World Rabbit Science

World Rabbit Sci. 2009, 17: 87 - 95

PERFORMANCE, DIGESTIVE DISORDERS AND THE INTESTINAL MICROBIOTA IN WEANING RABBITS ARE AFFECTED BY A HERBAL FEED ADDITIVE

Krieg R.[*], Vahjen W.[†], Awad W.[†], Sysel M.[†], Kroeger S.[†], Zocher E.[†], Hulan H.W.[‡], Arndt G.[§], Zentek J.[†]

[*]Kleintierzucht Ronald Krieg, Merseburger Strasse 3. D 06255 Mücheln/Wünsch. Germany.

[†]Institut für Tierernährung, Fachbereich Veterinärmedizin, Freie Universität Berlin, Brümmerstr. 34. D 14195 Berlin. Germany.

[‡]Department of Biochemistry, Memorial University of Newfoundland, St. John's, NL. A1B 3X9. Canada

[§]Institut für Biometrie und Informationsverarbeitung, Fachbereich Veterinärmedizin, Freie Universität Berlin, Oertzenweg 19b, D 14163 Berlin. Germany

Abstract: A herbal feed additive (Digestarom®, containing a mixture of onion, garlic, caraway, fennel, gentian, melissa, peppermint, anise, oak bark and clove) was fed to rabbit does and kits to study its impact on performance, post-weaning digestive disorders and intestinal microbiota. Two groups of 9 doe rabbits and their offspring, after weaning, were fed a standard diet without or with the addition of 300 mg Digestarom®/kg diet. Forty kits from each group were weaned at 28 d of age weighing 0.614±0.005 kg. They were caged in groups of four rabbits (10 cages/treatment) and fed the same diet as their mothers for 13 d. Weight gain and feed intake of the kits fed Digestarom® was 18 and 14% higher, respectively, than those fed control diet (P<0.001), with no differences in the feed conversion. Rabbits were killed 13 d after weaning and 10 healthy animals from the Digestarom® group and 10 healthy and 10 diseased animals from the control group were dissected. Healthy rabbits fed control diet and those fed Digestarom® showed closer intestinal digesta dry matter, pH and volatile fatty acid (VFA) profiles, compared to diseased animals. VFA concentration in the small intestine was higher (P=0.030) in the diseased animals of the control group compared with the healthy and Digestarom® fed rabbits. However, no differences were observed in VFA concentration in stomach or caecum contents. The fermentation profile of diseased animals was characterised by a higher proportion of propionic, i- and n-valeric acids in the caecal contents (P<0.001), and an increased i-butyric acid concentration in the stomach and caecum contents (P=0.014), whereas n-butyric acid was reduced (P<0.033) compared with the healthy or Digestarom® fed rabbits. Denaturing gradient gel electrophoresis indicated a higher caecal bacterial diversity in the control rabbits compared with kits fed Digestarom® (P=0.008). The reduced evenness factor (P<0.010) also indicated that the bacterial composition included more dominant species in the Digestarom® group. Under our experimental conditions, the tested herbal feed additive Digestarom® had protective effects in rabbit kits after weaning, making it an interesting alternative for establishing nutritional strategies.

Key Words: rabbits, kits, Digestarom®, diarrhoea, weaning, microbiota.

INTRODUCTION

Herbal feed additives comprise of a wide variety of herbs, spices, and essential oils. Apart from enhancing the taste and improving the flavour of the feed, such feed additives are believed to have positive effects on digestion and intestinal health. Some of the important aspects associated with herbal additives are the prevention of digestive disturbances, improved feed utilisation and enhanced animal performance.

