World Rabbit Science

World Rabbit Sci. 2016, 24: 95-105
doi:10.4995/wrs.2016.4069

DIETARY SUPPLEMENTATION OF DIGESTAROM® HERBAL FORMULATION: EFFECT ON APPARENT DIGESTIBILITY, FAECAL AND CAECAL MICROBIAL COUNTS AND LIVE PERFORMANCE OF GROWING RABBITS

CELIA C.*†, CULLERE M.†, GERENCSÉR ZS.*, MATICS ZS.*, GIACCONE V.†, KOVÁCS M.*, BÓNAI A.*, SZENDRŐ ZS.*, DALLE ZOTTE A.†

*Faculty of Agricultural and Environmental Sciences, Kaposvár University, 40, Guba S. str., H-7400, Kaposvár, Hungary.
†Department of Animal Medicine, Production and Health, University of Padova, Agripolis, Viale dell'Università, 16, 35020 Legnaro, Padova, Italy.

Abstract: The experiment aimed to study the effect of Digestarom® dietary inclusion (herbal formulation containing a mixture of essential oils, herbs, spices and extracts) on apparent digestibility and digestive ecosystem of growing rabbits, as well as the effects of its supplementation before and after weaning on growth performance. At kindling, rabbit does and litters were divided into 2 dietary groups (51 does/group) and fed either a control diet (C) or a diet supplemented with 300 mg Digestarom®/kg diet (D) until weaning, which occurred at 35 d (before weaning supplementation). Each group was further divided into 3 dietary groups: CC received the control diet and DD received the D diet from 5 to 12 wk of age, and DC were fed with D (from 5 to 8 wk of age) and C diets (from 8 to 12 wk of age) (after weaning supplementation; 54 kits/group). An *in vivo* digestibility trial and a faecal microbial count were carried out on growing rabbits that received only the C or D diets during the trial. The C group showed higher DM intake than D group (215 *vs*. 196 g/d; $P<0.05$). The faecal digestibility of ether extract (75.9 *vs.* 59.8%; $P<0.001$), cellulose (25.9 *vs*. 20.6%; $P<0.05$) and gross energy (51.8 *vs*. 49.1%; $P<0.05$) was higher for C than for D group, whereas that of starch (98.9 *vs.* 98.8%; $P<0.001$) and the digestible protein to digestible energy ratio (13.9 *vs.* 13.2 g digestible protein/MJ digestible energy; $P<0.01$) was the highest for rabbits fed D diet. Stomach and caecal pH, caecal and faecal microbial counts were independent of the dietary treatment. The only exception was the stomach pH in 8 wk-old rabbits, which had the lowest value in C rabbits ($P<0.05$). The D supplementation before weaning improved feed conversion ratio throughout the growing phase (4.3 *vs.* 4.4 for D and C, respectively; $P<0.05$), whereas significant differences in daily weight gain, feed conversion ratio and mortality were observed only in the first period after weaning. Based on the results obtained, dietary supplementation with Digestarom® does not seem to confirm the positive results previously reported for growing rabbits.

Key Words: rabbit, Digestarom®, faecal digestibility, microbial count, performance.

INTRODUCTION

Digestarom® 1315 is a herbal formulation designed as rabbit feed supplement, consisting of a mixture of essential oils, herbs, spices and extracts of 10 different ingredients (Colin *et al.,* 2008): onion (*Allium cepa* L.*)*, garlic (*Allium sativum* L.), caraway (*Carum carvi* L.), fennel (*Foenicum vulgarae* L.), gentian (*Gentiana lutea* L.), melissa (*Melissa officinalis* L.), mint (*Mentha arvensis* L.), anise (*Pimpinella anisum* L.), oak bark (*Quercus cortex*) and clove (*Syzygium aromaticum* L.).

Studies on single plant extracts constituting Digestarom® feed supplement have reported several positive effects on animal health and live performance. Dehydrated onion, at 5 or 10% inclusion level, showed cholesterol-lowering and antioxidant effects in hyper-cholesterolemic experimental rats (Vidyavati *et al.,* 2010). Histological and biochemical

Correspondence: Zs. Szendrő, szendro.zsolt@ke.hu. Received *August 2015* - Accepted *February 2016.*
doi:10.4995/wrs.2016.4069

studies using suitable dosage of garlic according to the body weight found positive effects of different in-feed garlic extracts on hepatic coccidiosis in infected rabbits (Toulah and Al-Rawi, 2007), as well as cholesterol levels of blood and oxidative status of the hepatic tissue in cholesterol-fed rabbits, treated with 1,5 mL/kg day of garlic extract for 3 mo (Arhan *et al.,* 2009). In addition, dietary fermented garlic demonstrated a beneficial effect on the immune response during an inflammatory challenge in growing pigs, reporting a linear immune response as fermented garlic increased from 1, 2 to 4 g/kg (Wang *et al.,* 2011). Garlic meal positively affected intestinal mucosal morphology of broiler chickens when supplemented at 1 or 2% inclusion level, thus potentially improving nutrients absorption (Adibmoradi *et al.,* 2006). A simultaneous supplementation of garlic (50 g granulate powder) and aniseed (25 g) was reported to improve feed intake in post-weaned piglets (Langendijk *et al.,* 2007).

In growing rabbits, mortality was reduced with the supplementation of 0.05% essential oil of fennel and thyme (Benlemlih *et al.,* 2014). Furthermore, a diet supplemented with 0.5% fennel seed increased the digestibility of organic matter, crude fibre and ether extract, and final weight and body weight gain improved (Omer *et al.,* 2013).

An essential oil of *Melissa officinalis* contributed to a lipid-lowering action in cholesterol-fed rabbits (Karimi *et al.,* 2010), whereas a dietary addition of peppermint improved crude protein digestibility (Ibrahim *et al.,* 2000).

Oak bark is traditionally used for human consumption as a decoction and powder to treat gastrointestinal problems, such as diarrhoea, gastritis and ulcer, and 10 µL of extract impregnated in sterile discs showed antibacterial activity against reference strains *in vitro* (Berahou *et al.,* 2007).

Clove, caraway and gentian were reported to provide appetite-stimulant effect (Baytop, 1984; Loo and Richard, 1992; Wichtl, 1994).

