Proceedings of the

7th World Rabbit Congress

4-7 july **2000** – Valencia Spain

These proceedings were printed as a special issue of WORLD RABBIT SCIENCE, the journal of the World Rabbit Science Association, Volume 8, supplement 1

ISSN reference of this on line version is 2308-1910
(ISSN for all the on-line versions of the proceedings of the successive World Rabbit Congresses)

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Volume C, pages 451-457

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ABSTRACT

A preliminary study was carried out to characterise the fermentation kinetics of hare caecal contents using the *in vitro* cumulative gas production technique (Theodorou *et al*., 1994). Alfalfa meal, beet pulp, sunflower meal and two commercial diets for rabbits (high fibre content and low fibre content) were used as substrates to test. The cumulative gas profile gave a good description curve, fitting the model: low and high fibre diets had the highest potential gas production (300 and 294 *ml/g*), while beet pulp and alfalfa hay showed lower values (218 and 223 *ml/g*). The two concentrates and sunflower organic meal were degraded more efficiently than alfalfa hay and beet pulp. The maximal fermentation rate was very variable in all feedstuffs. Comparison between kinetic parameters from hare and rabbit showed many differences. Further studies of hare feedstuffs are required, using caecal contents from animals with known diets. It would be of great interest to compare the two species (rabbit and hare) using subjects fed similar diets as donor animals.

INTRODUCTION

In the last few years, hare breeding has greatly increased because of the greater demand for live subjects (hunting and repopulating) and improved breeding techniques, which generally provide for the use of gates and dry feed. The diet, similar to that of rabbits, consists of commercial feed administered as pellets *ad libitum.* Such kinds of diet do not completely satisfy consumer expectations. Much research has been carried out to study the capacity of digestive utilisation of feedstuffs and nutritive requirements of hare. However, this has not yet permitted the formulation of diets able to respond very well to the demands of subjects bred in captivity. This holds particularly for captured animals used for controlled reproduction to increase the number of farm-reared animals.

Moreover, as further investigation is required to improve our current knowledge of hare caecal physiology and microbial activity and to obtain information about feedstuff nutritive characteristics, it seemed worth studying the *in vitro* fermentation characteristics of five feedstuffs with the gas production technique using caecal content from hare as inoculum.

The cumulative gas production technique (GPT) is a method to evaluate the nutritive value of ruminant feeds (Pell & Schofield, 1993; Cone *et al.*, 1994; Theodorou *et al*., 1994), applied successfully using other animal species, such as horses (Macheboeuf *et al.*, 1997), chicken (Kwakkel *et al*., 1997) and rabbits (Calabrò *et al*., 1999, Calabrò *et al*., 2000). So far, no information has been reported concerning the fermentability of hare according to the GPT.

Therefore, in this preliminary study, we report results using the GPT to characterise the extent and fermentation kinetics of hare caecal contents. Moreover, a comparison was made of the results between kinetic parameters of hare and rabbit.

MATERIALS AND METHODS

Substrates. Three simple feedstuffs (alfalfa meal, beet pulp and sunflower meal) and two commercial diets for rabbits (high fibre content and low fibre content) were used as substrates. They were the same as those used by Calabrò *et al.* (2000).

Inoculum. Two adult hares which died during afternoon capture at the Calandriello Wild Animal Production Farm in southern Italy (February 1999) provided the microbial inocula. After death, the caecum were isolated by tying up the two extremities with a nylon string to prevent losses of digesta. The caecal content was frozen at -18°C for 1 month; then it was defrosted, diluted with a medium (Theodorou, 1993) 1:1 (v/v), and squeezed through six layers of gauze to obtain the inoculum. During the above procedures, the microbial suspension was kept at 39°C under a stream of CO_2.

In vitro fermentation. Fermentation kinetics were measured using the cumulative gas production technique according to the procedure reported by Theodorou *et al*. (1994). The adopted procedures are the same as those reported by Calabrò *et al.* (2000). In particular, about 820 mg of sample was weighed in a 120 ml serum bottle, containing 74 ml of a medium, 3.5 ml of reducing solution and 5 ml of inoculum. Each substrate was replicated 4 times. All the bottles were incubated at 39°C for 96 h; the pressure and volume of gas produced were recorded 20 times at 3-24 h intervals. At the end of the incubation time the residual OM, pH and VFA concentration were determined.

