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The effects of natural substances and "Xtract" on *Eimeria* spp. oocysts in broiler rabbits

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Summary

There is growing interest in the use of natural substances (NS, *e.g.* herbal extracts/phytoadditives or probiotic bacteria and their products) in rabbit husbandry. NS are being used in the prevention/elimination of diarrhoeal diseases in rabbits, especially in the post-weaning period, with many NS reported to have antibacterial, antiprotozoal, anti-inflammatory and immunomodulatory effects. Coccidiosis is one of the most serious protozoal diseases in rabbits, causing major losses through damage to the liver and intestine and affects on the nasal tract. We have compared the antiprotozoal effects of sage, oregano, a bacteriocin-producing, probiotic strain of *Enterococcus faecium* CCM 4231 and its bacteriocin enterocin 4231 with those of the commercial product "Xtract" on *Eimeria* spp. in broiler rabbits. We used the zootechnical parameters weight gain and feed conversion as indicators of the presence of fewer or more pathogenic oocysts. We recorded a significant reduc-

Zusammenfassung

Wirkung natürlicher Substanzen und des Produktes „Xtract" auf *Eimeria* spp. Oozysten bei Mastkaninchen

Einleitung

Die Eimeriose ist eine der schwerwiegendsten und am weitesten verbreiteten endoparasitären Protozoenerkrankungen bei Kaninchen, und verursacht wirtschaftliche Schäden. In den letzten Jahren stieg das Interesse an der Verwendung natürlicher Substanzen (NS) (z.B. Kräuterextrakte/Phytoadditive oder probiotische Bakterien) zur Unterstützung der Kaninchenhaltung. Zu den innovativen Methoden zählt der Einsatz von NS zur Vorbeugung/Beseitigung von Durchfallerkrankungen bei Kaninchen, insbesondere in der Zeit nach dem Absetzen. Es wird berichtet, dass viele natürliche Substanzen antibakterielle, antiprotozoäre, entzündungshemmende und immunmodulatorische Wirkungen haben.

Material und Methode

Das Ziel dieser Studie war es, die Wirkung von Futterzusätzen auf die Ausscheidungsintensität von *Eimeria* spp. Oozysten bei natürlich infizierten Broilerkaninchen zu vergleichen. Bei den fünf Futterzusätzen handelte es sich um Salbei, Oregano, und den Bakteriocin-produzierenden *Enterococcus faecium* CCM 4231 mit probiotischen Eigenschaften und sein Bakteriocin Enterocin 4231 sowie das Handelsprodukt „Xtract". Gewichtszunahme und Futterverwertung wurden dokumentiert. Die Kaninchen (n = 144, Hy-plus-Rasse, 5 Wochen alt) wurden in 5 Versuchsgruppen (EG 1–5; je n = 24) und eine nicht medikierte Kontrollgruppe (ohne die Zugabe eines Coccidiostatikums; CG; n = 24) mit 2 Tieren je Käfig geteilt. Die NS sowie „Xtract" wurden den Tieren in den ersten 21 Tagen in EG 1–5 verabreicht. Der Versuch dauerte 42 Tage. Kotproben für die koproskopischen Untersuchungen wurden am Tag 21 (3 Wochen Anwendung von NS) und dann am Tag 42 (3 Wochen nach Beendigung der NS-Zufütterung) gesammelt.

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tion in the number of *Eimeria* spp. oocysts (p<0.001) after the administration of sage and oregano as well as after the application of *Enterococcus faecium* CCM 4231 and after administration of "Xtract". We found a significant decrease in the number of *Eimeria* oocysts after three weeks of application (p<0.001) and noted a significant and prolonged antiprotozoal effect at the end of the experiment (three weeks after cessation of feed supplementation with all NS and Xtract; p<0.001). However, we observed no improved weight gain or feed conversion in the treated groups compared to the control group, probably due to the lower pathogenicity of the coccidia naturally present in the faeces. Further studies are recommended to ensure that this magnitude of reduction of oocysts can be achieved for highly pathogenic coccidia species, as the effect of NS seems to be more pronounced than that of a commercial anticoccidicum.