Digestarom® feed additive was tested only in 3 experiments in rabbits, all considering an inclusion level of 300 mg Digestarom®/kg feed. Colin *et al.* (2008) found a reduction in mortality rate in a field trial with 19000 rabbits (13.4 *vs.* 14.2%; $P<0.01$), and Krieg *et al.* (2009) observed a positive effect on the performance and health of weaned rabbits for the 13 days observation period, whereas Abd El-Hady *et al.* (2013) observed an improvement in growth performance, some blood constituents and carcass characteristics of growing rabbits.

The present study aimed to investigate in depth the effects of dietary supplementation of Digestarom® on the total tract apparent digestibility, faecal and caecal microbial counts, live performance and health status of growing rabbits measured at different times during the growing period. For the first time, the effects of before and after weaning supplementation on the live performance of growing rabbits were considered. Reproductive performance scores of rabbit does were also evaluated, but results are presented elsewhere (Celia *et al.,* 2015).

MATERIAL AND METHODS

The study was approved by the Institutional Animal Welfare Committee as the animal-welfare body of the Kaposvár University. All animals were handled according to the principles stated in the EC Directive 86/60963/2010 EU regarding the protection of animals used for experimental and other scientific purposes.

Animals and experimental design

The experiment was carried out in the experimental farm of Kaposvár University. The animals derived from a previous part of the experiment which also aimed to evaluate the effect of dietary supplementation with Digestarom® on the reproductive performance of rabbit does (Celia *et al.,* 2015). At kindling, does and litters were divided in 2 dietary groups and fed with balanced pelleted diets (Table 1): the first group (51 does/group) received a commercial diet (group C), whereas the other one (52 does/group) was fed the same diet supplemented with 300 mg/kg of Digestarom® (group D). However, the litters were fed experimental diets from 21st d of life onward. This represented the Before Weaning phase (BW), described in a previous article (Celia *et al.,* 2015). At weaning (35 d), each group was further divided into 3 feeding groups: CC rabbits received the C diet and DD ones received the D diet from 5 to 12 wk of age. Differently, DC rabbits were fed with D and C diets from 5 to 8 wk of age and from 8 to 12 wk of age, respectively (Figure 1). This represented the After Weaning phase (AW). The experiment involved 372 growing rabbits of the Pannon breeding programme (Pannon Ka maternal line). Among them, 324 rabbits were used to evaluate the growth performance (54 rabbits/diet), whereas 48 rabbits were used

for gastrointestinal pH and caecal microbial count analyses. From the 48 rabbits, 12 were slaughtered at 5 wk of age (6 rabbits/diet), and 36 were reared separately, then 24 were slaughtered at 8 wk of age (6 rabbits/diet). Remaining rabbits were not considered for the study. The kits were housed in wire-mesh cages (3 rabbits/cage, size of cage: 61×32×30 cm). The temperature and the photoperiod were 15-18°C and 16 h light:8 h dark, respectively.

Performance data collection and management

Body weight of rabbits was measured at 5, 8 and 12 wk of age, feed intake for 5-8 and 8-12 wk periods was recorded and the daily weight gain and feed conversion ratio were then calculated. Body weight and daily weight gain were evaluated based on individual data, whereas feed intake and feed conversion ratio were based on the cage unit. When calculating feed intake, it was assumed that morbid rabbits did not consume any pellets for the 2 d preceding their death. Mortality was recorded daily.

pH of the stomach and caecal content and caecal microbial count

From 13:00 to 14:00 h six healthy rabbits per experimental group were slaughtered at 5 (6 C and 6 D) and at 8 wk of age (6 C-C, 6 C-D, 6 D-C, 6 D-D). The digestive tract of each animal was removed immediately and the stomach, small intestine and caecum were separated. The pH values of the stomach and caecal contents were determined using an OP-110, Radelkis pH-meter (Hungary).

Table 1: Chemical composition and mineral profile of the experimental diets (g/kg as fed).

	Experimental diets	
	C	D
Dry matter	905	905
Ash	65	70
Acid insoluble ash	0.9	0.2
Crude protein	158	158
Ether extract	30	30
Starch	123	129
Crude fibre	181	165
Neutral detergent fibre	466	448
Acid detergent fibre	231	223
Acid detergent lignin	60	58
K	7.21	7.33
P	5.93	6.16
Ca	5.77	6.21
Mg	2.55	2.62
Na	1.02	1.44
S	0.59	0.57
Fe	0.09	0.09
Zn	0.08	0.07
Ca/P	0.97	1.01
Gross energy (MJ/kg)	16.64	16.50

C: control diet; D: C diet supplemented with 300 mg Digestarom®/kg.

From the 1 g sample taken from the caecal digesta of each rabbit (serial dilutions were made: 1 g caecal sample+9 mL diluent [0.9% NaCl]), and used for microbiological determination. Anaerobic conditions were ensured by the use of carbon dioxide.

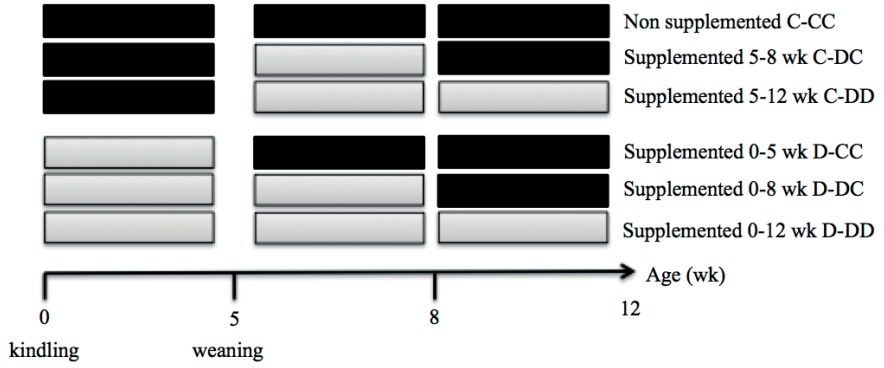

Figure 1: Experimental design (n=54 rabbits/treatment). ▒ Diet D, supplemented with 300 mg Digestarom®. ■ Diet C.

