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In vitro fermentation of olive oil mill wastewaters using sheep rumen liquor as inoculum: Olive mill wastewaters an alternative for ruminant's nutrition

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ABSTRACT

Olive oil mill wastewaters (OMWW) are the main liquid effluents generated by the olive oil production industry. This liquid, considered pollutant and toxic, is characterised by its high content of organic matter including mainly sugars and fats, and phenols compounds, which can be used in ruminants feeding. The purpose of this study is to valorise this agricultural by-product in ruminant feeding by estimation its *in vitro* degradability in presence of ovine ruminale microbiota comparatively to vetch-oat hay, using *in vitro* gas production technique coupled with NH_3-N and protozoa measurements. Cumulative gas production was recorded at 3, 6, 9, 24, 48, 72 and 96 hours of incubation. The determination of gazes produced (carbon dioxide and methane) was recorded at 6, 9, 24, 48 and 96 hours. However, Ammonia and protozoa number were recorded after 24 hours of incubation. Fermentation profile was fitted to the exponential model $y = a + b (1 – e^{-kt})$.

The OMWW are characterized by their high sugars content (39.91%) and their low content in ash (1.99%) and crude protein (2.70%). This by-product is also characterized by its high concentration in total phenols (7.2%) and tannins (4.5%). However, they contain a very small amount of condensed tannins (0.89%). Comparatively to vetch-oat hay, OMWW produced low amount of gas (-23.6 units). Furthermore, its *in vitro* fermentation generates low volume of methane (9.83%, V/V), suggesting that the OMWW nature enhanced the efficiency of ruminale microbiota towards microbial biomass production and inhibition of ruminale methanogenesis pathway. This result is reinforced by the reduction of ammonia production (-0.35 units) and protozoa proliferation (-1 unit) comparatively to vetch-oat hay.

The anaerobic biodegradation of OMWW reveal their significant use by the rumen microbiota, allowing us to strongly recommend its use as a supplement in feed ruminant. In addition, it allows considering using this residue as a feed additive in diets of ruminants for the reduction of ruminal methanogenesis.

Key words: olive mill wastewaters, nutritive value, phenolic compounds, ruminal microbiota, anaerobic biodegradation

Introduction

Olive oil mill wastewaters (OMWW) are the main liquid effluents generated by the olive oil production industry. The great majority of the world's olive oil production is concentrated around the Mediterranean basin which produces around 1-2.5 10^6 tons (Mantzavinos & Kalogerakis, 2005). Its high production during a short time generated approximately

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10 to 30 million m^3 of OMWW (Niaounakis & Halvadakis, 2006). This liquid effluent is in fact both pollutant and toxic due to its high content of organic matter including mainly sugars, fats and phenols compounds (Ben Sassi et al., 2006, El-Abassi et al., 2012).

Algeria is classified fourth after CEE, Tunisia and Morocco. Production on Algeria soil corresponds annually to 64000 tons (COI, 2010). In Algeria, a new governmental program has been lanced for enhancing olive oil production by olive tree cultivation (which rich 10^6 trees in 2014) and by the increase of the number of oil extraction units. This development should be accompanied imperatively by environmental impact limitation. So, the modernization of ancient units or the installation of the new oil extraction station with a two-phase decanter, which decrease considerably the volume of plant effluent and the disposal problems (Lessage-Meessen et al., 2001), need a high investment and did not be efficient before a long time. Therefore, it appears primordial reducing pollution of this agro-industrial residue. However, few techniques have been applied on an industrial scale due the high cost of the treatment plant (Papadimitriou et al., 1997; Zenjari et al., 2006). Another alternative may be the valorisation of OMWW in animal feeding. To our knowledge, many research investigations have shown the possibility of using this wastewater in animal feeding as beverage water for poultry (Roig et al., 2006) and as supplement to alfalfa hay for ewes (Gasa et al., 1991). These authors mentioned that inclusion of OMWW did not affect feed intake and *in vivo* digestibility. Although, its toxic effect towards aerobic bacteria, OMWW contain useful compounds that can be used as energy source for ruminale microbiota and consequently ruminants. In this context, this study aimed to valorise this by-product (crude OMWW) in ruminant's nutrition by evaluation its *in vitro* anaerobic degradation in presence of ovine ruminale microbiota comparatively to vetch-oat hay commonly consumed by ruminants.

