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EFFECT OF INULIN SUPPLEMENTATION AND AGE ON GROWTH PERFORMANCE AND DIGESTIVE PHYSIOLOGICAL PARAMETERS IN WEANED RABBITS

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Abstract: Three diets were formulated, a control diet (C, 40.7% NDF, 15.1% CP), the same diet but medicated (M, 500 mg/kg oxytetracycline and 50 mg/kg thiamulin), and a third diet obtained substituting 4% of barley with inulin (Frutafit) in C diet (I). Pannon White does and their litters were randomly allocated into three groups (8/group) at 21 d of lactation and diets offered to the does and kids from 21 d of lactation onwards. After weaning (28 d), growing rabbits (30 cages/treatment, 2 rabbits/cage) were fed the same diet as before weaning. At 28, 35 and 42 d of age, 6 healthy animals from each group (1 animal/cage) were slaughtered. Live body weight, feed intake and feed conversion ratio increased and growth rate decreased with age (P<0.001). Feed intake decreased in rabbits fed I diet compared to those fed M diet (by 11%, P<0.05), with those fed C diet showing an intermediate value. Growth rate from 28 to 35 d of age was not affected by diets, but decreased from 36 to 42 d in rabbits fed I diet compared to those fed C and M diets (P<0.05), with no effect on feed conversion ratio. Inulin did not affect mortality, which was low (≤ 3.3%), but increased morbidity compared to C and M diets (11.7 *vs.* 2.5%, P<0.05). Diets did not affect caecal weight, pH, cellulase and pectinase activity or microbial counts. Inulin diet decreased caecal xylanase activity (P<0.05) compared to C and M diets, reduced propionic and butyric acid and increased acetic acid concentration compared to M diet, whereas C diet showed intermediate values. Caecal pH and counts of *E. coli* and total aerobic bacteria increased and pectinase activity decreased (P<0.05) at 35 d of age (compared to 28 and 42 d of age). The number of the strictly anaerobic bacteria decreased and cellulase and xylanase activity increased (P<0.05) at 42 d of age compared to 28 and 35 d. Propionic acid concentration decreased with age from 28 to 42 d (P<0.05) but VFA concentration and acetic and butyric acids proportions did not change. In conclusion, the inclusion of 4% of inulin in the diet of weanling rabbits showed no positive effect.

Key Words: inulin, medication, growth traits, caecal bacteria, fermentation, rabbit.

INTRODUCTION

Mortality in rabbit production is primarily due to diseases of the digestive tract (Gidenne and Fortun-Lamothe, 2002) which have a major impact on the growth performance of animals. In connection with the ban on using antibiotics as growth promoters in the EU, several studies have been carried out on different

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feed additives as alternatives to antibiotics. Many of these results have been summarised by Falcao-e-Cunha *et al*. (2007).

Inulin-type fructans (fructo-oligosaccharide, FOS) are soluble dietary fibres which are present in amounts in various fruits and vegetables and can be used as prebiotics. According to the definition of Gibson *et al*. (2004, 2005) a prebiotic is a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microbiota, which confer benefits upon host well-being and health. Inulin is a polydisperse GFn (glucosyl-[fructosyl]n-1-fructose) molecule, built as a linear chain of fructose units by means of β(2-1) bonds, having one terminal glucose molecule linked by an α(1-2) bond. Because of the β(2-1) configuration of the linkages, it cannot be digested by rabbit endogenous enzymes but is fermented by the intestinal microbiota (Niness, 1999).

The effect of inulin-type fructans on performance has been thoroughly studied in pigs, poultry, calves and pets (Verdonk *et al*., 2005). Reported effect on performance of rabbits varied from little or no effect on weight gain (0.75-3.75 % inclusion, Luick *et al*., 1992; 4% inclusion, Volek *et al*., 2005, 2007), live weight (0.25% inclusion, Morisse *et al*., 1993; 2% inclusion, Maertens *et al*., 2004) and mortality (Volek *et al*., 2005, 2007), whereas inulin increased caecal volatile fatty acid concentration (Volek *et al*., 2005, 2007).