The obligate anaerobe microorganisms 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 iron ammonium citrate (Sharlan Chemie, Barcelona, Spain). Gamma sterile Petri dishes (Biolab, Budapest) were placed into Anaerocult culture system (Merck, Darmstadt, Germany), in which the anaerobic conditions were ensured by an "Anaerocult A" (Merck, Darmstadt, Germany) gas-producing bag. Subsequently, the samples were incubated in an LP 104 type thermostat (LMIM, Esztergom, Hungary) at 37°C for 96 h.

Total aerobic bacteria were cultured on media supplemented with 5% calf blood. The samples were incubated at 37°C for 72 h. *E. coli* and other *coliform bacteria* were 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 according to standard methodology (ISO 4833:2003) with Acolyte colony counter (Aqua-Terra Lab, Veszprem). The colony counts were expressed in $\log_{10}$ colony-forming units (CFU) related to 1 g of sample.

Digestibility trial

An *in vivo* digestibility trial was carried out according to the European standardised method (Perez *et al.,* 1995). To this end, twenty 50 d-old growing rabbits were used to determine the total tract apparent digestibility (TTAD) of C and D diets (10 rabbits/diet). These rabbits received the C or D diets during the digestibility trial, only. Animals were equally distributed by gender and live weight (average live weight of 1478±142 g) into the 2 dietary groups and individually caged. After 1 wk of adaptation to the new diets, faeces were collected for a 4-d period. Morbid and/or dead rabbits were excluded from the trial; they were not replaced and not considered in the statistical analysis.

The TTAD of dry matter (DM), organic matter, crude protein, ether extract, starch, neutral detergent fibre, acid detergent fibre, cellulose, hemicelluloses and gross energy of the experimental diets (C and D) was measured.

The day after the end of the digestibility trial, the rabbits continued to be fed the same experimental diets. Samples of hard faeces were collected from each animal and immediately submitted to the quantitative determination of coliforms, lactic acid bacteria and spore-forming aerobes (*Bacillus* spp.). Coliforms were counted using the same procedure previously reported for caecal content. The lactic acid bacteria load was measured by plating on MRS agar (Scharlan Chemie, Barcelona, Spain) after anaerobic incubation at 37°C for 48 h. The count of spore-forming *Bacillus* spp. was determined by plating on Bacillus Selective Agar (Oxoid LTD, Basingstoke, Hampshire, England) after aerobic incubation at 37°C for 24 h. Colony counts were expressed in $\log_{10}$ CFU related to 1 g of sample.

Chemical analyses

Chemical composition of the experimental diets and faeces was analysed in duplicate by AOAC (2000) methods to determine the concentrations of dry matter (Method no. 934.01), crude protein (Method no. 2001.11), crude fibre (Method no. 978.10) and ash (Method no. 996.11). Ether extract was determined after acid-hydrolysis (EC, 1998). Neutral detergent fibre (NDF without sodium sulphite), acid detergent fibre (ADF) and acid detergent lignin (ADL) were analysed according to Mertens (2002), AOAC (2000, Method no. 973.187), and Van Soest *et al.* (1991), respectively, using the sequential procedure and filter bag system (Ankom Technology, New York). The gross energy (GE) was measured with an adiabatic bomb calorimeter (ISO, 1998). The mineral profile (Ca, P, K, Mg, Na, S, Fe, Zn) of the experimental diets was analysed by ICP-OES (Spectro Ciros Vision EOP) after microwave digestion (AOAC 2000, 999.10).

Statistical analysis

Digestibility data, faecal microbial count during the digestibility trial and caecal microbial count of rabbits at 5 wk of age were analysed by one-way ANOVA of the GLM procedure of the Statistical Analysis System (SAS Institute, 2004). Experimental diets (C, D) were considered as fixed effect. Live performance and caecal microbial count of rabbits at 8 wk of age data were subjected to another ANOVA in which a PROC MIXED procedure tested the effect of dietary supplementation before weaning (BW), after weaning (AW) and their interaction (BW x AW) on the studied variables. Microbial count data were also analysed by one-way ANOVA with age (5 and 8 wk of age) as fixed effect. Mortality

data were analysed by chi-square test according to the Marascuilo (1966) procedure. Post hoc pairwise contrasts were evaluated by Bonferroni adjustments.

RESULTS AND DISCUSSION

Digestibility trial and faecal microbial count

Dry matter intake (DM, g) as well as DM intake/live weight (g/kg LW) were higher in C compared to D rabbits ($P<0.05$. Table 2). The TTAD of cellulose was higher in C than D diet ($P<0.05$), as was that of ether extract ($P<0.001$), the latter explaining the better energy digestibility ($P<0.05$) and nutritive value of the C diet in terms of digestible energy (DE, MJ/kg; $P<0.05$). Conversely, digestible protein (DP) to DE ratio was in favour of D diet ($P<0.01$). Also starch TTAD was higher in D diet ($P<0.001$). The DE of both dietary treatments was in the normal range recommended for growing rabbits, but under 10-10.5 MJ/kg, which ensures maximum average daily growth (Xiccato and Trocino, 2010).

Results from this study found partial confirmation in the work considering the digestibility coefficients of 63 d-old Alexandria rabbits supplemented with 300 and 400 mg Digestarom®/kg of feed (Abd El-Hady *et al.,* 2013). In fact, crude fibre digestibility worsened as Digestarom® supplementation increased. However, organic matter digestibility was the best in supplemented animals, whereas no effect of the dietary treatment on this trait was observed in our experiment. In general, as a probable effect of age, TTAD scores in our experiment tended to be lower than those presented in the work of Abd El-Hady *et al.* (2013), especially when considering the DM (–27%, on av.).

The lower DM intake of D rabbits compared to C ones might be explained by the tannin-like substances included in Digestarom® which could have negatively influenced the palatability of the feed, as was observed in a study testing a dietary supplementation of a tannin extract derived from quebracho trees in growing rabbits, and in another one in which calves' diet was supplemented with a dry pomegranate extract (Dalle Zotte and Cossu, 2009; Oliveira *et al.,* 2010). In fact, tannins are known to form complexes with salivary glycoproteins generating the astringency sensation,

Table 2: Effect of Digestarom® dietary supplementation on total tract apparent digestibility (TTAD) of 50 d-old growing rabbits and nutritive value of diets.