Materials and Methods

Origin of the olive mill wastewaters

The OMWW used in the present study was obtained from an olive oil production plant located in the district of Jijel (East of Algeria), which uses a three-phase process for extraction of olive oil. The samples were collected at the outlet of a three-phase centrifugation plant in 5l dark cans and then stored at 4°C. The processed olive fruits are a mixture of different varieties (*Chemlal, Dardi* and *Zebouche*) which have been conserved for 15 days before extraction and without salt supplementation.

Chemical analysis

Vetch-oat hay was ground through a 1 mm sieve before analysis. Standard methods as described in AOAC (1990) were used for substrates chemical determination. Dry matter (DM) content was determined by drying to a constant weight in a forced draught oven at 105°C until constant weight (DM, method no. 930.15); Organic matter (OM) was calculated from the ashes content, determined by ashing in a muffle furnace for 6 h at 550°C (method no. 924.05), crude protein (CP) was determined by Kjeldahl (CP, method no. 984.13). Soluble sugars were estimated as mentioned by Dubois et al. (1956).

OMWW and vetch-oat extract

Liquid–liquid extraction with ethyl acetate was carried out on OMWW samples. First 20 ml of OMWW were centrifuged at 3200 g for 10 min. In order to remove the lipid fraction, 10 ml of supernatant were mixed with 20 ml of n-hexane, the mixture was vigorously shaken and centrifuged for 15 min at 2500 rpm. The phases were separated and the washing was repeated successively two times. Extraction of phenolic compounds was then carried out with ethyl acetate: 1.5 ml of OMWW samples preventively washed was mixed with 2 ml of ethyl acetate, the mixture was vigorously shaken and centrifuged for 15 min at 2500 rpm. The phases were separated and the extraction was repeated successively four times. The ethyl acetate phase was evaporated under vacuum at 40°C in rotary evaporator (Buchi Instruments 4030). The dry residue was dissolved in methanol and this solution was used for quantification of phenolic compounds.

For vetch-oat hay, extraction of phenolic compounds was done as described by Makkar (2000). Dried ground plant material (200 mg) is taken in a glass beaker of approximately 25 ml capacity. 10 ml of aqueous acetone (70%) is added and the beaker is suspended in an ultrasonic water bath (Branson 3210) and subjected to ultrasonic treatment for 20 min at room temperature. The contents of the beaker is then transferred to centrifuge tubes and subjected to centrifugation for 10 min at 3000 g and at 4°C. The supernatant was kept on ice and the pellet was suspended a second time in 10 ml of aqueous acetone (70%) and again the mixture was subjected to ultrasonic treatment for 20 min. Then, the content was centrifuged and the supernatant was collected as described above.

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Total phenol content and tannins determinations

Total phenols and total tannins in the extract were determined as described by Makkar (1993).

For total phenols, the phenolic extract (50µl) was diluted with distilled water (950 µl). Folin-Ciocalteau reagent (1N, 250 µl) was added and the content was mixed thoroughly. After 3 min, 1.25 ml of an anhydrous carbonate solution (20%, W/V) was added and then the mixture was allowed to stand in dark and at ambient temperature for 40 min. The optical density of the blue-coloured samples was measured at 725 nm. Total phenols content was measured as acid gallic equivalents and values were expressed as g of gallic acid per 100 g of OMWW.