The aim of the present experiments was to study the effect of age and dietary supplementation with inulin on growth performance and certain digestive physiological parameters, especially the caecal ecosystem and the fermentation in weaned rabbits.

MATERIALS AND METHODS

Experimental diets

A control diet (C) was formulated with no supplementation (40.7% neutral detergent fibre and 15.1% crude protein). A second diet (medicated, M) was obtained supplementing C diet with 2 antibiotics (500 mg/kg oxytetracycline and 50 mg/kg tiamulin). A third diet (I) was obtained substituting 4% inulin (Frutafit, HD, Brenntag, Budapest) for barley in C diet (Table 1). All diets contained Clinacox anticoccidial feed additive (0.5% diclazuril). Frutafit contained 93.2% inulin (degree of polymerisation=12), 2.9% fructose and 3.3% saccharose. The starch content of C and M diets was 14 and 16%, respectively, but 11% in diet I. Sugar content of diets C and M was lower (7%) than in diet I (10%). The ingredients and chemical composition of the diets are shown in Table 1.

Experimental animals and design

Twenty four Pannon White does and their litters were randomly allocated into three groups (C, M and I) at 21 d of lactation (8 litters/treatment and an average of 8 rabbits/litter). The does and kits were housed in flat-deck cages (85×55 cm) in a closed building, with 16 h/d light. After weaning (28 d of age), rabbits were caged in pairs (30 cages/treatment, 2 brothers/cage and 16 rabbit/m^2) in wire mesh cages until slaughter at 42 d of age. Average temperature ranged from16 to 18°C, lighting cycle was 16 h light: 8 h dark, and the farm had overpressure ventilation. The experimental diets were first given to the does and the kids from 21 d of age (lactation) onwards, also after weaning.

In the experimental period (between 28 and 42 d of age) body weight was measured twice a week and feed consumption weekly. Mortality was checked daily, while morbidity was assessed weekly through an individual control of all clinical signs of digestive troubles (transitory diarrhoea, presence of mucus in excreta, abnormal intake behaviour).

Table 1: Ingredients and chemical composition of experimental diets (%).

	Experimental diets		
	Control	Medicated	Inulin
Inulin	-	-	4.00
Oxytetracycline 50%	-	0.1	-
Tiamutin 10%	-	0.1	-
Barley	12.0	12.0	8.0
Soybean meal 46%	3.0	3.00	3.0
Sunflower meal 37%	15.0	15.0	15.0
Alfalfa meal 19%	34.0	34.0	34.0
Sugar beet pulp	12.0	12.0	12.0
Molasses	1.0	1.00	1.0
Sunflower oil	1.0	1.00	1.0
Wheat bran	18.0	18.0	18.0
Limestone	0.1	0.1	0.1
Monocalcium phosphate	1.7	1.7	1.7
NaCl	0.4	0.4	0.4
Zeolite universal	1.2	1.1	1.2
Rabbit 0.5% Clinacox[1]	0.5	0.5	0.5
Premix [2]	0.2	0.2	0.2
Chemical composition			
Dry matter	91.6	91.5	91.8
Crude protein	15.1	14.3	15.0
Crude fat	2.6	2.1	2.5
Crude fibre	18.7	18.7	18.7
Ashes	7.8	7.7	7.9
Nitrogen free extractable matter	47.4	48.7	47.8
Neutral detergent fibre	40.7	40.4	39.3
Acid detergent fibre	22.6	22.0	22.7
Acid detergent lignin	3.5	3.2	3.6
Starch	13.7	16.1	11.2
Total sugar	6.9	6.9	10.2

[1]5 g / kg diclazuril. [2] Dry matter: 93.1%, Ca: 29.86%, Fe: 8000 mg/kg, Mn: 3000 mg/kg, Cu: 1000 mg/kg, Zn: 6000 mg/kg, Se: 20 mg/kg, Co: 200 mg/kg, J: 200 mg/kg, A vitamin (E672): 2400000 IU/kg, D-3 vitamin (E671): 240000 IU/kg, α-tocopherol: 8000 IU/kg, K-3 vitamin: 200 mg/kg, B-1 vitamin: 300 mg/kg, B-2 vitamin: 1000 mg/kg, B-6 vitamin: 500 mg/kg, B-12 vitamin: 4000 mg/kg, Pantothenic acid: 2800 mg/kg, Folic acid: 100 mg/kg, Biotin: 24 mg/kg, Niacin: 10000 mg/kg, Colin chloride: 800010 mg/kg, Diclazuril: 200 mg/kg.

