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ADDITION OF PROBIOTIC TO FEEDS WITH DIFFERENT ENERGY AND ADF CONTENT IN RABBITS.

2. EFFECT ON MICROBIAL METABOLISM IN THE CAECUM

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ABSTRACT : Effects of feed composition, addition of the probiotic Acid-Pak 4-Way (AP4W), sex, age at slaughtering (53 or 67 d.)and interactions between feed and the addition of AP4W, age and feed, age and AP4W and age and sex on the fermentation pattern in the caecum of 84 weaned New Zealand White rabbits were studied. Three complete feed mixtures with different levels of digestible energy (DE, in MJ/kg of feed) and ADF content (in g/kg dry matter-DM) were tested (feed I had recommended DE and high ADF content: 9.22 MJ and 299 g ADF; feed II had 11.60 MJ and 254 g ADF; feed III had high DE and recommended ADF content: 13.14 MJ and 187 g ADF), each with and without the addition of 0.5 % AP4W. High-energy/recommended-ADF diet (feed III) significantly ($P \leq 0.05$) decreased the pH value (I : 6.39^a, II : 6.21^b, III : 6.09^b), increased the molar proportion of n-butyric acid (I : 10.3^a, II : 11.2^{ab}, III : 12.6^b %) and decreased molar % of propionic acid (I : 7.0^a, II : 6.2^{ab}, III : 5.7^b %) in the caecum contents. A decreased DM content (I : 226.1^a, II : 212.2^b, III : 207.9^b g/kg) and an increased ammonia level (I : 13.3^a, II : 13.8^{ab}, III : 16.6^b mmol/kg) had no negative influences on zootechnical parameters. The addition of AP4W had no influence on the parameters tested. Age at slaughter influenced the dry matter caecum content (53 days: 210.9^A, 67 d.: 219.9^B g/kg, $P \leq 0.10$). In older animals (67 days) differences in the proportions of VFA between female and male rabbits were significant (mol% of acetic acid: females 78.3^a, males 81.0^b %; mol% of propionic acid: f. 7.3^a, m. 5.6^b %), indicating that there were some differences in the microbial fermentation pattern of the caecum between females and males.

RÉSUMÉ : Etude chez le lapin de l'addition d'un probiotique à des aliments composés à niveaux énergétiques et d'ADF différents. 2 - Effet sur le métabolisme microbien dans le caecum.

Les effets sur le profil fermentaire caecal de la composition de l'aliment, de l'addition d'un probiotique Acid-Pak 4-Way (AP4W), du sexe, de l'âge à l'abattage (53 ou 67 jours) et les interactions entre l'aliment et l'addition de AP4W, l'âge et l'aliment, l'âge et AP4W et l'âge et le sexe ont été étudiés sur 84 lapins Néo Zélandais Blancs sevrés à 32 jours Trois aliments composés avec des niveaux d'énergie digestible (ED : MJ/kg) et contenu d'ADF (g/kg MS) différents ont été testés (aliment I : 9.22 MJ et 299g ADF ; aliment II : 11.60 MJ et 254g ADF ; aliment III : 13.14 MJ et 187g ADF) chacun d'entre eux étant doublé par le même aliment additionné de 0.5 % d'AP4W. Le régime haute énergie & ADF faible (aliment III) diminue significativement ($P \leq 0.05$) la valeur du pH caecal (I : 6.39^a ; II : 6.21^b ; III : 6.09^b), augmente la proportion moléculaire d'acide n-butyrique (I : 10.3^a ; II : 11.2^{ab} ; III : 12.6^b %) et diminue le % d'acide propionique (I : 7.0^a ; II : 6.2^{ab} ; III : 5.7^b %) dans le contenu caecal. Une diminution du contenu en matière sèche (I : 226.1^a ; II : 212.2^b ; III : 207.9^b g/kg) et une augmentation du taux d'ammoniaque (I : 13.3^a ; II : 13.8^{ab} ; III : 16.6^b mmol/kg) ont été les conséquences regrettables de l'aliment III. L'addition de AP4W n'a pa eu d'influence sur les paramètres enregistrés. L'âge à l'abattage influence la teneur en MS caecale (53 jours : 210.9^A ; 67 jours : 219.9^B g/kg ; $P \leq 0.10$). Chez les animaux les plus âgés (67 jours) les différences entre mâles et femelles pour les taux d'acides gras volatils ont été significatives (acide acétique : femelles 78.3^a ; mâles 81.0^b %, acide propionique : femelle 7.3^a; mâles 5.6^b %) indiquant une différence dans le schéma de fermentation caecale entre mâles et femelles.

