

SHORT COMMUNICATION

Effect of Digestarom® dietary supplementation on the reproductive performances of rabbit does: preliminary results

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Abstract

The study investigated the effect of Digestarom® dietary supplementation on the reproductive performances of rabbit does. Pannon Ka (maternal line) multiparous does were randomly divided into two dietary groups since insemination and fed *ad libitum*. In the first group (n=51), rabbit does were fed with a commercial diet (C), whereas in the other group (n=52) they received the same diet supplemented with 300 mg/kg of Digestarom® (D). The experiment lasted for two reproductive cycles (kindling 1=K1; kindling 2=K2). Body weight of does and litter size (kits born total, alive, stillborn) were recorded at kindling. Litter size and litter weight were registered at 7, 14, 21 days of age after nursing, and the average individual weight of kits was calculated. Kits' mortality was recorded daily. At K1, rabbit does performances were unaffected by dietary treatments. During K2, D does were significantly lighter than C ones ($P<0.05$) and displayed a lower kindling rate ($P<0.05$). Digestarom® did not improve the reproductive performance of rabbit does. They seemed to dislike the D diet in K1 and such behavior could have led to the negative results in K2. Further studies should focus on feed acceptance, dose-dependent effect, physiological adaptation and *in vivo* oxidative status of does. Finally, several consecutive reproductive cycles are recommended to test the efficacy of new feed supplements.

Introduction

An increasing consumers awareness towards food safety as well as health safety concerns regarding the use of antibiotics as growth promoter feed additives in animal farming led to the EU ban in 2006 (De Marco *et al.*, 2015). On the one hand, consumers' interest for natural alternative animal feeding strategies has been steadily growing (Hosseini *et al.*, 2013). On the other hand, as a consequence of this new scenario, feed industry started looking for alternative feed additives, which could replace antibiotics in guaranteeing satisfactory productive performances. Therefore, to help to meet this demand, a new research direction towards natural feed additives suitable for animal nutrition has been taking place (Darabighane *et al.*, 2011; Tosi *et al.*, 2013; Mahmoudi *et al.*, 2015).

Strategies to reduce antibiotic use in rabbit production include the use of probiotics, prebiotics, synbiotics, (in)organic acids, plants and their extracts, enzymes and immune modulators (Falcão-e-Cunha *et al.*, 2007; Maertens, 2011; Bovera *et al.*, 2012). Regarding plants and their extracts, in general they can improve the productive performances mainly acting as antimicrobial agents, antioxidants, immune and digestion stimulants (Lee *et al.*, 2014). According to Bingal and Farnsworth (1991) there are over 400 plant species that have been used in a variety of settings and some of them can facilitate, promote and maintain lactation in women (Gabay, 2002).

Digestarom® 1315 is an example of herbal formulation for rabbits which contains a mixture of ten different herbs, spices and plant extracts. The ingredients are: onion (*Allium cepa*), garlic (*Allium sativum*), caraway (*Carum carvi*), fennel (*Foeniculum vulgare*), gentian (*Gentiana lutea*) melissa (*Melissa officinalis*), peppermint (*Mentha arvensis*), anise (*Pimpinella anisum*) oak bark (*Quercus cortex*) and clove (*Syzygium aromaticum L.*), as described by Colin *et al.* (2008). Such herbal formulation was previously tested only by some authors on growing rabbits (Colin *et al.*, 2008; Krieg *et al.*, 2009; Abd-El-Hady *et al.*, 2013; Abd-El-Hady, 2014), whereas it has never been studied in rabbit does.

According to Albert-Puleo (1980) and Hosseinzadeh *et al.* (2013) fennel and anise had a positive effect on milk secretion. Fennel seeds and anise essential oil are rich in anethole, an active estrogenic agent which, in the traditional medicine, has a long history of galactagogue effect (Saddiqi and Iqbal, 2001). Other spices are known for their stimulant

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effect on appetite such as clove, caraway and gentian (Baytop, 1984; Loo and Richard, 1992). Particularly, due to its bitterness, gentian root increased saliva and digestive juices secretions thus alleviating digestive disorders in dogs (Meier and Meier-Liebi, 1993). Peppermint, oak bark, onion and garlic are known for their antibacterial activity. In addition, garlic possesses antifungal, antiparasitic and antioxidant effects (Ankri and Mirelman, 1999; Inouye *et al.*, 2001; Ye *et al.*, 2013).