At 28, 35 and 42 d of age 6 healthy animals from each group (1 animal/cage) were randomly selected and slaughtered at 11:00 a.m. The digestive tract was removed immediately and the caecum was separated. Caecal content was homogenised at room temperature. One gram of caecal digesta was used immediately

after sampling for microbiological determination and anaerobic condition was ensured by carbon dioxide. The rest of the caecal content was weighed, frozen and stored at -80°C.

The research protocol was reviewed by the Animal Use and Care Administrative Advisory Committee and approved by the Agricultural Administrative Authority (Protocol No. 00618/007/SOM/2003).

Laboratory analyses

Chemical composition of the diet was analysed following the recommendations of the Association of Official Analytical Chemists (AOAC, 2000): dry matter (DM; 930.15), crude protein (Kjeldahl method, 976.05), crude fat (920.39), ash (942.05), crude fibre (978.10) and total starch (996.11). Neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) content were determined according to ISO 16472:2006 and ISO 13906:2008. The total sugar content was analysed according to EC 152/2009. The pH value of the fresh caecal content was measured by a pH meter (OP-110, Radelkis, Hungary). From one gram of caecal digesta, serial dilutions (with 0.9% NaCl) were made immediately after sampling and used for microbiological determination. The obligate anaerobe organisms were cultured on Schaedler's agar (Sharlan Chemie, Barcelona, Spain), the selectivity of which was increased by the addition of esculin (Merck, Darmstadt, Germany), neomycin (Merck, Darmstadt, Germany) and Fe-ammonium citrate (Sharlan Chemie, Barcelona, Spain). The gamma sterile Petri dishes (Biolab, Budapest) were placed into Anaerocult culture dishes (Merck, Darmstadt, Germany), in which the anaerobic conditions were ensured with the help of an "Anaerocult A" (Merck, Darmstadt, Germany) gasifying bag. Subsequently, the samples were incubated in an LP 104 type thermostat (LMIM, Esztergom, Hungary) at 37°C for 96 h. *E. coli* was cultured on a Chromocult differentiation medium (Merck, Darmstadt, Germany). The samples were incubated at 37°C, under aerobic conditions, for 24 h. After the incubation time had elapsed, the colonies were counted (ISO 4833:2003) with Acolyte colony counter (Aqua-Terra Lab, Veszprem, Hungary). The colony counts were expressed in $\log_{10}$ colony forming units (CFU) related to 1g of sample.

The fibrolytic activity of the caecal bacteria was analysed by measuring the activity of cellulase, xylanase and pectinase. The method described by Gidenne *et al*. (2002) was used with minor modification: the reducing sugars were quantified spectrophotometrically at 540 nm using dinitrosalicylic acid instead of p-hydroxybenzoic acid hydrazide. The quantity of released sugars was expressed as: µmol of reducing sugar/g DM caecal digesta /h.

Approximately 3 g of caecal digesta was homogenised with 4.5 mL metaphosphoric acid (4.16%), then centrifuged at 10.000 *g* for 10 min and filtrated. The concentration of volatile fatty acids (VFA) was measured by gas chromatography (Shimadzu GC 2010, Japan. Nukol 30 m×0.25 mm×0.25 µm capillary column -Supelco, Bellefonte, PA, USA-, FID detector, 1:50 Split ratio, 1 µL injected volume, helium 0.84 mL/min. Detector conditions: air 400 mL/min, hydrogen 47 mL/min, temperature: injector 250ºC, detector 250ºC, column 150ºC). 2-etil-butyrate (FLUKA Chemie GmbH, Buchs, Switzerland) was used as internal standard.

