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Effect of Endophyte-infected Diets on Selected Blood Parameters and the Histopathological Picture of the Kidney and Liver of Lambs

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The aim of the study was to find out if the presence of endophytic fungi and thus of the alkaloid ergovaline in feed affects blood plasma parameters and the histological picture of the kidney and liver of reared lambs. Sixteen growing lambs were divided into 2 groups: control (K) and experimental (D). Animals were fed with meadow hay (0.4 kg), concentrate feed (0.4 kg) and silages (2 kg). Group K received silage from endophyte-free fescue forage while group D received silage from fescue infected with the endophyte fungus *Neotyphodium uncinatum* (high level of seed infection – 52%, and forage infection – 47%). Blood was taken from the lambs three times (at the start, on the 45th day and at the end of the study – on 79th day) to determine the levels of aspartate aminotransferase (AspAT), alanine aminotransferase (ALAT), creatinine, triglycerides, cholesterol and bilirubin. Kidney and liver samples were taken from each animal postmortem. H+E staining was performed in order to disclose histopathological changes in the interstitial tissue of the kidneys and renal corpuscles. The degree of fatty liver was determined using Oil Red staining. Feeding silages from endophyte-infected meadow fescue forages did not have a clear effect on the plasma parameters of growing lambs. The levels of AspAT and triglycerides were high but did not exceed the upper permissible limit. In the group of animals receiving endophyte-infected silage, the degree of fatty liver was slightly higher with more histopathological changes in the interstitial tissue and renal corpuscles than in the animals fed with endophyte-free diets.

Key words: Lambs, silages, endophytes, blood parameters, kidney, liver.

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Some *Festuca* and *Lolium* grasses are infected with endophytic fungi. These organisms develop inside plant cells rather than on the surface of plants. Fungal cells increase the resistance of grasses to adverse environmental factors, such as drought and cold. However, depending on the level of infection, they can be detrimental to animals ingesting the infected fodder (EMILE *et al.* 2000; OLIVER *et al.* 2000). The main alkaloids produced by endophytes are neurotoxins (lolitrems) in ryegrasses and ergot alkaloids (ergotamine, ergosine, ergovaline and others) in fescue (KALAČ & MIKA 1997). Attempts have been made to limit the adverse effect of mycotoxins produced by endophytic fungi to prevent their detrimental effects on the health and productivity of animals.

Metabolites produced by fungi have a negative effect on animal health. Fatty liver, i.e. intracellular lipid deposition in liver hepatocytes, affects many livestock species such as dairy cows, fattening cattle, hens and geese (MARCZUK and FILAR 2003; MIŚKOWIAK *et al.* 1976; OPRZĄDEK *et al.* 2000). These changes are most often caused by hypoxia, unbalanced diet (e.g. excess dietary energy) and toxin contamination of feeds. Nephrotoxic agents produced by fungi, e.g. ochratoxin, lead to necrosis of the proximal nephron cells (SCHWERDT *et al.* 2003) or proliferative processes in endothe-

The studies were carried out in accordance with the permission of the Ethical Committee in Bydgoszcz (No. 9/2004).

lial cells and cell degeneration in renal tubules (AYDIN *et al.* 2003).

The aim of the study was to find out if the presence of endophytic fungi (*Neotyphodium* species) and thus of the alkaloid ergovaline in feed affects plasma parameters and the histological picture of the kidney and liver of reared lambs.

Material and Methods

Forage of meadow fescue for silage making was harvested at the Mochełek Research Station belonging to the Department of Animal Science of the University of Technology and Agriculture in Bydgoszcz. Seeds originating from the Plant Breeding and Acclimatization Experimental Station in Bartążek were sown in the spring of 2003 with a barley companion crop and in the first year they were subjected to crop production practices such as fertilizing, trimming and weeding. One-hectare fields were sown with fungus-free fescue and fescue infected with the endophytic fungus *Neotyphodium uncinatum* (seed infection rate of 52%). In each case, 10 kg of grass and 100 kg of barley per ha was sown. Fertilization rate was 51 kg N/ha, 15 kg P/ha and 24 kg K/ha (following barley harvest on 20 July). In the second year of use, first-cut forage (1 June 2004) was ensiled in concrete silos of 6 m^3 capacity, each in two variants: K – control silage made of forage from an endophyte-free field and D – silage made of forage infected with the fungus *N. uncinatum*. The infection rate of plants infected with *N. uncinatum* was 47%.

The level of seed and plant infection with endophytes was determined using the Rose Bengal test (SAHA *et al.* 1988). Chemical composition of the forages and silages were determined using the Weende and Van Soest methods (DYMNICKA & SOKÓŁ 2001), and their nutritive value was calculated using WinWar software.

