

Consequences of reduced fibre intake on digestion, rate of passage and caecal microbial activity in the young rabbit

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(Received 10 November 1994 – Revised 9 May 1995 – Accepted 27 June 1995)

The present work was undertaken to study *in vivo* fibre degradation, rate of passage and caecal fermentation activity (CFA) in the young rabbit (7 weeks old) receiving *ad lib.* a control (C) or a low-fibre (LF) diet (400 and 220 g neutral-detergent fibre (NDF)/kg respectively). As a consequence of the 50% reduction in the dietary fibre level, the voluntary food intake of the rabbits decreased by 25%, and the daily fibre intake was reduced by 60% (26.7 and 10.8 g NDF/d for groups C and LF, respectively). In spite of a longer mean retention time of the fibre particles, the quantity of fibre digested daily was significantly lower ($P < 0.01$) for the LF than for the C group (4.0 and 7.8 g NDF/d respectively). The circadian distribution of the faecal excretion (as a percentage of the total DM output) did not differ between diets ($P = 0.52$) and no interaction was found ($P = 0.96$) between diet and time of excretion. Also, variables describing the CFA showed no interactions between diet (C or LF) and time of sampling (during caecotrophy or during hard faeces excretion). Our results indicated no direct relationship between the quantity of fibre digested and the total short-chain fatty acid concentration in the caecum, but the fermentation pattern indicated lower proportions of acetate for the LF diet. Higher levels of diaminopimelic acid (DAPA) and ATP were found for the LF diet associated with an improved dietary fibre digestibility, suggesting a higher microbial activity. However, this effect was balanced by a lower caecal digesta turnover rate and the microbial biomass output estimated through the faecal DAPA output did not vary significantly.

Caecal fermentation: Dietary fibre: Short-chain fatty acids: Rabbit

The rabbit, a single-stomached herbivorous animal, is widely used as a model for man in nutritional studies. Its digestive physiology is adapted to high intakes of dietary fibre (DF) which is fermented in the hindgut (caecum and proximal colon). A reduced DF intake results in digestive disturbances such as changes in the caecal fermentative activity (CFA) (Peeters & Maertens, 1988; Bellier, 1994), and slow transit (Gidenne, 1994) could favour the occurrence of diarrhoea especially in the young rabbit. Many studies have investigated the effects of fibre intake on digestion, but the effects on CFA and on transit have been studied separately. In addition, very few studies have been able to separate the effect of the DF content from that of the nature of the cell wall (CW) (Champe & Maurice, 1983; Gidenne, 1992), because complex dietary models are often used. Thus, in the present experiment the effect of the DF level (without variations in the proportions of the different CW fractions) on caecal microbial activity, digestibility and rate of passage was studied in the young rabbit.

In addition, rabbit digestive physiology follows a circadian rhythm characterized mainly by the practice of caecotrophy (Gallouin, 1983) and variations in CFA (Gidenne, 1986; Vernay, 1987). More recently, the circadian variation of CFA *in vivo* was described

* For reprints.

according to the age of the rabbit (Bellier *et al.* 1995). However, possible interactions between sampling time and treatment were not assessed, as dietary effects on rabbit CFA were usually assessed from one sample taken at one time during the day. The present study was designed to determine whether there were interactions between diet and sampling time for two diets differing in their fibre level.

MATERIALS AND METHODS

Diets and feeding

In order to study the effect of the fibre level, two diets were prepared (Table 1) in a pelleted form using the same source of CW. The low-fibre (LF) diet differed from the control (C) by a proportional reduction in the level of the CW materials which were replaced mainly by wheat and soyabean meal (Table 1). Consequently, the DF content decreased without changes in the proportions of the CW fractions. Feed and water were available *ad lib*.

Animals, housing and experimental protocol

Two groups of twelve New Zealand White rabbits were allocated (after controlling for the effect of litter origin and weaning weight) at weaning (28 d old) to the C and LF diets. They were housed in individual metabolism cages (550 × 400 mm) and submitted to a 12 h light (07.00–19.00 hours) and 12 h dark schedule.