Abbreviations: CG = control group; EG = experimental group(s); NS = natural substances; OPG = oocysts per gram of faeces

Ergebnisse

Nach Verabreichung von Salbei und Oregano sowie von *Enterococcus faecium* CCM 4231, und nach Verabreichung des kommerziellen Produkts „Xtract" ergaben sich statistisch signifikante Reduktionen von *Eimeria* spp. Oozysten (p<0,001). Eine statistisch signifikante Abnahme der *Eimeria*-Oozysten wurde nicht nur am Tag 21 (3 Wochen Anwendung; p<0,001), sondern auch am Ende des Versuchs (3 Wochen nach Beendigung der Gabe der natürlichen Substanzen und von „Xtract"; p<0,001) festgestellt.

Gewichtszunahmen und Futterverwertung waren bei den behandelten Gruppen aber nicht besser als bei den Tieren der Kontrollgruppe, was wahrscheinlich auf eine geringere Pathogenität der natürlich vorhandenen Kokzidien zurückzuführen war.

Schlussfolgerung

Weitere Studien sollten durchgeführt werden, um einerseits sicherzustellen, dass dieses Ausmaß der Reduktion auch bei pathogeneren Kokzidienarten erreicht werden kann, da die Wirkung von NS stärker zu sein scheint als die des kommerziellen Anticoccidicums und andererseits um die Wirkungsmechanismen von NS bei der Kanichenkozidiose zu verstehen.

Introduction

Life cycle of *Eimeria* sp.

Coccidiosis is caused by protozoan parasites of the subclass Coccidia, genus *Eimeria*, family Eimeriidae (MAYER and DONNELLY, 2013). The development of Eimeriae is complex and involves alternating sexual and asexual reproduction, which takes place partly within the host organism (internal/endogenous phase) and partly in the environment (external/exogenous phase). Animals are infected via the oral route, *e.g.* by contaminated feed, water or animal fur or by cage licking. In the gut of rabbits (under suitable conditions and pH), infectious sporozoites are released from oocysts into the lumen of the intestine. The parasitic stages multiply in the cells until they damage the host cell membranes and the parasite can invade another cell (SIVAJOTHI et al., 2016). After several generations, unsporulated oocysts are formed and excreted in the faeces into the environment, where they mature and become infectious to another host (LÓPEZ-OSORIO et al., 2020).

Coccidiosis in rabbits

Coccidiosis is one of the most serious and widespread endoparasitic protozoal diseases in rabbits (SHI et al., 2016), affecting animals of all ages and causing financial losses in rabbit farms. Coccidiosis in a rabbit farm can take two forms: hepatic (caused by *Eimeria (E.) stiedae*) and intestinal (caused by *E. magna, E. media, E. perforans, E. piriformis, E. intestinalis, E. exigua or E. irresidua*). The combination of the two forms is characterized by rapid and malignant development of infection (RENAUX et al., 2003; SZABÓOVÁ et al., 2011; HERICH et al., 2018). The life cycle of rabbit coccidia has some peculiarities, the most prominent of which is the migration of sporozoites from the site of entry to the target site. This is also seen in hens, goats and other animals but rabbit coccidia, especially *E. coecicola* and *E. stiedai*, exhibit unique features (PAKANDL, 2009). The second peculiarity is the presence of two types of meronts and merozoites. The pathogenicity seems to be connected, at least partially, to the localization of the coccidia. The most pathogenic rabbit coccidia, *E. intestinalis* and *E. flavescens*, parasitize the crypts of the lower part of the small intestine and the caecum, respectively (PAKANDL, 2009). The intestinal epithelium is apparently more seriously damaged if the parasite destroys the stem cells in the crypts (PAKANDL et al., 2003; PAKANDL, 2009). The intestines of naturally infected rabbits show characteristic lesions manifested in a severe degree of necrosis of the intestinal cells, characterized by pyknotic and tiny nuclei with acidophilic cytoplasm along the intestinal tissues, as well as desquamation of the epithelial lining at the lumen. Atrophy of the intestinal villi and various developmental stages of *Eimeria* spp. are seen in the crypts (ELSHAHAWY and ELGONIEMY, 2018). *E. stiedae* infection in rabbits (three-month-old, New Zealand White females) produced significant changes to the

red and white blood profile parameters: there were decreased numbers of erythrocytes and lower values of haemoglobin and haematocrit with an increased mean concentration of corpuscular haemoglobin and higher numbers of leukocytes, granulocytes, monocytes and lymphocytes. The blood profile could be indicative for the diagnosis of liver coccidiosis in rabbits (PETROVA et al., 2018).

