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Effect of the substrates nature on their *in vitro* fermentation kinetics using rumen fluid of slaughtered dromedary as inoculum

Effet de la nature de la ressource alimentaire sur la cinétique de fermentation des substrats par la microflore rumina le de dromadaires

Hacène BOUSSEBOUA, R. ARHAB & M. RIRA
Laboratoire de Génie Microbiologique et Applications, Département des Sciences de la nature et de la Vie/Faculté des Sciences/ Université des frères MENTOURI,
Route de Ain-El-Bey BP 325, 25017 CONSTANTINE (ALGERIE).
Tél/fax: + 216 (0)31 81 87 56, E-mail adress: hblgma@yahoo.fr

SUMMARY

The in vitro fermentation kinetics of dates, orange and olive residues by the dromedary ruminal microflora is comparatively studied with hay used as standard. The results indicate the greater hydrolytic activity of the dromedary ruminal microflora towards dates and orange residues than olive residues and vetch-oat hay ($P<0.05$). The fermentation reaches the stationary phase after 24 and 48 hours for dates and orange residues, and olive wastes respectively. It is marked by two phases; corresponding to the degradation of soluble fraction (soluble sugars) and that of the insoluble one (cellulose). The results showed also that the substrate nature is a determining factor for in vitro gas production. The degradation of substrate rich in cellular content (dates and orange residues) is characterised by a fast fermentation that moves towards CO_2 production, and it is marked by a long latency phase. Whereas, the fermentation of the fibrous substrate (olive residues and vetch-oat hay) generates CH_4 production and it is tributary of less long latency period.

The degradation level observed indicate that the dates and orange residues might represent an acceptable source of energy for dromedary, but the olive residues, in spite of their chemical composition rich in organic matter, cannot be used in animal feeding.

Key words: dromedary, ruminal microflora, agro-industrial by-products, *in vitro* gas production, fermentative CO_2, fermentative CH_4.

RESUME :

La cinétique de fermentation in vitro des résidus de dattes, d'oranges et de grignons d'olives par la microflore ruminale d'un lot de dromadaires est étudiée comparativement au foin de vesce avoine, pris comme substrat de référence. Les résultats montrent que les résidus de dattes et d'oranges sont nettement plus fermentescibles que le foin et les grignons d'olives ($P < 0,05$). La fermentation atteint la phase stationnaire après 24 heures et 48 heures pour les résidus de dattes, d'oranges et les grignons d'olives, respectivement. Elle est tributaire de deux phases distinctes, correspondant respectivement à la dégradation de la fraction soluble de l'aliment (les glucides simples) et à celle de la fraction insoluble (cellulose).

Le profil fermentaire des résidus de dattes, riches en sucres solubles, s'oriente vers une production de CO_2, tandis que celui des résidus fibreux (grignons d'olives et foin) produit du CH_4.

Le niveau de dégradation constaté indique que la microflore ruminale de dromadaires est fortement active, il montre aussi que les résidus de dattes et d'oranges possèdent un important potentiel énergétique, alors que les grignons d'olives s'avèrent faiblement fermentescibles.

Mots clés : dromadaire, microflore ruminale, sous-produits agro-industriels, production de gaz *in vitro*, CO_2 fermentaire, CH_4 fermentaire.

1- INTRODUCTION

The lignocellulosic biomass, composed of residues of harvest and agro-industrial by-products, represents a considerable volume. It remains unexploited or very little so, because it is generally considered as weakly degradable and therefore without a real commercial value. However, it provides a potential source to ruminant feeding (JAYASURYA, 1993; NGUYEN and *al*., 2001; PHAM and *al*., 2001), notably in developing countries, such as Algeria.

Among herbivores, camels are considered particularly as able to convert any type of biomass into energy thanks to their presumably specific microflora activity and their ability to adapt to difficult environmental conditions (ENGELHARDT and *al*., 1986). The available data deals essentially with the physiological properties of the animal, such as their resistance to heat and thirst. Its digestive physiology has been illustrated only during the last decade (KAYOULI and *al*., 1991; 1995; JOUANY and *al*., 1995; DULPHY and *al*., 1995).

The use of non conventional substrate in its feeding has not yet been subjected to significant studies. For this reason, we tried in the present study to establish the fermentation capacity of dromedary ruminal microflora towards some agro-industrial by-products retained for their availability in our country, and to examine the effect of the nature of the feeding resource on its *in vitro* gas production kinetics.