After a 7 d period of adaptation to the diet, six rabbits from each group were surgically fitted with T-cannulas in the caecum as described by Bellier *et al.* (1995). They were subjected to *in vivo* digesta sampling and digestibility measurements for six consecutive days (42–48 d of age). Live weight was measured at 42 and 48 d of age, and mean values are reported in Table 2. For each rabbit, samples of caecal material were collected at 3, 11 and 15 h after the start of caecotrophy (about 08.00 hours), i.e. at 11.00, 19.00 and 23.00 hours. The total duration of the collection procedure did not exceed 15 min (Gidenne & Bellier, 1992). Portions of caecal digesta (5–10 g fresh matter) were placed in tubes containing 0.03 M-H₃PO₄ or 0.035 M-H₂SO₄ storage solution (1 and 2 ml/tube respectively) for further analyses of short-chain fatty acids (SCFA) and NH₃-N, and stored at –18°. In addition, portions were kept at –80° in tubes containing perchloric acid–EDTA (2 M-HClO₄, 10 mM-EDTA) storage solution for ATP analyses.

Circadian faecal excretion pattern and rate of passage measurements were recorded on the remaining six rabbits of each group, during four consecutive days (from 50 to 54 d old), using an automatic faecal sampler (Automatic Sampler; API, Castanet, France). Digesta mean retention time in the whole tract was obtained by following the excretion of CW particles labelled with ¹⁶⁹Yb given orally at 21.00 hours (Gidenne, 1994).

Biochemical analyses

Analyses were performed in duplicate on freeze-dried samples of feeds and faeces. DM was determined by heating at 103° for 24 h. Organic matter (OM) was determined by ashing at 550° for 5 h. Water-insoluble CW material was determined on feeds according to the method of Carré & Brillouet (1989). Measurements of Van-Soest fibre fractions (neutral-detergent fibre (NDF), acid-detergent fibre (ADF) and acid-detergent lignin (ADL)) were made according to Van Soest *et al.* (1991), and N was measured by a Kjeldahl procedure and converted to crude protein using the factor 6.25. Gross energy was measured using an adiabatic calorimeter (Parr Instrument; Moline, IL, USA). The concentration of diaminopimelic acid (DAPA) in faeces was determined (for four animals out of six) after an acid hydrolysis (Hirs *et al.* 1954) by ion-exchange chromatography (Autoanalyser Beckman 6300, Beckman, Gagny, France).

Table 1. *Composition of the experimental diets*

Diet...	Control	Low-fibre
Ingredients (g/kg diet)		
Soyabean meal	90	175
Wheat	290	562
Wheat bran	200	65
Dehydrated lucerne (<i>Medicago sativa</i>) meal	290	140
Wheat straw	110	30
Minerals and vitamins*	20	28
Chemical composition (g/kg DM)		
Organic matter	920	932
Starch	230	396
Crude protein (N × 6.25)	172	194
Water-insoluble cell walls	380	227
Neutral-detergent fibre	396	217
Acid-detergent fibre	200	106
Acid-detergent lignin	44	22

* Contained (g/kg diet): calcium carbonate 7, dicalcium phosphate 1, D,L-methionine 2, sodium chloride 7, retinol 540 mg/kg, cholecalciferol 5 mg/kg, α -tocopherol 3.3, robenidine 13.2.

The pH of the caecal digesta was taken with a glass-electrode pH meter (pH 95, WTW, Weilherm, Germany) immediately after collection. SCFA were measured by gas chromatography (CP9000, Chrompack, Middelburg, The Netherlands) by the method of Jouany (1982) adapted to a semi-capillary column and NH_3 concentrations were measured by the technique of Weatherburn (1967) with an autoanalyser (Technicon, Domont, France). The concentration of ATP in caecal digesta was estimated by the luciferin-luciferase (EC 1.13.12.7) method (McElroy, 1947; Komisarczuk *et al.* 1984) (ATP dosing kit, BioOrbit, Turku, Finland).

Calculations

Apparent faecal digestibility was calculated from individual and daily total collections of faeces performed during six consecutive days. Results are given for five animals out of six, because one rabbit from the LF group died and one rabbit from the C group was omitted because of its abnormality low (> 2 SD) feed intake. The digesta mean retention time (MRT) in the whole tract was calculated according to Faichney (1975):

$$\text{MRT} = \Sigma M_i \times t_i / \Sigma M_i,$$

where t_i is the time that elapsed between marker administration and the i^{th} defecation and M_i is the quantity of marker excreted in faeces. MRT includes the transit time (TT), which is the time that elapsed between dosing and the first marker appearance in the faeces. TT reflects the retention time of digesta without a delay in the mixing compartments, so it represents the rate of passage in the tubular segment of the tract, i.e. mainly in the small intestine but also in the distal colon (Gidenne, 1994).

