EFFECT OF THE NATURE OF THE FEEDING RESOURCE ON ITS *IN VITRO* GAS PRODUCTION KINETICS USING RUMEN FLUID OF SLAUGHTERED DROMEDARY

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ABSTRACT

Degradation aspects in terms of kinetics of the tested samples namely dates, oranges and olive residues by the dromedary ruminal microflora is comparatively studied with vetch-oat hay as a standard. The results indicate greater hydrolytic activity of the dromedary ruminal microflora towards dates and orange residues than both olive residues and hay. Fermentation of dates and orange residues reaches their stationary phase after 24 hours and olive wastes after 48 hours. However, fermentation process was marked by two phase; namely the degradation of soluble fraction and that of cellulosic one. The results showed also that types of substrate is a determining factor for *in vitro* gas production. In fact, 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. On contrary, the fibrous substrate degradation (olive residues and hay) is tributary of less long latency period and generates CH_4. The degradation level observed indicates that the dates and oranges residues might represent an acceptable source of energy for dromedary. On the other hand, the olive residues, in spite of being rich in organic matter, cannot be used in animal feeding.

Key words: Dromedary, Ruminal microflora, Agro-industrial by-products, In vitro gas production, CO_2 and CH_4.

INTRODUCTION

The lignocellulosic biomass that are composed of residues of harvest and agro-industrial by-products, represent considerable volume. It remains unexploited or degraded very slowly because it is generally considered as weakly degradable and therefore being without a real commercial value. However, such biomass provides a potential source to ruminant feeding

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(Jayasuriya, 1993 and Pham *el 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 due to their presumably specific microflora activity and their ability to adapt to different and severe environmental conditions **(Engelhardt *et al* 1987).** The available data deals essentially with the physiological properties of the camels, such as their resistance to heat and thirst. Its digestive aspects and physiology pattern have been illustrated only during the last decade **(Kayouli *et al* 1991; Kayouli *et al* 1995; Jouany *et al* 1995 and Dulphy *et al* 1995).**

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

MATERIAL AND METHODS

Substrates

Substrates used in this experiment were dates, oranges, olive residues and hay (standard substrate). These substrates had a known chemical composition (Table 1). Samples were taken from an industrial firm of transformation and conservation of dates (relegated dates). Orange residues were obtained 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 oranges residues were dried at 45°C (in order to ovoid the Maillard reaction) and olive residues and hay at 105°C until constant weight. Samples were ground to pass a 1-mm sieve.

In vitro gas production

The substrates were incubated with rumen fluid in 100 ml calibrated glass syringes following the technique of **Menke *et al* (1979) and Menke & Steingass (1988).** The syringes were incubated at 39°C in an electrically heated, isothermal oven equipped with a rotor, which rolled continuously at nine rotations/min for 72 hours.

Rumen fluid was collected for each trial from three healthy dromedaries immediately after slaughter and stored in Thermos containers, at 39°C under saturated CO_2. After straining with four layers of gauze, the rumen fluid was

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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 (medium mixture solution) were incubated in triplicate. Under the same conditions, controls; hay (as standard substrate) plus blank syringe (without substrate) 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 McDonald (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. Software developed by **Chen (1997)** was used to calculate the data.

Statistical analysis

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

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 intensive 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 of the tested substrate and the second to the insoluble but potentially fermentable fraction. The examination of the specific fermentation curves shows that the by-products of dates and oranges were more fermented than hay and olive residues ($P < 0.05$) and their degradation occurs mainly during the first ten hours. Whereas, in the case of hay and olive residues, the fermentation is tributary of a latency phase.

The difference in the kinetics aspects of gas production between the dates and oranges residues from one side and those of hay is certainly a function of their chemical composition (Table 1), which indicates that the dates and oranges residues are rich in soluble sugars. In addition, their cell walls are less lignified compared to hay which is rich in cellulose **(Gihad *et al* 1989).**

Table 1. Chemical composition of the tested dates, oranges and olive residues, in relation to hay as a control samples.

