IN VIVO AND *IN VITRO* STUDY OF THE CAECAL FERMENTATION PATTERN IN RABBITS BETWEEN 22 AND 56 DAYS OF AGE

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Abstract - The age dependent caecal fermentation pattern was studied in young rabbits both *in vivo* and using *in vitro* batch incubations. Sequential slaughterings were performed in 6 conventional litters in order to collect caecal contents of rabbits from 22 till 56 days of age. In the present paper, *in vivo* and *in vitro* observations are linked in order to check correspondence between the two series of data.

Age had a significant effect ($P<0.01$) both on *in vivo* total VFA concentration and on *in vitro* VFA production. A significant correlation ($P<0.01$) was found between *in vivo* and *in vitro* total VFA values ($R^2=0.48$) and between the latter and daily weight gain ($R^2=0.20$, $P<0.05$). *In vivo* and *in vitro* VFA values showed a sharp drop on day 36, followed by a plateau. This decrease reflected the significant ($P<0.05$) drop in daily weight gain that occurred on day 36.

Molar proportions of both *in vivo* and *in vitro* VFA showed a shift from propionate to butyrate which occurred on day 25. *In vivo* and *in vitro* acetate, propionate as well as butyrate were significantly ($P<0.01$) correlated ($R^2=0.31$; $R^2=0.78$ and $R^2=0.61$, respectively). *In vivo* molar proportions of isobutyrate, isovalerate and valerate were negligible whereas significant amounts were produced *in vitro*.

In vitro NH_3-N showed a significant ($P<0.001$) decrease with age, whereas *in vivo* an irregular pattern was obtained. *In vivo* lactic acid concentration as well as *in vitro* production were significantly ($P<0.01$) affected by age. However, both for NH_3-N and lactic acid no significant ($P>0.05$) correlation between *in vivo* and *in vitro* data was observed.

In conclusion, *in vitro* production rates and *in vivo* concentrations showed significant correlations for those end-products that are not utilized by microbes (VFA), but not for those end-products that are continuously produced and utilized (NH_3-N, lactate). Therefore, the applied *in vitro* incubation technique is of valuable interest in studies related to rabbit digestive physiology.

INTRODUCTION

In intensive rabbit production the pre-weaning period is a very critical phase because of the change from milk to an exclusively solid feed intake within a period of 10-12 days. Such changes affect the caecal fermentation pattern as recently shown *in vivo* (PADILHA *et al.*, 1994 and 1995; PIATTONI *et al.*, 1995) and *in vitro* (PIATTONI *et al.* 1996). In the rabbit, the fibre degrading activity takes place mainly in the caecum. The main end-products of such microbial activity are the volatile fatty acids (VFA), found as acetate, propionate and butyrate and the gases methane and carbon dioxide. Acetate is the major acid present and the rate of butyrate exceeds that of propionate. The latter feature appears to distinguish the rabbit from the other mammalian species (JOUANY, 1991).

For the study of the caecal fermentation pattern, comparative slaughterings or the cannulation technique are used. Caecal cannulation, however, is only possible from the age of 6 weeks on (BELLIER & GIDENNE, 1992; GIDENNE & BELLIER, 1992). For this reason sequential slaughterings were performed in order to collect caecal contents of rabbits from 22 till 56 days of age. An *in vitro* rumen technique was adopted because it is known to reflect the fermentation pattern with rumen contents (DEMEYER, 1991).

The aim of the present paper is to link *in vivo* and *in vitro* observations obtained in our laboratory (PIATTONI *et al.*, 1995; PIATTONI *et al.*, 1996) in order to check correspondence between the two series of data.

MATERIAL AND METHODS

Animals and diet

Young, conventional rabbits from the experimental strain of the Research Station for Small Stock Husbandry (MAERTENS, 1992) were used. At 21 days of age the 6 most homogeneous litters with 8 youngs were chosen for the experiment. Weaning was performed at 28 days of age and litters were kept together in a fattening cage. Caecotrophy was not prevented. To avoid any change induced by feeding in the intestinal environment, the same

standard reproduction diet was fed *ad libitum* to the does and their youngs both before and after weaning. The proximate composition of the pellet was in accordance with the recommendations of LEBAS (1989) and contained on DM basis: 19.8% crude protein, 16.8% crude fibre, 35.2% NDF, 4.9% crude fat, 22.0% starch and 11.95 MJ metabolizable energy/kg.