Correspondence: R. Krieg, ronald.krieg@t-online.de
Received *March 2008* - Accepted *December 2008*

Weaning is the most critical period and it is associated with a higher risk of digestive disorders in growing rabbits (Lebas *et al*., 1998; Gidenne *et al*., 2005; Gidenne and Garcia, 2006). One predisposing factor is the limited digestive capacity of weaned rabbits. Diets with low acid detergent fibre concentrations are at risk of inducing digestive disorders during the weaning period (Gidenne *et al*., 1998). The precise mechanisms of rabbit post-weaning diarrhoea are still not completely understood. Changing microbial population dynamics and increased fermentation of undigested nutrients by the small intestinal and caecal microbiota seem to be of major significance. The prevalence of gastrointestinal problems is high during the weaning period and antibiotics are used as standard treatment on many commercial farms. However, the routine use of antibiotic drugs is restricted, not only due to the ban on antibiotic growth promoters in the European Union, but also by the negative public and political awareness of these practices. Resistance of enteropathogens such as *Escherichia coli* to antibiotics occurs on rabbit farms (Cerrone *et al*., 1999), restricting the use of these drugs for prolonged periods. Therefore, feed additives with the potential to prevent digestive problems are considered as a promising alternative. Several substance classes have been evaluated in rabbits, recently reviewed by Falcão-e-Cunha *et al*. (2007). A zeolite-based adsorbing substance (Grobner *et al*., 1982), mannan oligosaccharides (Mourao *et al*., 2006), insulin type fructans (Volek *et al*., 2007), probiotics (Simonova *et al*., 2005; Trocino *et al*., 2005, Pinheiro *et al.*, 2007), and tannin supplements (Maertens and Struklec, 2006) have been proven to be effective to some extent against digestive diseases in kits. Potential benefits of herbs and spices and plant extracts as feed additives have not yet been studied in the post-weaning period of rabbits. Herbs and spices contain a large number of antimicrobial, spasmolytic and anti-secretory compounds, raising interest in them as feed ingredients during the weaning period. The aim of this study was to study the influence of the herbal feed additive (Digestarom®) on the intestinal digesta and microbiota 13 d after weaning.

MATERIAL AND METHODS

Animals and housing

Pregnant doe rabbits, (ZIKA®- Hybrid, 73453 Abtsgmünd-Untergröningen) with a body weight of 5.595±495 g (mean±standard deviation), were randomly subdivided into a control group (n=9) fed a standard diet without the addition of Digestarom®, and an experimental group (n=9) fed a standard complete diet into which was incorporated 300 mg/kg of the herbal feed additive, (Digestarom® 1315, MICRO-PLUS Konzentrate GmbH, Stadtoldendorf, which contained a mixture of onions, garlic, caraway, fennel, gentian, melissa, peppermint, anise, oak bark and clove). The artificial light schedule for females was from 5 a.m. until 9 p.m. A closed breeding room with consequent low pressure ventilation and warmed environment between 18-24 °C was used. After farrowing, the litters were balanced and each feeding group consisted of 80 newborn rabbits. The does and their offspring were kept housed on flat decks following a randomised block design. The rabbits were weaned on 28 d, and 40 animals in each feeding group (20 male/20 female) remained on the respective diets of their does' group for another 13 d. All weaned rabbits were mixed within the group and caged independently of their litter or weight. Each cage (Meneghin srl., Italy, 4500 cm² bottom) housed 4 males or 4 females, and each feeding group included 10 cages with equal sex distribution. The identifying code for each kit was a consecutive black coloured number inside the right auricle. Individual body weights and feed intake were measured weekly. The kits were kept on a daily lighting schedule of 12 h light and 12 h dark, and feed was offered *ad libitum.* The experimental procedures were approved by Landesverwaltungsamt Halle according to the 'Tierschutzgesetz' animal protection act.

Diet

The control group was fed a commercial diet for rabbits (Reikanin Zucht, 058600, Kraftfutterwerk Zwickau, Reinsdorf; containing the following standard composition analysed by the manufacturer: 10.4

MJ digestible energy/kg dry matter (calculated from crude nutrients), 18.9 % crude protein, 3.4 % crude fat, 15.1 % crude fibre, 43.0 % nitrogen free extracts (all on dry matter (DM) basis) (VDLUFA, 1976). The experimental feed had the same composition and was supplemented by Digestarom®. The major essential oils supplied by Digestarom® were menthol, trans-anethol and thymol (1.8/ 0.76/ 0.41 mg/kg pelleted feed, respectively, according to the chemical analysis of pelleted feed). Both diets were offered *ad libitum* to both does and kits, in the form of pellets with a diameter of 2 mm.