	Experimental diets			
	C	D	MSE	Significance
n	5	7		
Live Weight (g)	2018	1976	72.1	NS
Dry Matter intake (g/d)	215	196	49.0	*
TTAD (%)				
DM	49.9	48.6	1.9	NS
Organic matter	50.5	48.6	1.9	NS
Crude protein	71.9	71.4	1.1	NS
Ether extract	75.9	59.8	1.4	***
Starch	98.8	98.9	0.04	***
Neutral detergent fibre (NDF)	28.6	26.7	2.7	NS
Acid detergent fibre (ADF)	16.6	13.9	3.2	NS
Cellulose (ADF-Acid detergent lignin)	25.9	20.6	2.9	*
Hemicelluloses (NDF-ADF)	40.3	39.4	2.3	NS
Gross energy	51.8	49.1	1.9	*
Nutritive value:				
Digestible protein (DP) (g/kg)	125.7	124.6	1.9	NS
Digestible energy (DE) (MJ/kg)	9.52	8.96	0.3	*
DP to DE ratio (g/MJ)	13.21	13.92	0.3	**

C: control diet; D: C diet supplemented with 300 mg Digestarom®/kg; MSE: mean squared error.
Levels of significance: *: $P<0.05$; **: $P<0.01$; ***: $P<0.001$; NS, non-significant.

Table 3: Effect of Digestarom® dietary supplementation on faecal microbial count during the digestibility trial.

	Experimental diets			
	C	D	MSE	Significance
n	5	7		
Coliforms, $\log_{10}$ CFU/g	7.34	8.06	1.02	NS
Lactic acid bacteria, $\log_{10}$ CFU/g	5.92	6.19	1.00	NS
Bacillus spp., $\log_{10}$ CFU/g	8.54	8.15	0.71	NS

C: control diet; D: C diet supplemented with 300 mg Digestarom®/kg; MSE: mean square error; NS: no significant.

thus reducing feed intake (Gidenne *et al.,* 1998). In contrast, chestnut hydrolysable tannins added as a supplement to growing rabbit diets did not impair the nutritive value of diets (Dalle Zotte *et al.,* 2012).

The negative effect of Digestarom® dietary supplementation on ether extract and cellulose TTAD could be explained by some constituents of plant polyphenols also present in Digestarom®, as they could have inhibited the activity of certain digestive enzymes. In fact, some polyphenol components can inhibit protease activity thus affecting protein digestion, whereas others can exert a lipase-inhibition activity, thus negatively affecting fat digestion (McDougall *et al.,* 2008). In this sense, when considering food producing animals such as rabbits, one of the most challenging aspects concerning natural feed additives is to find the optimum inclusion level that can guarantee satisfactory performance without impairing nutrient digestibility and absorption.

As a confirmation of this potential negative effect of specific components of plant polyphenols on the digestibility of nutrient fractions, Peiretti and Meineri (2008), Dalle Zotte *et al.* (2013) and Gerencsér *et al.* (2014) also observed a negative effect of different levels of spirulina and thyme dietary supplementation in growing rabbits on TTAD of DM, NDF, ADF, crude protein, starch, ether extract and minerals.

Table 3 depicts faecal microbial count of rabbits used for the digestibility trial and fed C or D diets. Even if no statistical differences were found between the 2 dietary groups, an unexpected situation was observed: the quantity of coliforms in the faeces was high in both treatments (7.34 and 8.06 $\log_{10}$ CFU/g for C and D faeces, respectively). The flow of caecal matter through the colon could have increased the specific charge of coliforms, leading to the high amount found.

Placha *et al.* (2013) observed that the dietary inclusion of 0.5 g/kg of thyme essential oil was able to limit the colonisation of coliforms in the caecum (<1.0 $\log_{10}$ CFU/g), compared to the control diet (2.4 $\log_{10}$ CFU/g); however, a higher coliforms content was found in faeces of rabbits fed with the thyme essential oil supplement (4.81 $\log_{10}$ CFU/g).

Lactic acid bacteria, which are not considered regular inhabitants of the digestive tract of rabbits by some authors (Gidenne and Fortun-Lamothe, 2002; Combes *et al.,* 2013), were also found in the faeces of both dietary treatments. However, they are reported to positively affect the health status of rabbits, as noted in a study in which *Lactobacillus plantarum* was sprayed on the litters (5 mL/rabbit) in the pre-weaning period (Bovera *et al.,* 2012). In our study, high counts of *Bacillus* spp. were found in rabbits fed both C and D diets. It should be noted that *Bacillus* spp. is a normal member of the rabbit intestinal microflora, as well as *Bacteroides* spp., and these high counts may have a positive effect on regular gut function because they are inducers of gut-associated lymphoid tissue development (Mage *et al.,* 2006; Hanson and Lanning, 2008; Carabaño *et al.,* 2010). High levels of lactic acid bacteria and *Bacillus* spp. could have played a role in preventing the mortality of rabbit after weaning (5-8 wk of age; Table 6).

Gastrointestinal pH and caecal microbial count of 5 and 8 wk-old rabbits

In 5 wk-old rabbits, a dietary supplementation with Digestarom® had no influence on stomach and caecal pH, total anaerobic and aerobic bacteria and counts of *E. coli*, Coliforms and *Bacteroides* (Table 4). An identical situation was observed in 8 wk-old rabbits in which the BW and AW supplementation with Digestarom® did not affect the studied traits (Table 5). The only exception was the pH of the stomach content of AW rabbits, which was higher in D than C dietary group (1.93 *vs.* 1.63, for D and C, respectively; $P<0.05$). However, these values were within the physiological range in accordance with the age (pH=1.5-2.0, Fortun-Lamothe and Gidenne, 2009).

Table 4: Effect of Digestarom® dietary supplementation before weaning on gastrointestinal pH, and caecal microbial count of rabbits at weaning (5 weeks of age).

