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G. Fonty, J.-P. Jouany, P. Thivend, Ph. Gouet, J. Senaud. A descriptive study of rumen digestion in meroxenic lambs according to the nature and complexity of the microflora. Reproduction Nutrition Développement, 1983, 23 (5), pp.857-873. hal-00898098

HAL Id: hal-00898098

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Submitted on 11 May 2020

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Reprod. Nutr. Dévelop., 1983, **23** (5), 857-873.

A descriptive study of rumen digestion in meroxenic lambs according to the nature and complexity of the microflora.

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Summary. We studied in meroxenic lambs, *i.e.* in lambs with a simplified digestive microflora, the effect of the microflora on the quantities of solid feed intake and on the main digestive parameters in the rumen.

Axenic lambs were inoculated with a more or less complex flora, obtained by diluting (10^{-6}, 10^{-7}, 10^{-8}) a pool of rumen fluid taken either from young conventional lambs before weaning from adult sheep (Pool A) or from meroxenic lambs (Pool B). A few of these lambs then were inoculated with a genus of protozoa (*Endodinium sp.* or *Polyplastron multivesiculatum*).

The results show that the main digestive parameters depended on the nature of the inocula which the lambs had received. Food consumption and volatile fatty acid concentration of the rumen fluid, low in lambs inoculated with the 10^{-8} dilution, were higher in lambs inoculated with a more complex microflora (10^{-6} and 10^{-7} dilutions). The VFA concentration measured in these lambs however was approximately two times lower than that observed in conventional animals at the same age and fed the same feed. Food intake and the development of the fermentation pattern were favoured by an early inoculation of the animals.

The complexity of the microflora appears to have influenced the composition of the VFA mixture. The latter was found to consist mainly of acetic acid in lambs inoculated with the 10^{-8} dilution. In lambs which received the 10^{-6} dilution, the composition of the VFA mixture was similar to that observed in conventional lambs.

In all animals, except in lambs 10^{-8}, the ammonia nitrogen concentration of the rumen fluid was found to be higher during the first month after birth (between 100 and 200 mg/l). A subsequent decrease in ammonia nitrogen concentration was observed at two and a half months of age (20 to 40 mg/l).

The establishment of protozoa ciliates in the rumen of these lambs was followed by an increase in butyric acid and ammonia nitrogen concentration.

Introduction.

In conventional animals a study of the specific role of microorganisms as well as of their interactions is difficult to achieve, given the complexity of the microbial ecosystem of the rumen. To understand the digestive mechanisms

involved in the rumen, the microbial conponents need to be simplified by using animals with a reduced number of bacterial and protozoal species.

The gnotobiotic conditions used to study the relationship between host and digestive microflora in monogastrics can also provide an interesting approach to the study of host-microflora-microfauna interactions in ruminants. Up until now, studies involving gnotobiotic ruminants have remained limited in scope. In the main works (Mann and Stewart, 1974 ; Lysons *et al.,* 1971, 1976 a et b ; Cheng and Wallace, 1979 ; Barr *et al.,* 1980 ; Hobson *et al.,* 1981), lambs were inoculated with a number of bacterial species varying from five to eleven according to the animals. With these lambs, the functions for which the bacteria were chosen could be verified and some aspects of the role of rumen bacteria in physical development could be shown. These studies demonstrated the extreme difficulty in successfully establishing cellulolytic bacteria in the rumen of gnotobiotic animals, and good growth in gnotobiotic lambs on a fibrous diet has not been obtained (Mann and Stewart, 1974). More success has been achieved in rearing lambs and reproducing rumen function when starchy feeds are used (Hobson *et al.,* 1981). Difficulties in establishing cellulolytic bacteria were also reported by Males (1973) who failed to established *Bacteroïdes succinogenes* in sheep raised in isolation from birth. This is why we used « meroxenic lambs », *i.e.* axenic lambs inoculated with a fraction of the rumen microflora of conventional animals, to study the ecological factors determining the establishment of cellulolytic bacteria and protozoa in the rumen. This fraction of the microflora which was inoculated, although not completely known, was obtained by simplifying the microflora of conventional animals by successive dilutions (Fonty *et al.,* 1983). The long-term aim is to determine the minimal flora that must be present in the rumen to allow cellulolytic bacteria and ciliate protozòa to be established. The kinetics of the establishment of cellulolytic bacteria (*B. succinogenes*) and of two genera of protozoa (*Entodinium* and *Polyplastron*) in the rumen of these meroxenic lambs has been described in a previous study (Fonty *et al.,* 1983). The aim of the present paper was to evaluate the effect of flora complexity on changes in the main parameters of digestion in the rumen of these meroxenic lambs. The results are compared with those obtained on conventionally-reared lambs.

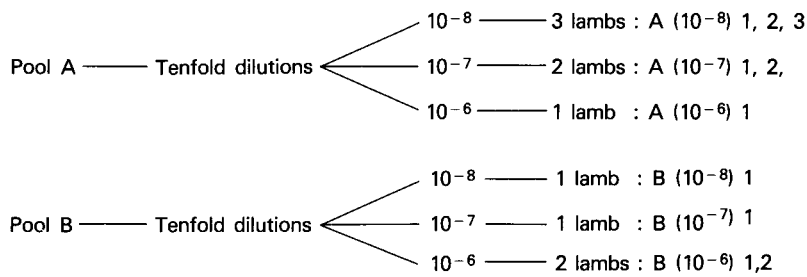

FIG. 1. — *Origin and composition of inocula used for axenic lambs.* Pool A was prepared from rumen contents of two conventional lambs (at age 4, 7, 9, 11, 15, 16, 18 and 21d) and from four adult sheep. Pool B was prepared from rumen contents of two meroxenic lambs (at age 60, 72 and 80 d) that had previously received the 10^{-7} dilution of Pool A.