The experiment was carried out with 16 lambs, which were reared semi-intensively at the Mochełek Research Station of the Department of Animal Science of the University of Technology and Agriculture from 5 August 2004 to 23 October 10 2004 (79 days). There were two groups of three-month-old lambs – control (K) and experimental (D), each with 8 animals (four ram lambs and four ewe lambs). Twice a day, all the animals received meadow hay (0.4 kg), concentrate feeds (0.4 kg) and silages (2 kg). Group K received silage from endophyte-free fescue forage, and group D silage from fescue infected with *N. uncinatum*. The initial body weights of the lambs were 23.13 kg in group K and 23.00 kg in group D.

Blood for biochemical analysis was collected before morning feeding (at the start, on the 45th day and at the end of the experiment). Blood serum (aspartate aminotransferase and alanine aminotransferase activity, the levels of creatinine, triglycerides, cholesterol and bilirubin) was analysed using a Technicon RA 2000 automatic analyser.

At the end of the experiment, the lambs were slaughtered and liver and kidney samples were taken from each animal for histopathological tests. Liver samples were taken from the right lobe, and kidney samples from the cortex. The tissue samples were collected 45 min postmortem and frozen in liquid nitrogen. Frozen samples were sectioned at 8 μm on a Leica cryostat and stained using the H+E method to analyse changes in the interstitial tissue of the kidney and renal corpuscles and the Oil Red method to analyse the degree of fatty liver (ROMEIS 1989).

The results were analysed statistically using two-way analysis of variance (SAS/STAT 1995) and significant differences between the means were verified by the Scheffe test at $P \leq 0.05$ and $P \leq 0.01$.

Results and Discussion

The nutritive value of the feeds is given in Table 1. The level of nutrients complied with the French requirements for growing lambs. The final weight was 31.8 kg in group K and 34.9 kg in group D. No significant differences in body weight were found between the groups.

Table 1

Chemical composition of the feeds

Ingredient	Silage K	Silage D	Meadow hay	Concentrate feed
Dry matter, %	25.46	27.86	87.66	86.73
Content in dry matter				
Organic matter, %	92.97	93.90	91.89	91.81
Crude protein, %	9.70	12.88	11.16	19.95
Crude fat, %	5.11	4.95	1.95	4.67
Crude fibre, %	38.85	36.76	39.81	8.79
N-free extractives, %	39.32	39.30	40.08	58.40
UFL	0.80	0.82	0.63	0.94
PDIN, g	56.44	74.96	69.48	126.00
PDIE, g	55.65	60.96	77.15	109.00

The determinations of serum parameters are shown in Table 2. The activity of liver enzymes is used as a major diagnostic indicator to determine the functional activity of the liver (JANIAK 1989). In our study, AspAT activity only slightly ex-

Table 2

Mean values of the biochemical parameters of lamb serum

Parameter	K	D	Reference values (WINNICKA 2002)
AspAT (U/l)	96.58±13.95	99.46±11.84	40-96
ALAT (U/l)	13.42^{A}±4.61	14.54^{B}±4.95	5-17
Bilirubin (μmol/l)	2.83±0.46	2.86±0.32	1.7-6.0
Creatinine (μmol/l)	95.54±9.51	94.96±8.79	79.6-160.9
Cholesterol (μmol/l)	1.75±0.29	1.63±0.24	1.1-2.1
Triglycerides (μmol/l)	0.29^{A}±0.09	0.30^{B}±0.11	0.1-0.3

Notes: A, B. ($P<0.01$) – statistically significant differences between the groups are marked with the same letters

ceeded the upper limit of the normal range (JANIAK 1989; WINNICKA 2002). Higher AspAT concentration was found in group D, but the differences were not statistically significant. Some authors (BRONICKI & DEMBIŃSKI 1994) established that the AspAT level reflects the degree of fatty liver. They reported the activity of this enzyme to increase in animals with fatty liver while challenging the diagnostic usefulness of AspAT as the only indicator of fatty liver syndrome. Analysis of the results of ALAT activity showed that they were uniform and within the permissible range for this group of animals (WINNICKA 2002), although differences between the groups were statistically significant.

Triglyceride and cholesterol levels are closely related to the body's lipid metabolism (GRABOWICZ *et al.* 2004). The level of triglycerides in animals showed statistically significant differences. No differences were revealed in cholesterol levels between the control and experimental groups. The accepted reference values were not exceeded in any of the groups (WINNICKA 2002).