Individual species of rabbit coccidia differ in pathogenicity and intestinal coccidia cause more or less severe disease in rabbits, depending mainly on the species, the infective dose and the immune status and age of animals. Characteristic symptoms include diarrhoea, loss of weight, reduced feed conversion and sometimes death (PAKANDL, 2009; RENAUX et al., 2003). Less pathogenic species *(E. perforans, E. exigua, E. vejdovskyi)* cause symptoms such as a slight loss in weight gain without diarrhoea. Rabbits are very sensitive to coccidiosis at the time of weaning, i.e. at the age of 35 days (VODIČKA, 2007).

Therapy of coccidiosis in rabbits

Treatments of natural origin, i. e. natural substances, against coccidiosis are attracting increasing attention. Alternative coccidiostats can be essential oils of plant origin, such as thymol, carvacrol, thujone or camphor, or plant extracts from oregano, sage, rosemary, basil or cinnamon, which affect the immune system through their stimulating and immunomodulatory effects (KOWALSKA et al., 2012). There is also increased interest in the use of microorganisms with probiotic properties and their products on rabbit farms. Innovative methods involve the use of natural substances (NS) to prevent/eliminate diarrhoea and other diseases, especially in the post-weaning period. NS may have a wide range of biological activities, such as antibacterial (SZABÓOVÁ et al., 2011; POGÁNY SIMONOVÁ et al., 2013; SPIŠÁKOVÁ et al., 2013; LAUKOVÁ et al., 2016, 2017), antiprotozoal (STROMPFOVÁ et al., 2010), anti-inflammatory and immunomodulatory (MAJOR et al., 2011; LEVKUT et al., 2012). The natural phytoadditives, sage and oregano, belong to the family *Lamiaceae* and show antimicrobial activity (ROYO et al., 2010), so may be able to reduce coccidiosis in rabbits. Probiotic strains are commonly used to improve the intestinal microbiome (AL-SHAWI et al., 2020) and are considered to be a safe and viable way to increase the performance of farm animals (ALAYANDE et al., 2020). We now report a comparison of the antiprotozoal effects of selected NS (plant extracts from sage and oregano developed by Calendula a. s., Nová Ľubovňa, Slovak Republic; *Enterococcus faecium* CCM 4231 and its enterocin 4231 selected and characterized by LAUKOVÁ et al., 1997) and the commercially available feed additive "Xtract" (Pancosma, Switzerland; the authors were not involved in its preparation/development) on *Eimeria* spp. oocysts in rabbit husbandry. "Xtract" contains phytocomponents from oregano, cinnamon and Mexican pepper.

Materials and Methods

Animals

Broiler rabbits, n = 144, male Hy-plus breed, aged five weeks (after weaning), were randomly divided into five experimental groups (EG1–EG5; n = 24; *i.e.* without the addition of an anticoccial drug in feed) and one control group (CG; n = 24; *i.e.* without the addition of a coccidiostat in feed). The rabbits had been reared in a large-capacity hall at the National Agricultural and Food Centre in Nitra – Lužianky (Slovak republic) and the experiment was conducted in co-operation with this institution.

The rearing conditions were intentionally similar to those on farms that practise intensive rabbit husbandry. Rabbits after the weaning period (aged 35 days) came from litters of the same breed and were kept in the same conditions at the same breeding facility. The initial, natural infection with *Eimeria* spp. was used as the starting point for the experiment. All animals were placed in standard cages (0.61 m x 0.34 m x 0.33 m) of type D-KV-72 (Kovobel company, Domažlice, Czech Republic), two per cage, and the groups separated from one another by two empty cages to obtain samples of faeces from the animals in the individual groups.

Ethical approval

The experiment was carried out in accordance with established standards for the use of animals (Guide for the Care and Use of Laboratory Animals) and the experimental schedule was approved by the Ethics Committee of the University of Veterinary Medicine and Pharmacy in Košice, Institute of Animal Physiology, Slovak Academy of Sciences and approved by the Slovak Veterinary and Food Administration. The experiment was performed in co-operation with National Agricultural and Food Centre in Nitra – Lužianky (Slovak republic).

