4, L/Kg DMI	43.9^{a}	28.6^{ab}	24.1^{b}	15.7^{b}	1.38	0.027	0.004	0.551

†PTS: propyl propane thiosulfinate; BCM: Bromochloromethane. ††Doses I, II and III in PTS were 5.5, 11 and 22 mg/kg BW respectively. Doses I, II and III in BCM were 5.5, 11 and 17.6 mg/kg BW respectively. †††Linear (L) and quadratic (Q) effect of dose. ††††Average of weighing prior and after measurements. $^{a-b}$ Within a row doses means without a common superscript letter differ, P<0.05. (Multiple comparisons LSD test).

Based on our observations made from in vitro trials, PTS and BCM have been further tested in vivo in our group using goats fed at maintenance level (Martinez *et al.*, submitted). As shown in Table 8, methane emissions, expressed per unit of dry matter intake decreased over 33% with PTS at the highest dose (22 mg/kg BW per day). This is equivalent to the reduction (17%) observed in vitro with 160 µl/L dose and far less than the reduction (87%) achieved with a higher dose (320 µl/L) in vitro. On the other hand, the reduction observed with BCM in vivo (34 to 64 %) was not as high as obtained in vitro (96%); however, it was similar to the decrease (30%) achieved in our lab with dairy goats treated with the same compound and similar dosage over two months (Abecia *et al.*, 2012). The disagreement observed between the same doses in vitro and in vivo data strongly supports the need of testing in vivo what it is previously observed in the lab and may be explained by a number of factors: the compounds used in this study had very low solubility in water and therefore the homogenous distribution across the rumen compartments might have not been fully achieved. In addition, the direct extrapolation of concentrations from in vitro to in vivo did not take into account the rumen outflow, which in our conditions is around 3%/h (Yáñez Ruiz *et al*., 2004). This would require an increase of the daily dosage of about 80% in vivo and would explain the proportionally lower reduction achieved in vivo as compared to in vitro.

2. Antimethanogenic additives in dairy goats

As pointed above, several technologies have been tested to reduce enteric methanogenesis, but very few have been successfully used in practical conditions for livestock. Furthermore, the consequences of reduced rumen methane production on animal performance and milk quality are poorly understood.

As reported in Abecia *et al.* (2012), an in vivo trial was conducted in our group to investigate the effect of feeding bromochloromethane (BCM), a halogenated aliphatic hydrocarbon with potential antimethanogenic activity, to dairy goats on rumen methane production, fermentation pattern, the abundance of major microbial groups, and on animal performance and milk composition. Eighteen goats were allocated to 2 experimental groups of 9 animals each: treated (BCM+) or not (BCM−) with 0.30 g of BCM/100 kg of body weight per day. The BCM was administered in 2 equal doses per day from parturition to 2 wk postweaning (10 wk). As shown in table 9, the treatment with BCM reduced methane production by 47% (19.3 vs 10.1 L/kg DMI), although it did not affect the abundance of rumen bacteria, protozoa and total

methanogenic archaea. The observed improvement in the efficiency of digestive processes was accompanied by a 45% increase in milk yield, probably due to the more propionic type of rumen fermentation. Positively, the increase in milk yield did not affect its concentration of fat, protein or lactose. Moreover, there were only minor changes in milk fatty acid profile, which suggests that the substantial decrease in methane production reported here did not seem to alter ruminal biohydrogenation pathways either. Compounds with similar mode of action deserve therefore further development for future application in the dairy sector.

Table 9. Methane emissions, total VFA, microbial numbers in the rumen and milk production by goats treated (BCM+) or not (BCM-) with bromochloromethane (n = 9 per treatment)

	BCM-	BCM+	*SED*	*P-value*
Methane emissions				
CH_4 L/kg DMI	19.3	10.1	3.30	0.013
CH_4 L/kg of milk	24.1	13.7	4.86	0.051
VFA concentration, mmol/L	58.5	74.4	8.79	0.216
Acetate:propionate	5.6	3.9	0.26	<0.001
log gene copies/g fresh matter				
Bacteria	10.3	10.02	0.116	0.124
Protozoa	9.57	9.54	0.077	0.810
Methanogens	7.88	7.96	0.161	0.753
Milk, g/d	901	1324	164	0.021
g/100 g milk				
Fat	5.46	4.98	0.701	0.505
Protein	3.73	3,52	0.335	0.551
Casein	3.18	3.05	0.252	0.606
Lactose	4.63	4.73	0.131	0.476
Total solids	14.4	13.9	0.909	0.570

Conclusions

The results obtained showed that replacement of concentrate with feed blocks based on by-products decreased (22-36%) feeding cost and methane production (37-39%) both in nonproductive and lactating goats and improved the fatty acids profile in milk. However, it compromises N metabolism which could be overcome by improving the formulation of the block;On other hand, some essential oils and organosulphur compounds show in vitro antimethanogenic effect without affecting overall ruminal fermentation; In vivo studies confirm the antimethanogenic effect of PTS in the short term (7 days) and a significant reduction of methane emissions by BCM in lactating goats implies an increase in milk yield production and no effect on milk composition. The use of either feed blocks with by-products/wastes or plant extracts does not essentially modify the numbers of the main microbial groups in the rumen.

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