Slaughtering and sampling

Sequential slaughterings were performed in each litter at the age of 22; 25; 28; 32; 36; 42 and 56 days, respectively. However, at the age of 22 days 2 rabbits were slaughtered from each litter, in order to collect enough caecal contents. The rabbits were sacrificed one by one between 8 and 12 a.m.. Immediately after slaughtering and dissecting, the caecum was isolated by tying off the two extremities with a nylon string, to prevent movement of the digesta. The caecum was weighed, the contents squeezed out into a beaker under CO_2 flushing and carefully mixed with a spatula. Part of it was used for the *in vivo* study (PIATTONI *et al.*, 1995) and the rest was used for *in vitro* incubations (PIATTONI *et al.*, 1996).

Zootechnical Performances

The live weight (LW) development of each single animal was determined on every slaughtering day and daily weight gain (DWG) of the slaughtered rabbits was calculated (PIATTONI *et al.*, 1995).

Incubations and analyses

Immediately after slaughtering, the caecum was removed, weighted and the contents collected. Nine grams of caecal contents from each rabbit were divided into 3 subsamples of equal weight. Caecal contents were incubated in double within 30 min. after slaughtering. They were fivefold diluted with a buffer solution (pH 6.9) (BURROUGH *et al.*, 1950) under CO_2 flushing. NH_4HCO_3 (705.7 mg/l) was added to the solution to allow microbial growth. Then 15 ml of the dilution were transferred into gaslight incubations flasks, filled with CO_2 and incubated in a shaking water bath at 39°C/24h. Incubations were stopped by injection of 0.3 ml of H_2SO_4 10N. One sample was not incubated and acted as a blank. All samples were immediately centrifuged (10 min. 15.000 *g*). The supernatant was filtered and the filtrate used for gas liquid chromatography (SHIMADZU; GC 14 A) (MARTY & DEMEYER, 1973). The net amount of VFA produced *in vitro* was calculated by substraction of VFA present in the blank. Ammonia nitrogen (NH_3-N) and lactic acid (LA) were analyzed using the microdiffusion method of Conway (CONWAY, 1957) both before and after incubation.

Statistical analysis

In vivo and *in vitro* data were submitted to analysis of variance using the Statgraphics Package version 5 (1991). Correspondence between the two series of data was done using multiple regression analysis.

RESULTS AND DISCUSSION

Body weight of the rabbits at the different slaughterings was 455, 609, 664, 757, 859, 1068 and 1809g. Data are presented in graphs as Least Square Means, referring to the original papers for detailed information (PIATTONI *et al.*, 1995; PIATTONI *et al.*, 1996).

In vivo total VFA concentration, *in vitro* production and DWG are shown in Figure 1. *In vivo* as well as *in vitro,* age had a significant effect ($P<0.01$) on total VFA. Initially a significant ($P<0.05$) increase was observed between 22 and 32 days. This pattern, however, was disturbed by a sharp decline on day 36 both *in vivo* and *in vitro,* followed by a plateau after day 42. Also DWG dropped significantly ($P<0.05$) between 32 and 36 days of age. The correspondence between the reduced DWG and the *in vivo* and *in vitro* VFA results is expressed by the significant correlation between *in vivo* and *in vitro* data ($R^2=0.47$; $n=40$; $P<0.01$) and between the latter and DWG ($R^2=0.20$; $P<0.05$). The significance of the drop in VFA, both *in vivo* and *in vitro* could probably be related to the bacterial status of the rabbits. Rabbits showed a quite unregular growth curve, characterized by a decrease in DWG already from day 28 on. A significant lower ($P<0.05$) DWG was recorded on day 36. A significant drop of both *in vivo* and *in vitro* VFA values was recorded on the same day. As two rabbits died from enterotoxaemia, an infection with *Clostridium spiroforme* could have interfered and be responsible for the drop in DWG on day 36. Recently, PEETERS *et al.* (1995) have demonstrated that an infection with *Clostridium spiroforme* tends to decrease caecal VFA concentration.

VFA molar proportions are presented in Figure 2. *In vivo* molar proportions of acetic acid were significantly higher ($P<0.001$) than *in vitro* and the correlation between both was significant ($P<0.01$; $R^2=0.31$). Both *in vivo* and *in vitro* molar proportions of propionate and butyrate showed significant and opposite changes. A shift from propionate to butyrate was observed on day 25. From day 25 on, butyrate exceeded propionate. In the oldest rabbits (56d of age) butyrate was almost three times higher than propionate, which feature is already longtimes known as typical of the rabbit caecum (HOOVER & HEITMANN, 1976). PADILHA *et al.* (1995) obtained the inverted C3/C4 ratio already on day 22. However, they used "germ-free" rabbits, resulting in lower total VFA caecal content (only 50% of our *in vivo* data).