2- MATERIALS AND METHODS

2-1- Substrates

Substrates used in this experiment were dates, orange, olive residues and vetch-oat hay (standard substrate), and had a known chemical composition (Tab.1). Samples were taken from an industrial firm of transformation and conservation of dates (relegated dates). Orange residues were sampled from an industrial firm for jam and juice production (pulps and seeds). Olive waste was taken from traditional olive oil refinery (crushed olive).

Dates and orange residues were dried at 45°C (in order to ovoid the MAILLARD reaction) and olive residues and vetch-oat hay at 105°C until constant weight. Samples were ground to pass a 1-mm sieve.

2-2- *In vitro* fermentation

The fermentation was conducted according to the procedure described by MENKE and *al*., (1979); MENKE and STEINGASS (1988). The substrates were incubated with rumen fluid in 100 ml calibrated glass syringes which were incubated at 39°C in an electrically heated, isothermal oven, equipped with a rotor, which rolled continuously at 9 rpm for 72 hours.

Rumen fluid was collected for each trial from three healthy dromedaries immediately after slaughter and stored in the Thermos containers, which were heated to 39°C and were CO_2 saturated. After straining with four layers of gauze, the rumen fluid was mixed with the medium mixture solution of MENKE and STEINGASS (1988) in a 1:2 ratio (v/v) and saturated with CO_2.

For each substrate and each series of incubation, about 200 mg of dried samples plus 30 ml of rumen fluid and buffer were incubated in triplicate. Under the same conditions, blank syringes (rumen fluid plus buffer solution) were also incubated in triplicate. Gas production was recorded at 2, 4, 6, 10, 24, 48 and 72 hours.

The quantitative analysis of gas production was obtained by direct reading of the level of piston displacement in the syringe and then the qualitative one is carried out using the procedure of JOUANY (1982).

Net gas volume at each incubation period was calculated by subtracting the mean gas volume of the blank from the volume of gas in syringes with samples.

The volume of gas was not corrected according to a standard substrate.

Data for gas production (mean of three observations) were fitted to the exponential model proposed by ORSKOV and Mc DONALD (1979) and adapted for gas production by BLÜMMEL and ORSKOV (1993): $p = a + b\,(1 - e^{-ct})$, where p represents the net gas production at time t, $(a+b)$ potential gas production and c the rate of gas production. Neway exel software developed by CHEN (1997) was used to calculate the data.

2-3- Statistical analysis

The data were analyzed by one factor variance analysis (effect of substrate) using STAT-ITCF program (version 1987).

3- RESULTS AND DISCUSSION

The kinetics of gas production is showed in Fig.1a. It follows an ascending pattern for the different substrates. The fermentation is relatively intense during the first 24 hours of incubation, after which it reaches a stationary phase, however with certain substrates, it already started to decline. The kinetic of gas production appears to be determined by two distinct phases; the first one corresponds to the degradation of the soluble fraction and the second to the insoluble but potentially fermentable fraction. The examination of the specific fermentation curves shows that the dates and orange residues were more fermented than vetch-oat hay and olive residues ($P < 0.05$). Their degradation occurs mainly during the first 10 hours. However, the vetch-oat hay and olive residues fermentations are tributary of a latency phase.

The difference in the kinetics of gas production between the dates and orange residues, and that of vetch-oat hay resulted certainly from their chemical composition (Tab.1), which indicates that the dates and orange residues are rich in soluble sugars. Besides, their cell wall is less lignified

compared to vetch-oat hay which is rich in cellulose (GIHAD and *al*., 1989; NEFZAOUI, 1999).

The weak gas production, observed with olive residues, has also been mentioned by THERIEZ and *al*., (1970); NEFZAOUI and *al*., (1999). This result could be explained by the fact that olive residues contain probably some anti-nutritional factors (tannic substances) which insolubilize the protein and inhibit the microbial activity. It seems that their effect is mainly important during the first hours of incubation (LEINMÜLLER and *al*., 1991). According to ERNEST and *al*., (1987), the olive residues pressed traditionally contain a part of pulp and around 40% of nucleus which are rich in fatty acids. These fatty acids are converted into calcic salts in the presence of calcium and magnesium (compounds of the buffer solution). These ions are primordial for the adhesion of cellulolytic bacteria to the cellulose (TAMMINGA and *al*., 1991). This situation could also be an explanation for the weak gas production.