Material and methods

1. *Meroxenic lambs.* — The techniques for obtaining and raising the animals used have been described previously (Fonty *et al.,* 1983). The origin and composition of the inocula used is shown in figure 1 and the time of the inoculation (with complex flora, *Bacteroïdes succinogenes* culture, *Entodinium sp.* culture, and *Polyplastron multivesiculatum* culture) in figure 2.

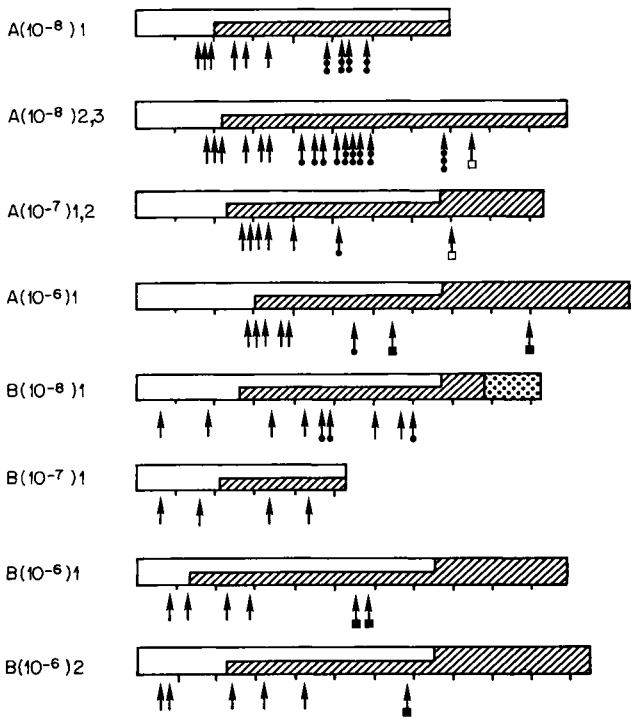

FIG. 2. — *Rearing and inoculation of lambs.* Feeding regime is described in Methods : milk (□) ; solid food formula I (▧) ; solid food formula II (▩). Lambs were inoculated with : a dilution from pool A or pool B (↑) ; *B. succinogenes* (↑), *B. succinogenes* plus sterile rumen fluid (↑); *B. succinogenes* plus sterile rumen fluid plus cellulose powder (↑) ; *Entodinium sp.* (↑) or *P. multivesiculatum* (↑).

From birth to three weeks of age, the lambs received sterilized cow's milk (UHT) exclusively, then, simultaneously, milk and a solid feed sterilized by γ-irradiation (4,5 Mrads) until eleven weeks of age. During this time, the amount of milk offered decreased regularly as solid feed intake was increased. At eleven weeks of age, lambs were fed on solid food only except for lambs A (10^{-8}) which had a reduced intake level and consequently continued receiving milk. The solid feed used was ground and pelleted and given twice daily. The composition

of this feed is given in table 1. One lamb B (10^{-8}) was given a second feed of dehydrated alfalfa hay at the end of the experiment.

TABLE 1

Diet composition ; Mineral and vitamins supplement

Diet components (%)		*Minerals* (added)		*Vitamins* (added)			
Meadow hay	28	$CoSO_4$, $7H_2O$	31 mg/kg	Vit. A	=	12,000	U.I./kg
Dehydrated alfalfa	5	$FeSO_4$, $7H_2O$	66 mg/kg	D_3	=	2,000	
Barley	15	$MnSO_4$, H_2O	20 mg/kg	E	=	37.5	mg/kg
Oats	4	ZnO	20 mg/kg	K	=	22.0	mg/kg
Dried beet pulp	20	IK	2 mg/kg	C	=	3,300	mg/kg
Peanut cake	24			Thiamin	=	13	mg/kg
Glucose	3			Riboflavin	=	13.7	mg/kg
Molasses	1			Niacin	=	101	mg/kg
				Panthotenic acid	=	39.5	mg/kg
	100			Choline	=	2,920	mg/kg
				Histine	=	1.4	mg/kg
Chemical composition of diet (%)				Folic acid	=	1.3	mg/kg
				Pyridoxine	=	11.7	mg/kg
Organic matter	92.8			Inositol	=	0.5	mg/kg
Crude fiber	18.7			Vit. B_{12}	=	0.04	mg/kg
Starch	17.7						
Nitrogen (N × 6,25)	13.1						

Between the second and third weeks of age, the lambs were fitted with a permanent rumen cannula by which digesta was sampled before the morning meal (TO) and two hours after (T2), except during the period preceding weaning. During the latter period, feed intake was intermittent and consequently samples was taken only at TO. From these samples, measurements were made of pH, volatile fatty acids (concentration and composition percentage), ammonia nitrogen concentration and lactic acid concentration.

2. *Conventional lambs.* — Six control lambs, reared with their dams (two lambs/dam) in conventional conditions, were weaned at six weeks of age. From the second week, they were fed the same solid feed as meroxenic lambs. One lamb was eliminated at two months because it had kidney lithiasis. Three lambs remained in the experiment until they were 95 days old, and two until they were 120 days old.

Rumen samples were taken either through a stomach tube (in 2 lambs) or through a permanent rumen cannula (in 4 lambs). Sampling was done before the morning feeding (To) and two hours thereafter (T2). At To, rumen samples were taken only from two lambs.

3. *Analysis.* — Volatile fatty acids were determined by the method of Jouany (1982), ammonia nitrogen (NH_3N) by the method of Weatherburn (1967) modified by Michel (1971) and lactic acid by the Boehringer method ([1]).