The aim of the experiment was to investigate for the first time the effects of Digestarom® dietary supplementation on the reproductive performances of does in two consecutive kindlings.

Materials and methods

Animals and experimental design

The study was performed in the rabbit farm of Kaposvár University (Kaposvár, Hungary) on Pannon Ka (maternal line) multiparous does (n=103) with an average of 4 parturitions. The does were individually housed in wire mesh cages, under controlled conditions (temperature: 15-20 °C, photoperiod: 16L:8D,

relative humidity: 55-65%). Does received an intramuscular injection of 1.5 µg GnRH analogue (Ovurelin; Reanal[®], Budapest, Hungary) and were artificially inseminated (AI) 11 days after kindling with diluted semen of a single buck using one batch system. After parturition, the litters were equalized to ten kits and controlled nursing was applied. The experiment started at the day of insemination and finished after two reproductive cycles (kindling 1=K1 and kindling 2=K2), at weaning.

Does were fed balanced pelleted diets and divided in two feeding groups which were homogeneously formed considering similar body weight, parity order and status of production (lactating, non-lactating). The first group (n=51) was fed a commercial diet (C), whereas in the other group (n=52) the animals received the same diet supplemented with 300 mg/kg of Digestarom[®] (D). Experimental diets were available *ad libitum* and were isoprotein, isoenergy and had similar chemical composition (Table 1).

Rabbit does were weighed at kindling and kindling rate was calculated as kindling/AI (%). Litter size data (number of kits born total, alive, stillborn) were recorded at the day of kindling. During the experiment, litter size and litter weight were registered after nursing at 7, 14, 21 days of age, and the individual weight of kits was calculated (litter weight/litter size). Feed intake was measured weekly, but it was not taken into account due to the large amount of pellet wasted by some D does. Kit's mortality was recorded daily, until weaning. The does that died or were culled during the experiment (inadequate body condition or health problem) were not replaced.

The protocol has been approved by a suitably constituted Institutional Animal Welfare Committee of the Kaposvár University, in accordance with the provisions of the Declaration of Helsinki (revised in Seoul) - Ethical Principles for Medical Research Involving Human Subjects.

Chemical analyses of the diets

Analyses of the experimental diets were performed in duplicate according to AOAC (2000) methods to determine the concentrations of dry matter (Method n. 934.01), crude protein (Method n. 2001.11), crude fibre (Method n. 978.10), ash (Method n. 996.11) and starch (amyloglucosidase-alpha-amylase, Method n. 996-11). Ether extract was determined after acid-hydrolysis. Acid detergent fibre (ADF), neutral detergent fibre (NDF, without sodium sulphite), and acid detergent lignin (ADL) were analysed according to AOAC procedure (2000; method n. 973.187), Mertens (2002)

and Van Soest *et al.* (1991), respectively, using the sequential procedure and the filter bag system (Ankom Technology, New York, NY, USA). Gross Energy content of the diets was as measured with an adiabatic bomb calorimeter (ISO, 1998). Mineral analyses (Ca, P, Na, K, Mg, Fe, S, Zn) were performed by ICP-OES (Spectro Ciros Vision EOP; SPECTRO Analytical Instruments GmbH, Kleve, Germany) after microwave digestion (999.10; AOAC, 2000).

Statistical analysis

Data were analysed using the General Linear Model Procedures of the SAS 9.1 statistical analysis software for Windows (SAS, 2004). All does and kits performances were subjected to a one-way ANOVA in which experimental diets (C, D) were a fixed effect and the number of kindlings was considered as random effect. A Chi squared test was performed to evaluate kindling rate and mortality. Differences were considered significant when $P < 0.05$.