In vivo and *in vitro* propionate as well as butyrate were significantly ($P<0.001$) correlated ($R^2=0.78$ and $R^2=0.61$, respectively). *In vivo* molar proportions of isobutyrate, isovalerate and valerate were negligible whereas significant amounts were produced *in vitro*. They significantly ($P<0.001$) decreased from 9.5% on day 22 to 3.0% on day 56. Their presence could be related to the intensive microbial activity during the incubation period.

No significant ($P=0.32$) correspondence between *in vivo* content and *in vitro* NH_3-N production was found (Figure 3). NH_3-N concentration showed an irregular pattern: after an initial decrease in early stage a non significant increase (day 36) was observed. An explanation could probably be related to the already mentioned pathological problem. Instead, *in vitro* NH_3-N production, showed a significant ($P<0.001$) age dependent decrease. Especially in early stage (day 22) a high production was recorded, which was more than two times higher ($P<0.01$) than in 8 weeks old rabbit. As NH_3-N is mainly derived from protein metabolism through amino-acid deamination, this decrease could be related to a lower availability of protein substrate in caecal contents with age. On day 22, the intake of the youngs is still mainly based on the protein rich mother-milk and is progressively replaced by solid feed. NH_3-N production found *in vitro* is a consequence of the overnight microbial activity in a closed system and is the result of a balance between production and utilization. Hence, it is not unlikely that there is no correspondence between *in vivo* NH_3-N concentration and *in vitro* NH_3-N production.

In vivo as well as *in vitro* LA concentration and production was significantly different according to the age of the rabbits (Figure 4) but no significant correlation ($P=0.20$) between them was found. As for NH_3-N this is maybe due to the fact that lactic acid is produced by some strains of caecal bacteria and thus its production after incubation depends on the balance between lactate-producing and lactate-utilizing microorganisms.

CONCLUSION

The *in vitro* technique used here has been applied over the last 20 years in our laboratory for the study of the rumen metabolism, and was adapted for incubations with rabbit caecal contents. *In vitro* production rates and *in vivo* concentrations showed significant correlations for those end-products that are not utilized by microbes (VFA), but not for those end-products that are continuously produced and utilized (NH_3-N, lactate). Therefore, for some parameters, the applied *in vitro* incubation technique is of valuable interest in studies related to rabbit digestive physiology.

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Etude *in vivo* et *in vitro* de l'activité fermentaire caecale chez les lapereaux de 22 à 56 jours d'âge - L'effet de l'âge sur l'activité fermentaire caecale a été étudié chez les lapereaux *in vivo* et *in vitro*. Des abattages successifs de lapins ont été effectués dans 6 nichées conventionnelles de 22 à 56 jours d'âge pour en échantillonner le contenu caecal. Dans le présent travail, les resultats obtenus *in vitro* ont été comparés avec ceux obtenus *in vivo*.

La production d'AGV aussi bien *in vitro* qu'*in vivo* a évolué significativement ($P<0,01$) avec l'âge. Une corrélation significative ($P<0,01$) a été observée entre les concentrations AGV *in vivo* et les productions d'AGV *in vitro* ($R^2=0,48$) et entre ces derniers et le gain moyen quotidien ($R^2=0,20$, $P<0,05$). La concentration en AGV totaux obtenue *in vivo* et la production d'AGV *in vitro* revèlent toutes les deux une chute au 36è jour, suivie d'un plateau. La diminution des AGV *in vivo* et *in vitro* reflète la chute du gain moyen quotidien observée au 36è jour.

Aussi bien *in vivo* qu'*in vitro*, le rapport des proportions molaires propionate/butyrate s'inverse au 25è jour au profit du butyrate. Les proportions molaires d'acétate, du butyrate et du propionate *in vivo* sont significativement correlées ($P<0,01$) avec celles observées *in vitro* ($R^2=0,31$; $R^2=0,78$; $R^2=0,61$, respectivement). Les proportions molaires d'isobutyrate, d'isovalérate et du valérate sont négligéables *in vivo,* tandis qu'une quantité significative a été observée *in vitro*.

L'azote ammoniacal ($N\text{-}NH_3$) dosé *in vitro* montre une diminution progressive ($P<0,001$) avec l'âge, alors qu' *in vivo* la chute n'est pas prononcée. La concentration en lactate *in vivo* et sa production *in vitro* sont significativement ($P<0,01$) influencées par l'âge. Mais, entre le taux *in vivo* et *in vitro*, aussi bien pour l'$N\text{-}NH_3$ que le lactate, il n'existe pas de corrélation significative ($P>0,05$).