	Experimental diets			
	C	D	MSE	Significance
n	6	6		
Body weight (g)	908	937	0.05	NS
pH of stomach content	1.38	1.46	0.30	NS
pH of caecal content	6.44	6.36	0.21	NS
Total aerobic bacteria[a]	5.51	5.54	0.58	NS
Total anaerobic bacteria[a]	9.58	9.31	0.22	NS
Escherichia coli[a]	3.44	3.77	1.87	NS
Coliforms[a]	1.90	1.97	0.12	NS
Bacteroides[a]	8.80	8.93	0.40	NS

C: control diet; D: C diet supplemented with 300 mg Digestarom®/kg; MSE: mean squared error; NS: non-significant.
[a]Germ counts expressed in $\log_{10}$ colony-forming units/g caecal content.

When Digestarom® dietary supplementation was tested in 41 d-old ZIKA® hybrid rabbits, reduced bacterial diversity in the caecum and increased relative abundance of the more dominant species compared to rabbits fed with a non-supplemented diet were observed (Krieg *et al.,* 2009). In addition, Abd-El-Hady (2014) also found that a dietary supplementation with Digestarom® reduced the caecal microbial count of total bacteria, as well as those of *Clostridium* spp. and *E. coli*. The latter study, however showed a higher count for *E. coli* (6.09 $\log_{10}$ CFU/mL caecal content for 63 d-old rabbits, on av.) compared to that in our study (*E. coli*: 3.21 $\log_{10}$ CFU/g caecal content for 8 wk-old rabbits, on av.).

Increasing rabbit age from 5 to 8 wk resulted in a proportional lower density of anaerobic bacteria (9.45 *vs* 8.22 $\log_{10}$ CFU/g; (P<0.001), as well as *Bacteroides* (8.86 *vs.* 7.84 $\log_{10}$ CFU/g; P<0.001). In an experiment testing the effect of spirulina and thyme dietary supplementation on digesta traits and caecal microbiota, Bónai *et al.* (2012) observed the same decreasing trend of total anaerobic bacteria with increasing age of rabbits. The higher (P<0.01) stomach pH of 8 week-old rabbits compared to 5 week-old ones (1.78 *vs.*1.42) was also in agreement with the above mentioned study and within the normal range reported in the literature (Gidenne and Lebas, 2005).

Table 5: Effect of Digestarom® dietary supplementation on body weight, gastrointestinal pH, and caecal microbial count of growing rabbits (8 weeks of age).

	Experimental diets							
	Before weaning (BW)		After weaning (AW)			Significance of diet		
	C	D	C	D	MSE	BW	AW	BW×AW
n	6	6	6	6				
Body weight (g)	1858	1803	1824	1837	110	NS	NS	NS
pH of stomach content	1.72	1.85	1.63	1.93	0.33	NS	*	NS
pH of caecal content	6.12	6.24	6.25	6.11	0.16	NS	NS	NS
Total aerobic bacteria[a]	5.38	5.54	5.47	5.44	0.23	NS	NS	NS
Total anaerobic bacteria[a]	8.28	8.17	8.26	8.19	0.50	NS	NS	NS
Escherichia coli [a]	3.20	3.30	3.12	3.39	1.66	NS	NS	NS
Coliforms[a]	2.13	2.22	2.33	2.01	0.66	NS	NS	NS
Bacteroides[2]	7.75	7.93	7.86	7.82	0.52	NS	NS	NS

C: control diet; D: C diet supplemented with 300 mg Digestarom®/kg; MSE: mean squared error.
Level of significance: *: P<0.05; NS: non-significant.
[a]Germ counts expressed in $\log_{10}$ colony-forming units/g caecal content.

Table 6: Effect of Digestarom® dietary supplementation on live performance of growing rabbits.

	Experimental diets								
	Before weaning (BW)		After weaning (AW)				Significance		
	C	D	CC	DC	DD	MSE	BW	AW	BW×AW
n	162	162	108	108	108				
Body Weight (g)									
5 wk	887	880	881	883	886	6.9	NS	NS	NS
8 wk	1776	1784	1743	1802	1795	10.6	NS	NS	NS
9 wk	2019	2013	1993	2022	2032	12.5	NS	NS	NS
12 wk	2643	2663	2645	2654	2659	15.9	NS	NS	NS
Average weight gain (g/d)									
5-8 wk	42.3	43.0	41.0^{a}	43.8^{b}	43.3^{b}	0.3	NS	**	NS
8-12 wk	30.8	31.3	32.1	30.7	30.4	0.4	NS	NS	NS
5-12 wk	35.8	36.4	36.0	36.3	36.0	0.3	NS	NS	NS
Feed intake (g/d)									
5-8 wk	130.5	128.4	130.1	129.7	128.6	1.0	NS	NS	NS
8-12 wk	176.0	174.5	179.2	172.7	173.8	1.8	NS	NS	NS
5-12 wk	156.5	154.8	158.1	154.3	154.5	1.2	NS	NS	NS
Feed conversion ratio									
5-8 wk	3.1	3.0	3.2^{b}	3.0^{a}	3.0^{a}	0.0	*	***	NS
8-12 wk	6.0	5.8	5.9	6.0	5.8	0.1	NS	NS	NS
5-12 wk	4.4	4.3	4.4^{b}	4.3^{ab}	4.3^{a}	0.0	*	*	NS
Mortality (%)									
5-8 wk	0.0	1.9	2.8^{b}	0.0^{a}	0.0^{a}	-	NS	*	-
8-12 wk	8.6	8.0	10.2	6.5	8.3	-	NS	NS	-
5-12 wk	8.6	9.9	13.0	6.5	8.3	-	NS	NS	-

C and CC: control diet; D and DD: C diet supplemented with 300 mg Digestarom®/kg; DC: between 5 and 8 wk D diet and between 8 and 12 wk C diet; MSE: mean squared error.

Level of significance: *: $P<0.05$; **: $P<0.01$; *: $P<0.001$; NS: non-significant.

^{ab}Means in the same row with different superscript letters are significantly different ($P<0.05$).

Live performance depending on Digestarom® supplementation before and after weaning

Digestarom® dietary supplementation had a positive effect on feed conversion ratio from 5 to 8 and from 5 to 12 wk of age ($P<0.05$), whereas it did not affect the body weight, average daily weight gain, feed intake and mortality of the rabbits (Table 6). Although TTAD of some nutrients was lower in 50 d-old Digestarom®-fed rabbits compared to the C group, no negative effects on live performance were observed. Average daily weight gain improved when rabbits consumed the D diet from 5 to 8 wk of age ($P<0.01$). Consequently, feed conversion ratio was also better in DC and DD animals compared to CC ones ($P<0.001$). Moreover, supplementation with Digestarom® during AW did not show mortality from 5 to 8 wk of age, which is a good outcome in the most critical phase of growing rabbits (Rashwan and Marai, 2000). No interaction between before and after weaning supplementation was observed for growth traits.

