Acta fytotechn zootechn, 25, 2022(3): 194–199
http://www.acta.fapz.uniag.sk

Original Paper

The effects of dietary supplementation of common mugwort (*Artemisia vulgaris*) meal on growth and excreted *Eimeria* spp. oocysts incidence in Dwarf Lop rabbit kits

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Article Details: Received: 2022-03-03 | Accepted: 2022-05-18 | Available online: 2022-09-30

https://doi.org/10.15414/afz.2022.25.03.194-199

The goal of the study was to evaluate the effect of dietary inclusion of common mugwort (*Artemisia vulgaris*) herb on selected growth and health traits of young dwarf rabbits. The mugwort species represent regular botanical species with proven favourable effects on health state of many animal species. However, a dietary effect of *Artemisia vulgaris* (AV) on health of dwarf rabbit genepool has not been studied yet. The study was performed on a total of 12 kits of the Dwarf Lop breed. These kits were divided into 2 groups (control, C; experimental, E). From the age of 21 days up to the age of 112 days the kits in the experimental group received a complete diet specifically designed for the dwarf rabbits with 10% supplementation of the AV meal. There was found no significant adverse effect on live weight and average daily weight gain in rabbits of the E group. Concerning the anticoccidial effect, the 112-day-old rabbits fed the E diet showed a significantly lower (P <0.05) number of excreted oocysts (1766 OPG) as compared to those in the C group (6845 OPG). Based on the results of the present study, there can be suggested that 10% dietary inclusion of the AV meal represents a safe share and it showed a positive effect on the excreted coccidiosis count in dwarf rabbits. However, it would be suitable to perform further studies, when the optimization of a mugwort dietary level during specific life periods of dwarf kits and the selection of other *Artemisia* botanical species will take a place. In addition, monitoring of more growth traits and biological effects of particular substances contained in *Artemisia* sp. would be studied on dwarf pet rabbits as wel.

Keywords: pet rabbit, diet, coccidian infection, *Artemisia vulgaris*

1 Introduction

Rabbits including companion pets have a specific requirement for housing, nutrition and handling (Rooney et al., 2014; Bradbury and Dickenes, 2016). These requirements and good health are basic assumptions for their normal behaviour and welfare (Rioja-Lang et al., 2019). But some stages in the life of rabbits can be stressful and weaning technique influence rate of weaning distress. Kits during weaning are exposed changes of feed and its environment and during this period, young rabbits are susceptible to various diseases, including coccidiosis (Kudělková and Šimek, 2021). Previous studies showed, that coccidiosis is a major endoparasitic disease in small scale rabbit stock and also in pet rabbits (Mäkitaipale et al., 2017). Infection is caused by protozoa of the genus *Eimeria* (Apicomplexa, Eimeridae). There are least 14 species of *Eimeria*, which affect the rabbits and vary in pathogenicity (Varga, 2014). Numerous *Eimeria* infect rabbits´ intestines and *Eimeria stiedae* invades rabbit liver (Pakandl, 2009). Acording to Varga (2014) overcrowding and poor hygiene are predisposing factors of gastrointestinal disease in rabbits. Rabbits are infected by ingestion of sporulated oocysts from environment, while suckling rabbits are not infected before age of 20 days (Pakandl and Hlásková, 2007). One of the most applied methods of prevention is the incorporation of anticoccidial substances into rabbit feed (or water), whereas synthetic anticoccidial drugs are main products used in the treatment of this infection (Hrženjak et al., 2021). However, frequent and

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long-term use of these drugs has led to the development of resistance. In addition, they have some disadvantages, such as the negative impact on environment and toxicity risk for hosts (Pakandl, 2009). Therefore is important search new alternatives for prevention and treatment of coccidiosis which are safer for animals and more environmental friendly. According to the toxicological findings, Bisht et al. (2021) state that the *Artemisia* species plants administered at low dose and short duration show non or low toxic effects. In connection with this issue chosen medicinal plants with anticoccidial and antioxidant potential can be considered as alternative feed components (Numerous et al., 2017).

