

Pathological changes in the liver and thyroid in broiler chickens fed by rapeseed cake

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ABSTRACT

The aim of this study was to investigate pathological changes in the liver and thyroid gland, to determine the possibility of using different levels of rapeseed cake in diets for chickens. The experiment was carried out on three hundred and sixty day-old male Cobb hybrid chickens, were fed by three different feeding regimens (T-0 = 0%; T-5 = 5%; T-10 = 10% of rapeseed cake, "double low" cultivar Bristol). The rapeseed cake contains 2.86 µmol/g of glucosinolate (GIs) and the ratio of erucic acid was 0.08% of total fatty acids. Introduction of 5% rapeseed cake to chicken feed increased mortality, but it was still an acceptable mortality rate. The average liver mass increased in groups T-5 and T-10 compared to the control group. The thyroid glands of the treated animals showed no significant increase in weight compared to the control group. Histopathologically, liver and thyroid lesions were more prominent in treated animals compared to the control group. In the treated group a significantly higher rate of perivascular necrosis of hepatocytes, and perivascular mixtocoelular cell infiltration in the liver was found in the T-5 group. In the liver, there was a significantly higher rate of congestion, degeneration of blood vessels, vasculitis, and hydropic degeneration in the T-10 group. The thyroid glands of the treated animals showed a significantly higher rate of scattered proliferation of follicular epithelial cells, and mild interstitial fibrosis in the T-5 group. Severe interstitial fibrosis was pronounced only in the T-10 group, with a significantly higher rate. The highest rate of follicular haemorrhages was found in the T-10 group, followed by the T-5 group. The relation between these rapeseed cake specific changes, and the undesirable effects specific to rapeseed cake remain to be elucidated. However, the existence of causative factors other than erucic acid and glucosinolates cannot be excluded. In conclusion, the introduction of 5% and 10% rapeseed cake (Bristol-

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“double low” cultivar) to broiler chickens caused liver and thyroid changes, but these changes were not severe enough to have any major impact on production results.

Key words: rapeseed cake, broiler chickens, liver, thyroid gland, pathological changes

Introduction

In recent years, a rise in the price of soybean meal has been recorded on the world market, which, together with the prohibition of the use of processed animal protein, has led to a significant increase in the price of feed for poultry. Therefore, a great deal of effort by nutritionists and feed producers is being aimed at finding a favourable source of protein in poultry nutrition that would partially or completely replace the above mentioned feeds. In past decades, by-products in the production of biodiesel have been used as an alternative source of protein in poultry nutrition.

Rapeseed cake, as a by-product in biodiesel production, is characterized by high nutritional value, consisting of approximately 35.0 crude protein, 14.0 to 15.0 % of crude fat, 1736 kcal kg⁻¹ of metabolic energy, 8.0 to 9.0 % of omega-3 (PUFAn3) and 20.0 to 24.0 % of omega-6 (PUFAn6) fatty acids. It contains less protein than fish meal, but the amounts of omega-3 and omega-6 acids are approximately equal (PROSKINA et al., 2011). However, the limiting factors for use of a higher proportion in feed mixtures are high amounts of crude fibre, and antinutritional factors such as: glucosinolates (Gls), synapine, erucic acid, tannin, phytates, and electrolyte balance (SMULIKOWSKA et al., 1998; CHIBOWSKA et al., 2000; KHAJALI and SLOMINSKI 2012; AHMED et al., 2015). The Gls are a large group of sulphur-containing secondary plant metabolites. The ingestion of substantial amounts of glucosinolates may be deleterious to animal health and production. Upon ingestion, the intact Gls and/or their breakdown products are absorbed from the intestinal lumen and/or are converted into other products (LEMING et al., 2004; TRIPATHI and MISHRA, 2007). The fodder and seed meals of the genus Brassica are the chief source of Gls in animal diets. Gls have long been known to reduce the intake of feed (HILL, 1991), induce iodine deficiency (BUREL et al., 2000), and hypertrophy of the liver, kidneys and thyroid (MANDIKI et al., 1999; BUREL et al., 2000; MABON et al., 2000; TRIPATHI and MISHRA, 2007) and cause higher levels mortality (CSWRI, 2002). But the use of rapeseed cake derived from double zero, low-glucosinolate and low-erucic varieties of rapeseed could effectively substitute soybean meal in poultry diets (MIKULSKI et al., 2012). Double zero varieties of rapeseed contain less than 2 % erucic acid and less than 30 µmoles Gls in the defatted meal (ZEB, 1998).

Therefore, the aim of this study was to investigate the pathological changes in the liver and thyroid glands of broiler chickens fed with different proportions of rapeseed cake, to determine the possibility of using rapeseed cake derived from double zero varieties of rapeseed in diets for chickens.

Materials and methods

Three hundred and sixty day-old male Cobb hybrid chickens, vaccinated against Newcastle disease, were randomly divided into 3 groups, each group with 4 replications (n = 30). Each group was placed in a cage with floor grazing throughout the test duration of 42 days. The trial facility, a few days before the date of receipt of the chickens, was thoroughly cleaned, disinfected, and on the day before the chickens were brought in, it was heated to a temperature of 27 °C (in the occupied zone 32 °C) (according to MUŽIĆ and JANJEČIĆ, 2002). Control groups of broilers were fed with a mixture (pellets) that did not contain any rapeseed cake (T-0), and the experimental groups were fed with mixtures that contained rapeseed cake (“double low” cultivar) in proportions of 5 % and 10 % (T-5 and T-10 respectively) (Table 1). Each feeding regimen had four replicates. Diets were formulated as isoenergetic and isonitrogenous, according to the National Research Council (1994). Rapeseed cake used in the study was derived from rapeseed cultivar Bristol (“00”), originating from Croatia, harvest 2009. It was obtained by cold pressing, where the temperature of heating the grain does not exceed 50 °C. The rapeseed cake contained 2.86 µmol/g of GlS and the ratio of erucic acid was 0.08 % of total fatty acids. The chemical compositions of the diets and rapeseed cake are shown in Tables 2 and 3.