([1]) Boehringer, 2, avenue du Vercors, 38240 Meylan.

Results.

I. *Lambs inoculated with Pool A*

1) *Lamb A (10^{-6}).* — The food intake of this lamb was irregular (fig. 3). Solid food intake increased up to ten weeks of age. Appetite declined over the following three weeks, after which there was a gradual increase. Until three months of age, the animal consumed its ration intermittently and not as a true meal. This made it difficult to carry out a postprandial kinetic digestion study.

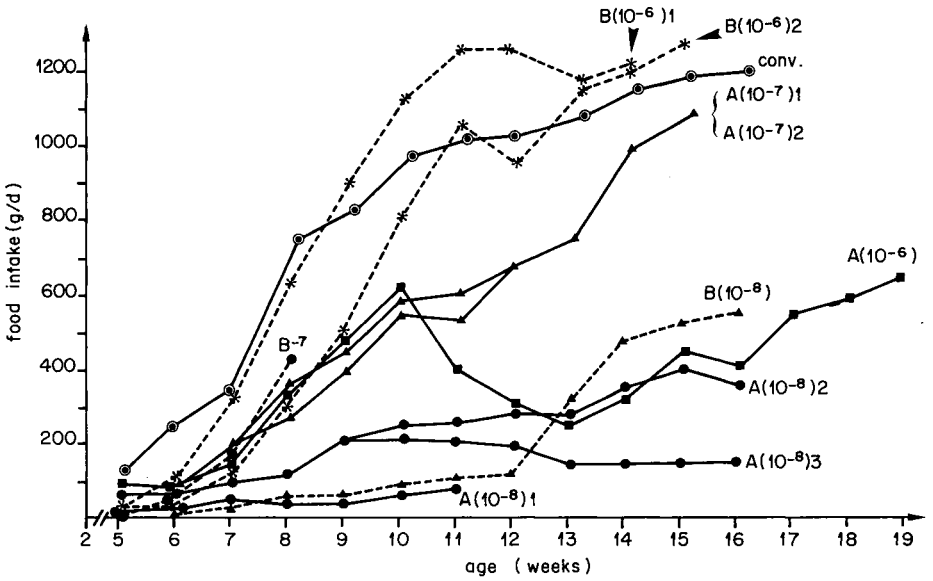

FIG. 3. — *Solid food intake by meroxenic and conventional lambs.* Between the third and fifth week food consumption was negligeable (< 100 g/d) and is not included in the figure.

Abnormalities in the feeding pattern and appetite probably explain the variations observed in the different digestion parameters measured (fig. 4). pH. values varied from 5.7 to 7.0, VFA concentration from 10 to 100 mM/l and ammonia nitrogen from 15 to 220 mg/l. Lactic acid concentration ranged from 0 to 6 mM/l and 0 to 22 mM/l for the D and L forms, respectively. The percentage composition of the different VFA (acetic acid : 55 to 65 %, propionic acid : 20 to 40 %, butyric acid : 10 to 20 %, other acids : 1 to 3 %) did not differ from those of animals with conventional flora.

Establishment of *Bacteroïdes succinogenes* (60th day) does not seem to have modified the nature and the concentration of digestion end products, but variations in the amounts consumed could have masked the effect of this bacterium.

2) *Lambs A (10^{-7}).* — The consumption of solid food was satisfactory in both lambs (fig. 3). It increased regularly during the experimental period and attained values close to those found in conventional lambs.

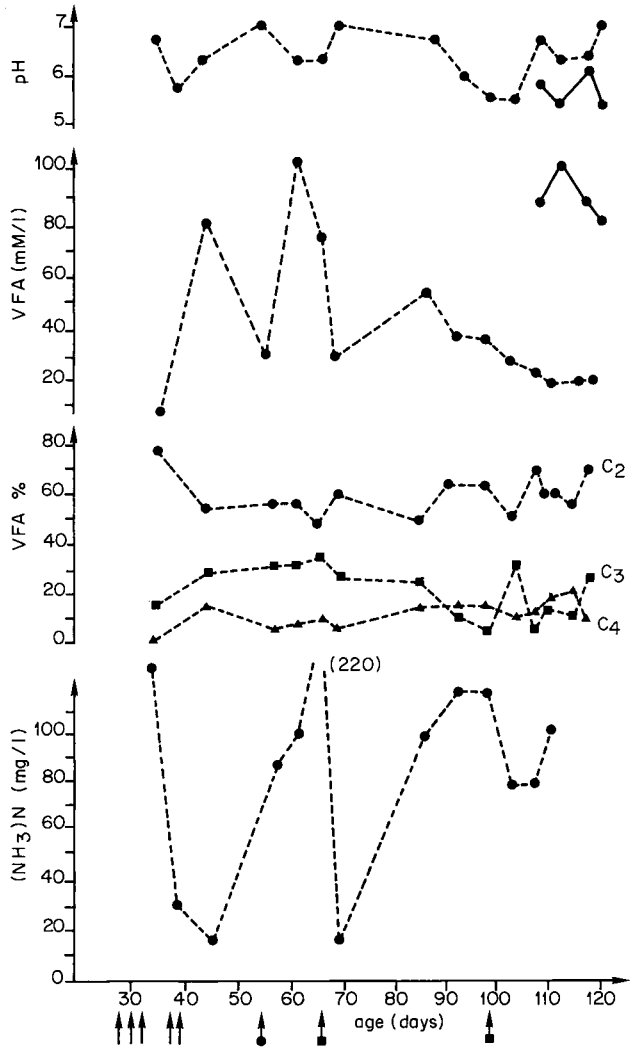

FIG. 4. — *Changes in pH; concentration and centesimal composition of VFA; ammonia nitrogen concentration in the rumen of lamb A(10^{-6})*. Dashed lines : values before feeding (T_0) ; solid lines : values two hours after feeding (T_2). The molar composition of VFA is that measured before feeding (T_0). Inoculations were made with material from pool A (↑), with *B. succinogenes*(↑) and with *P. multivesiculatum*(↑) as described in figure 2.