Results and discussion

Reproductive performances of the K1 are reported in Table 2. In general, diet did not affect the considered traits. At K1, body weight of does was similar in the two dietary groups (4538 and 4516 g for C and D does, respectively). Despite D does exhibited a kindling rate of 86.5%, which was 13% higher than that of C animals, however no statistical difference was evidenced. Litter size, litter weight as well as individual weight of kits were overall satisfactory. Specifically, even if no statistical differences were evidenced, litter weight as well as individual weight of kits at 7, 14 and 21 days were always numerically lower in D animals compared to C ones. Mortality percentages were overall acceptable and comparable in both experimental groups.

Overall kindling rate at K1 was 76.5 and 86.5% for C and D rabbit does, respectively, which was higher than the fertility rate of another study on multiparous rabbit does (Elkomy and El-Speiy, 2015). Kindling rate of D multiparous does in K1 was higher than that reported in another study considering Pannon White rabbits (Szendr *et al.*, 1999). Litter size (total alive) of the purebred genotype used in our study was similar in both experimental groups and high if compared to the prolificacy of some hybrids (Xicato *et al.*, 2004; Costa *et al.*, 2004; Pascual *et al.*, 2004).

Regarding K2 (Table 3), results of the reproductive performances were different from

those evidenced at K1. In this case, body weight of does at kindling statistically differed between the two groups with D does being lighter than C ones (4246 *vs* 4495 g, respectively; $P < 0.05$). As a consequence of the body condition of does at parturition, also kindling rate resulted different between the two dietary groups with D does showing lower performances compared to C ones (49.0 *vs* 74.4 %, for D and C does, respectively). However, the lower body weight at kindling as well as kindling rate of D does compared to C ones, did not statistically affect neither litter size, weight of kits, nor their mortality rate whose differences were always below the significance threshold.

The study presented an interesting scenario in which, looking at reproductive results of K1, Digestarom[®] seemed to provide similar and overall satisfactory results compared to the C diet. However, the situation consistently changed in K2 where body weight of does at kindling, and consequently their kindling rate, resulted significantly lower in D group compared to C one. Nevertheless, to better understand this phenomenon, it would have been interesting to study the results of further consecutive kindlings applying the same dietary

Table 1. Chemical composition and mineral profile of the experimental diets.

	Experimental diets	
	C	D
Dry matter	905	905
Crude protein	158	158
Ether extract	30	30
Ash	65	70
Crude fibre	181	165
NDF	466	448
ADF	231	223
ADL	60	58
AIA	0.9	0.2
Starch	123	129
NFE	470	481
Ca	5.77	6.21
P	5.93	6.16
Na	1.02	1.44
K	7.21	7.33
Mg	2.55	2.62
Fe	0.09	0.09
S	0.59	0.57
Zn	0.08	0.07
Ca/P	0.97	1.01
Gross energy, MJ/kg	16.64	16.50

C, commercial diet; D, Digestarom[®] diet; NDF, neutral detergent fibre; ADF, acid detergent fibre; ADL, acid detergent lignin; AIA, acid insoluble ash; NFE, nitrogen free extract. Values are expressed as g/kg as fed, unless otherwise stated.

treatments.

Results of weaning rabbits born from does which had been fed with Digestarom® with the same inclusion level of our study (Krieg *et al.*, 2009) evidenced that final body weight, as well as feed conversion ratio and clinical records of digestive disorders, were in favour of Digestarom®-fed rabbits compared to a control group. Better results in terms of body weight and feed conversion ratio were also observed in supplemented rabbits *vs* those fed with a control diet (Abd-El-Hady *et al.*, 2013). Similar results were observed in other two studies on growing rabbits (Colin *et al.*, 2008; Abd-El-Hady, 2014).