The qualitative analysis of gas produced during fermentation is illustrated by Fig.1b and 1c. It reveals that the degradation patterns of dates and orange residues are similar. In the first hours of incubation, the dominant gas released is CO_2, but beyond 24 hours of incubation, an inverse tendency takes place and CH_4 becomes dominant. Concerning vetch-oat hay, both CO_2 and CH_4 are produced with a little disequilibrium in favour of CH_4. However, the degradation of olive residues produces exclusively CH_4. In the same way, it is noted that the CO_2 and CH_4

production, observed *in vitro* for dates and oranges residues, evolves the other way around during fermentation. This result is in agreement with those mentioned *in vivo* by VERMOREL (1995).

The gas production is correlated with both the quantitative and qualitative production of volatile fatty acids (ORSKOV and RYLE, 1990). Numerous authors suggest that the degradation of substrates rich in starch and soluble sugars favours the propionic and butyric acids (ORSKOV and *al*., 1988; ORSKOV, 1991). According to the WOLIN equation (1975), this production is related to CO_2 production. Otherwise, the fermentation of fibrous substrates produces acetic acid itself, being associated with an important production of H_2 which induces an increased production of gas in the form of CH_4. This lets us deduce that the degradation of dates and orange residues, which are rich in soluble sugars, might favour the production of these two acids, but that of fibrous substrates (olive residues and vetch-oat hay) favours the production of acetic acid.

The gas production characteristics deduced from the exponential model are shown in table 2. The results reveals that the values of soluble fraction (*a*), obtained from the exponential model after 72 hours of incubation, are positive as well as negative. The negative values have also been reported by other authors working under the same conditions or *in sacco* (ORSKOV and RYLE, 1990; BLÜMMEL and *al*., 1993). They are associated to more less long latency phase and they could be explained by the necessary time to ruminal microflora to degrade soluble fraction and then to adhere to the cellulosic fraction of the substrate. Furthermore, the dates and orange residues are characterised by a fast fermentation than vetch-oat hay and olives residues ($P < 0.05$). This is due mainly to their wealth in soluble sugars.

4- CONCLUSION

The results of the present study complement the important studies made on dromedary by scientist in the last decade, and indicate the greater hydrolytic activity of the dromedary ruminal microflora against substrates rich both in soluble fraction and cell wall compounds.

The results show also that the substrate nature is a determining factor for in vitro gas production. In fact, the substrate rich in cellular content is characterised by a fast fermentation that moves towards CO_2 production, and it is marked by a long latency phase. Whereas, the fermentation of fibrous substrates is tributary generates CH_4 production and occurs with less long latency period. Concerning the nutritive value of the studied substrates, our results indicate that the dates and orange residues might represent an acceptable source of energy for dromedary. However, the olive residues, in spite of their chemical composition rich in organic matter, cannot be used in animal feeding. Their use as a constituent of feeding ration can be considered perhaps after a treatment aimed to eliminate the inhibitory factor of the ruminal microflora and increase the solubility of protein.