Traits

The health status of the rabbits was recorded by the same person twice daily. Each individual animal was observed for signs of diarrhoea and constipation, and the incidence and duration (d) of such problems, and the rate of mortality was recorded. On 13 d after weaning (under our housing conditions this is the best time to collect diseased animals), 20 animals from the control group were randomly selected, 10 with and 10 without digestive disorders (acute diarrhoea from 10 to 13 d), and 10 animals from the Digestarom® group (without digestive disorders) were killed between 9 and 12 a.m. Only 2 animals with digestive problems were present in the Digestarom® group. The animals were killed by injection of 1 mL/kg body weight (BW) embutramide (T61, Hoechst Veterinär, Unterschleißheim), after anesthesia with 35 mg/kg BW Ketamin (Ketanest 50, Parke-Davis, Berlin) and 5 mg/kg BW Xylazin (Rompun 2%, Bayer, Leverkusen). The animals were positioned on their back, and the abdominal cavity was carefully opened by making an incision along the total length of the abdominal cavity, and the gastrointestinal tract was carefully removed. Samples of the stomach digesta were taken after mixing the total contents. Samples of digestive contents from the small intestine and caecum were collected by stripping out the entire small intestinal and caecal digesta. Samples were taken after opening the abdominal cavity of the animal in lying position within 5 min of death. After being carefully mixed, they were subdivided in 2 g portions and immediately stored on dry ice and transferred to the laboratory at −70 °C (dry ice) for subsequent analysis for volatile fatty acids (VFA) and lactate concentrations and analysis of the microbial community in the intestinal contents by PCR-DGGE.

Chemical analyses

Immediately after collection, the fresh digesta samples were diluted with distilled water (1:10) and the pH of the samples was determined by using an electronic pH meter (Beckman Coulter, Inc, Fullerton, CA, USA). For the analysis of lactate, the samples were diluted with 1M perchloric acid (1:5) and centrifuged at 1400 g for 15 min. The extracts for lactate were stored at −20°C until subsequent enzymatic analyses using commercial kits (Boehringer, Mannheim, Germany). For VFA analysis, after thawing the intestinal contents, 300 mg of digesta (n=10/group) were collected and diluted in distilled water, homogenised, and centrifuged (Heraeus Instruments, Düsseldorf, Germany) at 13,000 rpm for 15 min. Hexanic acid was used as an internal standard (0.5 mmol/L). The sample was injected into a gas chromatograph (Model 19095N-123, Agilent Technologies, CA, USA) fitted with a HP-INNOWax column A, (length 30 m, internal diameter 530 µm with film thickness of 1.0 µm). The initial temperatures of the oven, injector and FID-detector were 70, 230 and 250°C, respectively. Hydrogen gas produced by a gas generator (Parker ChromGas, Parker Hannifin Corporation, MN, USA) was the carrier gas, with a flow rate of 30 mL/min.

Analysis of intestinal contents by PCR-DGGE

Genomic DNA extraction and purification of total cellular DNA from the jejunal and caecal digesta for PCR-DGGE was carried out as described (Kraatz *et al*., 2006). Briefly, an aliquot of 1 g of sample was added to 3 g of glass beads (diameter 0.25–0.50 mm) and 10 mL of guanidinium isothiocyanate (GITC) solution (75 g GITC/100 mL citrate sarcosine buffer) in a cryotube, incubated at 60 °C for 5 min and vortexed (F. Retsch GmbH, Haan, Germany) for 2 min. The sediments were again diluted with the GITC solution (7.0 mL), incubated again at 60 °C for 5 min followed by renewed bead beating for

another 2 min. The nucleic acids were extracted with phenol-chloroform, chloroform-isoamylalcohol, and precipitated in 80% isopropanol. The nucleic acids were washed with 70% ethanol and purified on Macherey-Nagel columns (Nucleo-Spin-Tissue kit; Macherey-Nagel, Düren, Germany). Amplification of the variable V6-V8 region of bacterial 16S rDNA was performed using primer pairs F-0968-GC (ATTACCGCGGCTGCTGG) and RW-1401 (CTTACGGGAGGCAGCAGCCGGGGCGCGCC CGGGCGGGGCGGGGGCACGGGGGGAC) (Kraatz *et al*., 2006). PCR was performed using a Multiplex PCR kit (Qiagen, Hilden, Germany).

