THESIS OF DOCTORAL (PhD) DISSERTATION

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TESTING OF SOME HERBS AND SPICES ON GROWING RABBITS AND MEAT QUALITY

Written by

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1.ANTECEDENTS OF THE RESEARCH, OBJECTIVES

The health status influences the growing phase of the rabbits. Particularly the pre- and post-weaning period is the most critical phase: milk is substituted with solid feed, the kits' immune system is still immature and the kits are separated from their mothers, (Carabaño et al., 2006; Gidenne et al., 2005). Digestive disturbances are the main cause of the morbidity and mortality that create important economic losses for rabbit farmers (Marlier et al., 2006; Licois, 2004). For this reason some antibiotic growth promoters have been practiced in the United States and some other countries, but their usefulness was contested, since some similar antibiotics are used in human medicine and their use contribute to the pool of antibiotic resistant bacteria. Thus, in 2006 the use of antibiotics as growth promoters for farmed animals has been banned in the EU due to safety issues, health concerns as well as increasing demand of consumers for more natural products (Barug et al., 2006 Falcão-e-Cunha et al., 2007). Therefore, in order to keep ensuring satisfactory performances as well as low morbidity and mortality of farmed animals, were contemplated other potential substitutes of natural origin to improve health status and productive performance of the animal.

Herbs, spices, and botanicals are classified by habitat, part used, therapeutic value, and type of administration (Dalle Zotte et al., 2016). Vaunting a wide range of activities, some have been associated with improvements in animal performance and increased nutrient availability. Plants have developed a range of low molecular weight secondary metabolites, called phytochemicals, that help to prevent physiological and environmental stress and oppose pathogens (Wenk, 2003). The utilization of herbs and spices in animal nutrition focuses on the potential benefit

effect given by the phyotochemical compounds on the digestive system, as antimicrobial, antioxidant and as a growth promoter. Phenolic compounds are the biggest group of secondary metabolites identified in plants; they include simple phenols, flavonoids, lignins and lignans, tannins, xanthones and coumarins (Huang *et al.*, 2010). Phenolic substances presented in plants and plant products are also capable of oxidative action. They are used for multiple purposes as protecting animal feeds during storage, defending the tissues in the alive animals, and diminishing oxidative reaction in meat and meat products (Vekiari *et al.*, 1993; McCarthy *et al.*, 2001; Botsoglou *et al.*, 2004; Kulisic *et al.*, 2004; Shan *et al.*, 2005; Collin, 2006; Coma, 2008; Soultos *et al.*, 2009; Zinoviadou *et al.*, 2009; Eid *et al.*, 2011; Dal Bosco *et al.*, 2014; Dalle Zotte *et al.*, 2014; Cardinali *et al.*, 2015).

These natural additives have received closer attention from the feed industry in recent years. Many studies have described herbal plants as additives in rabbit feeding, but the in vivo studies are still limited (Dalle Zotte and Szendrő, 2011; Dalle Zotte et al., 2016). Moreover,

some plant extract has showed to possess a certainly toxic effect (Samson et al., 2012).

Aims

The aim of this PhD thesis was to study the effect of the dietary supplementation of a single herb, or of a mix of selected herbs and spices on the productive performances, health status and meat quality of growing rabbits, indeed:

• The first study focused on the effect of a commercial product (Digestarom®) on productive performances, nutrients balance and gastrointestinal microbiota when administered to growing rabbits.

- The second study aimed at exploring the effect of the Digestarom® on the rabbit carcass and meat quality.
- The third study involved a single plant, *Silybum marianum*, which was included in the growing rabbit's diet from their third week of age, with the aim to improve their health status, productive performances, carcass traits, antioxidant effect and sensory trait.

RESULTS AND CONCLUSIONS

All experiments were published in peer reviewed scientific journals, therefore the aims, material and methods, conclusions of the experiments were summarised based on these papers. The results of the experiments were summarized according to those publications.