Even if no studies have tested the Digestarom® dietary supplementation in both BW and AW periods, the literature reports few studies where Digestarom® has been tested on live performance, exhibiting results not always comparable to the present experiment. In 41 d-old rabbits, Krieg *et al.* (2009) observed a higher daily weight gain, daily feed intake and higher final body weight in Digestarom® fed rabbits (300 mg/kg diet) compared to the control group. In addition, Digestarom®-supplemented rabbits had fewer digestive disorders. Similarly, Abd-El-Hady *et al.* (2013) and Abd-El-Hady (2014) found higher final body weight and better feed conversion ratio in Digestarom®-supplemented rabbits (300 mg/kg diet) than those fed with a control diet (from 4 to 9 wk of supplementation in both experiments). Moreover, Colin *et al.* (2008) showed an improved feed conversion ratio and lower mortality in rabbits fed with Digestarom® (300 mg/kg diet) compared to the untreated ones.

The positive results on the live performance of growing rabbits observed in the studies testing Digestarom® dietary supplementation were generally attributed to the substantial reduction in digestive disorders of farmed rabbits. In fact, the phenolic components of essential oils possess antimicrobial activity against several microorganisms by altering the permeability of the cytoplasmic membrane to hydrogen ions (H^+) and potassium (K^+), leading to the disruption of essential cellular processes (Costa *et al.,* 2013). Chemically, essential oils are complex mixtures of several different components such as terpenoids and many low molecular weight aliphatic hydrocarbons, which often make it difficult to explain their activities (Brenes and Roura, 2010). A work by Stein and Kil (2006) on weanling pigs showed that the hydrophobic constituents of essential oils allowed them to disintegrate the outer membrane of *E. coli* and *Salmonella*, thus inactivating these pathogens. A reduction in the number of pathogenic bacteria would thus change the microbial ecology in favour of beneficial species (Michiels *et al.,* 2009).

However, when essential oils are added to animal diets, results can vary greatly and the reason could be attributed to differences in the type and dose of the essential oils used (Li *et al.,* 2012). In animals with a well-developed sense of smell, for example, if the dose used is too high, the strong smell and/or taste can negatively affect feed intake, thus compromising live performance. Digestarom® had a medium-term negative influence on the reproductive performance of rabbit does and a negative effect of smell on feed palatability was hypothesised to explain these results (Celia *et al.,* 2015). Another important aspect which could strongly affect final outcomes is the stability of essential oils during pelleting, as Maenner *et al.* (2011) showed a substantial loss of activity when essential oils were pelleted at a temperature of 58°C.

CONCLUSION

The inclusion of 300 mg/kg of Digestarom® in a diet for growing rabbits was mainly effective when administered after weaning (from 5 to 8 wk of age), as it was able to increase the growth rate, improve feed efficiency and reduce mortality rate. When considering the whole growing period, Digestarom® supplement had no effect either on the live performance of rabbits or on the microbial counts of the caecal and faecal content, whereas it impaired nutrient digestibility. On the whole, this study did not provide convincing evidence of the efficacy of the Digestarom® dietary supplement.

Acknowledgements: The authors thank Barbara Contiero, Riccardo Miotti Scapin, Francesco Endrici, and Péter Farkas for their technical support.

REFERENCES

Abd El-Hady A.M. 2014. Performance, physiological parameters and slaughter characteristics in growing rabbits as affected by a herbal feed additive (Digestarom®). *Agric. Food, 2: 353-365.*

Abd El-Hady A.M., El-Ghalid A.H., EL-Raffa A.M. 2013. Influence of a herbal feed additives (Digestarom®) on productive performance and blood constituents of growing rabbits. *Egyptian J. Anim. Prod., 50: 27-37.*

Adibmoradi M., Navidshad B., Seifdavati J., Royan M. 2006. Effect of dietary garlic meal on histological structure of small intestine in broiler chickens. *J. Poultry. Sci., 43: 378-383. doi:10.2141/jpsa.43.378*

Arhan M., Ozturk H.S., Turhan N., Aytac B., Güven M.C., Olcay E., Durak I. 2009. Hepatic oxidant/antioxidant status in cholesterol-fed rabbits: Effects of garlic extract. *Hepatol. Res., 39: 70-77. doi:10.1111/j.1872-034X.2008.00401.x*

AOAC. 2000. Official methods of analysis 17th Ed. *Association of Official Analytical Chemists, Arlington VA, USA.*

Baytop T. 1984. Phytotherapy in Turkey, past and present. *In: Phytotherapy in Turkey (Eds. T. Baytop) Istanbul University publications 3255, Istanbul, 194-195.*

Benlemlih M., Aarab A., Bakkali M., Arakrak A., Laglaoui A. 2014. Effect of dietary fennel and thyme essential oil supplementation on zootechnical parameters and caecal microflora of growing rabbit. *Rev. Microbiol. Ind. San et Environn., 8: 16-25.*

Berahou A., Auhmani A., Fdil N., Benharref A., Jana M., Gadhi C.A. 2007. Antibacterial activity of *Quercus ilex* bark's extracts. *J. Ethnopharmacol., 112: 426-429. doi:10.1016/j.jep.2007.03.032*

Bónai A., Dalle Zotte A., Kametler L., Vántus V., Morsy W.A., Matics Zs., Dal Bosco A., Szendrő Zs., Kovács M. 2012. Dietary supplementation of spirulina (*Arthrospira platensis*) and thyme (*Thymus vulgaris*). Part 2: effect on gastrointestinal growth, caecal microbiota and fermentation in rabbits. *In Proc.: 10th World Rabbit Congress 3-6 September, 2012. Sharm El-Sheikh, Egypt. 707-711.*