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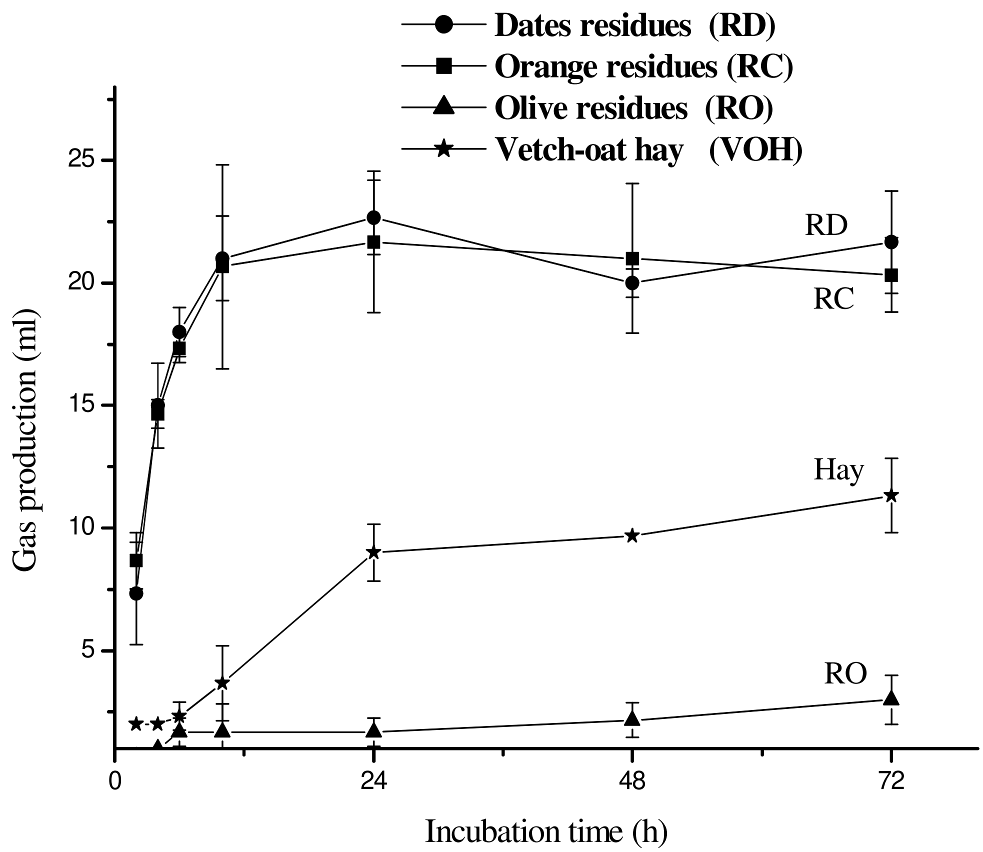
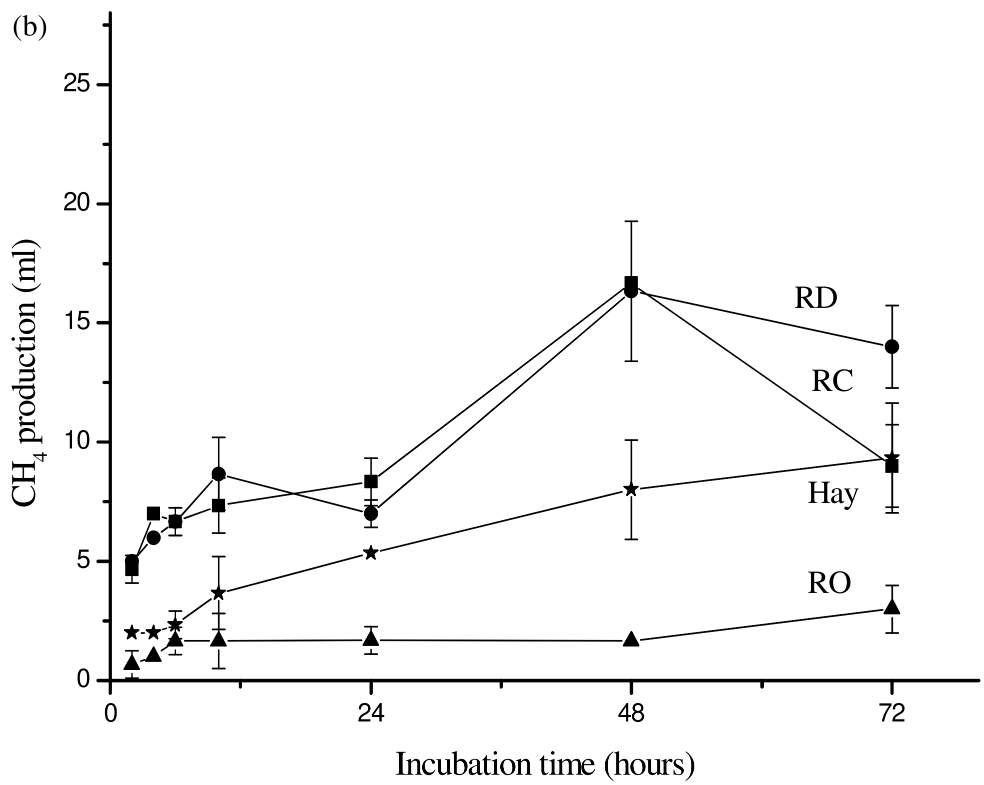
(a)


Dates residues (RD)
Orange residues (RC)
Olive residues (RO)
Vetch-oat hay (VOH)
Gas production (ml)
Incubation time (h)
RD
RC
Hay
RO
0
24
48
72
5
10
15
20
25

(b)


CH_4 production (ml)
Incubation time (hours)
RD
RC
Hay
RO
0
24
48
72
0
5
10
15
20
25

(c)