Paper 1

Dietary supplementation of Digestarom® herbal formulation: effect on apparent digestibility, faecal and caecal microbial counts and live performance of growing rabbits



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

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Aim - 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.

Material and Methods - 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) (Figure 1). 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.

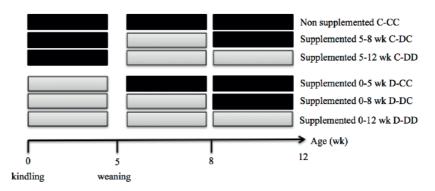


Figure 1: Experimental design (n=54 rabbits/treatment). ☐ Diet D, supplemented with 300 mg Digestarom®. ☐ Diet C.

Results - 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 (Table 1).

Table 1. Effect of Digestarom[®] dietary supplementation on total tract apparent digestibility (TTAD) of 50 day-old growing rabbits and nutritive value of diets

	Evnori	mantal			
		mental	N CODI	g: :«	
	d1	ets	MSE ¹	Significance	
	C	D			
n	5	7			
Live Weight (LW), g	2018	1976	72.1	ns	
Dry Matter (DM) intake, g/d	215	196	49.0	*	
<u>TTAD, %:</u>					
DM	49.9	48.6	1.9	ns	
Ether extract	75.9	59.8	1.4	***	
Starch	98.8	98.9	0.04	***	
Cellulose (ADF- ADL)	25.9	20.6	2.9	*	
Gross energy	51.8	49.1	1.9	*	
Nutritive value:					
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 Levels of significance: *, P < 0.05; **, P < 0.01; ***, P<0.001; ns= non-significant;

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.

Conclusions - 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

¹MSE= Mean Squared Error;

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.

Paper 2 Effect of pre- and post-weaning dietary supplementation with Digestarom® herbal formulation on rabbit carcass traits and meat quality

Meat Science 118 (2016) 89-95



Effect of pre- and post-weaning dietary supplementation with Digestarom® herbal formulation on rabbit carcass traits and meat quality



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Aim – The aim of this study was to evaluate the effect on carcass traits and rheological and sensory meat quality produced by including Digestarom[®] in the feed given to growing rabbits.

Material and Methods - Maternal line rabbits of the Pannon breeding programme (maternal line: Pannon Ka) were used in this study. At kindling, rabbit does and litters (9-10 kits/litter) were divided into two dietary groups (n=162 kits/dietary group) and fed either a control diet (C) or the C diet (crude protein:158 g/kg, ether extract: 30 g/kg, starch: 123 g/kg, crude fibre:181 g/kg) supplemented with Digestarom® (D: 300 mg/kg) herbal formulation. At weaning, which occurred at 35 days of age, both dietary groups were further divided into 3 dietary groups: CC received the C diet and DD the D diet from 5 to 12 weeks of age. Differently, the DC dietary group was fed D and C diets from 5 to 8

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weeks of age and from 8 to 12weeks of age. Overall, 6 feeding groups (54 rabbits/group)were created: C-CC, C-DC, C-DD, D-CC, D-DC, and D-DD. The animals were housed (3 rabbits/cage) in wire-mesh cages (61 × 32 cm); the temperature and photoperiod were 15–18 °C and 16 L: 8D, respectively.

At 12 weeks of age, rabbits were transported to a slaughterhouse located 200 km from the experimental farm. After fasting and electro-stunning, rabbits were slaughtered and carcasses were dissected according to World Rabbit Science Association (WRSA) recommendations as described by Blasco and Ouhayoun (1996). Raw left LTL and HL pH was measured 48 h post-mortem using a Mettler Toledo FE20 pH-metre. Colour values of lightness, redness, yellowness, chroma and hue were measured using a RM200QC colorimeter. Right LTL and HL meat samples were allowed to thaw overnight at +4 °C, removed from plastic bags, weighed, and subsequently used for thawing and cooking loss determinations. Shear force was assessed with a TA-HDi Texture Analyzer. Left HL were thawed and deboned in order to determine the meat/bone ratio. Femur and tibia were separately weighed, and then length and minor diameter were measured with a digital calliper. The sensory test was carried out in three consecutive days in which 30 samples/day were evaluated (5 samples × 6 treatments) by trained panel members.