Bovera F., Iannaccone F., Mastellone V., Nizza S., Lestingi A., De Martino L., Lombardi *P.*, Mallardo K., Ferrara M., Nizza A. 2012. Effect of spray application of *Lactobacillus plantarum* on *in vivo* performance, caecal fermentations and haematological traits of suckling rabbits. *Ital. J. Anim. Sci., 11: 145-149. doi:10.4081/ijas.2012.e27*

Brenes A, Roura E. 2010. Essential oils in poultry nutrition: Main effects and modes of action. *Anim. Feed Sci. Technol., 158: 1-14. doi:10.1016/j.anifeedsci.2010.03.007*

Carabaño R., Piquer J., Menoyo D., Badiola I. 2010. The digestive system of the rabbit. *In: De Blas C., Wiseman J. (Eds). The Nutrition of the Rabbit. CABI Publishing. CAB International, Wallingford Oxon, UK, 1-18. doi:10.1079/9781845936693.0001*

Celia C., Cullere M., Gerencsér Zs., Matics Zs., Dalle Zotte A., Giaccone V., Szendrő Zs. 2015. Effect of Digestarom® dietary supplementation on the reproductive performance of rabbit does: preliminary results. *Ital. J. Anim. Sci., 14: 700-705. doi:10.4081/ijas.2015.4138*

Colin M., Atkári T., Prigent A.Y. 2008. Efectos de la incorporación de una mezcla de extractos vegetales en los piensos por engorde: resultados en granja experimental y en granjas comerciales. *XXXIII Symposium de ASESCU October 30-31. Calahorra, Spain. 62-56.*

Combes S., Fortun-Lamothe L., Cauquiland L., Gidenne T. 2013. Engineering the rabbit digestive ecosystem to improve digestive health and efficacy. *Animal, 7: 1429-1439. doi:10.1017/S1751731113001079*

Costa L.B., Luciano F.B., Miyada V.S., Gois F.D. 2013. Herbal extract and organic acids as natural feed additives in pig diets. *S. Afr. J. Anim. Sci., 43: 181-193.*

Dalle Zotte A., Cossu M.E. 2009. Dietary inclusion of tannin extract from red quebracho trees (*Schinopsis* spp.) in the rabbit meat production. *Ital. J. Anim. Sci., 8: 784-786. doi:10.4081/ijas.2009.s2.784*

Dalle Zotte A., Matics Zs., Bohatir P., Sartori A., Gerencsér Zs., Szendrő Zs. 2012. Effect of dietary supplementation of chestnut hydrolysable tannin on digestive efficiency, growth performance and meat quality in growing rabbits. *In Proc.: 10th World Rabbit Congress, 3-6 September, 2012. Sharm El- Sheikh , Egypt. 961-965.*

Dalle Zotte A., Sartori A., Bohatir P., Rémignon H., Ricci R. 2013. Effect of dietary supplementation of Spirulina (*Arthrospira platensis*) and Thyme (*Thymus vulgaris*) on growth performance, apparent digestibility and health status of companion dwarf rabbits. *Livest. Sci., 152: 182-191. doi:10.1016/j.livsci.2012.12.017*

Fortun-Lamothe L., Gidenne T. 2009. Recent advances in digestive physiology of the growing rabbit. *In: Martens L., Coudert P. (Eds). Recent Advances in Rabbit Science. Institute for Agricultural and Fisheries Research (ILVO) Animal Science Unit Melle, Belgium, 201-210.*

EC. 1998. Commission Directive 98/64/EC of 3 September 1998 establishing Community methods of analysis for the determination of amino acids, crude oils and fats, and olaquindox in feeding stuffs and amending Directive 71/393/EEC. *Official J. European Union 19.9.1998 L257/14-L257/28.*

Gerencsér Zs., Szendrő Zs., Matics Zs., Radnai I., Kovács M., Nagy I., Cullere M., Dal Bosco A., Dalle Zotte A. 2014. Effect of dietary supplementation of spirulina (*Arthrospira platensis*) and thyme (*Thymus vulgaris*) on apparent digestibility and productive performance of growing rabbits. *World Rabbit Sci., 22: 1-9. doi:10.4995/wrs.2014.1351*

Gidenne T., Carabaño R., García J., de Blas C. 1998. Fibre digestion. *In: De Blas C., Wiseman J. (Eds). The Nutrition of the Rabbit. CABI Publishing. CAB International, Wallingford Oxon, UK, 241-253.*

Gidenne T., Fortun-Lamothe L. 2002. Feeding strategy for young rabbits around weaning: a review of digestive capacity and nutritional needs. *Anim. Sci., 75: 169-184.*

Gidenne T., Lebas F. 2005. Le comportement alimentaire du lapin. *In Proc.: 11èmes Journées de la Recherche Cunicole, 29-30 novembre, 2005. Paris, France. 183-196.*

Hanson N.B., Lanning D.K., 2008. Microbial induction of B and T cell areas in rabbit appendix. *Dev. Comp. Immunol., 32: 980-991. doi:10.1016/j.dci.2008.01.013*

Ibrahim S.A.M., El-Ghamry A.A., El-Mallah G.M. 2000. Effect of some medicinal plants of *Labiatae* family as feed additives on growth and metabolic changes of rabbits. *Egypt. J. Rabbit Sci., 10: 105-120.*

ISO. 1998. Animal feeding stuffs, animal products and faeces or urine. Determination of gross calorific value- Bomb calorimetric method. *Reference number 9831.*

Karimi I. Hayatgheybi H., Razmjo M., Yousefi M., Dadyan A., Hadipour M. 2010. Anti-hyperlipidaemic effects of an essential oil of *Melissa officinalis*. L. in cholesterol-fed rabbits. *J. Appl. Biologic. Sci., 4: 17-22.*

Krieg R., Vahjen, W., Awad W., Sysel M., Kroeger, S., Zocher L., Hulan, A.W., Arndt G., Zentek J. 2009. Performance, digestive disorders and the intestinal microbiota in weaning rabbits are affected by an herbal feed additive. *World Rabbit Sci., 17: 87-95. doi:10.4995/wrs.2009.662*

Langendijk P., Bolhuis J.E., Laurenssen B.F.A. 2007. Effects of pre- and postnatal exposure to garlic and aniseed flavour on pre- and postweaning feed intake in pigs. *Livest. Sci., 108: 284-287. doi:10.1016/j.livsci.2007.01.083*