INTRODUCTION

Rabbits in intensive breeding management are susceptible to digestive disturbances, especially to so-called non-specific digestive disorders; this seems to be related to microbial dysbiosis in the caecum. In such conditions irregular microbial fermentation and proliferation of potentially pathogen micro-organisms may develop, leading to enteritis, which is one of the main reasons for mortality in fattening rabbits, consequently leading to serious economic losses.

The caecum fermentation pattern can be estimated using acidity (pH), volatile fatty acids (VFA) and ammonia concentrations in the caecum content. These values together with dry matter (DM) of caecum content are different in the caecum of rabbits with diarrhoea from those of healthy ones: diarrhetic animals have lower VFA and DM concentrations, higher pH values and ammonia contents, a lower molar proportion of butyric and a higher proportion of propionic acid in the caecum (PROHASZKA, 1980; MORISSE *et al.*, 1982, 1989).

The influence of nutrition, especially of dietary fibre and readily available carbohydrates (RAC) in feed mixtures on the microbial fermentation pattern was reported by many researchers. There are various opinions about the influence of feed on pH, ammonia and VFA concentrations and VFA proportions in the caecum of rabbits. Some authors (POTE *et al.*, 1980; MORISSE *et al.*, 1985; CARABAÑO *et al.*, 1988) reported that a higher VFA concentration in caecum was caused by feed with lower dietary fibre content. On the other hand, some authors reported a higher VFA content when feed with either a higher ADF (MORISSE *et al.*, 1990), NDF (GIDENNE, 1992a), lignin (GIDENNE, 1986) or a low energy level (KRAJNC, 1989) was used. Other authors reported the independence of VFA concentrations on dietary fibre levels (CHAMPE and MAURICE, 1983, PARIGI-BINI *et al.*, 1995).

According to some researchers high CF feed caused lower pH value (MORISSE *et al.*, 1989, 1990) and to others (MORISSE *et al.*, 1982, 1985; PARIGI-BINI *et al.*, 1995) such feed resulted in higher pH. De BLAS *et al.* (1986) and POTE *et al.* (1980) found no influence of dietary fibre on the pH value in the caecum, except when feed with very low ADF content (under the recommended limits) was employed. This contradictory results can confirm the inconvenience of feed mixtures either with too low or too high fibre content (LEBAS, 1991). It is also difficult to expose main effects, when contents of fibre, energy, protein, physical structure of feed etc., all have the important influence on metabolism in caecum.

The findings of recent studies (YAMANI *et al.*, 1992; CHMITELIN, 1992; MORISSE *et al.*, 1993) indicate that the fermentation pattern in the caecum and consequently the digestive process and zootechnical parameters may be favourably affected by using various feed additives with probiotic activity. Such additives may help in maintaining

Table 1 : Analysis of variance: significance (P) of all effects for caecum parameters tested

	Main effects				Effects of interactions				Regression	
Tested parameter	**Feed**	**AP4W**	**Sex**	**Age**	**Feed* AP4W**	**Age* Feed**	**Age* AP4W**	**Age* Sex**	**covar.**	**lin.**
Dry matter	**0.005**	0.948	0.292	**0.071**	0.548	0.522	0.777	0.258	-	
pH in caecum	**0.001**	0.387	0.201	0.746	0.793	0.256	0.790	0.773	Dry matter	**0.000**
pH in appendix	**0.047**	0.232	0.120	0.408	**0.016**	0.921	0.970	0.355	Caecum weight	**0.015**
Total VFA	0.320	0.519	0.834	0.696	0.476	0.507	0.607	0.577	-	
Acetic acid	0.327	0.646	0.910	0.905	0.478	0.442	0.638	0.649	-	
Propionic acid	0.781	0.229	0.199	0.126	0.225	0.237	0.862	**0.069**	-	
n-butyric acid	**0.059**	0.182	0.823	0.200	0.574	0.648	0.886	0.609	-	
Mol % of acetic a.	0.544	0.125	0.226	0.379	0.173	0.535	0.824	**0.099**	Dry matter	**0.006**
Mol % of propionic acid	**0.031**	0.269	0.162	0.495	0.210	**0.013**	0.415	**0.021**	-	
Mol % of n-butyric acid	**0.055**	0.156	0.879	0.896	0.175	0.761	0.726	0.336	Dry matter	**0.001**
Ammonia	**0.050**	0.965	0.608	0.909	0.978	0.338	0.958	0.977	-	

optimal conditions for development of balanced microflora in the rabbit caecum.