Results - Digestarom[®] supplementation improved carcass yield and body mid part proportion only when it was administered before weaning Table 2).

Table 2. Effect of the dietary Digestarom® supplementation on rabbit carcass traits

Periods	Before Weaning (BW)		After Weaning (AW)			MS E ¹	Significance		
Experimental diets	C	D	CC	DC	DD				•
							B W	A W	BWx AW
No. Animals	162	162	108	108	108				
Slaughter weight (SW), g	2613	2604	258 5	261 5	2626	15. 2	ns	ns	ns
Chilled Carcass (CC), g	1553	1556	154 3	155 6	1564	10. 3	ns	ns	ns
Reference carcass (RC), g	1279	1289	127 2	128 5	1295	9.0	***	ns	ns
Reference carcass yield, % CC	82.3	82.8	82.3	82.5	82.7	0.0 7	***	ns	ns
HLLTO ² , % CC	1.45	1.42	1.45 a	1.47 a	1.38 b	0.0	ns	**	ns
Liver, % CC	5.15	4.82	5.09	4.88	4.99	0.0 5	**	ns	ns
Kidneys, % CC	1.14	1.13	1.17 a	1.14 ab	1.09 b	0.0	ns	**	ns
Fore part, % RC	28.3	28.0	28.2	28.1	28.2	0.0 7	*	ns	ns
Mid part, % RC	32.3	32.7	32.4	32.5	32.6	0.0 8	*	ns	ns
Hind part, % RC	37.6	37.7	37.7	37.7	37.5	0.0 7	ns	ns	ns
Perirenal fat, % RC	1.35	1.22	1.28	1.23	1.34	0.0	ns	ns	ns

Level of significance: *, P < 0.05; **, P < 0.01; ***, P < 0.001 a, b, ;ns= no significant;

¹MSE=Mean Squared Error; ²HLTTO= Heart, lung, thymus, trachea and esophagus

Rabbits fed D before weaning had higher hind leg meat cooking losses. Loin meat spiciness and rancidity increased with D both before and after weaning supplementation (Table 3).

Table 3. Sensory analysis of Digestarom[®] *Longissimus thoracis et lumborum* (LTL) muscles

Periods	Wea	fore ining W)	After Weaning (AW)		MSE	Significance			
Experiment al diets	С	D	CC	DC	DD				
							B W	A W	BWxA W
No. of samples	45	45	30	30	30				
Olfactory rancidity	3.2 7	3.6 7	3.00^{b}	3.64 a	3.78 a	1.66	*	***	ns
Olfactory spicy	3.2	3.7 5	3.44	3.59	3.4	1.68	**	ns	ns
Flavour rancidity	3.0	3.9 5	3.26	3.46	3.71	1.61	**	ns	**
Flavour spicy	3.2 4	3.7 0	3.45 ^a	3.18 b	3.78 a	1.67	**	*	ns
Flavour overall	3.5 8	3.3 6	3.57	3.43	3.41	1.70	ns	ns	ns

Level of significance: *, P < 0.05; **, P < 0.01; *** $P \le 0.001$ level; ^{a, b} Means in the same row having different superscripts are

significant at $P \le 0.05$ level; ns=no significance; MSE 1 = Mean Squared Error; Ranking test

Conclusions - In this study, Digestarom® dietary supplementation appeared to be ineffective in improving growing rabbit carcass traits, especially when given after weaning. Furthermore, even without affecting meat tenderness, before weaning supplementation increased hind leg cooking losses. Moreover, despite the fact that overall flavour perception reached the same scores in all groups, panellists recorded higher scores for spiciness and rancidity descriptors in meat of rabbits fed D. On the basis of the considerations above, Digestarom® does not appear to be an effective natural feed additive for the improvement of carcass traits or meat quality in growing rabbits.