Statistical analyses

The statistical model included diet, age and their interaction as main effects, which were studied by using Multiway ANOVA. The significance of differences was tested by LSD post hoc test. The experimental unit was the cage in the case of feed intake and feed conversion, but the animal for growth rate and other measurements. When a significant ($P<0.05$) age×treatment interaction occurred, data were further subjected to two types of statistical analyses: within the same age (among the three diets) and within the same diet (among ages). Pearson's correlation was used to find a relationship between pH and *E. coli*. Mortality and morbidity of the groups was compared by chi-squared analysis. Data were analysed by using the GLM procedure of SPSS (2002), version 10.0.

RESULTS

Live body weight, feed intake and feed conversion ratio (FCR) increased and growth rate decreased with age ($P<0.05$, Table 2). Rabbits fed I diet decreased feed intake from 28 to 42 d of age (by 11%, $P<0.05$) compared to M diet. Growth rate from 28 to 35 d of age was not affected by type of diet, but rabbits fed I diet reduced it by 24% from 36 to 42 d of age (Figure 1), with no effect on FCR. Morbidity was higher in the inulin supplemented group ($P<0.05$), and between 35-42 d of age ($P<0.05$). Symptoms of morbidity were acute diarrhoea, low feed consumption and weakness. No effect of age and treatment on mortality was observed.

Diets had no effect on caecal pH, bacterial counts, and cellulase and pectinase activity (Table 3). Inulin diet decreased xylanase activity (by 18%, $P<0.05$) compared with C and M diets. Total VFA concentration was not affected by diet, except in group C, where a temporary decrease by 40% was observed at 35 d of age ($P<0.05$, Figure 2). Medicated diet showed lower proportions of acetic acid than C and I diets, and higher of propionic and butyric acids compared to I diet ($P<0.05$), whereas C diet showed intermediate values. Weight of caecal contents and its DM content increased with age, respectively ($P<0.05$), with no effect of treatments (Table 3). Caecal pH and counts of *E. coli* and total aerobic bacteria temporarily increased and pectinase activity decreased ($P<0.05$) at 35 d of age compared to 28 and 42 d of age. A positive correlation between caecal counts of *E. coli* and caecal pH was observed throughout the experiment (r=0.32, $P=0.019$, n=54), being higher at 35 d (r=0.612, $P=0.007$, n=18). The number of the strictly anaerobic bacteria decreased by 10% and cellulase and xylanase activity increased by 25 and 29%, respectively ($P<0.05$) at 42 d of age compared to 35 d of age, whereas these traits remained unchanged between 28 and 35 d. Propionic acid concentration decreased with age from 28 to 42 d by 23% ($P<0.05$).

DISCUSSION

The reduction of feed intake observed in rabbits fed inulin-containing diet led to a lower daily weight gain in the second week after weaning, without any change in feed conversion. In similar experiments, supplementation of the diet with 4% inulin had no effect on the weight gain of rabbits (Volek *et al*., 2005 and 2007). While inclusion of 4% inulin had no effect on feed intake (Volek *et al*., 2007), 4% inulin in

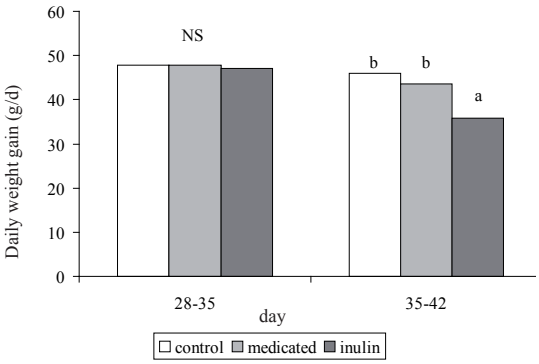

Figure 1: Effect of age and dietary treatment on daily weight gain of weaned rabbits (residual standard deviation=12.2 g/d).
Bars with different superscripts differ ($P<0.05$)
NS: not significant.