Feeding and environmental conditions during the experiment

The experiment lasted 42 days. During the entire period the animals received the standard pelleted diet (pellet length about 3.5 mm) for rabbit breeding (Anprofeed VKZ Bučany, Slovak Republic) as shown in Table 1, and had access to water *ad libitum*. A cycle of 16 h light and 8 h dark was used throughout. The temperature and the humidity in the building were recorded continuously with a thermograph at the same height as the cages. The heating and forced ventilation systems allowed the air temperature to be maintained at 16 ± 4 °C. The relative humidity was about 70 ± 5 %. The NS and "Xtract" were administered to the animals in EG1–EG5 for the first 21 days. Sampling was performed on day 21 (after three weeks of NS application) and on day 42 (three weeks after cessation of NS application at the end of the experiment).

EG1 received sage plant extract containing thujone 24.0 ± 1.0 %, borneol 18.0 ± 1.0 %, cineole 15.0 ± 1.0 % (gas chromatography analysis; density: 0.915 ± 0.001 g/cm^3; refractive index: 1.469 ± 0.001) at a dose of 10 µl/animal/day in the water (*Salvia officinalis* L.; Calendula a.s., Nová Ľubovňa, Slovak Republic).

The animals in EG2 received the oregano plant extract containing carvacrol 55.0 ± 3.0 % (gas chromatography analysis; density: 0.959 ± 0.002 g/cm^3; refractive index: 1.515 ± 0.001) at a dose of 10 µl/animal/day in the water (*Origanum vulgare*; Calendula a.s.).

EG3 was given the bacteriocin-producing strain *Enterococcus faecium* CCM 4231 with probiotic properties (prepared as described by LAUKOVÁ et al., 1997; rifampicin resistant variant Rif^R, 10^9 CFU/ml at a dose of 500 µl/animal/ day in the water).

Tab. 1: Composition of standard pelleted diet provided for rabbits during experiment (from day 1 to day 42) / Zusammensetzung des pelletierten Kaninchen-Standardfutters, das während des gesamten Experiments (von Tag 1 bis Tag 42) verfüttert wurde

Component (%)		Composition (g.kg^{-1})	
Red clover	27.00	Crude protein	197.00
Sugar extracted from crushed sugarbeet	10.00	Crude fibre	165.50
Barley	15.00	Fat	39.00
Oat	13.00	Ash	80.00
Wheat bran	6.00	Organic substances	921.00
Soy flour	7.50	Starch	178.00
Sunflower meal	14.00	Lysine	7.50
Monocalcium phosphate	0.60	Methionine + cysteine	6.50
Dicalcium carbonate	0.90	Choline chloride	0.80
Salt (NaCl)	0.30	Metabolic energy (MJ kg^{-1})	10.00
Carob - breadfruit *(Ceratonia Siliqua)*	2.50		
DL-methionine	0.10		
wheat bran	0.10		
Mineral and vitamin premix	3.00		

The rabbits in EG4 received bacteriocin produced by strain *E. faecium* CCM 4231 – enterocin 4231 with an activity of 3,200 AU/ml at a dose of 50 µl per animal/day in the water (prepared as described by LAUKOVÁ et al., 1997).

EG5 received feed supplemented with "Xtract" (producer PANCOSMA, Switzerland) at a dose of 15 g/100 kg feed. The mixture of plant extracts in "Xtract" consists of 5.4 % (wt/wt) carvacrol ($C_{10}H_{14}O$; from oregano – *Origanum* sp.), 3.2 % (wt/wt) cinnamaldehyde (C_9H_8O, from cinnamon – *Cinnamonum* sp.) and 2.2 % (wt/wt) *capsicum* oleoresin ($C_{18}H_{27}NO_3$ from Mexican pepper – *Capsicum annuum*).

Feeding and environmental conditions during the experiment

The health status and consistency of faeces of the rabbits were checked daily by a veterinarian throughout the experiment. Mixtures of randomly collected faecal samples (n = 5) were taken from each group in sterile sample bags on days 21 and 42, before the daily mechanical removal of faeces on a belt conveyor from the built-in waste chute, which was wider than the single-flat-deck cage arrangement above it, to ensure that all faeces could be collected.

Faecal samples were stored at 4 °C and examined for the *Eimeria* spp. oocysts by the flotation technique according to McMASTER (MAFF, 1986). Oocysts were not differentiated at species level, but designated as *Eimeria* spp.; they were counted microscopically and the intensity of infection was expressed as oocysts per gram of faeces (OPG).

We monitored weight gain in kg and feed conversion in kg/kg daily in each animal throughout the experiment. The values of group weight gain and group feed conversion were calculated as the average ± standard deviation over 42 experimental days.