Artemisia vulgaris (common mugwort) is native to temperate Europe and in the Czech Republic is mugwort a typical rum plant (Duke et al., 2002; Jursík et al., 2008). The plants of the genus artemisia have a different biological effect (Bisht et al., 2021). Common mugwort is a species with great importance in the history of medicine and was called as the "mother of herbs" in the Middle Ages (Ekiert et al., 2020). Mugwort plant contains essential oils (such as cineole, or wormwood oil, and thujone), flavonoids, triterpenes, and coumarin derivatives (Neelamma et al., 2016). In traditional herbal medicine parts of *Artemisia vulgaris* are being used as anti-helmint, antiseptic or antispasmodic (Duke et al., 2002). Moreover, study of El-Tantawy (2015) showed that *Artemisia vulgaris* extract has hypolipidemic, anti-inflammatory and antioxidant properties in hypercholesterolemic rats. Anticcocidial potencial of *Artemisia vulgaris* was studied as a feed supplement in diet for rabbit meat production (Popović et al., 2017). Although pet rabbits represent a very popular group, no studies evaluating the anticcocidial effect of *Artemisia vulgaris* in Dwarf Lop rabbits in common pet stocks were performed yet.

2 Material and methods

2.1 Animals, husbandry conditions

The study was performed on a total of 12 kits of the Dwarf Lop rabbit breed. The rabbits came from a common pet stock, which focuses on exhibition activity. The rabbits were housed in an outdoor hutch (pen size 70 × 70 × 60 cm), roofed for protection against unfavourable weather conditions. The pens were equipped with the plastic slatted floor and nest boxes with solid floor. All rabbits were housed and treated under identical conditions. The rabbit does and their kits at the age of 10 weeks were vaccinated against rabbit haemorrhagic disease (Castorex, Pharmagal, Slovak Republic) and myxomatosis (MXT, Dyntec, Czech Republic). The young kits were housed with the breeding does ($n = 4$) up to the age of 49 days. After weaning, from 56th day the young rabbits were housed individually. The health status of the rabbits was monitored once daily.

2.2 Nutrition and study design

Regarding the feed management, a total of 2 types of the pelleted complete feed mixture were used in the present study. The formulation of these diets was based on our previous work dealth with testing of diets intended for dwarf rabbit nutrition (Šimek et al., 2018). The ingredient composition of the basal diet (C group) is presented in Table 1. The experimental diet (E group) represented the basal diet and the 10% addition of fine-grounded dried common mugwort (*Artemisia vulgaris*, AV). The whole herb at the flowering phase was used for preparation of the AV meal. The chemical composition of the used diets is presented in Table 2.

Table 1 Ingredient composition of the basal diet used for the dwarf rabbit kits (as fed basis)

Item	Units	Content
Alfalfa meal	g/kg	300.0
Wheat bran	g/kg	200.0
Lupin seed	g/kg	200.0
Barley straw	g/kg	100.0
Sugar beet pulp	g/kg	65.0
Oat	g/kg	40.0
Barley	g/kg	30.0
Molasses	g/kg	30.0
Monocalcium phosphate	g/kg	15.0
Calcium carbonate	g/kg	10.0
Sodium chloride	g/kg	5.0
Mineral premix	g/kg	5.0

The pelleted diets were analysed in the laboratory of the Department of Animal Nutrition. As for chemical composition of the diets, we determined the content of crude protein, starch, crude fibre, acid-detergent fibre (ADF), neutral-detergent fibre (NDF), acid-detergent lignin (ADL), ether extract, gross energy, ash and selected minerals. Crude protein was determined by the Kjeldahl method using a Buchi analyser (Centec Automatika, Czech Republic). Starch was determined using the P3002RS automatic digital polarimeter (Kruss, Germany). Crude fibre, ADF, NDF and ADL were determined using the ANKOM 220 fibre analyser (O.K. Servis BioPro, Czech Republic). Ether extract was determined using the Soxhlet method. Gross energy was determined using the AC500 Calorimeter (LECO, s.r.o., Czech Republic). Digestible energy was calculated according to Villamide