Statistical procedures

Digestibility and rate of passage data were examined by one-way ANOVA using the general linear models (GLM) procedure of Statistical Analysis Systems (1988). CFA data (Table 4) were examined to evaluate the effects of sampling time, diet, and the sampling time × diet interaction. However, the effect of the individual was also controlled in this model to take into account the fact that three measurements (three sampling times) were

made on the same animals. The significance of the effect of the diet was thus calculated using the mean square of the intraindividual variations as an error term (split-plot design). The same procedure was used to analyse the faecal excretion pattern, and the SEM mentioned in Fig. 1 corresponds to residual variations after controlling for the effects of time, diet and individual.

RESULTS

Feed intake, faecal excretion pattern and digestibility of diets

As a consequence of the 50 % reduction in the DF level (Table 1), the voluntary feed intake of the rabbits decreased by 25 % (Table 2). Fibre intake was reduced by 60 % (mean 26.7 and 10.8 g NDF/d for groups C and LF respectively), whereas the intake of starch was increased by only 25 % (mean 15.5 and 19.6 g/d for groups C and LF respectively). Faecal DM output (Fig. 1) was reduced by more than 50 % as a result of the reduced DF content (mean 26.8 and 11.7 g DM/d for C and LF respectively) whereas the DM content of the faeces increased (mean 696 and 759 g/kg for C and LF respectively, SEM 0.99, $P < 0.01$). A significant interaction between the effects of diet and time of excretion was found ($P < 0.01$) with a higher excretion of DM during the night period for the control group (Fig. 1). However, if expressed as a percentage of the total DM output the circadian distribution of the faecal excretion did not differ between diets ($P = 0.52$) and no interaction between diet and time was found ($P = 0.96$). For the two groups of rabbits a period of very low faecal output (2–4 % of the total excretion) was found between 09.00 and 13.00 hours, which corresponded with the period of caecotrophy (soft faeces excretion and subsequent ingestion); the main period of faecal excretion (mean 46 % of the total excreted) was during the night between 19.00 and 23.00 hours.

Diet LF resulted in higher digestibilities of OM (+18 points), crude protein, and DF fractions (NDF, ADF) (Table 2). In contrast, the quantity of fibre digested daily was significantly lower for the LF group than for the C group (4.0 and 7.8 g NDF/d respectively, SEM 0.7, $P < 0.034$). Although the non-nitrogenous cellular content (NNCC) of the feed (sum of nutrients excluding fibre and N) was almost completely degraded, the replacement of fibre by starch in the LF diet improved its digestibility significantly.

Rate of passage

The feed intake of non-cannulated rabbits (Table 3) was 15 % higher than that found for cannulated rabbits (Table 2). However, no significant interaction with the diet was found. Compared with group C, NDF ingestion by rabbits fed on the LF diet was 60 % lower and the total MRT of the fibre particles doubled. TT was not significantly different between the two groups (mean 6.3 h), indicating therefore that the change in total MRT occurred in the mixing compartments (i.e. caecum and proximal colon).

Caecal fermentations and microbial activity

Whatever the variable, no significant interactions were found between the effect of sampling time (during caecotrophy or hard faeces excretion) and the effect of the diet (Table 4), so these two effects will be presented separately hereafter.

Effect of sampling time. The caecotrophy, corresponding to an absence of hard faeces excretion, occurred between 08.00 and 13.00 hours (Fig. 1), and the caecal pH decreased significantly between the caecotrophy period (sampling 3 h after caecotrophy start, at 11.00 hours) and the period of hard faeces excretion (sampling at 12.00 hours). Total SCFA concentration was significantly higher (mean +30 %) during hard faeces excretion, associated with a decrease (mean –10 %) in propionate molar proportion. No significant variations in caecal concentrations of NH_3 or ATP with the sampling time were found.