PCR amplification of the V6-V8 region was carried out by a hot-start and touch-down programme with a T1 Thermocycler (Biometra, Göttingen, Germany). The PCR programme consisted of 35 cycles: initial activation step at 95 °C for 15 min; a single cycle. Denaturation was achieved by carrying out a single cycle at 94 °C for 60 sec, followed by annealing at 66 °C for 90 sec and a 72 °C extension for 90 sec; 20 thermal cycles in which the annealing temperature was decreased 0.3 °C every other cycle; 14 cycles of 94 °C for 30 sec, 59 °C for 90 sec, and 72 °C for 90 sec, and final elongation at 72 °C for 10 min. An INGENYphorU vertical DGGE system (Ingeny International, Netherlands) was used for subsequent nucleotide sequence-specific separation of PCR amplicons. To separate PCR fragments, a 35-50% linear chemical DNA-denaturing gradient gel of urea and formamide was used. After electrophoresis, gels were silver-stained and developed. All samples for an intestinal segment were run in one gel.

Scanned DGGE banding patterns were analysed using computer software, Phoretix 1D (version 5.1, Phoretix International Limited, Newcastle upon Tyne, UK). Bands with an area of <1% of the gel were omitted. To determine microbial diversity, calculation of the Shannon index based on number (richness) and relative abundance (evenness) of bands (16S species) in a gel lane (sample) was performed. Sörensen's pair-wise similarity index (Cs), sometimes referred to as community similarity, is based on the total number and the number of common PCR-DGGE bands (Konstantinov *et al*., 2004; Kraatz *et al*., 2006; Kwak and Peterson, 2007).

Statistical Analysis

The Chi-(χ-) square test was used for the evaluation of the prevalence of digestive disorders. The Kolmogorov Smirnov's test was used to test the normal distribution of the data. Feed intake, weight gain and feed conversion were calculated considering the cage as replicate (n=10). The biochemical traits in the digesta were statistically analysed by one way ANOVA and Scheffé test (P<0.05), considering the animal as replicate. For the microbial analysis by PCR-DGGE, the same test was used to compare differences of ecological diversity indices, whereas similarity indices were assorted according to treatment in three groups, i.e. forming two intra-group and one inter-group similarity value group, respectively. The differences (P<0.05) between the groups were compared by one-way analysis of variance. The group differences were determined by the Duncan`s multiple range test (P<0.05). The data were analysed using the SPSS software (version 12.0, SPSS Inc., Illinois, USA). The sex was not considered in any of the statistical models used.

RESULTS

Daily weight gain and feed intake was 18 and 14 % higher (P<0.001) in the group fed Digestarom® (including healthy and sick animals; Table 1). Feed conversion was not different between treatments (P=0.25), being on average 1.95 g/g. Animals fed Digestarom® showed lower digestive disorders (diarrhoea and constipation) compared to the control group (P<0.001). Scores for days with pathologic diarrhoea and constipation were more than 5 fold higher (1.6/kit) for the control group compared to the group administered the feed additive (0.3/kit) (P<0.001). The clinical problems were observed from 4 d after weaning in the control group, and 8 d after weaning in the Digestarom® group (Figure 1). During

Table 1: Effect of Digestarom® supplementation on growth performance and prevalence of diarrhoea and constipation of the rabbits during the first 13 d post-weaning.