Table 2 Chemical composition of the diets used for the dwarf rabbit kits (in dry matter)

Item	Units	Basal diet	Basal diet +10% of AV meal
Dry matter	g/kg	1000.0	1000.0
Crude protein	g/kg	167.8	170.9
Crude fibre	g/kg	198.9	220.8
ADF	g/kg	253.5	260.8
NDF	g/kg	402.3	426.0
ADL	g/kg	39.8	42.7
Crude fat	g/kg	42.4	37.9
Crude starch	g/kg	135.9	103.4
Ash	g/kg	85.5	87.9
Calcium	g/kg	10.85	11.55
Inorganic phosphorus	g/kg	8.7	7.3
Sodium	g/kg	2.3	1.8
Potassium	g/kg	12.8	14.8
Magnesium	g/kg	2.1	2.18
Copper	g/kg	10.3	10.8
Iron	mg/kg	453.1	448.3
Manganese	mg/kg	148.3	142.3
Zinc	mg/kg	77.8	81.3
DE	MJ/kg	11.3	10.8

ADF – acid detergent fibre, NDF – neutral detergent fibre, ADL – acid detergent lignin, DE – digestible energy

et al. (2009). Ash was determined by weighing the sample after incineration at 550 °C under prescribed conditions. The calcium, sodium, copper, zinc and manganese contents were determined using the 240 AA atomic absorption spectrometer (Agilent Technologies, USA). The total phosphorus content was determined spectrophotometrically. An acidic solution of ash was treated with molybdovanadate reagent and the absorbance of the solution was measured at 430 nm (Helios Alpha UV-Vis spectrophotometer, Thermo Fisher Scientific Inc.).

The particular complete diet was offered to the breeding does one week prior to the expected parturition. The young kits received the diets up to the age of 112 days. Both of the diets C and E were offered to the rabbits at the daily dose of 25–30 g/kg of body weight according to recommendation stated by Lowe (2010). The rabbit received the pelleted diet once a day. Moreover, the meadow hay was offered to the rabbits three times weekly. Unlimited access to drinking water was provided.

The subdivision of the young kits into the C and E group was performed on the basis of coprological oocyst poer gram (OPG) examination of the breeding does. The faecal samples of the breeding does were collected and analysed 7th day after the parturition. Because the breeding does showed various OPG incidention of the oocysts, the kits of breeding does with higher OPG values results were included in the experimental E group with the assumption of better assessment of intrinsic anticcocial effect of dietary administered AV for pet rabbits. The dietary effect on the rabbits´ growth intensity was recorded. The live weight (LW) of the monitored rabbit kits was recorded and the age of 32, 56, 84 and 112 days. Subsequently, the average daily weight gains (ADG) were calculated.

The faecal samples of the kits were individually collected from the hutches of respective rabbits into a plastic packet sampler at the age of 32, 56, 84 and 112 days. McMaster counting technique was used to analyze the faecal samples. Four grams of the feacal matter was mixed with 56 ml of flotation Breza solution ($MgSO_4$ and NaS_2O_3). Then the mixture was sieved trough a metallic tea strainer (mesh size 0.9 mm) into a plastic tube. After that was used McMaster counting slide with two chambers. The chambers with feacal mixtures were examined under a light microscop at 100× magnification. Oocysts in both chambers were counted and then were recalculated according to Mäkitaipale et al. (2017); each of the oocyst counted in chambers represents the 25 *Eimeria* oocysts per gram of assessed faeces. The *Eimeria* species determination was not performed in the present study.

2.3 Statistical analysis

The obtained data were analysed using unpaired *t*-test in the statistical program Unistat for Excel 6.5. First, was used f-test to detect variances between two files. After that was significance determined by *t* test considered at $^*P < 0.05$; $^{**}P < 0.01$.

3 Results and discussion

The results of the use of the assessed diets on the selected growth traits of the dwarf rabbit kits are presented in Table 3.