Table 2. *Live weight, feed intake and apparent faecal digestibility values for rabbits given a control diet or a low-fibre diet**

(Mean values for five rabbits per treatment, with the pooled standard error of the mean)

Diet...	Control	Low-fibre	SEM	Statistical significance: $P =$
Live weight (LW; g)	1013	1081	57	0.30
DM intake (g/kg LW per d)	67.4	49.6	5.1	0.057
Apparent faecal digestibility:				
Organic matter	0.608	0.787	0.0086	< 0.001
Energy	0.596	0.775	0.0092	< 0.001
Crude protein ($N \times 6.25$)	0.734	0.827	0.0180	0.011
NNCC†	0.938	0.958	0.0049	0.017
Neutral-detergent fibre	0.286	0.341	0.0157	0.006
Acid-detergent fibre	0.169	0.213	0.0205	0.032

* For details of diets and procedures see Table 1 and pp. 354–356.

† Non-nitrogenous cellular content (organic matter – crude protein – neutral detergent fibre).

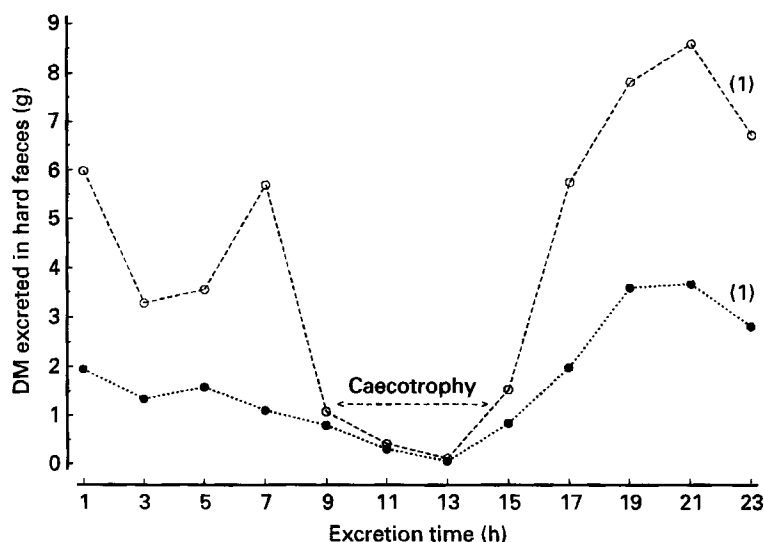


Fig. 1. Circadian pattern of the excretion of dry matter in hard faeces of rabbits fed on a control diet (○—○) or a low-fibre diet (●—●). Values are means for six rabbits per treatment. For details of diets, see Table 1. (1), SEM = 0.05.

Effect of the diet. Caecal pH was significantly lower for the LF than for the C group, but no dietary effect was found for total SCFA or NH_3 (Table 4). However, the lower consumption of fibre in the LF group was accompanied by a lower acetate molar proportion and by higher ($P = 0.12$) proportions of butyrate and minor SCFA. In addition, higher variability (+ 50%) of SCFA proportions was observed for animals in the LF group.

Although not statistically significant (high interindividual variations), caecal ATP tended to be higher for the LF group (Table 4). The faecal DAPA concentration almost doubled from C to LF animals (Table 5); however, the faecal excretion of DAPA remained slightly

Table 3. *Feed intake and rate of passage measurements of rabbits given a control diet or a low-fibre diet**

(Mean values for six rabbits per treatment with the pooled standard error of the mean)

Diet ...	Control	Low-fibre	SEM	Statistical significance: $P =$
DM intake (g/kg LW per d)	81.8	56.7	1.9	< 0.001
Rate of passage:				
Mean retention time (h)	17.3	33.4	1.8	< 0.001
Transit time (h)	5.7	6.3	0.49	0.37

LW, live weight.

* For details of diets and procedures, see Table 1 and pp. 354–356.

higher for the C group as a consequence of a higher faecal output. The faecal DAPA output was positively correlated with the total SCFA concentration in the caecum (r 0.72, P < 0.04) and negatively correlated with the propionate:butyrate ratio (r -0.85, P < 0.01), but no significant correlations were found between DAPA excretion and fibre degradation either in terms of quantity digested or in terms of digestibility coefficient.

