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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 does (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 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, synthiotics, (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 Hosseinizadeh 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 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, 1984; Abd-El-Hady et al., 2009; El-Hady, 2013; Abd-El-Hady et al., 2013). Peppermint, oak bark, onion and garlic are known for their antibacterial activity. In addition, garlic possesses antifungal, antiparasitic and antioxidant effects (Ankri and Mirelman, 1984; Abd-El-Hady et al., 2009; El-Hady, 2013; Abd-El-Hady et al., 2013). Peppermint, oak bark, onion and garlic are known for their antibacterial activity. In addition, garlic possesses antifungal, antiparasitic and antioxidant effects (Ankri and Mirelman, 1984; Abd-El-Hady et al., 2009; El-Hady, 2013; Abd-El-Hady et al., 2013). Peppermint, oak bark, onion and garlic are known for their antibacterial activity. In addition, garlic possesses antifungal, antiparasitic and antioxidant effects (Ankri and Mirelman, 1984; Abd-El-Hady et al., 2009; El-Hady, 2013; Abd-El-Hady et al., 2013). Peppermint, oak bark, onion and garlic are known for their antibacterial activity. In addition, garlic possesses antifungal, antiparasitic and antioxidant effects (Ankri and Mirelman, 1984; Abd-El-Hady et al., 2009; El-Hady, 2013; Abd-El-Hady et al., 2013).

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,
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 satisfac-
tory. Specifically, even if no statistical differences were evidenced, litter weight as well as individual weight of kits were overall satisfactory. Generally, the lower body weight of does at kindling, and consequently their kindling rate, resulted significantly lower in D group com-
pared 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

| 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 as 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 (Arafà 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; Courreard 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 the 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 sucking.

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   |
| 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                    | 12.7    | 12.5 | 0.823 | 2.72 |
| Born total                        | 11.6    | 11.7 | 0.971 | 3.21 |
| Born alive                        | 1.03    | 0.87 | 0.718 | 2.88 |
| Stillborn                         | 10.0    | 10.0 | 1.000 | 0.00 |
| After equalization                | 9.67    | 9.64 | 0.886 | 0.71 |
| 7 d                               | 9.62    | 9.62 | 0.965 | 0.71 |
| 21 d                              | 9.54    | 9.51 | 0.881 | 0.83 |
| Litter weight, g                  | 1489    | 1439 | 0.362 | 250 |
| 14 d                              | 2501    | 2431 | 0.447 | 418 |
| 21 d                              | 3346    | 3233 | 0.379 | 582 |
| Individual weight of kits, g      | 154     | 149  | 0.280 | 20.7 |
| 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.

| Experimental diets | C | D | P | MSE |
|--------------------|---|---|---|-----|
| AI/kindling, n      | 39/29 | 45/22 |     |     |
| BW at kindling, g   | 4495 | 4246 | 0.036 | 409 |
| BW difference (K1 to K2), g | -15.9 | -10.9 | 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 | 3.40 |
| 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  | 333  | 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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