Li P.F., Piao X.S., Ru Y.J., Han X., Xue L.F., Zhang H.Y. 2012. Effects of adding essential oil to the diet of weaned pigs on performance, nutrient utilization, immune response and intestinal health. *Asian Australas. J. Anim. Sci., 25: 1617-1626. doi:10.5713/ajas.2012.12292*

Loo A., Richard H. 1992. Nature, origine et propriétés des épices et des aromates bruts. *In: Epices et aromates. Richard H. (ed) TEC and DOC, Lavoisier, Paris: 18-22.*

Maenner K., Vahjen W., Simon O. 2011. Studies on the effects of essential-oil based feed additives on performance, ileal nutrient digestibility, and selected bacterial groups in the gastrointestinal tract of piglets. *J. Anim. Sci., 89: 2106-2112. doi:10.2527/jas.2010-2950*

Mage R.G., Lanning D., Knight K.L. 2006. B cell and antibody repertoire development in rabbits: The requirement of gut associated lymphoid tissues. *Dev. Comp. Immunol., 30: 137-153. doi:10.1016/j.dci.2005.06.017*

Marascuilo L.A. 1966. Large-sample multiple comparisons. *Phychol. Bull., 65: 280-290. doi:10.1037/h0023189*

McDougall G.J., Kulkarni N.N., Stewart D. 2008. Current developments on the inhibitory effects of berry polyphenols on digestive enzymes. *Biofactors, 34: 73-80. doi:10.1002/biof.5520340108*

Mertens D.R. 2002. Gravimetric determination of amylase-treated neutral detergent fibre in feeds with refluxing beakers or crucibles: collaborative study. *J. AOAC Int. 85: 1217-1240.*

Michiels J., Missotten J.A.M., Fremaut D., De Smet S., Dierick N.A. 2009. *In vitro* characterization of the antimicrobial activity of selected essential oil components and binary combinations against the pig gut flora. *Anim. Feed Sci. Technol., 151: 111-127. doi:10.1016/j.anifeedsci.2009.01.004*

Oliveira R.A., Narciso C.D., Bisinotto R.S. Perdomo M.C., Ballou M.A., Dreher M., Santos J.E.P. 2010. Effects of feeding polyphenols from pomegranate extract on health, growth, nutrient digestion, and immunocompetence of calves. *J. Dairy Sci., 93: 4280-4291. doi:10.3168/jds.2010-3314*

Omer H.A.A., EL-Nomeary Y.A.A., El-Kady R.I., Badr A.M.M., Ali F.A.F., Ahmed S.M., El-Allawy H.M.H., Ibrahim S.A.M. 2013. Improving the utilization of rabbit diets containing vegetable oil by using fennel (*Foeniculum vulgare)* and oregano (*Origanum vulgare* L.) as feed additives. *Life Sci. J., 10: 2625-2636.*

Peiretti P.G., Meineri G. 2008. Effects of diets with increasing levels of *Spirulina platensis* on the performance and apparent digestibility in growing rabbits. *Livest Sci., 118: 173-177. doi:10.1016/j.livsci.2008.04.017*

Perez J.M., Lebas F., Gidenne T., Maertens L., Xiccato G., Parigi-Bini R., Dalle Zotte A., Cossu M.E., Carazzolo A., Villamide M.J., Carabaño R., Fraga M.J., Ramos M.A., Cervera C., Blas E., Fernández J., Falcão-e-Cunha L., Bengala Freire J. 1995. European reference method for *in vivo* determination of diet digestibility in rabbits. *World Rabbit Sci., 3: 41-43. doi:10.4995/wrs.1995.239*

Placha I., Chrastinova L., Laukova A., Cobanova K., Takacova J., Strompfova V., Chrenkova M., Formelova Z., Faix S. 2013. Effect of thyme oil on small intestine integrity and antioxidant status, phagocytic activity and gastrointestinal microbiota in rabbits. *Acta Vet. Hung., 61: 197-208. doi:10.1556/AVet.2013.012*

Rashwan A.A., Marai I.F.M. 2000. Mortality in young rabbits: a review. *World Rabbit Sci., 8: 111-124. doi:10.4995/wrs.2000.427*

SAS Institute. 2004. SAS User's Guide: *Statistics Version 9.1 ed. SAS Institute, Cary, NC.*

Stein H.H., Kil D.Y. 2006. Reduced use of antibiotic growth promoters in diets fed to weanling pigs: dietary tools, Part 2. *Anim. Biotechnol., 17: 217-231. doi:10.1080/10495390600957191*

Toulah F.H., Al-Rawi M.M. 2007. Efficacy of garlic extract on hepatic coccidiosis in infected rabbits (*Oryctolagus cuniculus*): histological and biochemical studies. *J. Egypt. Soc. Parasitol., 37: 957-968.*

Van Soest P.J., Robertson J.B., Lewis B.A. 1991. Methods for dietary fiber, neutral detergent fiber and nonstarch polysaccharides in relation to animal nutrition. *J. Dairy Sci., 74: 3583-3597. doi:10.3168/jds.S0022-0302(91)78551-2*

Vidyavati H.G., Manjunatha H., Hemavathy J., Srinivasan K. 2010. Hypolipidemic and antioxidant efficacy of dehydrated onion in experimental rats. *J. Food Sci. Technol., 47: 55-60. doi:10.1007/s13197-010-0015-3*

Wang J.P., Yoo J.S., Jang H.D., Lee J.H., Cho J.H., Kim I.H. 2011. Effect of dietary fermented garlic by *Weissella koreensis* powder on growth performance, blood characteristics, and immune response of growing pigs challenged with *Escherichia coli* lipopolysaccharide. *J. Anim. Sci., 89: 2123-2131. doi:10.2527/jas.2010-3186*

Wichtl M. 1994. Herbal Drugs and Phytopharmaceuticals. *Boca Raton, CRC Press, FL, USA, 128-129.*

Xiccato G., Trocino A. 2010. Energy and protein metabolism and requirements. *In: De Blas, C., Wiseman, J., (Eds), Nutrition of the Rabbit 2nd ed., CABI Publishing, Wallinford. UK: 83-118. doi:10.1079/9781845936693.0083*