DISCUSSION

Effect of sampling time

Under physiological conditions including free access to feed and the normal practice of caecotrophy, the rabbit digestive physiology follows a circadian cycle (according to the light schedule) which could possibly interact with the effect of the diet, especially if the feed intake or the faecal output are modified. However, in many studies of caecal fermentation sampling has been carried out at one time of the day only. If important interactions exist between diet and sampling time then sampling at only one time could be misleading. The present study has provided some results to explore this area. The faecal excretion pattern found here (Fig. 1) was typical of rabbits fed *ad lib*. It included a low faecal excretion at the beginning of the light period corresponding to the excretion and ingestion of soft faeces and to a low feed intake, and the peak of the faecal excretion took place at the beginning of the night period after the feed intake had increased (Prud'hon *et al.* 1972; Lebas & Laplace, 1974; Hörnicke *et al.* 1984). The low-DF diets used here resulted in reduced feed intake and consequently reduced faecal output, but the time distribution of the faecal output was not affected, as also noticed by Grigorov (1989). It is also clear from the results in Table 4 that variables describing caecal fermentation showed no interaction between diet (C or LF) and time of sampling (during caecotrophy or during hard faeces excretion). Consequently, it seems reasonable to assume that CFA studies can be performed using one sampling time to compare dietary effects in the rabbit. Similarly, no interaction between diet and sampling time after feeding on rat CFA was observed by Mathers & Fotso Tagny (1994).

The caecotrophy period (09.00 to 13.00 hours) corresponded with a low CFA including higher caecal pH and lower concentrations of SCFA. In contrast, the hard faeces excretion period, corresponding to high intake and faecal output (Prud'hon *et al.* 1972; Lesault *et al.* 1991), was characterized in the present study by a high CFA. Similar time changes in CFA have been observed previously in growing (Gidenne, 1986) and adult rabbits (Leng & Hörnicke, 1975). The present results indicated a slight effect of the sampling time on SCFA

Table 4. Concentration and molar proportions of short-chain fatty acids (SCFA), ammonia concentration, pH and ATP concentration in the caecum of 6-week-old rabbits fed on a control diet or a low-fibre diet and sampled at three different times*
(Mean values for five rabbits per treatment with the pooled standard error of the mean)

	Diet		Sampling time			Statistical significance of:		
	Control	Low-fibre	11.00	19.00	23.00	SEM	Diet (D)	Time (T)
pH	6.45	6.03	6.47	6.22	5.97	0.11	0.04	<0.01
Ammonia (mmol/l)	7.2	9.0	8.1	9.4	7.1	1.0	0.30	0.13
Total SCFA (mmol/l)	64.9	63.2	56.6	61.5	74.8	3.9	0.65	0.001
Molar proportions of individual								
SCFA (mmol/mol):								
Acetate	866	812	832	843	836	9.6	0.055	0.47
Propionate	54	65	65	60	55	4.0	0.35	0.065
Butyrate	75	113	94	90	102	8.2	0.12	0.66
Minor SCFA†	6	9	9	8	6	0.26	0.12	0.23
Propionate:butyrate	0.80	0.64	0.79	0.76	0.60	0.067	0.55	0.97
ATP (µg/l)	68.3	83.1	75.1	—	76.4	12.7	0.18	0.79

* For details of diets and procedures, see Table 1 and pp. 354–356.

† Isobutyrate + valerate + isovalerate.

Table 5. *Diaminopimelic acid (DAPA) concentration and excretion in hard faeces of rabbits given a control diet or a low-fibre diet**

(Mean values for four rabbits per treatment with the pooled standard error of the mean)

Diet ...	Control	Low-fibre	SEM	Statistical significance: <i>P</i> =
DAPA concentration ($\mu\text{mol/g DM}$)	2.8	4.8	0.38	0.009
DAPA excretion ($\mu\text{mol/d}$)	70.6	59.0	15.0	0.60

* For details of diets and procedures, see Table 1 and pp. 354–356.

molar proportions (mainly on propionate), in agreement with previous studies reporting a higher propionate:butyrate ratio at the end of the caecotrophy period (Gidenne, 1986; Bellier *et al.* 1995).

The use of ATP concentration in the caecum as an indicator of the microbial activity has not been reported previously in the rabbit. Our results were similar to those observed in the caecum of the pig (Bach Knudsen *et al.* 1991). However, there was high interindividual variation and this was not consistent with the changes in SCFA with sampling time. Large interindividual variations in ATP concentration of rumen digesta have also been reported (Erfle *et al.* 1981; Komisarczuk *et al.* 1984). Thus, in contrast to previous results obtained in rats or pigs (Bach Knudsen *et al.* 1984, 1991), the validity of this indicator for the young rabbit remains to be confirmed.