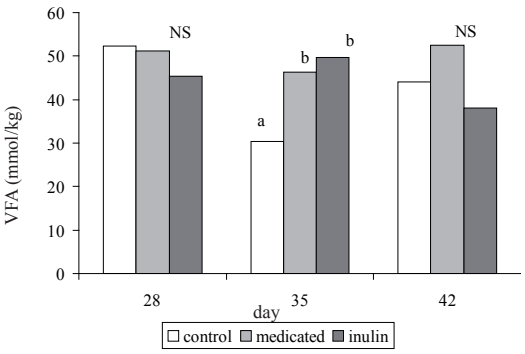

Figure 2: Effect of age and dietary treatment on caecal total volatile fatty acids (VFA) concentration in weaned rabbits (residual standard deviation=12.9 mmol/kg).
Bars within each age with different superscripts differ ($P<0.05$)
NS: not significant.

Table 2: Effect of medication and inulin inclusion on growth traits of weanling rabbits.

	Experimental diets			Age (d)				*P*-value		
	Control	Medicated	Inulin	28	35	42	RSD	Diet	Age	Diet×Age
Live weight (g)[1]	932	919	896	566^{a}	947^{b}	1240^{c}	305	0.10	0.001	0.220
				28-35 d		36-42 d				
Feed consumption (g/d)[2]	81.4^{AB}	84.6 ^{B}	75.5^{A}	68.7^{a}		92.7^{b}	17.8	0.002	0.001	0.100
Daily weight gain (g/d)[1]	47.0^{B}	45.6^{B}	41.5^{A}	47.6^{b}		41.9^{a}	12.2	0.003	0.001	0.015
Feed conversion (g/g)	1.77	1.95	1.97	1.46^{a}		2.33^{b}	0.70	0.110	0.001	0.410
Morbidity (%)	3.3^{A}	1.7^{A}	11.7^{B}	1.7^{a}		15.0^{b}		0.038	0.009	
Mortality (%)	0	0	3.3	1.7		1.7		0.130	1.000	

[1] n= 60 rabbits/treatment, [2] n= 30 cages/treatment
Different superscripts mean significant differences between a,b,c ages and A,B treatments

Table 3: Effect of medication and inulin inclusion on caecal digesta traits in weanling rabbits[1].

	Experimental diets			Age (d)				*P*-value		
	Control	Medicated	Inulin	28	35	42	RSD	Diet	Age	Diet×Age
Caecal content, % body weight	4.14	3.93	4.28	2.04^{a}	4.49^{b}	5.84^{c}	1.99	0.680	0.001	0.560
Caecal dry matter content, %	20.5	21.5	20.7	18.6^{a}	20.7^{b}	23.5^{c}	3.0	0.390	0.003	0.680
Caecal pH	6.48	6.40	6.57	6.23^{a}	6.71^{b}	6.51^{ab}	0.44	0.460	0.005	0.710
E. coli [2]	4.6	4.1	4.4	3.9^{a}	4.9^{b}	4.4^{ab}	1.1	0.290	0.022	0.100
Strictly anaerobic bacteria [2]	9.3	9.6	9.4	9.6^{b}	9.9^{b}	8.9^{a}	0.7	0.370	0.001	0.100
Total aerobic bacteria [2]	5.8	5.8	6.0	5.5^{a}	6.5^{b}	5.6^{a}	0.7	0.550	0.001	0.950
Cellulase [3]	78.2	72.4	70.2	69.9^{a}	67.1^{a}	83.7^{b}	14.6	0.160	0.001	0.330
Xylanase [3]	142^{B}	139^{B}	115^{A}	107^{a}	126^{a}	163^{b}	43	0.048	0.001	0.070
Pectinase [3]	105	108	98.8	102^{b}	67.6^{a}	142^{c}	41.1	0.600	0.001	0.350
Total volatile fatty acids (mmol/kg)	42.3	50.1	44.4	49.7	42.2	44.9	12.9	0.140	0.170	0.040
Acetic acid (%) [4]	79.9^{B}	75.2^{A}	81.6 ^{B}	77.9	79.6	79.3	5.2	0.001	0.510	0.290
Propionic acid (%) [4]	7.50 ^{AB}	8.88^{B}	7.15^{A}	9.09^{b}	7.45^{ab}	6.99^{a}	2.43	0.044	0.012	0.100
Butyric acid (%) [4]	10.3^{AB}	12.3^{B}	9.05^{A}	10.5	10.6	10.6	2.83	0.001	0.990	0.180