Compared to previous results, a more regular digestion pattern was noted in the rumen. The pH values remained more or less constant (7 at T_0 and 6 at T_2) with little variation in total acidity (fig. 5). The latter increased naturally two

hours after food consumption (from 50 to 90 mM/l) and reached a maximum ten days after the introduction of *Entodinium sp.* VFA composition varied markedly from that observed in lamb A (10^{-6}), i.e. acetic acid percentage was lower ; however there was a notable increase in propionic acid percentage. The introduction of *Entodinium sp.* brought about an increase in C_2 and C_4 concentrations ; however, there was a marked decrease in the percentage of propionic acid.

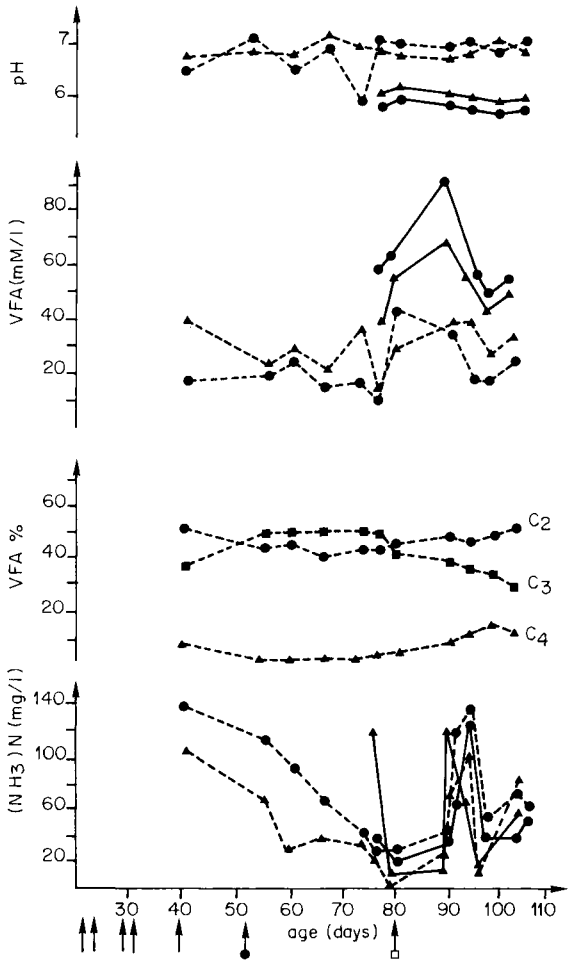

FIG. 5. — *Changes in pH ; concentration and centesimal composition of VFA ; ammonia nitrogen concentration in the rumen of lambs A(10^{-7})$_1$ (•) and A(10^{-7})$_2$ (▲).* Dashed lines : values before feeding (To) ; solid lines : values two hours after feeding (T_2). The molar composition of VFA is that measured in lamb A(10^{-7})$_1$ before feeding (To). Inoculations were made with material from pool A(↑), with *B. succinogenes* (↑) and with *Entodinium sp.* (↑) as described in figure 2.

The ammonia nitrogen concentration decreased regularly for the first 60 days of measurement, then rose abruptly when *Entodinium sp.* was established. The lactic acid concentration, which was zero prior to feeding, remained very low untill protozoa establishment (2 to 4 mM/l at T_2) ; it then rose slightly (4 to 10 mM/l). L-lactate was mainly involved.

3) *Lambs A (10^{-8}).* — Three lambs were used. Since one of these animals (lamb A $(10^{-8})_1$ refused practically all solid foods (daily consumption : < 50 g),

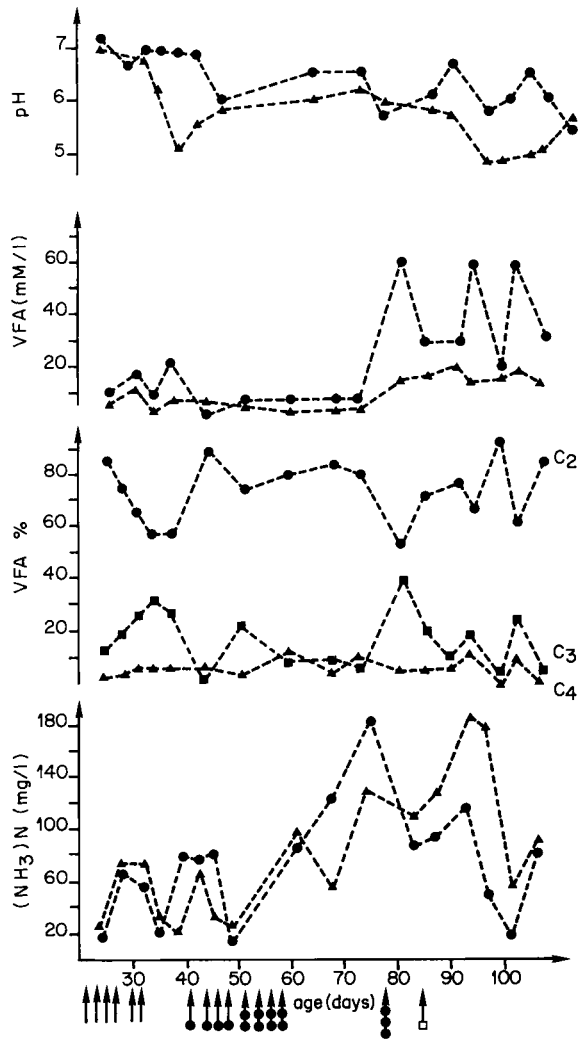

FIG. 6. — *Changes in pH ; concentration and centesimal composition of VFA ; ammonia nitrogen concentration in the rumen of lambs $A(10^{-8})_2$ (•) and $A(10^{-8})_3$ (▲).* The molar composition of VFA is that measured in lamb $A(10^{-8})_2$. Inoculations were made from material from pool A( ), with *B. succinogenes* ( ), and with *Entodinium sp.* ( ) as described in figure 2.