The aim of our study was to establish whether high energy feed can be fed to weaning rabbits without an increased risk of digestive disorders (measured by pH value, VFA and ammonia content in caecum), and how different fibre concentrations in feed together with the addition of a probiotic feed additive influenced the microbial fermentation pattern in the caecum of rabbits.

MATERIAL AND METHODS

The experimental diets are described in the first part of this study (KERMAUNER and STRUKLEC, 1996). Feeds differed regarding their contents of digestible energy (DE, MJ/kg) and acid detergent fibre (ADF, g/kg DM): feed I had recommended DE and high ADF content: 9.22 MJ and 299 g ADF, feed II had 11.60 MJ and 254 g ADF and feed III high energy and recommended ADF content: 13.14 MJ and 187 g ADF). Each feed was tested with and without the addition of 0.5 % probiotic Acid-Pak 4-Way (AP4W).

Rabbits were slaughtered at 10 a.m. Feed was withdrawn 2 hours before slaughtering. Immediately after slaughtering the caecum with its content was weighed and the pH in the caecum and in the appendix were measured. Total caecum contents were collected and deep-frozen at -18° C for further analysis. The following parameters of caecum content were measured (in fresh weight): dry matter content (DM, g/kg), ammonia concentration (mmol/kg) and concentrations of volatile fatty acids (VFA, mmol/kg): acetic, propionic and n-butyric acid; the molar proportions of separate VFA in the total VFA were calculated.

The VFA concentrations were analyzed by a standard gas chromatographic (GC) procedure (Anaerobe Laboratory Manual, 1975). Samples for GC were prepared by a modified ether extraction method (MARINSEK LOGAR, 1992) as follows. A homogenized sample (1 - 2 g) of caecal content was suspended in 5 ml of distilled water and centrifuged for 10 min at 3000 RPM. 1 ml of supernatant was withdrawn and its pH was adjusted to less than 2.0 by addition of 50 % H_2SO_4; then 1 ml was withdrawn and mixed with 0.4 g dried NaCl. At the same time 1 ml of standard solution (pH under 2) was prepared, which was treated in the same way as the sample. 100 µl of internal standard (crotonic acid, concentration 1 g/l) was added to both sample and standard sample. To each test tube 200 µl 50 % H_2SO_4 and 1 ml of ether were added; the mixture was shaken and centrifuged at 2000 RPM. The upper ether phase was withdrawn and 2 spatulas of $CaCl_2$ were added to each sample.

The extraction procedure was then repeated for each sample. The two ether phases were pooled and 1 µl was injected into a Shimadzu gas chromatograph (GC-14 A) with

Table 2 : Influence of feed mixtures on parameters tested in rabbits

Parameter	**Feed I$^{1)}$ LSM$^{2)}$ ± SE n = 28**	**Feed II LSM ± SE n = 23**	**Feed III LSM ± SE n = 24**
Dry matter (g/kg)	226.1^{a} ± *3.9*	212.2^{b} ± *4.4*	207.9b ± *4.2*
pH in caecum	6.39^{a} ± *0.05*	6.21^{b} ± *0.05*	6.09b ± *0.05*
pH in appendix	7.13^{a} ± *0.07*	6.98a^{b} ± *0.08*	6.86b ± *0.08*
Total VFA (mmol/kg)	53.5 ± *4.1*	57.8 ± *4.7*	62.7 ± *4.5*
Acetic acid (mmol/kg)	42.5 ± *3.4*	47.0 ± *3.8*	49.9 ± *3.7*
Propionic acid (mmol/kg)	3.8 ± *0.3*	3.5 ± *0.3*	3.7 ± *0.3*
n-butyric acid (mmol/kg)	5.8^{a} ± *0.6*	6.4^{ab} ± *0.7*	7.9b ± *0.6*
Mol% of acetic acid	80.0 ± *0.8*	80.8 ± *0.8*	79.5 ± *0.8*
Mol% of propionic acid	7.0^{a} ± *0.3*	6.2^{ab} ± *0.4*	5.7b ± *0.4*
Mol% of n-butyric acid	10.3^{a} ± *0.6*	11.2^{ab} ± *0.7*	12.6b ± *0.7*
Ammonia (mmol/kg)	13.3^{a} ± *0.9*	13.8^{ab} ± *1.1*	16.6b ± *1.0*