Total tannins (TT) were determined as the difference in total phenolics (measured by Folin-Ciocalteau reagent) before and after treatment with insoluble polyvinylpyrrolidone (PVPP). 100 mg of PVPP were introduced in a 100 x 12 mm test tube. Then, 1.0 ml of distilled water was added to 1.0 ml of the extract. The content was mixed thoroughly and it kept at 4°C for 15 min. The mixture was centrifuged at 3000 g for 10 min. The supernatant collected contain only simple phenolics other than tannins. The phenolic content of the supernatant was measured as mentioned above. Total tannins content was measured as tannic acid equivalent and values were expressed as g of tannic acid per 100 g of OMWW.

For total condensed tannins (TCT), the extract (0.5 ml) was treated with n butanol-HCl (3 ml, 95%, v/v) in the presence of ferric ammonium sulphate (0.1 ml, 2% ferric ammonium sulphate dissolved in 2N HCl). Reagents were heated in a boiling water bath for 60 min. Absorbance was read at 550 nm. TCT content was measured as quebracho equivalents and values were expressed as g of quebracho per 100 g of OMWW.

In vitro fermentation study

Inoculum

The rumen liquor for the experiment was collected from two sheep that were fed a daily ration of 700 g vetch-oat hay (chemical composition illustrated in table 1) and 300 g of concentrate (58% barley, 38% wheat bran, 1% mineral and vitaminic premix, 1% NaCl and 2% limestone) divided into two equal meals at 8:00 and 16:00 h. The concentrate was supplemented progressively with 10, 20 and 30% of OMW. After adaptation period (28 days), the experiment has commenced. The sheep had free access to water throughout the experiment. The rumen liquor was collected from the two sheep just before the morning feeding, brought in Thermos flasks and used as source of inoculum. In the laboratory, the rumen juice was strained through four layers of cheesecloth and maintained at 39°C under CO_2.

Inoculation and incubation

The estimates of gas production were obtained by the method of Menke and Steingass (1988) by incubation in rumen fluid. All incubations were completed in 60 ml calibrated propylene syringes containing 200 mg DM of each sample and 30 ml of buffered rumen fluid, consisting of 10 ml rumen fluid and 20 ml artificial saliva prepared as described by Menke and Steingass (1988). The piston was fitted precisely and lubricated using a small amount of Vaseline. The needle of the syringe was connected with a silicon rubber tube and closed using a metallic clip.

Table 1. *Chemical characteristics (g/100 g DM) of olive mill wastewaters from the three-phase process and vetch-oat hay (control).*

	DM	Ash	CP	ST	TP	TT	CT
OMWW	7,84^{b}	1,99^{b}	2.70^{b}	39,91^{a}	7,2^{a}	4,5^{a}	0,89^{b}
Vetch-oat hay	35,97^{a}	8,17^{a}	6.79^{a}	24,43^{b}	2,75^{b}	2,05^{b}	1,84^{a}
S.E.M.	1,86	0,20	0,20	1,76	0,02	0,01	0,02
Pr.	< 3‰	< 1‰	< 1‰	< 1‰	< 5‰	< 1‰	< 3‰

DM, dry matter; CP crude protein; ST, total carbohydrates; TP, total phenols expressed as gallic acid equivalents; TT, total tannins estimated by PVPP procedure and expressed as tannic acid equivalents; CT, condensed tannins expressed as quebracho equivalents; Means are values of triplicate repetitions and that with different superscripts in the same column are statistically different at P < 5%; S.E.M., standard error of means; Pr. Probability.