it was eliminated. The amounts eaten by the other two lambs were small : 200 to 250 g/day at ten weeks of age for one lamb and approximately 400 g/day at fifteen weeks of age for the second lamb. As indicated for lamb A(10^{-6}), feed consumption was intermittent throughout the day. This, no doubt, explains why pH variations were sometimes considerable, from one sampling to the next (fig. 6).

The VFA concentration was very low up to the age of ten weeks (10 to 15 mM/l) ; however, it significantly increased in lamb A $(10^{-8})_2$ after *B. succinogenes* establishment, but the amplitude of the variations was high. In the

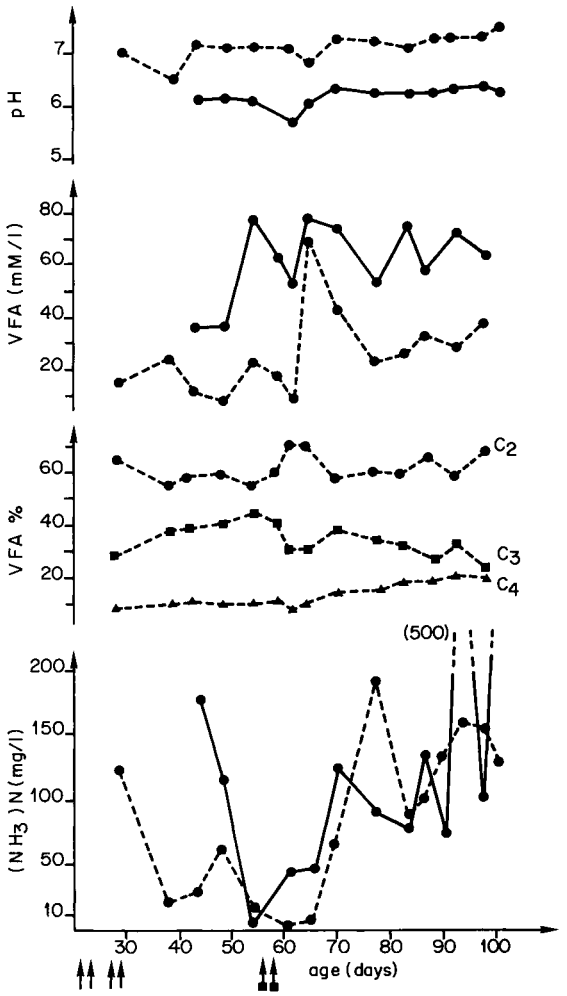

FIG. 7. — *Changes in pH ; concentration and centesimal composition of VFA ; ammonia nitrogen concentration in the rumen of lamb B(10^{-6})$_1$.* The molar composition of VFA is that measured before feeding (To). Inoculations were made with material from pool (B(↑) and with *P. multivesiculatum* (↕) as described in figure 2.

other animal (lamb A $(10^{-8})_3$, *B. succinogenes* was not established. The slight increase observed from the eleventh week onward was probably due to the presence of sterile rumen liquor introduced at the same time as the *B. succinogenes* strain (fig. 2).

The composition of the VFA mixture was very different from that of the lambs which received more concentrated inocula (*i. e.*) essentially acetic acid (80 %), propionic acid (10 to 12 %) and variable but small amounts of butyric acid were detected. The other acids never accounted for more than 1 % of the mixture and, in most cases, were even absent.

The ammonia nitrogen concentration of the rumen liquor was very low during the first two months (25 to 30 mg/l). It then increased, but as for other parameters, there were great variations from one sampling to the next.

II. *Lambs inoculated with pool B.*

Contrary to what was done with animals from pool A (inoculated from the age of three weeks on), those from group B were inoculated at an earlier age (from the first week on) in order to foster rumen development.

1) *Lambs B(10^{-6}).* — These two animals had little appetite until the sixth week (50 to 100 g/day). Then appetite increased markedly and food intake attained 1.2 kg per day during the twelfth week (fig. 3).

At T_0, pH varied little (6.8 and 7 on the average for lambs B(10^{-6})1 and B(10^{-6})2, respectively. Generally, pH decreased by one unit after feeding. *Polyplastron mutivesiculatum* establishment did not modify these values (fig. 7 and 8).

Before protozoa establishment, VFA concentration was relatively low before the meal, especially in lamb B$(10^{-6})_1$. It was twice as high two hours after feeding. Acetic acid predominated (50-60 %), with propionic acid accounting for 35 to 40 %. However, the percentage of butyric acid was low (5 to 6 %). The totality of the other acids accounted for approximately 2 %. After *P. multivesiculatum* was introduced, the VFA concentration rose in both animals, especially after feeding. The proportion of acetic and butyric acids increased while that of propionic acid decreased.

Before the introduction of *P. multivesiculatum* ammonia nitrogen concentrations decreased from 130 mg/l to 10 mg/l. With the introduction of this species, there was a significant increase in ammonia concentration both at T_0 and at T_2. However, there were great variations between samplings, especially for lamb B$(10^{-6})_1$. Moreover, the differences between concentrations measured before and after the meal were small. Lactic acid concentration, which was very low, increased after the establishment of *P. multivesiculatum* (5 to 10 mM/l). The D and L isomers were in equal proportions.