The statistical significance of differences between experimental groups and control and days 21 and 42 was determined using One-way-ANOVA and confirmed with Tukey *post-hoc* tests (MiniTab, Czech Republic). Normal distribution of data was confirmedd before applying ANOVA. The level of statistical significance was set at $p<0.05$.

Results

The initial average concentration of *Eimeria* oocysts in the faeces of all groups of rabbits in our experiment was 65.0 ± 22.8 OPG. On day 21 (after three weeks of NS administration), the numbers of oocysts were significantly lower than the CG in the experimental groups with sage and oregano, *Enterococcus faecium* CCM 4231 and "Xtract" ($p<0.001$; see Table 2). Significantly fewer oocysts were excreted after three weeks application of sage, *E. faecium* CCM 423 and "Xtract" than after three weeks of oregano ($p<0.001$; see Table 2).

The greatest reduction in *Eimeria* spp. oocysts throughout the experiment was observed in EG1 (sage extract). An anti-coccidial influence was also seen in EG2 (with oregano), in which the number of oocysts was reduced from day 21 to day 42. On day 42 (three weeks after cessation of NS application), we observed a prolonged and significant antiprotozoal effect ($p<0.001$) in all experimental groups. There were significantly fewer *Eimeria* spp. oocysts ($p<0.001$) in EG 1 (sage), EG 2 (oregano) and EG5 ("Xtract") than in EG3 (*E. faecium* CCM 4231) and EG4 (enterocin 4231) on day 42 (see Table 2). The probiotic strain *E. faecium* CCM 4231 and the enterocin 4231 significantly ($p<0.001$) reduced the numbers of oocysts in the faeces even three weeks after the cessation of application. The difference with *E. faecium* CCM 4231 was significantly greater ($p<0.001$) than that caused by the enterocin 4231 produced by the strain.

We noted a non-significant increase in average weight gain only in EG 3 (38.10 kg) compared with CG (36.40 kg) or with EG 1 (32.10 kg), EG 2 (34.20 kg), EG 4 (33.7 kg) or EG 5 (34.7 kg) on day 42. EG3 also showed a higher average feed conversion ratio (3.30 ± 0.56 kg/kg), as did EG 1 (3.50 ± 0.45 kg/kg), EG 2 (3.50 ± 0.47) and EG 5 (3.34 ± 0.53 kg/kg), compared with CG (3.02 ± 0.26 kg/kg) and EG 4 (2.81 ± 0.45 kg/kg).

The examination of the health status of the experimental animals and of the consistency of their faeces showed that the rabbits remained in good condition from the beginning to the end of the experiment and showed no symptoms of coccidiosis such as lethargy, weakness, dehydration or diarrhoea. All animals had a solid faecal consistency thoughout the experiment.

Tab. 2: Effects of natural substances and "Xtract" on number of *Eimeria* spp. oocysts in rabbit faeces expressed in oocysts per gram (OPG) ± standard deviation (SD) and on weight gain per group, individual weight gain and feed conversion / Die Wirkung der Verfütterung von Naturstoffen und von "Xtract" auf die Anzahl der *Eimeria* spp. Oozysten im Kaninchenkot, ausgedrückt in Oozysten pro Gramm (OPG) ± Standardabweichung (SD), und auf Gewichtszunahme pro Gruppe, individuelle Gewichtszunahme und Futterverwertung

	Day 21	Day 42	Total weight gain per group (kg)/ individual weight gain (kg)	Feed conversion (kg/kg)
EG1 (sage)	30.0 ± 2.6[a]	20.0 ± 2.0[d]	32.10/ 1.33 ± 1.09	3.50 ± 0.45
EG2 (oregano)	250.0 ± 48.6[b]	183.0 ± 6.0[d]	34.20/ 1.43 ± 0.73	3.50 ± 0.47
EG3 (*E. faecium* CCM4321)	33.4 ± 7.6	781.0 ± 32.1[e]	38.10/ 1.59 ± 1.19	3.30 ± 0.56
EG4 (enterocin 4231)	nd	2,250.0 ± 180.7[f]	33.7/ 1.40 ± 0.50	2.81 ± 0.45
EG5 (Xtract)	33.4 ± 7.9[a]	133.4 ± 12.4[d]	34.7/ 1.45 ± 0.96	3.34 ± 0.53
CG	1,184.0 ± 85.8[c]	5,640.0 ± 216.4[g]	36.40/ 1.52 ± 0.85	3.02 ± 0.26

EG = experimental group; CG = control group; nd = not determined; different superscripts indicate significant differences ($p<0.001$) within experimental days. / EG = Versuchsgruppe; CG = Kontrollgruppe; nd = nicht bestimmt; verschiedene Buchstaben zeigen statistisch signifikante Unterschiede innerhalb der Versuchstage ($p<0.001$) an.