On the basis of the above-mentioned studies, our results seemed surprising and hardly explicable. However, Abd-El-Hady (2014), interestingly observed that Digestarom®-fed growing rabbits consumed less feed than the control group. This situation could be deleterious when considering rabbit does. In fact, especially in intensive farming, they need to face challenging physiological situations due to high-energy metabolic expenses, which derive from foetus development, milk production and great litter size (Fortun-Lamothe and Gidenne, 2000). For example, pregnancy is considered a state of oxidative stress due to increased placental mitochondrial activity and production of reactive oxygen species resulting from high-energy and oxygen requirements (Arafa *et al.*, 2010). Approximately 80% of these energy requirements are covered by feed intake (Parigi-Bini *et al.*, 1992) thus, for the remaining 20%, the doe must mobilize body energy reserves, which cause weight losses (Xiccato *et al.*, 1999). It is widely known that energy deficit, consequent mobilization of energy and thus lower body condition, globally reduce the reproductive performances and generate hypo-fertility (Monget and Martin, 1997; Facchin *et al.*, 1999; Fortun-Lamothe and Prunier, 1999; Pascual *et al.*, 2004). Friggens (2003) demonstrated that feed refusal was correlated to the suspension of the doe reproductive cycle in order to avoid severe health problems, which would compromise the whole reproductive capacity.

Even if feed intake was not considered in our study, during the experiment a certain feed refusal by D-fed does was noticed which could have originated the lower reproductive performances observed during K2. As rabbits feed choice is affected by feed palatability (Gidenne *et al.*, 2010), it was hypothesized that such feed refusal expresses by rabbit does could be partly attributed to the particular flavour of Digestarom®. Rabbits are very sensitive to the smell, which is a well-developed

sense. It was shown in several experiments (Hudson, 1985; Hudson and Distel, 1999; Coureaud *et al.*, 2002, 2010; Moncomble *et al.*, 2005) that the closed eyes newborn rabbits are able to find their mother's teats quickly in the dark warren. They are directed to their mother's nipples by specialized odour cues, the so-called nipple-search pheromone. They learn it at very early age because the odour component of the feto-neonatal environment provides the ability of new-born kits to find the teats to suck milk. However, they are able to associate other odours, *e.g.* artificial substances on their mother with nipple-search behaviour and suckling.

Rabbit kits also learn odours originating from their mother's diet (Bilkó *et al.*, 1994; Altbäcker *et al.*, 1995; Hudson and Distel, 1999). This ability is very important in areas where most of the plant species are poisonous. Kits of does prefer the aromatic plants their mother had eaten previously. This learning is very strong because the mother's diet still demonstrates a preference in adult age.

In the present experiment rabbit does received D diet with high level of a novel smell.

According to the experimental results mentioned above, rabbits prefer odours which they meet earlier. Thus, it seems that refusing diets with novel smell is a typical behaviour of rabbits.

Even if the influence of dietary herbs and spices on the reproductive performances of the rabbit does is poorly known, a study by Eiben *et al.* (2004) found that anise and fenugreek supplementation reduced feed intake and consequently determined a loss of body weight, lower milk yield and higher mortality of kits. In another experiment on growing rabbits, Chrastinová *et al.* (2005) observed that a dietary supplementation with 10, 20 and 30 g/kg feed of a commercial additive made of different plant extracts, reduced feed consumption.

On the other hand, some plant extracts were reported to possess a certain toxic effect. For example, studies in other species, including humans, showed that garlic can cause irritation of the stomach lining, nausea, intestinal gas, diarrhea, heart burn and anemia, significant damage to the epithelial mucosal mem-

Table 2. Effect of Digestarom® dietary supplementation on rabbit does performances at kindling 1.

