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ASSESSING THE POSSIBLE INTERACTION BETWEEN *CARDUUS MARIANUS* AND DIETARY DEOXYNIVALENOL ON CAECAL MICROBIOTA AND FERMENTATION OF GROWING RABBITS

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Original scientific paper

SUMMARY

Contamination of feed with mycotoxins is a common problem encountered in animal farming. Mycotoxin exposure can affect adversely the health of animals. In rabbits caecal fermentation is an essential digestive process being indication of physiological alterations. Deoxynivalenol (DON) is one of the most frequent contaminants of grains which affect the growth of monogastric animals. Data about dietary DON and its effect in rabbits are scarce. Medicinal plants are often used as feed additives to enhance the performance of the animals. Carduus marianus (milk thistle) is known for its hepatoprotective and antioxidant effects (among others) but no data are available about the effect on rabbit caecum. Considering the aforementioned, the aim of this study was to assess the possible interactive effect of Carduus marianus and DON on the caecum of growing rabbits. 75 Pannon White rabbits were reared for six weeks from 35 (after weaning) till 77 days of age. Rabbits received the following diets: control (C), control with DON (CT), control supplemented with C. marianus in 0,5% (H1), control supplemented with C. marianus in 0,5% and DON (H1T), control supplemented with C. marianus in 1% (H2) and control supplemented with C. marianus in 1% and DON (H2T). On slaughter, caecum was collected for the analysis of total volatile fatty acids (VFA) and the microbiota of the caecum, pH of the caecum was also recorded. There was no significant difference in total VFA concentration or individual VFA. Number of aerobic bacteria significantly differed among toxin and non-toxin groups. DON affected adversely the number of aerobic bacteria. An interactive effect of DON and Carduus marianus on E.coli number was observed. There was no effect on total or individual VFA amounts.

Key-words: rabbit caecum, milk thistle, VFA, aerobic bacteria

INTRODUCTION

Mycotoxin contamination of animal feed can pose a serious problem for farm animals, such as pigs, poultry and rabbits, since it frequently affects adversely their health status. In rabbit physiology, caecal fermentation is a very important digestive process that begins around the weaning period (when solid feed is introduced in the diet). The short chain fatty acids produced by fermentation are beneficial as additional source of energy, and also because of their antibacterial effect (Bellier and Gidenne, 1996). The physiological composition of caecal microbiota is crucial for the preservation of the homeostasis (Bagóné Vántus et al., 2014).

Deoxynivalenol (DON) is a mycotoxin mainly produced by *Fusarium graminearum* and *F. culmorum* which

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are common contaminants of cereal grains (Bonnet et al., 2012). Pestka and Smolinski (2005) stated that monogastric animals' growth and weight gain was suppressed upon chronic or sub-chronic exposure of DON with swine being the most sensitive among farm animals. Data on the effect of dietary DON in rabbits are scarce (Khera et al., 1986; Hewitt et al., 2012). Mycotoxins are taken up mainly orally by consumption of contaminated feed. As such, it can be proposed that they may interact with the gut microbiota as it has been shown in the case of ochratoxin A (Guo et al., 2014).

Medicinal plants are often used to improve the health status of animals, especially after the antibiotics have been banned in European Union (European Union Commission, 2005). *Carduus marianus* (syn. *Silybum marianum*) is a member of Asteraceae (Compositae) family which contains several species used in herbal medicine e.g. *Echinacea* (Gao et al., 2010). One of the most popular colloquial names of *C. marianus* is milk thistle (Dunnick and Nyska, 2012). *C. marianus* is known for its hepatoprotective (Flora et al., 1998) as well as for its antioxidant effect (Henning et al., 2014). Some studies suggest a potential positive effect in inhibition of chemically induced diabetes (Shakeel and Yar, 2014) or anti-atherosclerotic effect (Białecka, 1997). As far as we are concerned there are no studies about the effect of *C. marianus* on caecal microbiota of rabbits.

Considering the aforementioned the aim of this study was to assess the possible interactive effect of *C. marianus* and DON on the caecal microbiota and fermentation of growing rabbits.