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The artificial saliva containing buffer solution ($NaHCO_3$, 39 g/l), macrominerals solution (Na_2HPO_4, 5.7 g; KH_2PO_4, 6.2 g and $MgSO_4.7H_2O$, 0.6 g per 1l of distilled water), microminerals solution ($CaCl_2.2H_2O$, 13.2 g; $MnCl_2.4H_2O$, 10 g; $CoCl_2.6H_2O$, 1 g; $FeCl_3.6H_2O$, 8 g per 100 ml of distilled water) and potential redox indicator (resazurine, 0.1 g / 100 ml) was prepared the day previous to incubation and stored at 39°C. The reducing agent ($Na_2S.9H_2O$, 0.625 g; NaOH 1N, 4 ml; distilled water, 95 ml) then prepared to allow incubation of samples. After inoculation, syringes were incubated at 39°C and 9 rpm. Gas production was recorded by displacement of piston in the syringe at 3, 6, 9, 24, 48, 72 and 96 h of incubation. Results were corrected for a blank incubation (*i.e.*, buffered rumen fluid without sample). Each sample was incubated in two run in triplicate. The rate and extent of gas production were calculated by non-linear regression using an exponential model $y = a + b\ (1-e^{-k*t})$, where y is gas volume at time t, $(a + b)$ is potential gas production and k is rate at which gas is produced (Orskov & McDonald, 1979). The *in vitro* organic matter (OM) digestibility (OMD), metabolizable energy (ME) content and short-chain fatty acids (SCFA) production were estimated from the net 24 h gas volume (GPT), CP and ash contents (Menke & Steingas, 1988) according to the equations:

In vitro OMD (g/kg DM)=14.88+0.889GPT+0.45CP+0.0651XA
In vitro ME (MJ/kg DM)=2.20+0.136GPT+0.057CP+0.029CP^2
Total volatile fatty acids SCFA (µmole/g DM)=0.0239GPT-0.0601

where OMD, OM digestibility (g/100 g); ME, metabolizable energy content (MJ/kg DM); GPT, net gas volume at 24 h fermentation (ml/0.2 g DM); CP, crude protein content (g/100 g); XA, ash content (g/100 g).

Fermentation parameters

After 24h of incubation, the medium of each batch was checked for pH using a calibrated pH meter (Hanna 2039). Determination of ammonia-N concentration was conducted as mentioned by Chaney and Marbach (1962). 10 ml sample of the fermentation medium was placed in centrifugal tubes, mixed uniformly with 2 ml of ortho-phosphoric acid (50 g/l), and then centrifuged at 11000 rpm and 4°C for 15 min. 2 ml of supernatant were then mixed with 5 ml of solution A (5g, phenol and 25 g sodium nitroprusside per 500 ml of distilled water) and solution B (2.5 g hydroxide sodium and 5 ml hypochlorite sodium per 500 ml of distilled water). The mixture was maintained at 37°C for 20 min. Absorbance was recorded at 660 nm. The ammonia concentrations were expressed as NH_4Cl equivalents and values were determined as µg of NH_4Cl per ml. Ammonia of blanks was subtracted from the measured N-NH_3 of samples to obtain the net production. Methane and carbone dioxide productions were measured chemically after 3, 9, 24, 72 and 96h according to procedure described by Jouany (1982). It consists to transfer of total gas in other syringes then 4 ml of hydroxide sodium (10N NaOH) was introduced, this later reacted with carbone dioxide which induces piston retraction. The remaining gas corresponds to volume of methane. For Protozoa count, the methodology described by Ogimoto & Imai (1981) was used. 100 µl of the syringe contents after 24 h of incubation was mixed with an equal volume of the methylgreen-formaldehyde-saline fixative solution containing methylgreen (0.06 g/100 ml), sodium chloride (0.8 g/100 ml) and 1:10 diluted formalin (35% w/v, HCHO in water). The mixture was shaken gently, held in dark for 30 min and then pipetted into a Malassez chamber. The protozoa were then counted using microscopy at 40 magnifications.

Statistical analysis

Data of *in vitro* fermentation test and chemical analysis were analyzed by one-way analysis of variance employing the SAS software (1990). The means were compared by Scott-Knott's test at the level of 5%, using the entirely random design.