The cumulative gas data were fitted to the monophasic modified Michaelis-Menten model (Groot *et al.,* 1996):

$$G(t) = \frac{A}{1+\left(\frac{B}{t}\right)^{C}}$$

where G (ml/g) is the cumulative gas production per g of OM incubated at time t after incubation, A (ml/g) represents the asymptotic gas production, B is the time after incubation at which half of A has been produced and C is a constant determining the sharpness of the curve. In addition, the fractional rate of substrate digestion and the time (t_{RM}) at which the maximum digestion rate occurs (R_M), were calculated from parameters B and C with the following formula (Groot *et al.,* 1996):

$$t_{RM} = B(C-1)^{1/C} \qquad\qquad RM = \frac{Ct_{RM}^{C-1}}{B^{C} + t_{RM}^{C}}$$

Statistical analysis. The effects of the substrates were tested for significance by analysis of variance using the model:

$$y_{ij} = \mu + S_i + \varepsilon_{ij}$$

where: μ = overall mean, S_i = substrates (i: 1-5) and ε_{ijk} = residual error. All the statistical analyses were performed using the GLM procedure of SAS (SAS Institute INC., 1989).

RESULTS AND DISCUSSION

The monophasic model used gave a good description curve obtained by incubating all the feedstuffs with buffered caecal content of hare. Figure 1 exemplifies gas production over time for the low fibre diet.

The fermentation characteristics of the substrates are summarised in Table 1. Low and high fibre diets had the highest potential gas production (300 and 294 *ml/g* for low and high fibre diets, respectively), while beet pulp and alfalfa hay showed lower values (218 and 223 *ml/g*, respectively).

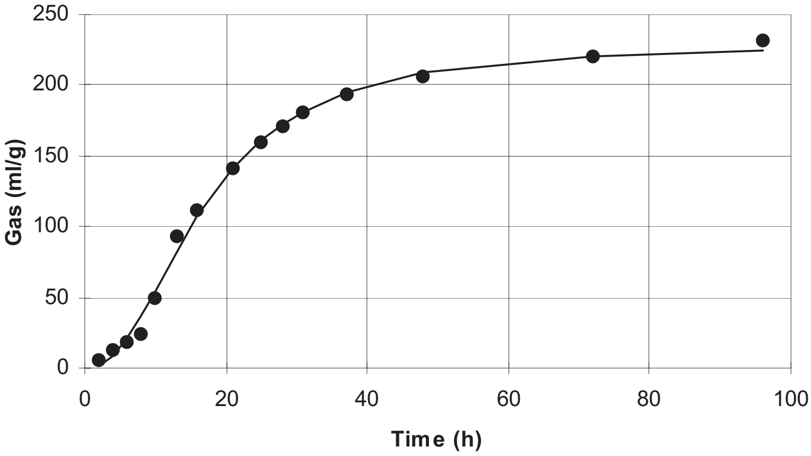
Table 1 - ***Fermentation parameters at 96 h***

Substrate	**Alfalfa meal**	**Beet pulp**	**Sunflower meal**	**High fibre diet**	**Low fibre diet**	s.e.
A, *ml/g*	223^{C}	218^{C}	240^{B}	300^{A}	294^{A}	5.28
B, *h*	19.6^{C}	38^{A}	28.8^{B}	13.2^{D}	10.1^{E}	0.78
$\mathbf{t_{RM}}$, *h*	15.9^{A}	4.68^{D}	3.55^{E}	15.4^{B}	10.1^{C}	0.10
$\mathbf{R_M}$, h^{-1}	0.044^{C}	0.022^{E}	0.028^{D}	0.096^{B}	0.099^{A}	0.0014
OM loss, *%*	63.2^{B}	47.8^{C}	70.8^{A}	69.6^{A}	69.6^{A}	2.14
OMCV, *ml/g*	123^{B}	91^{C}	121^{B}	215^{A}	213^{A}	1.89
Y, *ml/g*	232^{B}	242^{B}	198^{C}	361^{A}	358^{A}	11.66
PH	6.52^{AB}	6.48^{BCa}	6.56^{A}	6.44^{Cb}	6.49^{Bca}	0.03
VFA, *mM/g*	65.6^{B}	46.5^{Cb}	69.7^{B}	87.0^{A}	55.3^{Ca}	4.89
Acetate, *% tot. VFA*	81.2^{A}	77.75^{AB}	70.34^{C}	71.83^{C}	71.77^{C}	1.16
Propionate, "	10.26^{Aa}	7.84	5.74^{B}	6.78^{b}	5.42^{B}	0.96
Butyrate, "	6.38^{C}	11.95^{Bb}	15.69^{AB}	16.59^{ABa}	19.0^{A}	1.33

A: asymptotic gas production; B: time after incubation at which A/2 was formed; t_{RM}: time at maximum rate; R_M: maximum fractional rate; OM loss: organic matter degradability; OMCV: gas production related to incubated OM; Y: gas production related to degraded OM; VFA: volatile fatty acids related to incubated OM.
s.e.: standard error; A,B,C,D,E and a,b: samples with different letters in the same column are significantly different from each other ($P < 0.01$ and $P < 0.05$, respectively). ***: $P < 0.001$