Discussion

General and anti-coccidial effect of probiotic bacteria or their metabolic products as feed supplements for rabbits and other farm animals

Natural substances extracted from plants, fungi and microorganisms (prebiotics and probiotics) are currently being used to control coccidiosis (QUIROZ-CASTAÑEDA and DANTÁN-GONZÁLEZ, 2015). The past decade has seen a number of reports of the use of probiotics to control intestinal parasitic infections as well as some non-gut infections of human and veterinary importance. Probiotics are thought to increase digestive enzyme activity and decrease bacterial enzyme activity by maintaining the integrity of the intestinal microbiome by competitive mechanisms, as well as to modulate and stimulate the immune system (THANGARASU et al., 2016). The therapeutic use of probiotics could help reduce the risks of infestation by specific parasites or complement classical anti-parasite treatments (TRAVERS et al., 2011).

About 70 % of diseases in rabbits are caused by infectious diseases of the digestive tract (CARABANO et al., 2008). Enterococci added as probiotic and/or bacteriocin-producing strains with probiotic properties can colonize the intestinal tract of rabbits, independent of the source from which they were isolated, as confirmed with *Enterococcus faecium* AL41, *E. faecium* CCM 7420 and *E. durans* ED 26E/7 (POGÁNY SIMONOVÁ et al., 2009; LAUKOVÁ et al., 2016, 2017). The stimulative effects of probiotic strains on growth performance/ weight gain and feed conversion ratio have been noted not only in our study on broiler rabbits but also in broiler chickens (CHEN et al., 2017), pigs (AHASAN et al., 2015) and calves (FRIZZO et al., 2011).

Probiotics and microorganisms with a probiotic effect and their secondary substances (bacteriocins) have been shown not only to increase body weight gain in poultry and in rabbits but also to reduce coccidiosis (LEVKUT et al., 2009; MAJOR et al., 2011; LAUKOVÁ et al., 2016, 2017). In rabbits, the administration of probiotic *E. durans* ED 26E/7 (LAUKOVÁ et al., 2017) or of *E. faecium* CCM 7420 (POGÁNY SIMONOVÁ et al., 2009) has anticoccidial effects. We observed the same effect: the *E. faecium* CCM 4231 strain and its antimicrobial substance enterocin 4231 significantly ($p<0.001$) reduced the numbers of *Eimeria* oocysts in two experimental groups (in EG3 on days 21 and 42; in EG4 on day 42).

Avian coccidiosis poses a major problem in poultry farming, with a very high financial impact (LEE et al., 2007). The *in vitro* antiprotozoal potential of the lactic acid- and bacteriocin-producing strains *E. faecium* CCM 4231, *E. faecium* EF55 and *Lactobacillus fermentum* CCM 7421 was confirmed by a significant reduction in the numbers of oocysts of *Eimeria* spp. found in poultry after 32 hours of treatment (STROMPFOVÁ et al., 2010). The administration of Primalac, a commercially available *Lactobacillus*-based preparation, to hens from birth to three weeks of age stimulates intestinal intraepithelial lymphocytes and significantly de-

creases (by up to 75 %) the number of *E. acervulina* oocysts in the birds (DALLOUL et al., 2003).

General and anti-coccidial effect of plant extracts as feed supplements for rabbits and other farm animals