Paper 3

Effect of *Silybum marianum* herb on the productive performance, carcass traits and meat quality of growing rabbits

Livestock Science

Contents lists available at ScienceDirect

Livestock Science

journal homepage: www.elsevier.com/locate/livsci

Effect of Silybum marianum herb on the productive performance, carcass traits and meat quality of growing rabbits



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Aim - The present study aimed to test the effect of a dietary supplementation with *Silybum marianum* (SM), an herbaceous Mediterranean plant traditionally used to treat liver and gastrointestinal diseases and with antioxidant properties, on the productive performance, carcass traits and meat quality of growing rabbits.

Material and Methods - At weaning (5 weeks of age), a total of 144 Pannon Large rabbits were allocated to three experimental groups. The control group (C, n=51) was fed with a basal diet, whereas the other groups received the basal diet supplemented with SM herbal powder at two concentrations: 5 g/kg (SM1, n=48) and 10 g/kg (SM2, n=45). Rabbits were housed in wire-mesh cages (3 rabbits/cage) and fed ad libitum throughout the experiment. Productive performance and mortality were recorded weekly. Rabbits were slaughtered at 11 weeks of age, carcasses were dissected, and hind leg (HL) and Longissimus thoracis et lumborum (LTL) meat were analysed for meat quality (oxidative status,

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pHu and L*, a*, b* colour) traits. In addition, a sensory analysis on the LTL meat was carried out by a trained panel.

Results – The SM supplementation did not modified the growth performance (Table 4). However, mortality was significantly reduced in SM treatments compared to C group from week 6–7 (10.4 and 11.1 vs. 17.7%, for SM1, SM2 and C groups, respectively; P < 0.05), and in SM2 compared to C and SM1 considering the whole productive cycle (5–11 weeks).

Table 4. Effect of the supplementation of *Silybum marianum* on the live performance of growing rabbits

_	Expe	rimental d	MSE ²	Significance	
	С	SM1	SM2	- MSE	Significance
Initial n rabbits/cages	51/17	48/16	45/15		
Body weight at 11 wk	3067	3090	3067	62.3	ns
Body weight gain, g/d (5-11 wk)	52.3	52.5	51.5	1.35	ns
Feed intake, g/d (5-11 wk)	145	145	142	19	ns
Feed conversion ratio (5-11 wk)	2.88	2.86	2.85	0.17	ns
Mortality (5-11 wk)	25.6^{B}	20.9^{B}	15.5 ^A	-	**

 $^{^1}$ C: control diet; SM1: C diet supplemented with 5 g/kg of *S. marianum*; SM2: C diet supplemented with 10 g/kg of *S. marianum*; 2 MSE= Mean Square Error; ** P < 0.01 level; A,B Means in the same row having different superscripts are significant at $P \le 0.01$ levels; ns= non significant

The dietary inclusion of SM did not affect carcass traits and did not change neither colour nor oxidative status of LTL muscle. Differently, SM diet increased pHu of LTL muscle (5.98 vs. 6.03 vs. 6.10 in C, SM1 and SM2, respectively; P < 0.05). The sensory traits of LTL meat were affected by SM dietary inclusion: a higher herbaceous odour was observed in SM2 compared to C and SM1 (P < 0.001) treatments, whereas rabbit odour followed an opposite trend with C receiving a

higher score compared to SM1 and SM2 (P < 0.05). Panellists also perceived a stronger rabbit flavour in C than in SM1 and SM2 meat (2.40 vs. 1.90 and 1.70, P < 0.05; P < 0.001) (Table 5).