$^{1)}$ Feed regardless AP4W

$^{2)}$ LSM with different letters in the same row are significantly different (P≤ 0.05)

Table 3 : Influence of age at slaughter on parameters tested

Parameter	53 days LSM[1)] ± SE n = 40	67 days LSM ± SE n = 35
Dry matter (g/kg)	210.9^{A} ± *3.3*	219.9^{B} ± *3.6*

[1)]LSM with different letters in the same row are significantly different (P≤0.10)

flame-ionisation detector and Supelcowax capillary column (Supelco 4070, length 30 m, 0.25 mm ID, 0.25 µm film), split ratio 2:100. The carrier gas was argon (40 ml/min), and the detection gases were hydrogen (40 ml/min) and air (350 ml/min). The column was heated in a two step temperature programme: T_1 init. = 110° C, T_1 final = 158° C, rate = 5° C/min, T_2 init. = 158° C, T_2 final = 170° C, rate = 6° C/min. The injector temperature was 160° C and the detector temperature 170° C.

Data were subjected to statistical analysis using the GLM procedure in the SAS statistical program (SAS/STAT, 1990). The influences of feed composition, probiotic addition, age, sex and interactions between feed and probiotic addition, age and feed, age and probiotic addition as well as age and sex on the parameters tested were studied. Covariate analysis (regression on the caecum weight or DM content in the caecum) was included in the model wherever it was shown to be significant. The model was the same as in the first part of this study (KERMAUNER and STRUKLEC, 1996).

RESULTS AND DISCUSSION

The significance (P) of all the effects is shown in an Anova table (Table 1). One rabbit was eliminated due to scours (feed III) and one rabbit died (feed II+). Mortality was low, probably due to favourable conditions of individual housing and in the high ADF content in the trial feeds. Zootechnical parameters were the best in the group fed with feed III; these parameters were described in the first part of this study (KERMAUNER and STRUKLEC, 1996).

1 Effect of feed

The influence of feed composition on the tested parameters is shown in Table 2.

The highest **dry matter** was measured in the caecum of rabbits fed with recommended-energy/high-ADF feed I. There is a possibility that such a high DM content in the caecum of this group (feed I) was due to the water-holding capacity of fibre, since only small, non-fibrous particles with less water remain in the caecum. Larger particles with better water holding capacity may pass to the large intestine; a certain amount of water may flow backwards while the rest was excreted with larger particles in hard faeces. GIDENNE (1992b) found a lower DM content in hard faeces when feed with a high level of NDF was used. FRAGA *et al.* (1991) found a lower DM content in caecal digesta when highly digestible fibre sources (beet pulp) were used, which agrees with the results of our study. CARABAÑO *et al.* (1988) and KRAJNC (1989) reported no influence of feed on the DM of caecum content, but the concentrations were somewhat higher (220-260 g DM/kg) than our results.

Caecum content pH values, measured in our experiment, were all within physiological limits (under 7.0), assuring the antibacterial effect of VFA (PROHASZKA, 1980). The caecum pH was lower when feed III (high energy/recommended ADF) was fed what agree with results reported by MORISSE *et al.* (1982, 1985). PARIGI-BINI *et al.* (1995) also reported a lower pH in the caecum (6.0 *vs.* 6.3) when lower fibre feed was used (15.7 % *vs.* 22.4 % CF in DM of feed). GARCIA *et al.* (1993) found a significantly lower pH in the caecum when sugar beet pulp was included in feed instead of barley grain. Our results are in accordance with the conclusions of the above mentioned authors and suggest that lower pH is a consequence of high content of RAC in feed.

In the contrary POTE *et al.* (1980) reported the same pH value in the caecum when feed with 15.7 or 24.5 % ADF was given and GARCIA *et al.* (1995) no influence of dietary NDF level on pH value in caecum.

pH values in the appendix were also lower when feed III (high energy/recommended ADF) was used, which agrees with the results of KRAJNC (1989). STRUKLEC *et al.* (1995) found no influence of feed composition on the pH value in the appendix.