Results and Discussion

Chemical characteristics of OMWW and vetch-oat hay

Chemical composition and phenolic content of OMWW and those of control were presented in table 1. As expected, the dry matter (DM) of OMWW was lower; this high humidity is a consequence of the continuous washing during the extraction process in the three phase system (Piacquadiou et al., 1998). OMWW was also characterised by its low ash and crude protein contents. These results are in accordance with earlier data of Moussaoui et al. (2010) and Ben Othman et al. (2009) who recorded approximately the same levels (1.5-2%) and (1.25-2.4%) for ash and CP concentrations, respectively. However, total carbohydrates (ST) content was found to be higher than vetch-oat hay. As mentioned in literature, the main sugars reported in OMWW include fructose, mannose, glucose, saccharose, sucrose and pentose (Niaounakis & Halvadakis, 2006; McNamara et al., 2008). Effectively, theses sugars are very soluble in water and can play a significant role in supplying the ruminants by easily available source of energy (Molina-Alcaide & Yáñez-Ruiz,

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2008). In addition to its high ST concentration, OMWW was also characterised by its high total phenols content, estimated as gallic acid equivalents of dry residue. Although that the same result was observed by Ben Sassi et al. (2006) for Moroccan OMWW obtained from continuous process, this level is, however, different from those reported by Zenjari et al. (2006) and Blika et al. (2009). These discordances might be due to the different standards used for calibration curves, to the variety and the maturity stage of olive processed (Fiestas & Borja, 1992), but also to the technique used. Caponio & Catalano (2001) have showed that even the temperature of olives before and during crushing influence strongly the solubilization of phenols. For ruminants, this high level of phenols can contribute for inhibiting *archae* bacteria and therefore limiting energy loss via methane production. For tannins concentrations, the results obtained in this study were similar to that noted by El-Abbassi et al. (2012). The CT concentration (0.89% of DM) suggests that OMWW is rich in hydrolysable tannins. These later were efficiently degraded by ruminale microbiota and contribute to volatile fatty acids (AGV) production (main resource of energy for ruminant).

Biogas production and estimated exponential model parameters

Biogas production resulting from *in vitro* anaerobic fermentation of OMWW and the parameters calculated from the exponential model were illustrated in table 2. It indicates that the highest gas production recorded after 96h of incubation was noted for vetch-oat hay (68.33 ml/200 mg DM) and the lowest for OMWW (50.66 ml/200 mg DM) ($P < 1\%$). When the analysis of the kinetics of biogas production was done, it reveals that 63% and 82% of biogas was produced during the first 24 h of incubation for OMWW and control substrate, respectively. These differences were probably due to the chemical composition of the two substrates. Effectively, OMWW are rich in phenolic compounds and tannins than vetch-oat hay. These compounds have long been recognised as antinutritional factors that can reduce both palatability and digestibility of feedstuffs. Accordingly, to previous works, the presence of phenolic compounds reduced significantly gas production (Makkar et al., 1995; Hervas et al., 2000)**.** This reduction in gas production may be mediated through a decrease in microbial activity by phenolic compounds fixation to microorganisms or to their enzymes, as well as deprivation of substrate by formation of phenols-carbohydrate complexes. As a result, phenolic compounds reduced microbial degradation of carbohydrates, and subsequently gas production (Makkar et al., 1995; Akin et al., 1988). The exponential model parameters of two substrates were significantly different ($P<0.05$) (Table 2). For both substrates, values of gas production from soluble fraction (*a*) were positive which are associated to the absence of latency phase.

Table 2. *Biogas production resulting from in vitro fermentation of olive mill wastewaters in presence of sheep rumen liquor as inoculum.*

	OMWW	**Vetch-oat hay (control)**	**S.E.M.**	**Pr.**
	Biogas production at (ml/200 mg DM)			
3h	14.67^{h}	13.67^{i}	0.35	<1%
6h	17.34^{g}	24.01^{f}	4.71	<1%
9h	20.85^{d}	29.69^{e}	6	<1%
24h	32.14^{bc}	55.73^{b}	0.90	<1%
48h	50.66^{c}	68.33^{a}	0.90	<1%
72h	50.66^{c}	68.33^{a}	0.90	<1%
96h	50.66^{c}	68.33^{a}	0.90	<1%
	Parameters estimated from exponential model			
a	8.71^{a}	3.04^{b}	0.26	$<2\%_0$
b	44.83^{b}	68.69^{a}	1.12	$<2\%_0$
a + b	53.47	71.4	0.69	$<2\%_0$
k (%.h^{-1})	3.68^{b}	6.50^{a}	0.2	$<3\%_0$