Table 5. Effect of *Silybum marianum* dietary supplementation on the sensory analysis of *Longissimus thoracis et lumborum* (LTL) meat of rabbits

	Expe	rimental	MSE	Signific	
	С	SM1	SM2	2	ance
n	15	15	15		
Rancid odour	2.08	1.98	1.95	0.82	ns
Herbaceous odour	1.93 ^A	1.90 ^A	2.18^{B}	0.72	***
Rabbit odour	2.38^{b}	1.93 ^a	1.70^{a}	0.78	*
Rancid flavour	2.28	1.83	1.90	0.82	ns
Herbaceous flavour	2.03	1.88	2.10	0.82	ns
Rabbit flavour	2.40^{B}	1.90 ^A	1.70 ^A	0.73	***

¹C: control diet; SM1: C diet supplemented with 5 g/kg of *S. marianum*; SM2: C diet supplemented with 10 g/kg of *S. marianum*; ²MSE= Mean Square Error;

Silybum marianum seems to be a promising natural feed additive to improve the health condition of growing rabbits. Differently, the antioxidant activity of Silybum marianum was not confirmed when considering fresh meat of rabbits supplemented with the inclusion levels of the present experiment. The dietary supplementation with Silybum marianum changed then sensory characteristics of rabbit loin thus, in the future; consumer acceptability should be also carefully assessed.

Conclusions - *Silybum marianum* dietary supplementation reduced the mortality rate in growing rabbits under health stress, thus being a promising natural feed additive in improving the sanitary status of a

commercial rabbit farm. The dietary supplementation with Silybum marianum changed the sensory characteristics of rabbit loin thus, in the future; consumer acceptability should be carefully assessed. As the present study was the first attempt to test the dietary supplementation of *Silybum marianum* in the diet for growing rabbits, further studies need to implement the present results considering also digestibility of nutrients as well as the effect of this herb on the intestinal microbiota. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

GENERAL DISCUSSION AND CONCLUSIONS

Antibiotic were used for decades in the rabbit meat production, to diseases prevention and production enhancement. However, discovering of a theoretical connection on the development of resistant bacterial strains, have revealed doubts on their utilization. In 2006 they were finally banned from the European countries, opening the Era of the natural products.

Studies on the digestive disturbances of growing rabbit have revealed how nutrition plays an active role in maintaining a positive health status. Indeed, microbial colonisation of the rabbit gastrointestinal tract is directly related to a supply of balanced diets, and any alteration may provoke the colonisation of pathogenic bacteria, primary cause of digestive disturbances.

Different strategies were explored to reduce the use of the antibiotics, through feed restriction, modern management techniques, and natural feed supplements. Among the last category, the candidate might be probiotics, prebiotics, organic acids and, in particular, plants and their extracts.

Plants have played a significant role in maintaining human health and improving the quality of human life for thousand of years. It was estimated that more than 80% of the Earth population rely in traditional medicine for their primary health care need, and mostly the use of plant extract is involved. Thus, the aim of the experiments included in this PhD thesis was to find positive effects of some herbs and spices supplemented to rabbits diets, in particular on the health status, growth potential and meat quality of the growing rabbits.

The first study showed the single and/or synergistic effect of the phytochemicals included in the Digestarom®, a mixture of essential oils, herbs, spices and extracts of 10 different ingredients: 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.). Each ingredient contains different phytochemicals, mainly phenolics and flavonoids substances which produces a different effect due to different mechanisms. It was observed how phenolic substances present in the mix had influenced positively the live performances in the post-weaning period, improving feed efficiency and growth rate. On the other hand, tannins-like substances naturally present in oak bark had negatively

influenced the palatability of the diet, and impairing the nutrient digestibility.

The most important mechanism of phytogenic feed additives is claimed to be the beneficially effect on the ecosystem of gut microflora through controlling potential pathogens. Digestarom[®] supplementation lowered the mortality trend after weaning, but the microbial count analysis did not reveal positive change in the microbiota, differently from the results of the literature. The presence of phytochemicals in the Digestarom[®] had nearly no effect on carcass and meat quality traits in measured in the second study. Only flavour and taste perception was likely affected by the presence of the aromatic ingredients such as allicin, component of onion and garlic. The pungent aroma of allicin was not appreciated because the panelists associated it with olfactory and flavour rancidity.