In our experiment an increased **VFA content** in caecum occurred (but was not significant) in group with feed III (high energy/recommended ADF), where pH value in caecum was the lowest. This agrees with POTE *et al.* (1980), MORISSE *et al.* (1985) and CARABAÑO *et al.* (1988). PEETERS *et al.* (1995) found VFA by 22 % increased with lower indigestible fibre (10.2 % *vs.* 13.8 %). On the other hand, many authors reported different results: GIDENNE (1986) found higher VFA concentration with higher lignin content (39.6 *vs.* 32.2 % ADF), and CHAMPE and MAURICE (1983), PARIGI-BINI *et al.* (1995) and GARCIA *et al.* (1995) found no relations between feed composition and total VFA production in the caecum.

As to VFA, feed significantly influenced the **n-butyric** concentration in caecum content, which was higher when feed III (high energy/recommended ADF) was used, which agrees with the results of HOOVER and HEITMANN (1972), POTE *et al.* (1980) and STRUKLEC *et al.* (1994).

The **proportions** of propionic and n-butyric acid were affected by feed. The molar proportion of n-butyric acid increased from feed I to feed III, while the mol% of propionic acid decreased. KRAJNC (1989) and STRUKLEC (1990) also reported significantly a higher content and proportion of butyric acid in caecal content when feed with high energy (from carbohydrates) was used, but the proportion of propionic acid remained the same. TORTUERO *et al.* (1994) found that feed with a higher lignin content caused a higher proportion of propionic acid, which is in accordance with our results.

GIDENNE (1992a) reported that diet NDF influenced only proportion of acetic acid, GARCIA *et al.* (1994) and GIDENNE and JEHL (1995) reported no influence of feed on the proportions of particular VFA.

The content of ammonia was higher within the group fed high-energy/recommended-ADF feed (feed III) what is in accordance with findings of FRAGA *et al.* (1991) (higher ammonia contents when highly digestible sources of fibre (beet pulp) were used) and GARCIA *et al.* (1995): ammonia concentration in the caecum decreased linearly with increasing NDF level. Possible explanation for increased ammonia concentration in caecum content of rabbit in group III is lower pH value of caecum content in this group. At lower pH ammonia transfer to $NH4^{+}$ form being difficult to

Table 4 : Effect of interaction between feed and probiotic AP4W on parameters tested (LSM ± SE)

Parameter	Feed I[1)] without n = 14	Feed I[1)] +AP4W n = 14	Feed II without n = 12	Feed II +AP4W n = 11	Feed III without n = 11	Feed III +AP4W n = 13
pH in appendix	7.05 ± *0.10*	7.22 ± *0.10*	6.77[a] ± *0.11*	7.18[b] ± *0.12*	6.99 ± *0.12*	6.73 ± *0.11*

[1)]LSM with different letters are significantly different (P≤0.05)

absorb (DEMIGNE and REMESY, 1990). Therefore increased ammonia concentration in group III can be consequence of poor absorption of ammonia from caecum due to lower pH. N is not absorbed and excreted; it can be utilized by caecum micro-organisms.

Contrary to our findings, MORISSE (1985) found higher ammonia with a higher ADF content in feed. CARABAÑO *et al.* (1988), GIDENNE (1986) and GIDENNE and JEHL (1995) reported no correlation between ammonia content in caecum and feed fibre, i.e. lignin.

All effects of feed III on tested parameters were positive, except lower DM and higher ammonia content, but without negative effect on zootechnical parameters.

2 Effects of probiotic and sex

As to the two main effects neither probiotic AP4W nor the sex of experimental rabbits had an influence on the caecum parameters tested (Table 1). There not much in available literature dealing with probiotic influence on caecum fermentation in rabbits. In accordance with MAERTENS et al. (1994) who tested probiotic Paciflor, we found a similar caecal fermentation pattern in controls and in rabbits fed with the addition of AP4W.

3 Effect of age

Age at slaughter influenced only DM content in the caecum: in older rabbits the DM of caecum digesta was higher (Table 3). In accordance with our results BELLIER *et al.* (1995) found higher DM in the caecum of adult rabbits (20.3 % DM) in comparison with 42 day old rabbits (17.6 % DM); they established lower VFA, lower proportion of butyric acid and higher ammonia concentrations in the caecum content of younger rabbits. But PIATTONI *et al.* (1995) found no differences in caecum DM content between 22 and 56 days old rabbits; propionic acid decreased, butyric acid and total VFA increased with age.

These differences between young and adult rabbits can be connected with different feed intake pattern of this two categories. Adult rabbits have larger meals, they eat faster and more frequently (CHEEKE, 1987), therefore the substrate for caecal micro-organisms can be different.

4 Effect of interactions

Because of relatively small number of animals per group, the influences of particular interaction can not be unequivocal evaluated. The experiment should be repeated with large number of animal per treatment.