	Control group	Digestarom® group	SEM[1]	*P*-value
Initial individual body weight, kg	0.615	0.613	0.005	0.91
Final body weight, kg	1.20	1.32	0.022	0.002
Daily weight gain, g/d	45.7	54.5	1.44	0.001
Daily feed intake, g/d	90.6	103.7	2.13	0.002
Feed conversion	2.00	1.90	0.035	0.25
No cases with digestive disorders	18	4	-	0.001
No cases with a duration of disease of:				
1-2 d	6	1	-	0.001
> 2 d	12	3	-	0.001

[1] n=10 cages/treatment

the period of 13 d post-weaning, mortality for both groups was as follows: 3 animals in the control group and 1 animal in the Digestarom® group. All fatal cases suffered from diarrhoea during the second week after weaning.

The DM concentration of the stomach contents (Table 2) was 27% lower ($P<0.001$) in the diseased animals compared to the healthy animals of the control group and those fed Digestarom®. No effect was observed on DM concentration in the small intestine and caecum (86.7 and 20.1% on average, respectively). The pH of the digesta was 5% higher ($P=0.016$) in the caecal contents of the diseased animals compared to healthy rabbits from control and Digestarom® group. Total VFA and lactate were measured as indicators of intestinal microbial metabolism. The total concentration of VFA increased ($P=0.030$) by 127% in the small intestinal digesta of the diseased animals in the control group compared to healthy animals from the control group and rabbits fed Digestarom®. However, VFA concentrations in the stomach and in the caecum were not different among groups (15.0 and 49.0 mmol/L on average, respectively). The molar

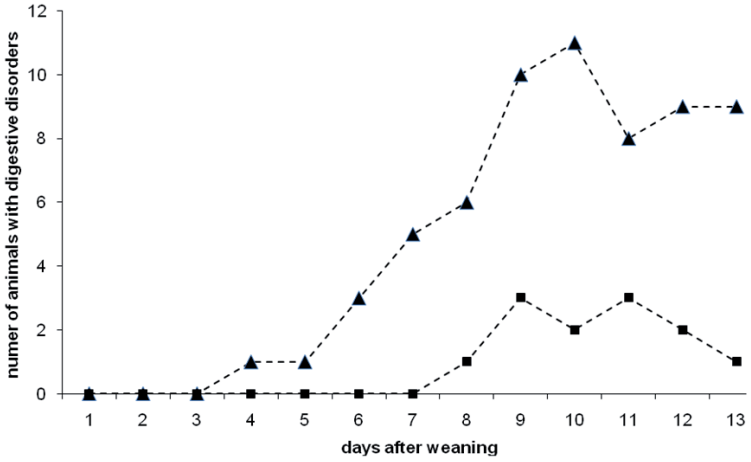

Figure 1: Prevalence of digestive problems (total number of animals with diarrhoea or constipation) of young rabbits after weaning (control group: ▲, Digestarom® group: ■; for group differences in prevalence and duration of digestive disorders see Table 1)

Table 2: Effect of Digestarom® supplementation and health condition on dry matter, pH, lactate and volatile fatty acids in the intestinal digesta.