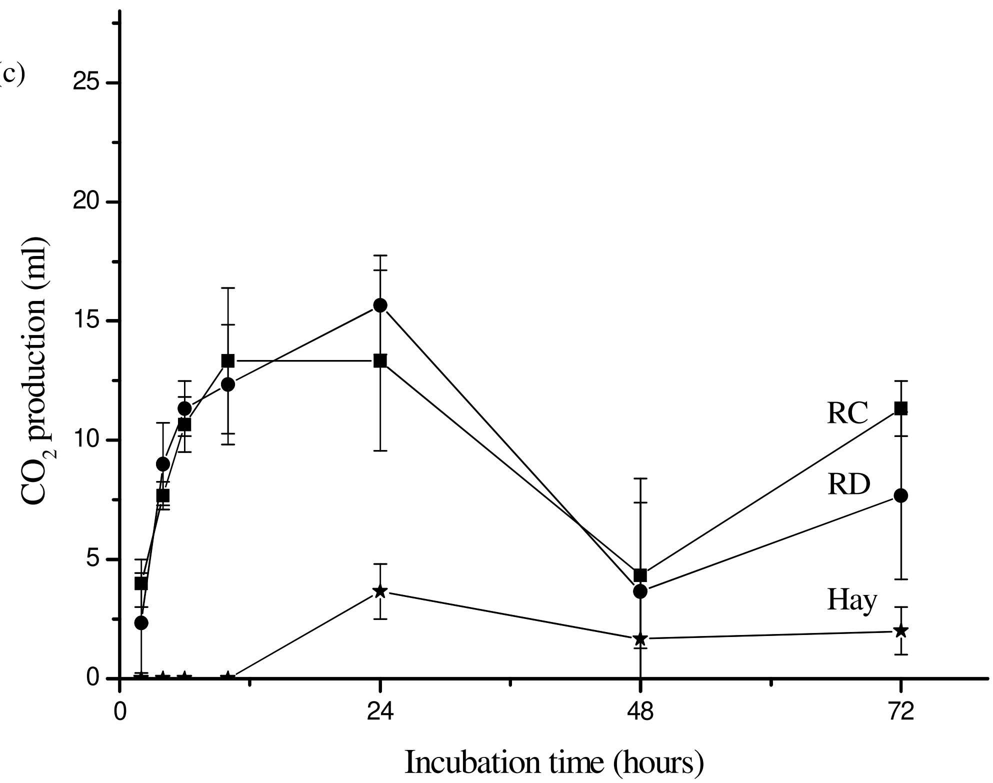


Figure 1: effect of the substrate nature substrate on *in vitro* gas production kinetics. (a), total gas production; (b), dioxide carbon (CO_2) production and (c), methane (CH_4) production.

Table 1: Dry matter content and chemical composition (% of dry matter) of the feedstuffs.

				% of dry matter			
Substrates	Abrev.	DM (%)	Total sugars	Crude protein	Crude fat	Crude fiber	Total ash
Dates residues	RD	91.1	82.6	2.85	0.54	2.93	2.4
Orange residues	RC	19.5	25.9	5.57	2.34	11.9	4.37
Olives residues	RO	68.2	5.14	0.97	15.6	40.9	1.61
Vetch-oat hay	VOH	90.1	2.9	6.1	1.3	51.3	5.6
	SEM	0.53	1.76	0.41	0.62	2.7	0.25

Table 2: Cumulative *in vitro* gas production (ml) and substrate fermentation characteristics defined by the exponential equation $p = a + b\,(1 - e^{-ct})$.

Substrates	Gas production after						
	48 hr	72 hr	b	a + b	C (%/h)	Lag time (hr)	RSD
RD	20.0	21.6^{a}	30.7^{a}	21.4^{a}	38.65^{a}	0.86	1.73
RC	21.0	20.3^{a}	25.2^{a}	21.0^{a}	35.26^{a}	0.50	1.98
RO	2.1	3^{c}	2.5^{c}	2.20^{c}	9.23^{b}	0.35	0.46
H	9.67	11.3^{b}	12.4^{b}	12.5^{b}	5.90^{b}	0.16	1.02
SEM	1.08	1.53	3.45	1.5	3.91		

[abc] Means in the same column without letter in common differ significantly ($P<0.05$); b, gas produced from the insoluble and potential fermentable fraction; c, rate of gas production; a+b, potential gas production; RSD, residual standard deviation; SEM, standard error of means.