MATERIAL AND METHODS

Animals and diets

Seventy five Pannon White rabbits were reared in mesh-wired cages from 35 till 77 days of age. The rabbits had free access to water (pacifiers) and feed (*ad libitum*). The temperature was 16-18ºC and the photoperiod was set to 16h of light and 8 hours of dark. The weight and feed intake were recorded on a weekly basis whereas the morbidity and mortality were daily monitored.

The animals received three different diets during the first period (3 weeks). The control (C) diet was a nonsupplemented basal diet formulated in such a way to meet the nutritional needs of weaned rabbits, control diet supplemented with the herb (*C. marianus*) in a concentration of 5 g/kg (H1) and control diet supplemented with the herb in a concentration of 10 g/kg (H2; Table 1). During the second period (3 weeks) the rabbits received six different diets after the subdivision of the three initial groups. The diets were formulated in a similar way of the first period but this time supplemented with the toxin (DON) as well (CT, H1 and H2T; Table 1). The experimental protocol was authorized by the Food Chain Safety and Animal Health Directorate of the Somogy County Agricultural Office, under permission number SOI/31/1679-11/2014.

Table 1. Chemical composition of the experimental diets

Chemical composition g/kg of DM	C	H1	H2	CT	H1T	H2T
Dry matter (g/kg of feed; DM)	91.2	90.4	91.0	90.8	90.9	93.9
Crude protein (CP)	18.5	18.8	18.1	18.6	18.3	18.4
Crude fat (EE)	2.8	2.9	2.8	2.9	2.8	2.7
Crude fibre	16.7	16.4	16.4	16.3	17.2	16.3
Ash (ASH)	8.2	8.6	8.3	8.2	8.3	8.3
Starch	18.8	18.6	19.8	18.5	18.0	18.6
Natural detergent fibre (NDF)	35.5	36.0	34.7	35.1	35.2	34.9
Acid detergent fibre (ADF)	22.3	22.4	21.6	22.1	22.0	21.9
Acid detergent lignin (ADL)	6.1	6.3	5.7	6.1	6.2	6.2
Acid insoluble ash (AIA)	1.2	1.4	1.1	1.0	1.6	1.0

Mycotoxin production and plant purchase

Toxin was produced using *Fusarium graminearum* strain number IFA 77 (from "Das Interuniversitäre Department für Agrarbiotechnologie", Tulln, Austria) fungal culture (7 days old), grown on Potato Dextrose Agar (PDA; Chemika-Biochemica, Basil, Switzerland). The inoculated cultures were stored and incubated at 28°C for 2 weeks. When the incubation time was complete the fungus-infected cereal was dried at room temperature and grounded.

LC-MS analysis was performed by a Shimadzu Prominence UFLC separation system equipped with a LC-MS-2020 single quadrupole (ultra-fast) liquid chromatograph mass spectrometer (Shimadzu, Kyoto, Japan) with electrospray source.

The homogenized fungal cultures contained DON at concentration of 7140 mg/kg. Fungal cultures were mixed into the feed of the experimental animals, based on the dose presented in Table 2.

Table 2. Concentration of the toxin in the experimental feeds in the different groups

Groups	DON (mg/kg)
CT	10.1
H1T	11.5
H2T	10.6

The mycotoxin production as well as the determination of its concentration, were performed at the Department of Physiology, Biochemistry and Animal Health of Kaposvár University. *Carduus marianus* was purchased from Parceval (Pty) Ltd Pharmaceuticals, South Africa in powder form (seeds).

Determination of composition of caecal microbiota and volatile fatty acids (vfa) concentrations

At 78 days of age 6 animals per group were slaughtered. The digestive tract was removed immediately and the caecum was separated. The caecal contents' pH values were determined using a pH meter (OP-110, Radelkis, Hungary). One gram of caecal chyme was used immediately after sampling for microbiological culture, and anaerobic conditions were maintained by the use of carbon dioxide. The rest of the caecal content was weighed, freezed and stored at -80ºC for further volatile fatty acids (VFA) analysis.