	Experimental diets		P	MSE
	C	D		
AI/kindling, n	51/39	52/45		
BW at insemination, g	4723	4738	0.872	484
BW at kindling, g	4538	4516	0.824	459
BW difference (insemination to K1), g	-171	-180	0.891	312
Kindling rate, %	76.5	86.5	0.188	-
Litter size, n				
Born total	12.7	12.5	0.823	2.72
Born alive	11.6	11.7	0.971	3.21
Stillborn	1.03	0.87	0.718	2.88
After equalization	10.0	10.0	1.000	0.00
7 d	9.67	9.64	0.886	0.71
14 d	9.62	9.62	0.965	0.71
21 d	9.54	9.51	0.881	0.83
Litter weight, g				
7 d	1489	1439	0.362	250
14 d	2501	2431	0.447	418
21 d	3346	3233	0.379	582
Individual weight of kits, g				
7 d	154	149	0.280	20.7
14 d	259	252	0.345	35.4
21 d	350	339	0.275	49.3
Mortality, %				
0-7 d	3.3	3.6	0.860	-
7-14 d	0.5	0.2	0.483	-
14-21 d	0.8	1.2	0.612	-
0-21 d	4.6	5.0	0.860	-

C, commercial diet; D, Digestarom® diet; MSE, mean square error; AI, artificially inseminated; BW, body weight; K1, kindling 1.

brane, resulting in bleeding, shrinkage and ulcers (Samson *et al.*, 2012). The irritating, acidic and oxidizing compounds in raw garlic can be affected by extraction techniques. In fact, many adverse reactions to garlic can be attributed to an excess of oil-soluble organosulfur constituents. For example, the lipid-lowering effects of some oil-soluble sulfur compounds in hepatocytes coincide with cytotoxicity. Differently, even if water-soluble sulfur compounds were effective in reducing cholesterol, they did not show cytotoxic effect. In addition, it was shown that garlic toxicity could be greatly affected by the period in which it is extracted (Amagase *et al.*, 2001). Consequently it is possible that rabbit does, which are in a permanent challenging physiological condition, could have been particularly sensitive and thus affected by a mild toxic/irritating effect of one or more Digestarom® constituents which would however require further investigations.

Overall, Digestarom® did not positively affect the reproductive performances of rabbit

does. In fact, they seemed to hardly accept the dietary supplement in the first kindling, maybe for the strong spicy flavour or as a physiological response to a bland toxicity, which caused a deficit in energy intake ultimately compromising their reproductive performances at K2.

Conclusions

On the basis of the results of our preliminary study, Digestarom® supplementation at dose of 300 mg/kg diet did not seem to be the best choice for rabbit does. However, further investigations are recommended considering feed acceptance, other inclusion levels (dose-dependent effect), physiological adaptation and oxidative status of does. Finally, in order to properly assess the effectiveness of novel feed additives for rabbit does, it is recommended to consider several reproductive cycles and to start the feeding at a younger age, some months before the first insemination.

Table 3. Effect of Digestarom® dietary supplementation on rabbit does performances at kindling 2.

	Experimental diets		P	MSE
	C	D		
AI/kindling, n	39/29	45/22		
BW at kindling, g	4495	4246	0.036	409
BW difference (K1 to K2), g	-15.9	-109	0.225	268
BW difference (insemination to K2), g	-208	-271	0.535	358
Kindling rate, %	74.4	49.0	0.020	-
Litter size, n				
Born total	12.6	12.4	0.851	3.54
Born alive	11.7	11.6	0.930	3.49
Stillborn	0.78	0.90	1.000	0.20
After equalization	10.0	10.0	1.000	0.00
7 d	9.35	9.50	0.547	0.90
14 d	9.0	9.0	1.000	1.25
21 d	8.83	8.86	0.923	1.31
Litter weight, g				
7 d	1442	1377	0.347	243
14 d	2366	2272	0.458	440
21 d	3126	2973	0.412	650
Individual weight of kits, g				
7 d	154	145	0.078	19.3
14 d	264	251	0.156	30.7
21 d	355	335	0.16	52.0
Mortality, %				
0-7 d	6.6	5.0	0.384	-
7-14 d	3.7	5.2	0.404	-
14-21 d	1.9	1.5	0.745	-
0-21 d	12.2	11.7	0.264	-

C, commercial diet; D, Digestarom® diet; MSE, mean square error; AI, artificially inseminated; BW, body weight; K1, kindling 1; K2, kindling 2.

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