a, gas production from the immediately soluble fraction; b, gas production from the insoluble fraction; (a +b), potential gas production; k, rate at which gas is produced; S.E.M., standard error of means; Pr., probability; Means with different superscripts in the same raw are significantly different (P < 0.05).

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The greatest value is observed for OMWW. This result can be explained by the highest concentration of OMWW in soluble sugars (39.91% DM). However, vetch-oat hay was faster degraded than OMWW (P < 2%$_0$). This situation can be due to the high level of crude protein in vetch-oat hay which enhances biomass production and therefore microbial activity.

Methane and carbon dioxide yields

The analysis of the gazes produced during 96h of incubation is illustrated in Figure 1. It reveals that the fermentation pattern of OMWW and the vetch-oat hay was similar and generate mainly carbon dioxide production. After 24 h of incubation, the proportions of methane (CH_4) and carbon dioxide (CO_2) were 9.85 and 89.65%, 11.2 and 88.8% for OMWW and control, respectively.

The gas production is correlated with both the quantitative and qualitative production of volatile fatty acids (Orskov & Ryle, 1990). Numerous authors suggest that the degradation of substrate rich in soluble sugars favors the production of propionic and butyric acids. While the fermentation of fibrous substrates produces acetic acid itself, being associated with an important production of hydrogen (H_2) which induces an increased production of gas in the form of CH_4. This leads us to deduce that the degradation of OMWW, which are rich in soluble sugars, might favor the production of propionic and butyric acids (major source of energy for ruminants).

The low methane production observed for OMWW comparatively to vetch-oat hay can be attributed to their high total phenols. These compounds act indirectly in reduction methane production (limit the H_2 transfer to CO_2) or directly by their inhibitory effects on methanogenic *Archaea* bacteria (Broudiscou et al., 2000).

In vitro fermentation parameters

The results obtained after 24 h of fermentation for pH, protozoa (cell/ml), ammonia (mg/ml) and estimated total volatile fatty acids (µmole/g DM), organic matter digestibility (g/100 g) and metabolisable energy (MJ/kg DM) are illustrated in table 3. The pH value (7.05) recorded for OMWW was lower than that of control (7.11, P < 1‰). These values are slightly superior to that reported for ruminale artificial saliva (6.8-6.9) by Menke et al. (1988). According to Arhab et al. (2009), the stability of pH in neutral zone in batch cultures can be explained by the excessive liberation of ions carbonates from the buffer solution for neutralization acidity resultant from volatile fatty acids production and/or high ammonia production.