Tested Substrates	Abrev.	DM	Values are % of dry matter		

			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
Oranges 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
Hay	H	90.1	2.9	6.1	1.3	51.3	5.6
Standard Error of means (SEM)		0.53	1.76	0.41	0.62	2.7	0.25

DM = Dry matter

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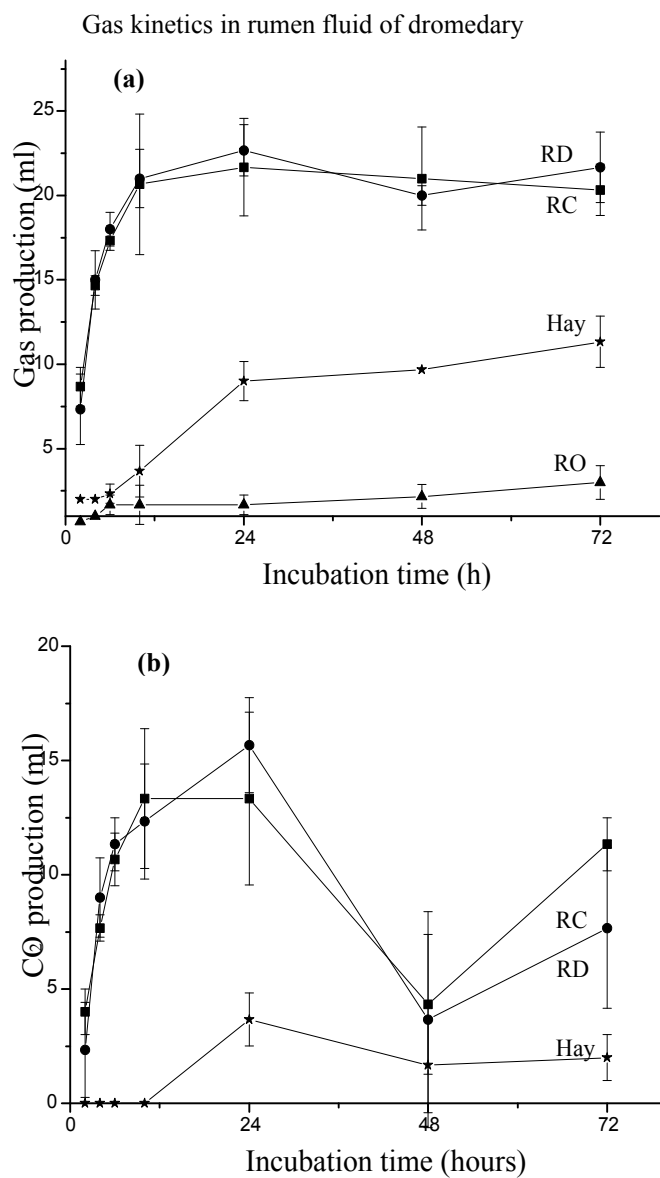Gas kinetics in rumen fluid of dromedary

Fig. 1. Effect of substrate nature on *in vitro* gas production kinetics.
(a) total volume, (b) volume of CO_2 and (c) volume of CH_4.
Rd: Dates residues,   Rc: Orange residues,   Ro : Olives residues
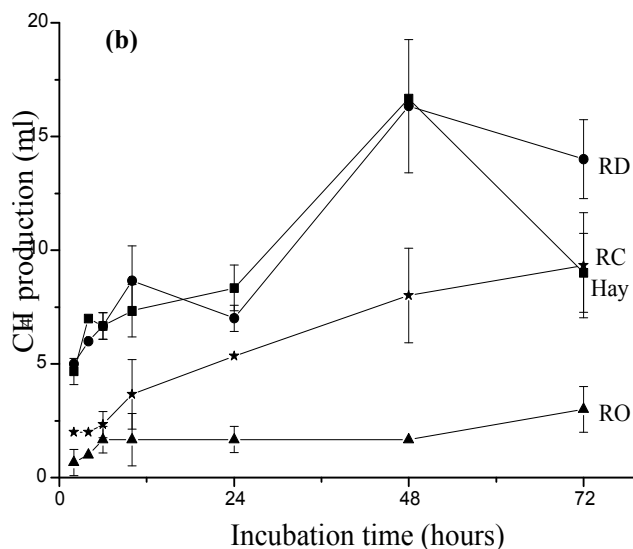
Fig. (1): Cont.

The weak gas production, observed with olive residues, has also been mentioned by **Theriez and Boule (1970).** This result could be explained by the fact that olive residues contain phenolic and tannic substances which are characterised by the unsolubilization of protein and the inhibition of microbial activity. Their effect is important mainly during the first hours of incubation **(Leinmüller *et al* 1991).** The pressed olive residues 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 Doreau, 1991).** This situation could also

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be an explanation for the weak gas production.