	Stomach					Small intestine					Caecum				
	Control		Digestarom®			Control		Digestarom®			Control		Digestarom®		
	healthy	diarrhoea	healthy	SEM[1]	*P*-value	healthy	diarrhoea	healthy	SEM[1]	*P*-value	healthy	diarrhoea	healthy	SEM[1]	*P*-value
Dry matter, g/kg	158[a]	121[b]	176[a]	9.37	0.001	79.2	75.6	105.2	6.25	0.22	209	189	205	4.35	0.15
pH	2.25	2.35	2.22	0.41	0.79	7.66	7.69	7.82	0.04	0.24	5.76[b]	6.06[a]	5.80[ab]	0.049	0.016
Volatile fatty acids mmol/L	14.8	12.5	17.6	3.82	0.54	6.30[b]	12.0[a]	4.25[b]	1.27	0.030	48.5	44.2	54.2	2.55	0.29
VFA molar proportions, %															
acetic acid	83.3	86.8	81.2	1.64	0.30	83.9	88.4	76.5	2.11	0.060	77.7	75.4	81.1	1.05	0.079
propionic acid	1.71	3.24	3.45	0.91	0.39	7.71	6.63	11.6	1.11	0.17	4.9[b]	10.0[a]	5.3[b]	0.67	0.001
i-butyric acid	0.49[b]	1.46[a]	0.19[b]	0.35	0.014	1.95	1.00	0.64	0.55	0.62	0.00[b]	0.68[a]	0.00[b]	0.11	0.014
n-butyric acid	8.65[ab]	5.08[b]	12.9[a]	1.12	0.033	4.79	2.04	4.91	0.55	0.051	16.6[a]	11.4[b]	13.2[b]	0.62	0.001
i-valeric acid	1.28	0.65	1.06	0.54	0.18	1.14[a]	0.66[a]	5.58[b]	0.87	0.033	0.10[b]	1.46[a]	0.00[b]	0.24	0.013
n-valeric acid	4.56	2.73	1.21	0.61	0.33	0.48	1.21	0.86	0.48	0.83	0.61[b]	1.02[a]	0.37[b]	0.10	0.023
d-lactate, mmol/L	0.17	0.13	0.15	0.03	0.72	-[2]	-	-	-	-	0.24	0.20	0.26	0.029	0.70
l-lactate, mmol/L	0.43	0.65	0.29	0.05	0.056	-	-	-	-	-	0.22	0.24	0.22	0.023	0.93
total lactate, mmol/L	0.60	0.78	0.44	0.06	0.19	-	-	-	-	-	0.46	0.44	0.47	0.048	0.95

[1]n=10/group; [2]insufficient quantity, no analysis performed

[a,b] Within the same row and segment, means with different letters are different ($P<0.05$).

Table 3: Effect of Digestarom® supplementation and health condition on ecological indices in the small intestine and caecum of the rabbits (DGGE analysis).

	Control		Digestarom®		
	healthy	diarrhoea	healthy	SEM[1]	*P*-value
Small intestine					
Richness	5.43	7.83	6.30	0.537	0.25
Shannon	1.01	1.06	1.02	0.046	0.91
Evenness	0.61	0.55	0.60	0.019	0.47
Caecum					
Richness	24.5	27.7	23.9	1.099	0.33
Shannon	1.99[a]	2.35[a]	1.53[b]	0.144	0.008
Evenness	0.63[a]	0.71[a]	0.49[b]	0.032	0.010

[1] n=10/group

[a,b] Within the same row means with different letters are different ($P<0.05$).

proportion of acetic acid was not affected by diet or health condition in any segment. In the diseased animals fed control diet, molar proportions of propionic acid increased ($P=0.014$) by 96% in the caecum, and that of i-butyric acid in the stomach (by 328%) and caecum contents (from 0 to 0.7%) as compared with healthy animals from the control group and rabbits fed Digestarom®. The relative concentrations of n-butyric acid tended to be lower in the stomach and small intestine contents of diarrhoeic animals (Table 2) compared to healthy rabbits or those fed Digestarom®. However, the highest caecal n-butyric acid concentration ($P=0.001$) was obtained for the healthy rabbits fed with control diet. In the caecal content the concentrations of i-valeric and of n-valeric acid increased in animals with diarrhoea ($P=0.023$), whereas in the small intestine i-valeric increased in the Digestarom® group ($P=0.033$). Although lactate concentrations were not affected ($P>0.05$) by the treatment, l-lactate values were lower ($P=0.056$) in the stomach content of rabbits fed Digestarom®.

The DGGE ecological indices indicated no differences in the small intestinal microbiota ($P=0.25$), while the caecal microbiota displayed some differences ($P<0.01$) between the Digestarom® and control group (healthy or not) (Table 3). The rabbits from the Digestarom® group had a lower bacterial diversity in the caecum ($P=0.008$). The reduced evenness factor ($P=0.01$) also indicated that the bacterial composition included more dominant species in the Digestarom® group.