[1] n=6 rabbit/age and treatment. [2] Germ counts expressed in $\log_{10}$ CFU/g caecal digesta. [3]expressed as µmol of reducing sugar/g dry matter of the caecal digesta/h. [4] Proportion within tVFA. Different superscripts mean significant differences between a,b,c ages and A,B treatments.

a diet rich in soluble fibre (of sugar beet pulp) seemed to reduce feed intake and feed conversion (Volek *et al*., 2005). The inclusion of 2% inulin in the feed fed for 66 d after weaning also reduced dry matter intake (by 14%), but increased daily gain (by 16%) in rabbits (Alvarado-Loza *et al*., 2009). Maertens *et al*. (2004) again found a tendency to decrease feed intake after the consumption of diet containing 2% inulin for 10 d, with no effect on live weight. It seems that a level of inclusion of 2-4% inulin can reduce feed intake, although data from the literature do not agree completely. In this work, mortality was low and differences were observed among groups. However, and in contrast with some works in the literature which found a favourable effect of dietary inulin in prevention of digestive disorders (morbidity in Volek *et al*., 2007, and mortality in Volek *et al*., 2005), inulin fed rabbits showed a higher morbidity. Lack of consistency in the results concerning the effect of prebiotics in rabbits may be explained by differences in the experimental conditions, especially those related to the hygienic status of the farm (Falcao-e-Cunha *et al*., 2007).

Concerning fermentation traits, our results with inulin fed rabbits were unexpected and do not fit with the increased total VFA concentration (Volek *et al*., 2007), molar proportion of butyrate (Maertens *et al*., 2004) and lower pH found by these authors. In our experiment, medicated rabbits had lower molar proportions of acetic acid and higher levels of propionic and butyric acids than those fed inulin diet, with those rabbits fed control diet showing intermediate values. The lower molar proportion of butyric acid in animals fed inulin diet was in accordance with their lower xylanase activity. This coincides with other findings according to which the end product of hemicellulose fermentation might be mainly butyric acid (Falcao-e-Cunha *et al*., 2004; Gidenne *et al*., 1998).

So, the effect of inulin on rabbit caecal fermentation is not clear, and might be influenced by the composition of the diet and the different large intestinal microbial populations among rabbits (Carabaño *et al*., 2006), not only in numbers but also in species composition. The ability to metabolise oligofructose and inulin is a strain-specific feature, so the effect of a prebiotic is influenced by the host's specific situation, as shown in case of lactobacilli and bifid bacteria. In addition, if a particular organism initiates metabolism of an oligosaccharide via extracellular hydrolysis, the products (mono- or disaccharides) that are released may then ''cross-feed'' other organisms (Huebner *et al*., 2007). The best known nutritional effect of inulin in humans (Niness, 1999) and in livestock (pig, poultry, calves, Verdonk *et al*., 2005) is its action to stimulate bifid bacteria growth in the intestine. This effect is thought to promote health of the host: inhibiting the growth of harmful bacteria, stimulation of the immune system, etc. According to several investigations, bifid bacteria are not dominant in rabbit caecum (Abecia *et al*., 2005; Monteils, *et al*., 2008). In rabbits, there is a clear prevalence of the strictly anaerobic, non-sporulated gram-positive bacteria (Carabaño *et al*., 2006, Monteils *et al*., 2008). In continuous anaerobic culture systems using human faecal slurries, bifid bacteria preferred inulin, whereas bacteroides could not grow on it. Wang and Gibson (1993) reported that after 12 h of incubation in the presence of 7 g/L fructose, starch, inulin or oligofructose, both of the chicory fructo-oligosaccharides had a negative effect on growth of the bacteroides. Other in vivo studies regarding the effect of the addition of inulin or oligofructose to the diet on the composition of the human colon microbiota reveal that bacteroides are neither stimulated nor depressed through administration of these prebiotics (Roberfroid, 2005). A possible explanation for this might be related to the mechanism of inulin degradation. In the case of bacteroides, it is presumed to be periplasmic or extracellular, causing loss of digestion products, while in contrast, in case of *Bifidobacterium* spp. enzymes are cell-associated or intracellular (Falony *et al*., 2009). As an important part of the rabbit caecal microbiota consists of bacteroides (Monteils *et al*., 2008), this result may also provide an explanation for the lack of effect of inulin supplementation.