2) *Lamb B(10^{-7}).* — This animal was withdrawn from the experiment at 52 days of age after an accident occurred inside the isolator.

During the measurement period, feed consumption and pH values before and 2 hours after feeding were very close to those noted in lambs B(10^{-6}). The

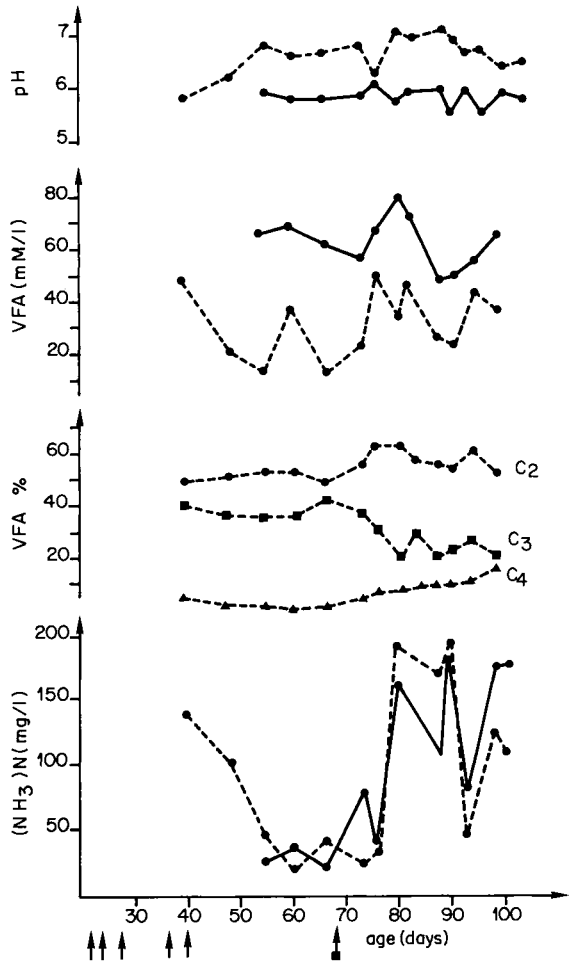

FIG. 8. — *Changes in pH ; concentration and centesimal composition of VFA ; ammonia nitrogen concentration in the rumen of lamb B(10^{-6})*$_2$. The molar composition of VFA is that measured before feeding (To). Inoculations were made with material from pool B (↑), and with *P. multivesiculatum* (↑) as described in figure 2.

VFA concentration was between 20 and 40 mM/l inclusively at T_0, and between 40 to 80 % at T_2. Acetic acid accounted for 30 to 52 %, propionic acid 40 to 45 %, and butyric 6 to 7 % of the VFA mixture. The ammonia nitrogen concentration was low (20 to 40 mg/l).

3) *Lambs B(10^{-8}).* — Until the ninth week, solid feed intake was almost zero, pH remained acidic (5.8), the ammonia nitrogen was low (30 mg/l) and VFA concentration negligible. At this time, the animal received a dilution of 7.5×10^{-8} from the same pool (fig. 2) and 5 days later a *Bacteroïdes succinogenes* culture.

The introduction of this more complex flora had an effect on all the parameters measured. Appetite increased, pH levelled off at 7.3 on the average before feeding and at 6.3 two hours after. The VFA concentration attained values between 30 and 70 mM/l (fig. 9). The centesimal composition of the VFA was also modified. When the animal hosted a simple flora, acetic acid proportion attained values of 80 to 90 %. With the introduction of the more complex flora, propionic and butyric acids proportions increased with a consequent decrease in the proportion of acetic acid. The change in the feed after the 85th day modified nothing in this composition.

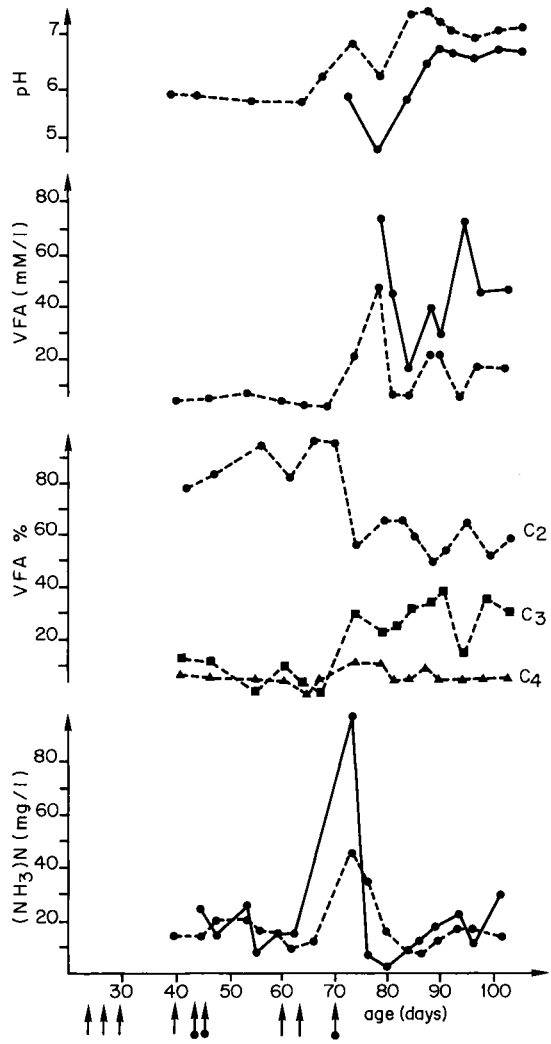

FIG. 9. — *Changes in pH ; concentration and centesimal composition of VFA ; ammonia nitrogen concentration in the rumen of lamb B(10^{-8})$_1$.* The molar composition of VFA is that measured before feeding (To). Inoculations were made with material from pool B (↑), and with *B. succinogenes* (↕) as described in figure 2.