The two concentrates and sunflower organic matter were degraded more efficiently (OM loss: about 70 %) than alfalfa hay (OM loss: 63.2 %) and beet pulp (OM loss: 47.8 %). The maximal fermentation rate (R_M), very variable in all feedstuffs, was higher using diets as substrate, although such feedstuffs and alfalfa reached the maximal rate in a very long time (t_{RM}: 15.9, 15.4 and 10.1 *h*, for alfalfa, high and low fibre diets, respectively). In a trial carried out using rabbit caecal content as a source of inocula, Calabrò *et al.* (2000) reported very different fermentation behaviour for the same feedstuffs. For example, with rabbit inoculum, beet pulp showed the highest potential gas production and the lowest maximal rate reached over a long period of time compared to the other substrate, allowing degradation of much organic matter during the fermentation process. The results obtained with caecal content from hare are highly questionable and hard to explain. However, it seemed appropriate to report the GPT as a method which obtains many inoculum characteristics and often supplies highly variable results (i.e. inoculum and preparation type). Thus, it is possible that the differences observed between the two species were also due to the initial characteristics of hare caecal content. Indeed, the hare caecal content was collected in the afternoon (5 p.m.) from captured, bound and hence highly stressed animals.

Figure 1 - ***An example of the raw data and resulting fitted curve for the low fibre diet***



The highest total VFA production was obtained with high fibre diet (87.0 *mM/g*), which also has the highest concentration of acetate, propionate and butyrate. Beet pulp produced low amounts of VFA. However, as a whole, the results disagree with data obtained in rabbit *in vivo* trials (Fraga *et al*., 1991; Bellier, 1994; Jehl & Gidenne, 1996) where the authors reported an increased caecal VFA concentration in diets with high beet pulp content.

At the end of incubation, all substrates recorded pH values normally found in hare caecum, and pH values over 6.3 did not negatively affect structural carbohydrate fermentation (Doane *et al*., 1997).

Table 2 shows a comparison between gas production parameters with hare and rabbit inocula. Data from rabbit come from a trial carried out with the same substrates using rabbit caecal content as a source of inoculum prepared with the same procedure (Calabrò *et al.,* 2000).

Table 2 - ***In vitro fermentation characteristics in rabbit and hare inocula***

Inoculum	**Rabbit**	**Hare**
A, *ml/g*	294	255
B, *h*	21.6	21.9
t_{RM}, *h*	6.49	9.92
R_M, h^{-1}	0.06	0.06
OM loss, *%*	61.5	63.8
OMCV, *ml/g*	170	153
Y*, ml/g*	274	282
PH	6.29	6.49
VFA, *mM/g*	51.4	64.8
Acetate, *% tot. VFA*	75.8	74.6
Propionate, "	14.4	7.2
Butyrate, "	4.9	13.9

For legend see table 1

However, no statistical comparison can be made between means, since hare caecal content came from captured animals experiencing very different nutritional and living conditions from those of donor animals of rabbit caecal content, reported fermentation parameters following the same tendency with the two inocula. It is worth noting the lower potential gas production (A: 255 *vs* 294 *ml/g*) and the higher t_{RM} value (9.92 *vs* 6.49, *h*) observed with the hare inocula, indicating a slower fermentation process. Such behaviour does not prevent production of more VFA or more OM degradation (VFA: 64.8 *vs* 51.4 *mM/g*, and OM loss: 63.8 *vs* 61.5 %, for hare and rabbit, respectively) as probably happens *in vivo* in the two species as reported by Mussa *et al.,* (1978) in digestibility trials comparing hare and rabbit. Considerable differences were observed also in butyrate production between the two inocula. It is difficult to interpret such results correctly, because no data relative to fresh hare caecal content are available.

There are a few possible reasons for such differences which could affect the fermentation activity of caecal microflora: the different diets of donor animals, the initial inoculum characteristics (i.e. collection time, animal stress conditions, etc.) and the differences inherent in the microflora species of rabbit and hare caeca. Further investigation is required to account for the differences in question.

CONCLUSIONS

Tested feedstuffs showed fermentation parameters which varied greatly and were not always in agreement with their chemical composition. However, these results allow us to propose the GPT for further studies of hare feedstuffs, perhaps using caecal contents from animals with known diets. Most of all, it would be of great interest to compare the two species (rabbit and hare) using subjects fed similar diets as donor animals.

Acknowledgement: Research supported by Salerno Province Administration, Hunting and Fishery Councillorship.

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