The relative lack of effect of inulin might also be accounted for by its partial hydrolysis in the upper intestine in rabbits, as observed previously by Maertens *et al*. (2004), due to the significant microbial

activity before the caecum (Marounek *et al*., 1995), as occurs with other less fermentable sources of fibre (Carabaño *et al*., 2001). However, whether the inulin had been partially hydrolysed before the hindgut was not investigated in our experiment.

Medication resulted in the lowest morbidity as could be expected, due to the inhibition of pathogenic bacteria (Commission on Antimicrobial Feed Additives, 1997), although no effect on microbial counts was detected. The increase in morbidity one week after weaning could be the result of the temporary increase in pH, *E. coli* and total aerobic bacteria number, which is a commonly observed accompanying feature of weaning and may predispose to digestive disorders (Lelkes and Chang, 1987). It has been shown in vitro that the antibacterial effect of VFAs on *E. coli* is detectable up to pH 6.6, while it is completely absent above 6.8 (Prohászka, 1986). This is because at lower pH, a higher proportion of VFA is in non-dissociated form, which exerts the antibacterial effect (Lewinson, 1978).

The increase in the relative weight and dry matter content of the caecal content with age is in accordance with the increasing importance to the large intestine after weaning, with increasing solid feed intake (Gidenne and Fortun-Lamothe, 2002). The enhanced fermentation activity in the caecum with age was shown by the increasing fibrolytic enzyme activities from 28 to 42 d of age. The fibrolytic potential of the caecal flora seems to evolve weekly, however these enzymatic parameters are variable (Gidenne *et al*., 2002). Fibrolytic activity is high for pectins and hemicellulose (xylane) and lower for cellulose (Gidenne *et al*., 2002; Marounek *et al*., 1995) as was also observed in the present experiment. It was also supported by microbiological enumeration as higher counts of pectinolytic and hemicellulolytic strictly anaerobic bacteria (mainly bacteroides) were observed (Boulharouf *et al*., 1991). We found higher xylanase than pectinase activity, contrary to Volek *et al*. (2007), but comparing the composition of the experimental diets, our diets contained higher hemicellulose (as NDF-ADF) (19 *vs*. 14%).

Total VFA concentration did not change with age, the VFA profile was characteristic of rabbit, with the predominance of acetate, followed by butyrate and then by propionate (Padilha *et al*., 1995). The propionate to butyrate ratio decreased by age from 0.9 to 0.6, in accordance with the literature (Gidenne *et al*., 2002).

Microbial counts were not in agreement with the changes observed in fibrolytic activity and VFA concentration. This may be due to the lack of information about VFA production and to the high uncertainty of culturing techniques in studying microbial community, because it reveals only 20-40% of the real bacterial richness (Suau *et al*., 1999). Analysis of 16S rRNA genes demonstrated that the rabbits' caecum harbours 80-90% of unknown bacterial species (Abecia *et al*., 2005; Monteils *et al*., 2008), which may have effect on fermentation processes.

In conclusion, after the inclusion of 4% of inulin in the diet of weanling rabbits no positive effects on growth, caecal microflora or fermentation pattern were observed.

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