In the third study, the supplementation of *Silybum marianum* (milk thistle) in the diet of growing rabbit was able to significantly reduce the mortality, mainly in the delicate post-weaning phase. Traditionally, milk thistle is used for protecting and restoring liver function, because of the high content of flavonoids are claimed to promote antioxydative and anti-inflammatory actions, and to help in reducing the risk of diseases. Surprisingly, antioxidative action was not detected in the meat of the rabbits fed with *Silybum marianum*, as occurred in other animal species. Possible factors, such as animal species, age, type of plant extract, and inclusion level, might have interfered with the effect of the phytochemicals, making useless its supplementation to this purpose. Differently, flavonoids affected positively some meat sensory traits, permitting the panelists to differentiate the meat of rabbits fed with or

without flavonoids-rich diet. Therefore, *Silybum marianum* might be considered a potential feed supplement for growing rabbits, considering its ability of lowering the mortality of the rabbits around weaning.

In all the studies included in this PhD thesis both positive and absence of effects the phytochemicals were found. To formulate diets using natural ingredients, it is preventively important to evaluate possibly side effects, as astringency, toxicity and tolerance level, however not always easy to determine. Indeed, when phytobiotic additives are added as feed supplements, different parameters can occour to modify the helpfulness: plant parts and physical properties, genetic variety of the plant, the level of dosage, harvest time and interaction with the other ingredients. In addition, the efficacy of the phytobiotic additives might be affected by the nutritional status of the animals, infections and diet composition.

It can be concluded that the future of using herbs and/or spices in rabbit feeding will, in great measure, depend on the knowledge of their chemical structure, economical value, and technological advancements for their use in pelleted diets.

Several herbs, spices, and botanicals products have been tested, as feed supplement, in the growing rabbits with disparate results. Some of them have shown beneficial effects in rabbit live performances as growth promoter, others exhibited antimicrobial and antioxidant properties, whereas others improved the meat sensory traits.

The administration of 300 mg/kg of Digestarom[®] in a diet for growing rabbits proved to be mainly effective after weaning (from 5 to 8 weeks of age), as it reduced the mortality rate, and improved feed efficiency and growth rate. However, it impaired nutrient digestibility and some meat

sensory traits. Also the dietary supplementation of *Silybum marianum* to growing rabbits had, as main effect, the reduction of mortality after weaning.

In conclusion, results of the present PhD thesis have demonstrated a weak effectiveness of the use of both supplements as natural feed additive for growing rabbits, and their use would be suggested around weaning, to improve the health status of commercial rabbits.

5. NEW SCIENTIFIC RESULTS

- 1. The dietary supplementation of 300 mg/kg of Digestarom[®] significantly reduced the DM intake. As the tannin content of Digestarom[®] is supposed to be responsible for that effect, it is suggested to exclude the oak bark in the commercial mix.
- 2. The dietary supplementation of *Silybum marianum* herbal powder at 5 and 10 g/kg inclusion level reduced the mortality rate of rabbits during post-weaning, thus being a useful natural feed additive in improving the sanitary status in commercial rabbit farms.
- 3. The use of 5 and 10 g/kg *Silybum marianum* in rabbit diets significantly increased the herbaceous odour (P<0.001), whereas it lowered the rabbit odour (P<0.05), and flavour (P<0.001). However, to evaluate the sensory traits of this herb, consumer acceptability should be carefully assessed