In all the animals, the bilirubin level assumed similar values that fell within the reference range (WINNICKA 2002). Other authors showed the concentration of total bilirubin to increase and the concentration of cholesterol to decrease due to hepatic dysfunction (JANIAK 1989; MARCZUK & FILAR 2003).

The degree of fatty liver was evaluated on a four-point scale (Table 3). No fatty liver degeneration was found in one animal of the experimental group. A high degree of fatty liver was shown in the same number of lambs (25%) in both groups. The slightly higher number of animals with a medium degree of fatty liver in the experimental group than in the control group is in line with other authors (MIŚKOWIAK *et al.* 1976) who found that the degree of hepatocyte resistance to fatty degeneration is to a great extent an animal specific reaction. The medium and high degree of fatty liver (Fig. 1) is supported by the biochemical parameters of blood, notably the level of serum triglycerides, which in all the groups reached the upper limit of the normal range for sheep (Table 2).

Table 3

Number of control and experimental lambs with fatty liver

Degree of fatty liver	K	D
–	0	1
+	4	1
++	2	4
+++	2	2
++++	0	0

(–) no change, (+) low, (++) medium, (+++) high, (++++) very high degree of fatty liver

Microscopic findings of the lamb kidneys (Table 4) showed small infiltrations in half of the control animals and in only one experimental animal. Large infiltrations were observed in group D

Table 4

Number of control and experimental lambs with inflammatory infiltration of the interstitial tissue or pathological changes of renal corpuscles

Item	K	D
No changes	3	3
Small infiltrations	4	1
Large infiltrations	0	2
Fibrosis of Bowman's capsule	0	1
Proliferation of mesangium cells	1	2

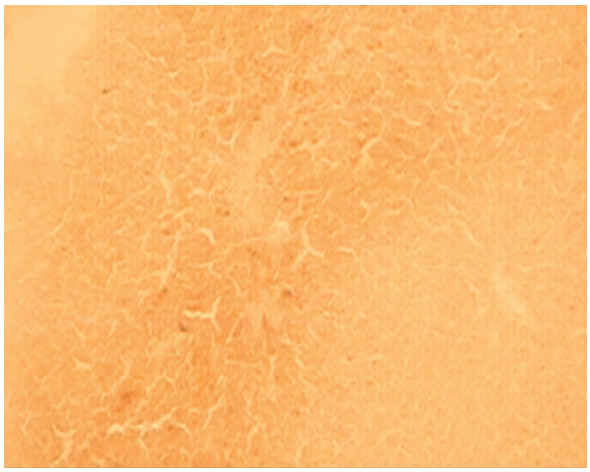

Fig. 1. Lipids filling of hepatocytes in the central and paracentral areas of lobule. Oil Red staining, ×200.

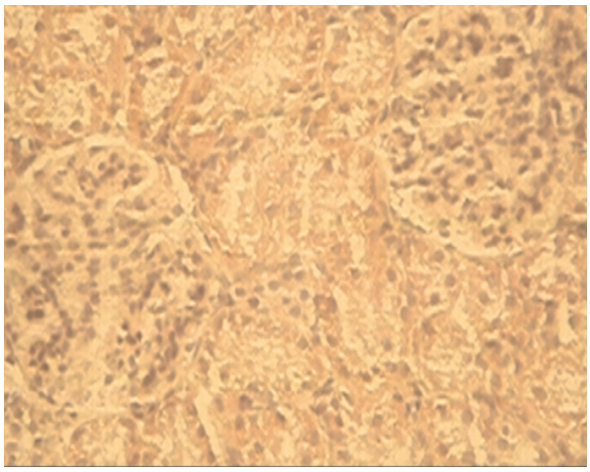

Fig. 2. Proliferation of mesangium cells in renal glomerules. H+E staining, ×200.

(25%) but not in the control group. In the experimental group, there was a greater degree (1/4 animals) of mesangium cell proliferation (Fig. 2) and fibrosis of Bowman's capsule was observed. According to DĄBROWSKA (2003), the concentration of creatinine increases in renal failure caused by toxemia. In the present study, the creatinine concentration remained constant within the physiologically normal range and no pathological changes in kidneys were found.

Feeding silages from endophyte-infected meadow fescue forages containing the ergopeptid alkaloid – ergovaline did not have a clear effect on the plasma parameters of growing lambs. The levels of AspAT and triglycerides were high but did not exceed the upper permissible limit. In the group of animals receiving endophyte-infected silage, the degree of fatty liver was slightly higher with more histopathological changes in the interstitial tissue and renal corpuscles than in the animals fed with endophyte-free diets.

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