4.1 Interaction between feed and probiotic

Probiotic addition increased significantly only the pH value in the appendix in the case of feed II (Table 4). Addition of AP4W to feed II increased pH value in appendix. The consequences of such changes on the process in GI tract are still not clear and this ought to be studied in the future.

4.2 Interaction between age and feed

Interaction between age and feed was noticed only for the molar proportion of propionic acid in the caecum content. In younger rabbits the influence of feed was different from in older animals, indicating that rabbits require a longer period to adapt to a certain feed. GIDENNE (1992b) found good adaptability of the digestive tract after 6 weeks of feeding. According to LELKES (1987) and LELKES and CHANG (1987) weaned rabbits show a lower ability of fermentation adjustment in the posterior parts of the intestine than older ones.

The different effect of feed on the caecal fermentation of younger and older rabbits probably indicates that feed composition should be adapted to different age groups (prestarter, starter, grower etc.).

4.3 Interaction between age and probiotic and age and sex

The interaction between age and probiotic addition was not significant.

Table 5: Effect of interaction of age and feed on parameters tested

Parameter	Age (days)	Feed I[1)] LSM[2)] ± SE	Feed II LSM ± SE	Feed III LSM ± SE
	53	n = 14	n = 13	n = 13
	67	n = 14	n = 10	n = 11
Mol % of propionic acid	53	7.1[a] ± *0.5*	5.1[b] ± *0.5*	6.2[ab] ± *0.5*
	67	6.9[a] ± *0.5*	7.2[a] ± *0.6*	5. 2[b] ± *0.6*

[1)] Feed regardless AP4W
[2)] LSM with different letters in the same row are significantly different (P≤0.05)

Table 6 : Effect of interaction between age and sex on parameters tested

Parameter	Age	Females LSM[1] ± SE	Males LSM ± SE
	53	n = 16	n = 24
	67	n = 13	n = 22
Propionic acid (mmol/kg)	53	3.3 ± *0.4*	3.5 ± *0.3*
	67	4.5^a ± *0.4*	3.3^b ± *0.3*
mol % of acetic acid	53	80.7 ± *1.0*	80.3 ± *0.8*
	67	78.3^a ± *1.1*	81.0^b ± *0.8*
mol % of propionic acid	53	5.9 ± *0.5*	6.4 ± *0.4*
	67	7.3^a ± *0.5*	5.6^b ± *0.4*

[1] LSM with different letters in the same row are significantly different (P≤0.05)

Differences between female and male rabbits were expressed only in older animals, where females had a lower proportion of acetic acid and a higher proportion of propionic acid in the caecum content. The concentration of propionic acid was higher in 67 days old females as well (Table 6).

Similarly STRUKLEC *et al.* (1994) observed differences between the proportions of acetic acid in males and females (males 67.6 %, females 64.6 %, P≤0.05), at the age of 84 days.

The caecum of female rabbits starts to develop earlier and reaches a higher final weight (LOPEZ *et al.*, 1988). This was confirmed by our results as well: the caecum weight of females was higher than of males and the proportion of caecum in younger rabbits was higher in females than in males (KERMAUNER and STRUKLEC, 1996). Differences in the growth of the caecum as a function of sex can also result in a different fermentation pattern in the caecum; in our trial this was occurred at the end of the growing period.

CONCLUSIONS

Feed (ADF and energy level) had an important influence on the caecum parameters tested in growing rabbits. The most favourable effect was obtained when feed with high energy and recommended ADF level (feed III) was given, resulting in the lowest pH value, the highest molar proportion of n-butyric acid, the lowest mol% of propionic acid and the tendency (not significant) for increasing VFA. The decreased DM content and increased ammonia level had no negative effect on zootehnical parameters.

As to the two main effects neither the addition of probiotic Acid-Pak 4-Way nor the sex of experimental rabbits had influence on the caecum parameters tested.

Age of slaughter influenced the dry matter in caecum content: in older rabbits the DM was higher.

The interaction between age and feed indicated a longer adaptation period to particular feed is required.

In older animals differences in the proportions of VFA between female and male rabbits were expressed, indicating that there were some differences in the microbial fermentation pattern of the caecum between females and males at the end of the fattening period (67 days). To confirm differences between sexes more experiments should be conducted. But even on the basis of our results together with the findings described in the available literature, we can recommend standardization of sex for nutritional trials with growing rabbits.

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