Scientific papers on the subject of the dissertation

Peer-reviewed papers published in foreign scientific journals

- Celia C., Cullere M., Gerencsér Zs., Matics Zs., Dalle Zotte A., Giaccone V., Szendrő Zs., 2015. Effect of Digestarom® dietary supplementation on the reproductive performances of rabbit does: preliminary results. Ital. J. Anim. Sci. 14, 700-705.
- Celia C., Cullere M., Gerencsér Zs., Matics Zs., Giaccone V., Kovács M., Bonái A., Szendrő Zs., & Dalle Zotte A., 2016. Dietary supplementation of Digestarom[®] herbal formulation: effect on apparent digestibility, faecal and caecal microbial counts and live performances of growing rabbits. World Rabbit Sci. 24, 95-105.
- Celia C., Cullere M., Gerencsér Zs, Matics Zs., Tasoniero G., Dal Bosco A., Giaccone V., Szendrő Zs., Dalle Zotte A., 2016. Effect of pre and postnatal dietary supplementation with Digestarom® herbal formulation on rabbit carcass traits and meat quality. Meat Sci. 118, 89-95.
- Dalle Zotte A., Celia C., Szendrő Zs., 2016. Herbs and spices inclusion as feedstuff or additive in growing rabbit diets and as additive in rabbit meat: a review. Liv. Sci. 189, 82-90.
- Cullere M., Dalle Zotte A., Celia C., Renteria-Monterrubio A.L., Gerencsér Zs., Matics Zs, Szendrő Zs, Kachlek M.L., Kovács M., 2016. Effect of *Silybum marianum* herb on the productive performances, carcass traits and meat quality of growing rabbit. Liv. Sci. 194, 31-36.

Conference proceedings published in foreign language

- Dalle Zotte A., Celia C., Szendrő Zs., 2014. Herbs and spices as feed additives in growing rabbit. Proceedings of the 26th Hungarian Conference on rabbit production. May 31th, 2014, Kaposvár, Magyarország pp. 47-48.
- Celia C. Gerencsér Zs., Farkas T.P., Dalle Zotte A., Cullere M., Szendrő Zs. Digestarom® kiegészítés hatása az anyanyulak termelésére. 27. Nyúltenyésztési Tudományos Nap May 20th, 2015, Kaposvár, Magyarország pp. 63-68.
- Celia C., Kachlek M.L., Gerencsér Zs., Matics Zs., Szendrő Zs., Dalle Zotte A., Giaccone V., Kovács M., 2015. Effect of Carduus marianum herb on the productive performances of growing rabbits. Proceedings of the 19th international symposium on housing and diseases of rabbits, furproviding animals and pet animals, May 27-28th 2015, Celle, Germany pp. 145-152.

Poster

Celia C., Szendrő Zs., Matics Zs., Gerencsér Zs., Cullere M., Tasoniero G., Dalle Zotte A., 2015 effect of Digestarom[®] feed additive on rabbit carcass traits and meat sensory attributes. Proceedings of the 61st International Congress of Meat Science and Technonlogy, August 23-28th 2015, Clermont-Ferrand, France 6.30.

Other publications not related to the topic of the dissertation

Peer-reviewed paper published in foreign scientific journal

Kachlek M., Szabó-Fodor J., Szabó A., Bors I., Celia C., Gerencsér Zs.,Matics Zs., Szendrő Zs., Tuboly T., Balogh-Zándoki E., GlávitsR., Dalle Zotte A., Kovács M., 2017. Subchronic exposure to

deoxynivalenol exerts slight effect on the immune system and liver morphology of growing rabbits. Acta Vet. Brno 86, 37-44.

Conference proceedings published in foreign language

Kachlek M., Szabó-Fodor J., Bonai A., Bors I., Celia C., Gerencsér Zs., Matics Zs., Szendrő Zs., Dalle Zotte A., Kovács M., 2015. Accessing the possible interaction between card*uus marianus* and dietary deoxynivalenol on caecal microbiota and fermentation of growing rabbits. Proceedings of the 23rd international symposium "Animal science days" 21-24th September, 2015, Istra-Neptun – National Park BRIJUNI, Croatia pp. 186-189.

Poster

Tasoniero G., Cullere M., Celia C., Giaccone V., Aquino J.L. López, Dalle Zotte A., 2016

Effectiveness of different plant sources in improving the shelf-life of chicken meat patties. Proceedings of the 61st International Congress of Meat Science and Technonlogy, 23-28th August 2015, Clermont-Ferrand, France 7.53.