The ammonia nitrogen concentration was also increased after the 7.5 × 10^{-8} dilution was introduced (95 mM/l), but this then decreased and levelled off at values close to those previously observed (25 mg/l). The lactic acid concentration was high during the first two months (25 to 35 mM/l) both prior to and two hours after feeding. This large amount of acid (L form) was due to the predominance of streptococci in the rumen flora (Fonty *et al.*, 1983). After inoculating the 7.5 × 10^{-8} dilution, the concentration dropped considerably (< 2 mM/l).

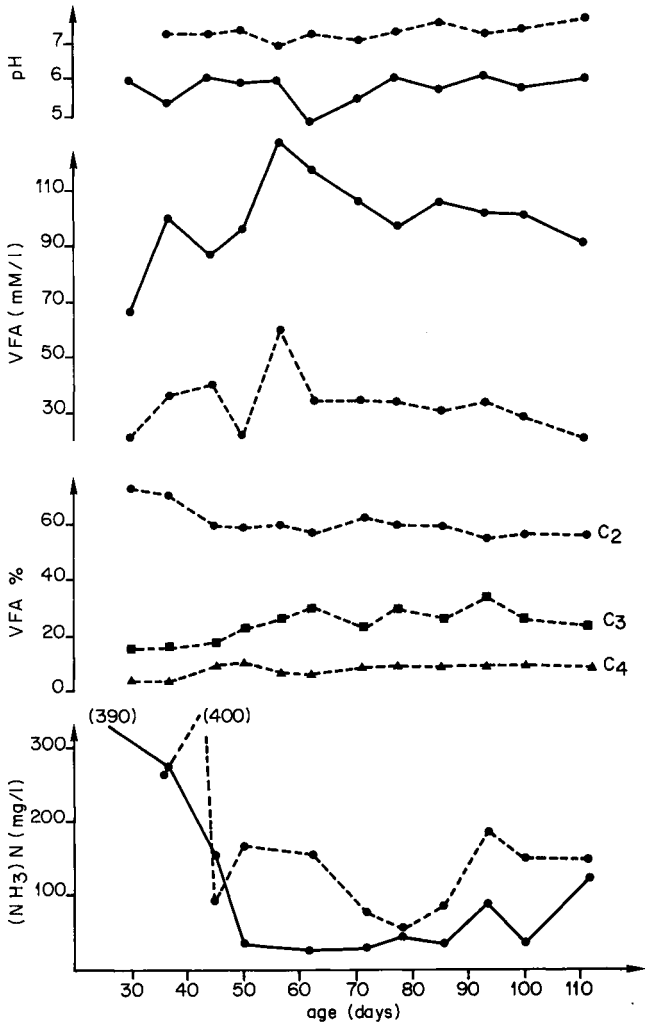

FIG. 10. — *Changes in pH ; concentration and centesimal composition of VFA ; ammonia nitrogen concentration in the rumen of conventional lambs.* The molar composition of VFA is that measured before feeding (To). Each value is the mean of two lambs at To and of six lambs at T_2.

III. *Conventional lambs.*

The food intake (fig. 3) of these lambs, which was about 100 to 150 g per day until fifth week, increased rapidly after weaning and reached 1 kg/day the ninth week, and 1.2 kg at 4 months.

Until the lambs were 40 days old, their VFA concentration (fig. 10) was low and variable, depending on the lambs. Then, it increased regularly and reached about 100 mM/l two hours after feeding at the age of 60 days. The percentages of the VFA mixture were the following : acetic acid (60-62 %), propionic acid (23 to 28 %), butyric acid (7 to 10 %), other acids (3 to 5 %).

The ammonia nitrogen concentration (fig. 10) was high during the first month (250 to 400 mg/l). Then it decreased markedly until the lambs were 50 days old (50 to 150 mg/l before feeding and 30 to 100 mg two hours afterwards).

Discussion.

The amounts of solid feed consumed by the animals varied, firstly, as a function of inoculum complexity and, secondly, depending on the age when inoculation was performed. In contrast, these amounts of solid feed were not modified by the presence of ciliate protozoa. On the whole, intake was high in animals that received inocula with complex bacterial compositions (10^{-6} and 10^{-7} dilutions) and was low, particularly up to 2 months of age, in lambs receiving simplified inocula (10^{-8} dilutions). In other respects, solid feed intake was both more rapid and quantitatively greater in lambs inoculated from the first week on (group B) than in lambs inoculated from the third week on (group A). Early inoculation of animals soon after birth thus favours the intake of solid food and the development of the rumen. This relationship between flora complexity and feed consumption is a particularity of the ruminant. Indeed, Lukey (1965) found no differences in food intake among comparable groups of germ-free and conventional rats. In some cases, intake was greater in germ-free rats (Combe *et al.,* 1965) or mice (Gordon, 1968), and Szylit and Charlet (1981) showed that gross energy intake was slightly higher in monoxenic and axenic chickens than in holoxenic ones.

There is also a good relationship between the development of rumen function and flora complexity. Thus, the VFA concentration, measured two hours after feeding, was much higher in animals which receved more complex inocula and consumed greater quantities of food lambs A(10^{-6}), A(10^{-7}) and B(10^{-6})). Nonetheless, the VFA concentrations were clearly lower than those measured in conventional animals of the same age receving the same feed (50 to 80 mM/l instead of 95 to 120 mM/l).