En conclusion, des corrélations significatives (*in vivo/in vitro*) ont été observées pour les produits finaux non utilisés par les microbes (AGV), mais non pour les produits continuellement utilisés et produits par les microbes du caecum ($N\text{-}NH_3$, lactate). Ainsi donc, la technique *in vitro* peut etre utilisée pour les études de la physiologie digestive du lapin.

Fig. 1 - Relationship between in vivo VFA concentration, in vitro production and DWG

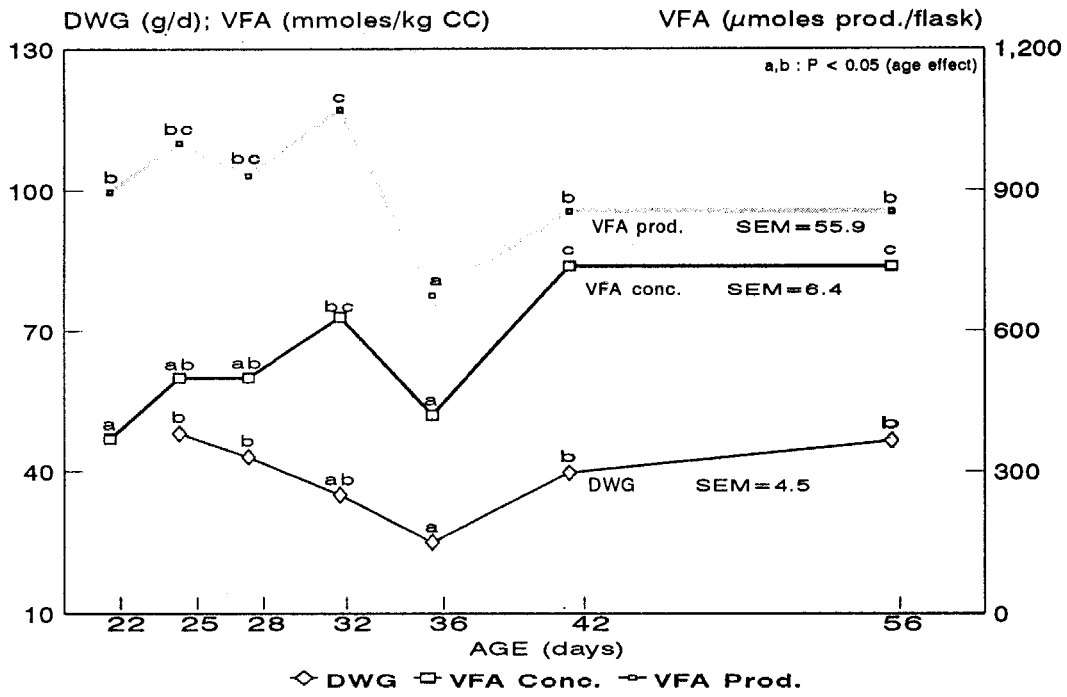

Fig. 2 - Age dependent VFA molar proportions: comparison of in vivo and in vitro pattern