Inclusion of the mugwort into diet used for the dwarf rabbit kits had no significant effect on their live weight and average daily weight gain. Average values of the kits´ live weight in the C as well as E group meet with the standard values for this genetics published in Rabbit Breed Standards Book (Šimek et al., 2020). The live weight of the suckled kits, as a principle health assessment factor, can be affected by many factors, including also the properly performed litter standardization (Zapletal et al., 2021). More specifically, at the end of the experimental period, the kits which received the experimental diet showed

Table 3 Live weight (LW) and average daily weight gain (ADG) of the dwarf rabbit kits in relation to the diet

Item (units)	Dietary group				Significance
	C		E		
	x	SEM	*x*	SEM	
LW at 32 days (g)	226.2	12.32	245.0	24.77	n.s.
LW at 56 days (g)	377.5	24.25	375.3	20.92	n.s.
LW at 84 days (g)	480.8	19.68	450.0	40.32	n.s.
LW at 112 days (g)	706.5	16.78	672.8	29.43	n.s.
ADG day 1 to day 32 (g/day)	7.07	0.385	7.66	0.774	n.s.
ADG day 33 to day 56 (g/day)	6.31	0.861	4.68	0.365	n.s.
ADG day 57 to day 84 (g/day)	3.70	0.476	3.31	1.913	n.s.
ADG day 85 to day 112 (g/day)	8.06	0.427	7.96	2.288	n.s.

C – control group fed basal diet, E – experimental group fed basal diet supplemented with 10% of *Artemisia vulgaris* meal, ns – not significant

non-significantly lower LW (P >0.05) as compared to the kits from the control group (+33.7 g). This finding can be caused by e.g. specific odour or taste of the experimental diet due to generally bitter tasting substances contained in meal of the common mugwort (Pandey et al., 2017). Thus, there can be suggested to use a slightly lower share of *Artemisia* spp. meal in intended further studies and to record also the feed intake.

Concerning the dietary use of the *Artemisia* sp. herbs for the rabbit nutrition, Popović et al. (2017) found a significantly higher LW in the New Zealand White rabbits after 10 weeks of feeding diets with 10% and 20% inclusion of dried common wormwood (*Artemisia absinthum*). Liu et al. (2019) observed that supplementation of the rabbit diet with species *Artemisia argyi* had no significant effects on the growth characteristics of the young growing hybrid HYLA rabbits but this diet significantly reduced diarrhoea signs and stimulated intestinal immune system. Also, Wang et al. (2019) recorded a significantly higher LW values in the fattened Rex rabbits when dietary supplementation of the mugwort meal ranged between 3 to 9%. Moreover, authors of the above-mentioned study found beneficial effects of *Artemisia vulgaris* supplementation on gut microflora, when *Lactobacili* and *Bifidobacteria* spp. increased in their concentrations.

The chemical composition of the both diets used in the present study was in concordance with nutritional recommendation (Lowe, 2010; Proença and Mayer, 2014). The supplementation of our basal rabbit diet with 10% of *Artemisia vulgaris* meal resulted in mild increase of the crude fibre and considerable decrease of the starch content. With respect to digestive disorders in young rabbit kits, simultaneously the higher crude fibre and lower starch content can be considered as effective dietary prevention (Carraro et al., 2007; Gidenne, 2015). Abousekken et al. (2015) studied *Artemisia annua* effects on growth characteristics, *Eimeria* ssp. oocysts excretion and gut microflora in the growing medium-sized New Zealand White breed. The authors found the administration route of the *Artemisia annua* has a great impact on the production performance traits as well as the rabbits´ health. The best results in coccidiosis controlling were achieved with the wormwood extract oral administration at the rate of 2.5 ml/rabbit for 3 days. Concerning the use of the *Artemisia annua* meal in the rabbit diet, authors of the above-mentioned study recommends that supplementation rate of 5% can be considered as the most effective as compared to the 10% supplementation rate.

Results of use of the complete diets on *Eimeria* spp. oocysts excretion in the dwarf rabbit kits at the age of 32, 56, 84 and 112 days are presented in Table 4.