The qualitative analysis of gas produced is illustrated by Figs. 1b and 1c. It reveals that the degradation patterns of dates and oranges residues are similar. In the first hours of incubation, the dominant gas released is CO_2; beyond 24 hours of incubation, an inverse tendency takes place and CH_4 becomes dominant. Concerning 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 was found 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 agree with those mentioned *in vivo* by **Vermorel (1995)**.

The production of gas during fermentation is correlated with both the quantitative and qualitative production of volatile fatty acids **(Orskov and Ryle, 1990).** Numerous authors suggest that the degradation substrate rich in starch and soluble sugars favours the production of propionic and butyric acids **(Orskov *et al* 1988).** These products are 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 leads us to deduce that the degradation of dates and oranges residues, which are rich in soluble sugars, might favour the production of propionic and butyric acids but that of fibrous substrates (olive residues and hay) favour the production of acetic acid.

Table (2) shows 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 & Ryle, 1990 and Blümmel & Orskov, 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 cellulosic fraction of the substrate. Furthermore, the dates and oranges residues are characterised by a fast fermentation than hay and olives residues ($P < 0.05$).

Table 2. Cumulative *in vitro* gas production (ml) after 72 hours of incubation and substrate fermentation characteristics defined by the exponential equation
$$p = a + b\ (1 - e^{-c*t})$$

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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 ($P<0.05$).
* For abreviants, sea Table, 1.

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CONCLUSION

The results of the present study complement the important studies made on dromedary by physiologists 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. On contrary, the fibrous substrate degradation is also tributary of less long latency period and generates CH_4.

Concerning the nutritive value of the studied substrates, the obtained results indicate that dates and oranges residues might represent an acceptable source of energy for dromedary. Whereas, the olive residues, in spite of being rich in organic matter, cannot be used in animal feeding. However, 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 its protein contents.

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تأثير وسط الصدئ الغذائي على حيوية خلايا الأنتاج باستخدام الخلايا الشاملة في *in vitro*

[46]

أرهاب رابخ- هدي ميعزوز2 – ديب وسيف بوصب حستاك

1- مخبر ودنستعمل الأحياء الدقيقة في التطبيق التقنية والعلوم قسم البيولوجيا والكيمياء جامعة منتوري طريق
عين البابص .ب 325، قسنطينة 25017، الجزائر
2- المعهد الفرني للتربية الوطنية والتعليم في خمن أو قليلن من قابلوا طليق الجزائر

تهدف هذه الدراسة إلى تحديد نشاط الميكروفلورا وعدد فاستعمل مواد للطلاء خلايا محتات الغذاء بقايا
الإجارة ويتهيئ حالة الأصناع الغذائية.
حيث مبحث كركية مخدم اللتمر من
البرتيكاكس لبزيت خوربي الميكرفلورا تتراكم على الجلدي حيوة بنانسلام،
بالمقارنة قيذأي الخليط كمادة ذخ أساسية.

بين نتيجتها تحسين نالأحيا باللبة عن الربتمر
والبرتيكاقليق بةلختمر مقارنة مزمة بوبكس
بالثبات بعدتع واليت خوبتثيص لعلمية مختم رملحة
24 ساعة ففي التخمير واللتمر بقلا على 48
ساعة ابن نعلاكساليل لوح نأظمر اللتمر
يخضع لمرحعصا لتمدتنتيهتيفلالحيم ماهاد مرحلة مدة
الجزاء الذلثىالأمثبتمىيرخر مرحلة مدة الجزء
السليلويز.

كما بين نتائطبحيجة مدنتة التفاعل
أساسيفيلتناغلزا *in vitro* ،

تحكيم: علمي محمد أمين عبداللّه

وعلهدا في الغلالاقيني تحملاخطلوبيا
والبرتقلاقين في يسكوطوليةفارق اهق
تخمر سري يعيدوا يإنتاج ثانيا إلى السلين كربون.
بين الفنيالمواد الغذية للأقينالألبسكياي بزيتلبسلنون
واتبزنزملطبلوأقلياري، فارقتافجات
الميثانا.

بين نستوتعلدمهسجلا لمسجل بأنابقاتي واالبرتقال
يمكن أن تكون ضصدرطراقة للجلملبيبني للفات
الزيتوفروزن غ من اهانا بالمدلقا على ضفيعقتنها
يمكن استعمالا فايغتيذلقي مجلم.

**الكلمات الدالة: ** الجمل العربي حيوة يد يدنسلام،
ميكروفلورا الأول الحي نانا التخمرت،
مخلفات الناعصاناذ غلخي الشثي ئنيسيد
الكربوي ثانيا انتاغلزا *in vitro*.