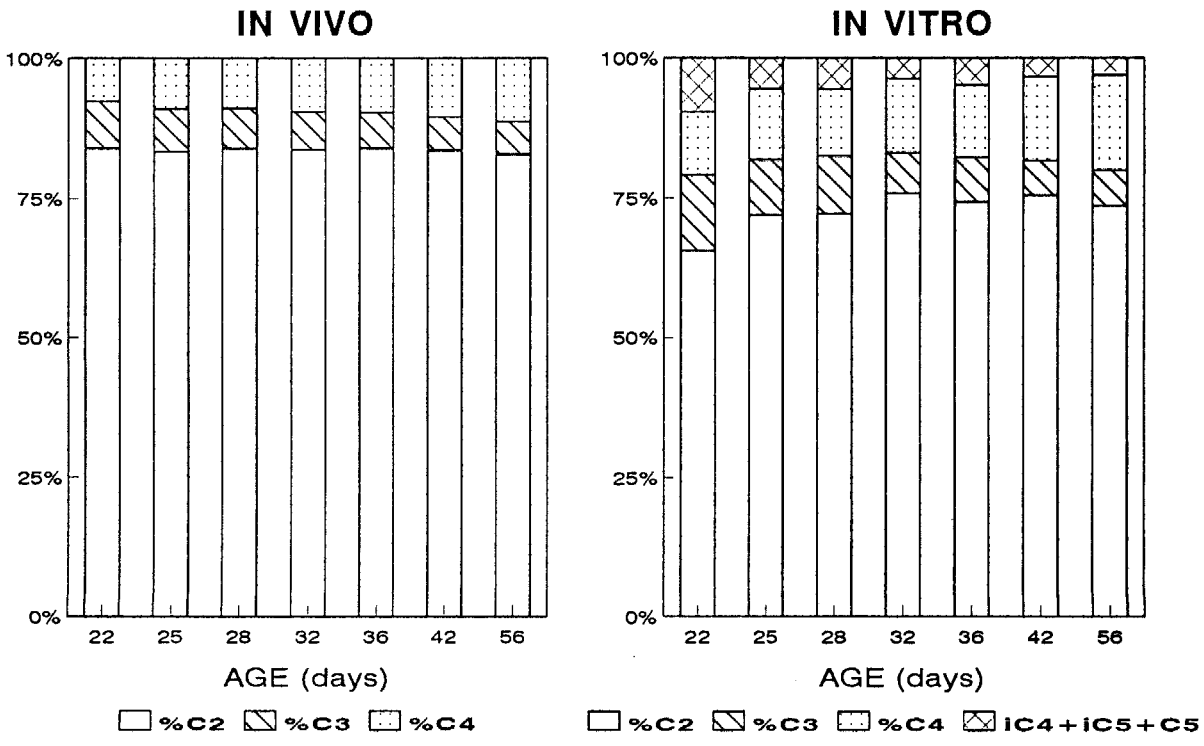

Fig. 3 - In vivo ammonia nitrogen concentration and in vitro production between 22 and 56 days of age

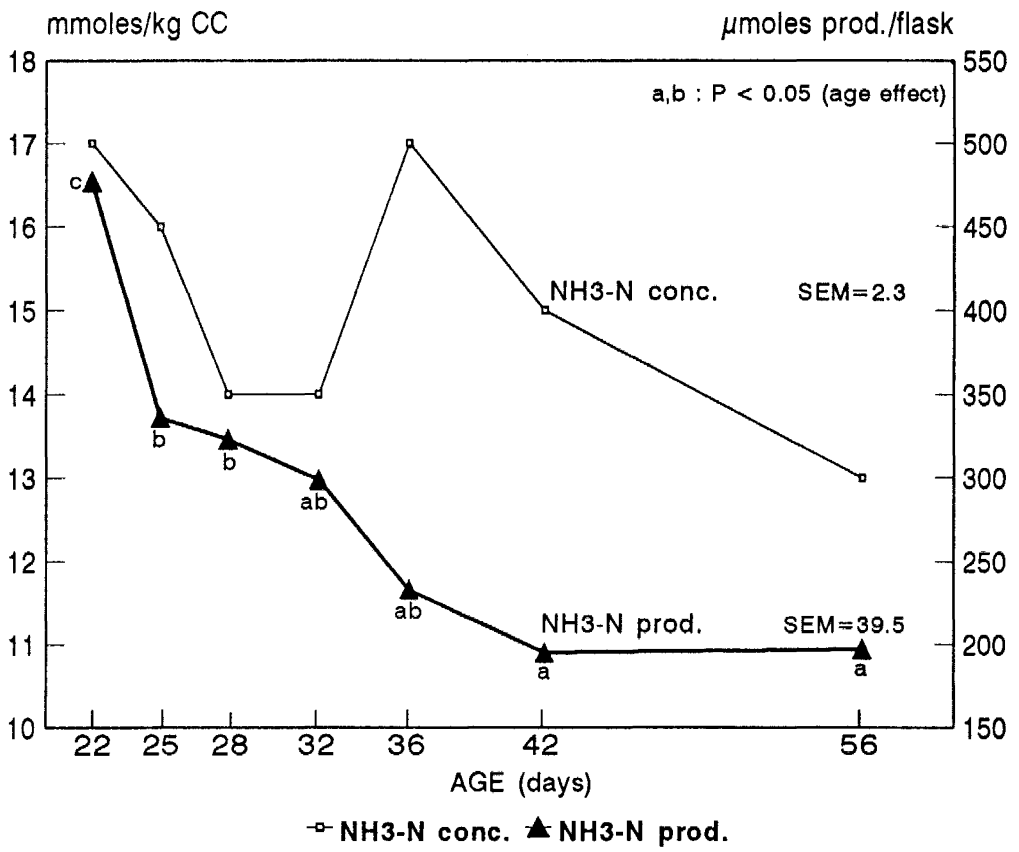

Fig. 4 - In vivo lactic acid concentration and in vitro production between 22 and 56d of age