At the first faeces sampling (32-day old kits), there was found a significant difference in the excreted oocysts in kits between the group C and E (P <0.05); this finding was caused by the different infection status of the rabbit does. Initially, at day 7 *post partum*, does of the group C excreted average OPG count at the value of 479, while the does of the group E excreted a higher oocyst count (2910 OPG). When sampling the rabbits´ faeces at the age of 56 and 84 days, there was no significant difference in the OPG count between the monitored groups (P >0.05). At the end of the experimental period (112-day old rabbits), rabbits of the group E showed significantly lower OPG count as compared to those in the C group (P <0.01); this decrease can be attributed to the anticoccidial effect of medicinal plant *Artemisia vulgaris* included in the experimental pelleted diet.

Pilarczyk et al. (2020) reported age effect of rabbits on the *Eimeria* infection. In generally it can be stated that rabbit

Table 4 Dietary effect of the 10% supplementation of the *Artemisia vulgaris* meal on the excretion of coccidial oocysts (OPG) in the dwarf rabbit kits

Sampling no.	Dietary group		Significance
	C	E	
1 (age 32 days)	10216	27712	*
2 (age 56 days)	23445	32533	ns
3 (age 84 days)	8775	6178	ns
4 (age 112 days)	6845	1766	**

C – control group fed basal diet, E – experimental group fed basal diet supplemented with 10% *Artemisia vulgaris* meal

at the age of 1 to 3 months are most susceptible to the disease (Pakandl et Hlásková, 2007; Papeschi et al., 2013).

In general, although the coccidiosis seriously impairs the rabbit growth traits (El-Akabawy et al., 2004), the infection level found in the present study doesn't affect clinical heath state of rabbit kits in both of the monitored groups. Zita et al. (2007) found that hybrid meat-type Hyplus rabbit kits weaned at the of 35 day showed a higher *Eimeria* spp. oocyst incidence as compared to the early-weaned rabbit kits, probably due to the prolonged period of mutual housing with their dams. However, the usual weaning age for broiler rabbits and for dwarf hobby rabbits considerably differ. The weaning age of the dwarf rabbit kits is typically prolonged and resemble an extensive broiler rabbit farming way where the weaning age up to 6 weeks take a place (Szendrö et al., 2012). Because of prevention of certain behavioural and health problems, there is recommended to wean the pet rabbit kits around 8 weeks of their age (Gendron, 2000). Generally, earlier weaning and separation of the doe increase susceptibility to enteral diseases in the pet rabbit kits (Varga, 2014).

With respect to the specific husbandry and nutrition requirements of the dwarf rabbit genepool, there can be recommended to use specific herb supplementation in the complete pelleted diet intended for these kits to reduce *Eimeria* incidence.

4 Conclusions

Proper feeding techniques, especially in weaning period, represents possible ways how to prevent some selected digestive problems in the rabbit kits. This study brings preliminary findings concerning the dietary use of the *Artemisia vulgaris* meal, for the first time used in the complete pelleted diet designed specifically for the nutrition of dwarf rabbit kits. Based on the results of the present study, there can be suggested that 10% dietary inclusion of the *Artemisia vulgaris* meal represents a safe share and it showed a positive effect on the excreted coccidiosis count in dwarf rabbit kits. The experimental pelleted diet with 10% share of the *Artemisia vulgaris* meal can be used for nutrition of the young dwarf rabbit kits as the first diet. However, it would be suitable to perform further studies, when the optimization of a mugwort dietary level during specific life periods of dwarf kits and the selection of other *Artemisia* botanical species will take a place. In addition, monitoring of more growth traits and biological effects of particular substances contained in *Artemisia* sp. would be studied on dwarf pet rabbits as well.

Acknowledgments

The study was supported by institutional resources of Department of Animal Breeding, Animal Nutrition and Biochemistry and Department of Animal Protection and Welfare and Veterinary Public Health, Faculty of Veterinary Hygiene and Ecology, University of Veterinary Sciences Brno, Czech Republic.

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