The percentage composition of the VFA in the rumen was also greatly influenced by the type of inoculum used. For example, until week 7 acetic acid alone was present in animals which received the 10^{-8} dilutions of inocula A and B (fig. 6 and 9). The presence of propionic acid was linked to the complexity

of the mixture of inoculated bacteria. The butyric acid content always remained low during the period prior to ciliate inoculation. It increased markedly when the animals were faunated, reaching values close to those observed in conventional animals, irrespective of the type of ciliate inoculated. This increase occured at the expense of propionic acid whatever the dilution of inoculum used. With the exception of animals A(10^{-8}) and B(10^{-8}), the ammonia nitrogen concentration in the rumen was high during the first month (between 100 and 200 mg/l). At two and a half months of age there was a considerable decrease. The high initial ammonia concentration could come from the break-down of desquamated epithelial cells by bacteria that adhere to these cells (Cheng *et al.,* 1979). Indeed, during this period, we noted that desquamation was intense and that the rumen was filled with a very thick mucous containing muco-peptides which could be hydrolysed by the proteolytic enzymes of micro-organisms present and produce ammonia. These adherent bacteria also showed an intense ureolytic activity with regard to the salivary urea and blood urea diffused throughout the rumen-wall. Ammonia absorption may also be limited in the preruminant, given the slightly acidic pH values of the rumen contents at that time. Lastly, as Jouany (1978) noted, the presence of ciliate protozoa brought about an increase in the ammonia concentration of the rumen. This can be explained either by the hydrolysing action of ciliates, by the decrease in the number of bacteria after ciliates are introduced (Eadie and Hobson, 1962 ; Kurihara *et al.,* 1968, Fonty *et al.*, 1983), or by the occurence of both simultaneously.

It would appear from our results that the presence of a simple flora cannot assure the digestive function as a complex flora can. To corroborate this, it has been shown (Fonty *et al.,* 1983) in these same lambs inoculated with dilutions greater than 10^{-7}, that the establishment of cellulolytic bacteria is very difficult and even impossible in some cases. That early inoculation of animals is a factor favouring fermentation and digestive activities in the rumen is probably related to the action of bacteria on papilla, rumen mucosa, and digestive tract development (Lysons *et al.,* 1976 b).

In this study we used an approach to host microflora relationships which consisted of inoculating axenic lambs with more and more simplified fractions of flora obtained from conventional animals. Although this approach is often used in monogastrics, it has never been applied, to our knowledge, to the preruminant. But in monogastrics, this model was used to study a specific microbiological problem (Ducluzeau *et al.,* 1977 ; Sacquet *et al.,* 1979), not a nutritional one.

Our study confirms the difficulties encountered in the management of gnotoxenic lambs. It also demonstrates the interactions between the host and its microflora as well as interactions between the diverse bacterial species which permit the rumen to function. This is not entirely due to the presence of a dominant flora but also to a number of other species which play an important symbiotic role in the rumen and are often underestimated.

Reçu en décembre 1982.
Accepté en avril 1983.

Acknowledgements. — We wish to thank J. Lefaivre who fistulated the animals, Marie-Paule Girard, Paulette Journaix, Marie-Thérèse Beauford and M. Chavarot, G. Andant, G. Vert for their valuable technical assistance.

Résumé. *Etude descriptive de la digestion dans le rumen d'agneaux méroxéniques selon la nature et la complexité de la microflore.*

Nous avons étudié chez des agneaux méroxéniques c'est-à-dire chez des animaux dont la microflore digestive était simplifiée par rapport à celle d'animaux conventionnels, l'effet de la microflore sur les quantités d'aliments solides ingérées par les agneaux et sur l'évolution des principaux paramètres de la digestion au niveau du rumen.

Les agneaux axéniques ont été inoculés avec des flores plus ou moins complexes obtenues par dilution (10^{-6}, 10^{-7}, 10^{-8}) d'un pool de jus de rumen, prélevé soit chez de jeunes agneaux conventionnels non sevrés et des moutons adultes (Pool A), soit chez des agneaux méroxéniques (Pool B). Certains animaux ont ensuite été également inoculés par un genre de protozoaire (*Endodinium sp.* ou *Polyplastron multivesiculatum*).

Les résultats ont montré que les principaux paramètres digestifs dépendent de la nature de l'inoculum qu'ont reçu les agneaux. En effet, la consommation d'aliment, la concentration en acides gras volatils du jus de rumen, très faibles chez les agneaux inoculés avec les dilutions 10^{-8}, ont été plus importantes chez les agneaux ayant reçu des flores plus complexes (dilutions 10^{-6} et 10^{-7}). Toutefois la concentration en AGV mesurée chez ces agneaux était environ 2 fois plus faible que celle d'animaux conventionnels de même âge et recevant le même aliment. Nous avons également noté que la consommation d'aliment solide et la mise en place des fermentations ont été favorisées par une inoculation précoce des animaux.

La composition centésimale du mélange d'AGV est en relation avec la complexité de la microflore. Il est constitué principalement d'acide acétique chez les animaux inoculés avec les dilutions 10^{-8}. Chez les agneaux ayant reçu les dilutions 10^{-6}, ce mélange a une composition qui se rapproche de celle observée chez les conventionnels.

Exception faite des agneaux ayant reçu les inoculums 10^{-8}, la concentration en azote ammoniacal du jus de rumen a été plus élevée pendant le premier mois (entre 100 et 200 mg/l) puis a diminué pour atteindre des valeurs très faibles à l'âge de 2 mois et demi (40 à 50 mg/l).

L'implantation des protozoaires ciliés dans le rumen de ces agneaux a entraîné une augmentation de la concentration en acide butyrique et de la concentration en azote ammoniacal.

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