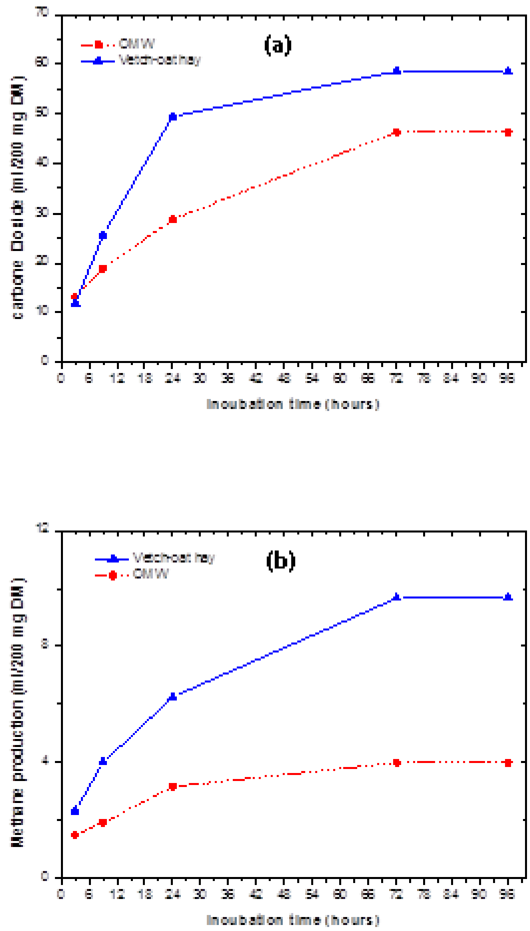

Figure 1. *Carbon dioxide (a) and methane (b) yields from in vitro fermentation of OMW and vetch-oat hay.*

The higher ammoniacal nitrogen concentration was recorded for control (4.16 mg/ml) and the lowest was observed for OMWW (3.81 mg/ml) (P < 1%). This result can be due to the low crude protein content of OMWW or to the presence of tannins in this substrate. Effectively, it has been demonstrated that tannins alter the tertiary structure of ammonia ligase and inhibited proteolytique bacterial growth (Bento et al., 2005). In counterpart, this ammonia should be incorporated in microbial biomass production. Min et al. (2005) showed that *Lotus corniculatus* tannins reduced the

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bacterial proteolysis and reduced the growth of proteolytic rumen bacteria which increase non-ammonia N flow in the intestine for absorption.

Protozoa count indicates that total protozoa population resulting from degradation of vetch-oat hay is higher (3×10^4 cell/ml) compared to that noted from fermentation of OMWW (2×10^4 cell/ml). This decrease in protozoa population observed in OMWW was attributed to their higher phenolic compounds and tannins concentrations. Although the mechanism of action of phenolic compounds on protozoa has not been studied. It is clear that phenolic compounds from some tree fodders do impact rumen protozoa, likely associated with their specific biological activity. Tannins have been shown to lower protozoal numbers which may also decrease protozoal-associated methanogenesis. The inhibitory effects of tannins on rumen methanogenesis have been ascribed to direct effects on methanogenic archaea, protozoal-associated methane production and indirectly through a depression of fibre digestion in the rumen (Tavendale et al., 2005).

Volatile fatty acids are produced in the rumen as end-products of microbial fermentation and represent the major source of energy for ruminants. In this study, their predicted concentration was different between OMWW and control diet. These values were 0.708 and 1.217 µmole/g DM for OMWW and vetch-oat hay, respectively. Organic matter digestibility (OMD) is also illustrated in table 3. In this case also the highest value was noted for control (68.01 g/100g DM) and the lowest for OMW (44.80 g/100 g DM). The predicted metabolizable energy is also presented in table 3. It was different between OMWW and control. The value of ME calculated in this study for control (11.5 MJ/Kg DM) was similar to that reported for alfafa hay with medium quality (NRC, 1989). However, the energy content of OMWW (6.93 MJ/kg DM) is acceptable for a by-product and it is distinctly superior to that recorded by Christodoulou et al. (2007).

Table 3. *In vitro fermentation parameters recorded after 24h of incubation (pH, ammonia concentration and protozoa count) and estimated total fatty acids (AGVt), organic matter digestibility (OMD) and metabolizable energy (ME)*

Substrates	pH	$N\text{-}NH_4$	Protozoa	AGVt	OMD	ME
OMWW	$7{,}05^b$	$3{,}81^b$	$2.0^b \times 10^4$	0.708	44.80	6.93
Vetch-oat hay	$7{,}11^a$	4.16^a	$3.0^a \times 10^4$	1.271	68.01	11.50
S.E.M.	0,01	0,18	0,29			
Pr.	< 1‰	< 1%	< 1‰			

Conclusion

The anaerobic biodegradation of OMWW reveal their significant use by the rumen microbiota, allowing us to strongly recommend its use as a supplement in feed ruminant. In addition, it allows considering using this residue as a feed additive in diets of ruminants for the reduction of ruminal methanogenesis.

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