Effect of the dietary fibre level

The levels of water-insoluble CW and NDF were similar for the two diets, confirming that NDF residue was, in this case, an appropriate way to recover total insoluble DF.

It is clearly established that a reduction of the DF level results in an increase in the digestible energy (DE) content of the diet (10.8 and 14.1 MJ/kg DM for C and LF diets respectively). This is associated with a reduction in voluntary feed intake (Lebas *et al.* 1982; Partridge *et al.* 1989; Gidenne *et al.* 1991) and with a greater reduction in fibre intake or faecal output. Here it explained, also, the increase in DF digestibility for a low DF intake, whereas the quantity of NDF degraded remained higher for control animals. This apparently contradicts previous results obtained in adult (Gidenne, 1992) or growing rabbits (Gidenne *et al.* 1991) where a lower DF content induced a lower fibre digestibility. However, the latter studies used semi-purified diets containing a higher level of lignocellulose (ADF). The small increase in fibre digestibility of the LF diet was associated here with a twofold longer rate of passage, which originated from a higher retention time in the caeco-colic segment as previously measured for the same diets in adult rabbits (Gidenne, 1994). Gidenne (1992) reported, also, higher fibre digestibility accompanied by shorter retention time in the caeco-colic segment. Therefore, in contrast to ruminants, the efficacy of fibre degradation in the caecum of the rabbit appears to be less controlled by retention time than by the nature of the fibre or the composition of the diet.

Although DF intake fell by 60% there was only a small fall in caecal pH and no change in the SCFA concentration; similar results were reported by Champe & Maurice (1983) for

weanling rabbits (6–8 weeks old). This was in contrast to results indicating a positive relation between caecal SCFA and fibre digestibility (Gidenne *et al.* 1991) obtained on older rabbits (over 11 weeks old) fed a semi-purified diet. Thus, an effect of the age and of the adaptation period to the diets on the caecal fermentations cannot be excluded.

Our results indicated no relation between quantity of fibre digested and concentration of SCFA in the caecum, whereas the fermentation pattern indicated lower proportions of acetate as previously observed by Herrman (1990) and Yu & Chiou (1992). McKay and Eastwood (1983) and Mathers & Fotso Tagny (1994) also noticed, in rats, that a reduction in DF level modified the molar proportion rather than the concentration of caecal SCFA, despite a longer rate of passage in the caecum. Therefore SCFA measurements seem to be more a qualitative indicator of the caecal fermentation than a means to evaluate the microbial activity in relation to fibre digestion.

Measurement of caecal ATP levels or faecal DAPA constituted a new approach for evaluation of the microbial activity in the rabbit caecum. Faecal DAPA concentrations were similar to those found in rat (Walter *et al.* 1988) or pig faeces (Rowan *et al.* 1992). Higher concentrations of DAPA and ATP were found when DF intake decreased in association with an improved DF digestibility, suggesting a higher microbial activity. However, this effect was balanced by the lower caecal digesta turnover rate (longer rate of passage) and the microbial biomass output estimated from faecal DAPA output did not vary significantly according to fibre level. On this point results from the literature are controversial, indicating in rats a positive relationship (Goodlad & Mathers, 1990) or no relationship (Walter *et al.* 1988) between DF level and faecal DAPA level.

In conclusion, measurements of the caecal fermentation pattern are insufficient to evaluate the microbial activity and the relationship with other variables of digestion (fibre degradation, rate of passage); complementary measurements (DAPA) are necessary and must be validated. SCFA pattern remains of interest as a qualitative indicator of caecal fermentation. Thus, the higher variability in the molar proportions of SCFA, occurring for low fibre intake, suggest an unsteadiness in fermentation pattern. In addition, adaptation of caecal fermentation to the fibre content of the diet has not yet been evaluated in the rabbit. Consequently, the caecal fermentative activity should be studied over a complete growth period of the animals to understand how it is controlled by nutritional factors.

The authors thank André Lapanouse and Muriel Ségura for their technical assistance (Station de Recherches Cunicoles, INRA, Toulouse), Dr C. Bayourte (Ecole Nationale Supérieure Agronomique, Toulouse) for DAPA measurements, and Dr Beauville and Dr Crampes (Centre Hospitalier Universitaire, Purpan, Toulouse) for assistance in ATP analyses. The present work was partly supported by a Ralston Purina grant.

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