The analyses for both procedures (microbiological culture and analysis of the volatile fatty acids) were performed as previously described respectively in Kovács et al. (2011).

Statistical analysis

Statistical analysis of the data was performed with SPSS (version 19) statistical software package using one way analysis of variance (ANOVA), LSD was used for the Post Hoc test. T-test was also performed. In all the cases the significance level was $p<0.05$.

RESULTS AND DISCUSSION

Production parameters were not affected adversely by the consumption of the toxin (data not shown).

There was no significant difference in the total VFA (mmol/kg) or the particular VFA (acetic, propionic and butyric; Table 4).

The composition of caecal microbiota showed no significant differences regarding anaerobic bacteria and bacteroides (Table 3). Coliforms' numbers were very low in all the samples (colonies<100; data not shown). On the other hand, there were significant differences in the number of aerobic bacteria. More specifically, groups C and H1 had significantly lower number of aerobic bacteria compared to all toxin groups (i.e. CT, H1T and H2T; Table 3) suggesting an effect of DON on aerobic bacteria regardless the supplementation with the herb. This is confirmed by the T test performed using as factor the consumption of mycotoxin solely (C, H1, H2 and CT, H1T, H2T respectively). *Escherichia coli* (*E. coli*) number significantly differed between H2 and all the toxin groups which can suggest a possible positive effect of the herb in 1% concentration (data not shown).

The pH of the caecal content didn't differ significantly among the groups (Table 4). There was a correlation of VFA and pH, the correlation coefficient was -0.593 and the significance $p=0.01$.

Table 3. Compositon of caecal microbiota (CFU log10/g, mean±SD, p<0.05)

Microbiota	C	CT	H1	H1T	H2	H2T
Aerobic	4.9 ± 0.3^{a}	5.8 ± 0.6^{b}	$5.3 \pm 0.6^{a,b}$	5.7 ± 0.6^{b}	$5.2 \pm 0.2^{a,b}$	5.7 ± 0.6^{b}
Anaerobic	8.9 ± 0.4	9.1 ± 0.4	8.8 ± 0.3	9.0 ± 0.6	8.7 ± 0.5	9.0 ± 0.5
Bacteroides	8.3 ± 0.5	8.5 ± 0.2	8.5 ± 0.3	8.4 ± 0.5	8.3 ± 0.5	8.6 ± 0.4

Table 4. Volatile fatty acids of the caecal chyme (% of total fatty acid content) and caecal pH (mean±SD)

Volatile fatty acids	C	CT	H1	H1T	H2	H2T
Total VFA mmol/kg	46.6 ± 7.7	48.9 ± 7.7	45.8 ± 11.9	39.8 ± 18.8	46.6 ± 16.0	43.4 ± 12.6
Acetic acid (%)	78.6 ± 1.5	78.7 ± 3.7	78.1 ± 2.9	77.2 ± 2.6	79.8 ±2.4	78.6 ± 2.8
Propionic acid (%)	7.2 ± 0.8	7.0 ± 1.2	6.2 ± 0.6	8.0 ± 1.1	7.1 ± 1.8	7.0 ± 1.1
Butyric acid (%)	14.3 ± 1.5	14.3 ± 3.7	14.3 ± 1.1	14.7 ± 2.1	13.2 ± 1.2	14.4 ± 2.1
pH	6.04 ± 0.15	6.05 ± 0.39	6.06 ± 0.14	6.08 ± 0.22	5.88 ± 0.15	6.01 ± 0.23

CONCLUSION

This was a preliminary study on the possible interactive effect of *C. marianus* and DON on caecal microbiota and fermentation. DON increased the number of aerobic bacteria in the caecum of rabbits which is an undesirable situation due to the anaerobic conditions (in physiological state) of caecum. The effect of DON was slightly influenced by the 1% herbal supplemantion regarding *E. coli*. More studies should be conducted with more animals to ensure if there is truly interactive effect. There was no interaction of DON and *C. marianus* on total VFA amount.

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