DISCUSSION

Digestive disorders and high morbidity and mortality are common problems in commercial rabbit production, causing significant economical losses (Maertens, 1999; Marlier *et al*., 2003) especially in newborn and in weaning rabbits (Richardson, 2000; Fortun-Lamothe and Boullier, 2004; Gidenne *et al*., 2005; Carabaño *et al*., 2006). In the present study using weaned rabbit kits, the tested herbal feed additive had an overall positive influence on animal performance with higher body weight gain and fewer cases of diarrhoea and related mortality. Accordingly, the effects on growth and performance of the tested feed additive Digestarom® can be explained by the substantially reduced prevalence and severity of digestive disorders after weaning.

The laboratory analysis indicated some changes in the VFA concentration and a reduced microbial diversity in the caecum in rabbits fed Digestarom®. The tested product Digestarom® contains a mixture of herbs, spices and essential oils. Several plant ingredients have been under research in poultry, pigs and

ruminants indicating variable, often beneficial effects on the intestinal microbiota and performance of animals (Jamroz *et al.*, 2003; Mitsch *et al*., 2004; Jamroz *et al*., 2005; Hume *et al*., 2006; Windisch *et al*., 2008). Due to the complexity of secondary plant products especially in mixed products such as the one tested in this study, it is almost impossible to define a specific active factor or ingredient. This is often considered as a major pitfall in the assessment of these substances. Antimicrobial effects, modulation of the intestinal immune response and a stabilised digestive function that could be the consequences, are considered major candidates for explaining intestinal effects (Fortun Lamothe and Drouet Viard, 2002; Wallace, 2004; Fortun Lamothe and Boullier, 2007). In the present study, the changes in the fermentation pattern of VFA indicate that both the group receiving the Digestarom® and healthy control rabbits seem to show a lower VFA concentration in the small intestine as compared with diseased animals. It might indicate either bacterial overgrowth, increased metabolic bacterial activity, or a lower absorptive capacity of the gut wall in the diseased status. Diseased rabbits also seem to reduce n-butyric acid concentrations in all parts of the gastrointestinal tract. Volatile fatty acids provide a considerable contribution to the energy requirement of rabbits (Parker, 1976). Butyric acid is an important fuel for enterocytes, especially in the lower digestive tract (Butzner *et al*., 1994). A decrease of n-butyric acid was observed independently, whether considered in absolute or relative values. This might induce energy deficiency in the colonocyctes and impair the absorptive capacity. Besides, the n-/i-butyrate ratio in stomach and caecum of diseased animals was lower compared to that of healthy control rabbits and rabbits fed Digestarom®. I-butyric acid is considered as indicator of increased intestinal proteloytic activity (Cardona *et al*., 2005) and the changed n-/i-butyric ratio indicates a shift in bacterial protein metabolism.

In spite of the effects observed in the VFA profile, there were no differences in ecological indices between healthy and diseased animals. DGGE has some limitations and data do not reflect the quantitative aspects of intestinal colonisation, and minor changes might be overlooked. Significant changes of the intestinal microbiota were determined in the caecum. DGGE results indicate a higher bacterial diversity in rabbits fed the control diet (healthy or not) compared to those fed Digestarom®. The tested feed additive obviously had a stabilising effect on the microbiota and this could explain its mode of action. We did not perform any identification for known pathogens as enteropathogenic *E. coli*, *Clostridium perfringens* and other clostridia. Those are important in the pathogenesis of post-weaning diarrhoea and can have similar effects on nutrient and electrolyte transport in the rabbit intestine as in other species (Nath *et al*. 1992; Baranyi *et al*. 2003; Marlier *et al*. 2003).

CONCLUSION

The tested botanical feed additive Digestarom® had a positive influence on performance and health in weaned rabbits. However, observation period was only 13 d post-weaning and the number of animals was limited. The mode of action is not fully clear. It might be related to effects on the intestinal microbiota. The changes observed in the VFA concentration might be considered an indicative. However, there was no shift in the ecological indices in the small intestine and caecum of the rabbits based on DGGE (in healthy *vs*. diseased rabbits). Further work is needed to elucidate this relationship. Interactions with the immune system and the function of the gut epithelium are further candidate mechanisms that have to be tested in future studies.

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