Our use of the "Xtract" preparation containing oregano compounds to supplement feed gave a significant reduction ($p<0.001$) on coccidia shed in the faeces of rabbits after the weaning period on days 21 and 42. Likewise, feeding of oregano and sage, another species from the *Lamiaceae* family, caused a reduction in numbers of oocysts excreted ($p<0.001$). Similar results in another study suggest that extracts of natural origin can be used as natural alternatives to coccidiostats in rabbit feed (KOWALSKA et al., 2012). Pure or mixed extracts from *Laminaceae* and *Amaryllidaceae* also have anti-coccidial effects. Two simultaneous experiments on a breeding farm for New Zealand White rabbits *(Oryctolagus cuniculus f. domestica)* to determine the feasibility of replacing artificial coccidiostats with garlic and oregano preparations showed a positive influence of the additives on the degree and course of coccidia infection, which helped maintain a good level of animal productivity, and suggested that these herbal extracts may be of value in coccidiosis prophylaxis (NOSAL et al., 2014). THANGARASU et al. (2016) reported an *in vitro* antimicrobial effect and an inhibitory effect against *E. tenella* sporulation of the garlic compounds sulfur, allicin, dialyl sulfide and allylcysteine. Experiments with the commercially produced Bell Gold and Bell Premium preparations (Bellako Ltd., Zabrze, Poland) containing biologically active compounds from garlic and oregano have confirmed the coccidiocidal effect of plant extracts administered to rabbits during the weaning period (the period in which rabbits are most affected by eimeriosis) (NOSAL et al., 2014). Based on production indicators and mortality status, the commercial preparation Emanox PMX (containing plant extracts from oregano, thyme, mint, rosemary, marjoram and garlic) can achieve results against coccidiosis that are comparable to those of the chemically based preparation Sulfacox (FIK et al., 2015). For oregano extracts, MAJOR et al. (2011) found an immunomodulatory effect in chicken infected with *Eimeria acervulina* and a beneficial effect on mucus production in the small intestine *(jejunum)*.

Extracts from plants of other families also show more or less pronounced anti-coccidial effects. CERVANTES-VALENCIA et al. (2015) evaluated the efficacy of an aqueous extract of curcumin *(Curcuma longa)* on the excretion of *Eimeria* spp. oocysts in New Zealand White rabbits, concluding that *C. longa* at a dose of 40 mg/kg of body weight decreased the excretion of *Eimeria* spp. oocysts from 63.7 % to 80.1 %, reducing the concentration of oocysts by about 15.6 to 20.1 per gram of faeces. A comparative study in the Mashonaland Central district of Zimbabwe on the effect of banana roots *(Musa paradisiaca)* and sulphadimidine sodium on tame rabbits infected with coccidiosis found a significant decrease in oocyst output in both groups ($p<0.05$), although the banana root was slightly more effective at reducing coccidiosis (MATEKAIRE et al., 2005). Rabbit farmers in Kenya apply *Aloe vera* and liquid paraffin to treat rabbit coccidiosis with varying efficacy and benefit (OGOLLA et al., 2017). The antiparasitic effects of various doses of an aqueous extract of *Curcuma longa* on the intestinal form of coccidiosis in New Zealand White rabbits have been confirmed through the reduced secretion of *Eimeria* oocysts (CERVANTES-VALENCIA et al., 2015). Zinc oxide nanoparticles, as chemical therapy, and propolis nanoparticles, as natural therapy, also have a protective role against infestation with *Eimeria* spp. in rabbits (ABD EL MEGID et al., 2018). *Artemisia annua* powder at a dose of 5 g/kg diet gave good results in terms of growth performance and coccidiosis prevention (ABOUSEKKEN et al., 2015).

Selected plant extracts applied during our trial showed similar effects. All types of extract used in our experiment (sage, oregano, "Xtract") led to an increase in feed conversion ratio at the end of the experiment, although the increase was in no case significant.

Conclusions

We suggest that the reduction in oocyst numbers observed in rabbits after the critical period of weaning might have been caused by a positive influence of the natural substances, including the commercially produced feed additive "Xtract" and the probiotic *E. faecium* strain CCM 4231. Their application decreased the numbers of excreted oocysts and thus the environmental contamination with these parasitic stages. It is noteworthy that we recorded effects not only during the application of various natural substances, including "Xtract", but also after cessation of their application. Nevertheless, we found no positive effect on weight gain or feed conversion, possibly because the experiment involved a very moderate infection with coccidian species that were probably non- or less pathogenic. No change in the consistency of the faeces (diarrhoea) was registered in the control group either. There is a need for further research, particularly with artificial infections and with pathogenic coccidial strains, on the effects of these natural additives, to understand how they work in animals and to achieve greater effectiveness in rabbit health and growth performance.

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Fazit für die Praxis:
Durch die Zufütterung von Pflanzenextrakten, Probiotika und probiotischen Produkten konnte die Ausscheidung von *Eimeria* Oozysten bei Mastkaninchen verringert werden. Dieser Effekt war auch nach dem Absetzen des Futterzusatzes nachweisbar. Die Zufütterung natürlicher Substanzen kann helfen, den Medikamenteneinsatz in der Kaninchenmast zu verringern.

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