Table 1. Feed ingredients of diets for chickens, %

Ingredients	Regimen					
	T-0		T-5		T-10	
	Starter	Finisher	Starter	Finisher	Starter	Finisher
Corn	52.60	58.50	51.00	56.70	49.75	54.90
Soybean meal	39.50	34.00	36.40	31.00	33.00	28.00
Rapeseed cake	0.00	0.00	5.00	5.00	10.00	10.00
Oil	2.80	2.30	2.70	2.30	2.50	2.30
Monocalcium phosphate	1.45	1.60	1.35	1.50	1.20	1.35
CaCO ₃	1.60	1.65	1.50	1.55	1.50	1.50
Salt	0.40	0.40	0.40	0.40	0.40	0.40
Vitamin and mineral premix	0.50	0.50	0.50	0.50	0.50	0.50
DL methionine	0.15	0.05	0.15	0.05	0.15	0.05
Binder	1.00	1.00	1.00	1.00	1.00	1.00

Table 2. Chemical composition of rapeseed cake, %

	Moisture	Ash	Crude protein	Crude fat	Crude fibre	N-free extractives	Ca	P	Starch
Rapeseed cake	6.68	6.93	30.62	7.44	12.37	35.96	1.07	1.22	0.30

Table 3. Chemical composition of diets for chickens, %

Nutrient	T-0		Regimen		T-10	
	Starter	Finisher	T-5		Finisher	Starter
			Starter	Starter		
Moisture	10.73	10.80	10.66	10.72	10.60	10.64
Crude protein	22.13	20.14	22.11	20.16	22.00	20.17
Crude fat	5.36	4.93	5.61	5.27	5.76	5.62
Crude fibre	2.55	2.44	2.92	2.81	3.30	3.19
Ash	6.58	6.43	6.45	6.31	6.36	6.19
Ca	0.98	1.00	0.97	1.00	1.00	1.00
P-total	0.69	0.71	0.70	0.71	0.70	0.71
P-usable	0.50	0.50	0.48	0.48	0.45	0.46
Na	0.19	0.19	0.19	0.19	0.19	0.19
Arginine	1.51	1.36	1.50	1.35	1.48	1.34
Methionine	0.49	0.37	0.50	0.38	0.51	0.39
Cystine	0.35	0.32	0.37	0.34	0.38	0.36
Met + Cys	0.83	0.69	0.86	0.72	0.89	0.75
Lysine	1.23	1.09	1.23	1.09	1.22	1.09
Tryptophan	0.27	0.24	0.27	0.24	0.27	0.24
Threonine	0.84	0.77	0.85	0.78	0.86	0.79
Metabolic energy, (kcal kg ⁻¹)	2870.93	2892.42	2870.93	2897.2	2868.54	2901.98
Lysine	1.23	1.09	1.23	1.09	1.22	1.09
Tryptophan	0.27	0.24	0.27	0.24	0.27	0.24
Threonine	0.84	0.77	0.85	0.78	0.86	0.79
Metabolic energy, (kcal kg ⁻¹)	2870.93	2892.42	2870.93	2897.2	2868.54	2901.98

On the basis of body weight gain and amount of feed consumed, conversion of feed mixtures were calculated. The mortality of the chickens was monitored and recorded daily. At the end of the experimental feed rs, 42 day old broilers were weighed and sacrificed under chloroform anesthesia immediately thereafter. Experimental animals

were treated according to the Croatian legislation on animal protection and in accordance with the recommendations of the European Community (86/609/EEC). The necropsy was performed and organ samples (liver and thyroid gland tissue) of 10 chickens (randomly chosen) from each group (including the control group) weighed and sampled for histopathological analysis by light-microscopy. The organs samples were fixed in 10 % neutral formalin, embedded in histosec (paraffin), 5 µm thick sections were cut on a microtome and stained with hematoxylin-eosin stain (ALLEN, 1994). Additionally, the liver tissue was stained with Sudan III (frozen sections) (PALLASKE and SCHMIDEL, 1959).

All data collected during the study were statistically analysed using the MEANS procedure and by one way analysis of variance (ANOVA), using the GLM procedure in the SAS software package (SAS 9. 2, 2008). The chi-square test was used to analyze histopathological changes in livers and thyroid glands. A P-value less than 0.05 in each comparison was defined as significant.

Results

Live body mass, feed conversion and mortality. The production results of the broilers are given in Table 4. The live body mass and total feed conversion were not significantly different between regimens. The mortality rate was within the limits of tolerance (Table 4).

Table 4. Production results (average ± standard error) of broilers chickens fed on three different feeding regimens with rapeseed cake (T-0, T-5, T-10) at the end of the experimental feed regimens (42 day old broilers)

Production trait	Regimen		
	T-0	T-5	T-10
Live body mass, g	2716.42 ± 27.14	2654.62 ± 20.96	2657.46 ± 23.22
Total feed conversion, kg/kg	1.85 ± 0.01	1.85 ± 0.02	1.85 ± 0.01
Mortality, %	1.64	3.28	1.64

Gross findings

Liver. Two livers from the T0 group were slightly enlarged, yellow-brown in color and friable. One chicken had liver necroses. In the T5 group in two chickens liver congestion was present, with suspected haemorrhages in one of them. It should be mentioned that hydropericardium was determined in the same animals. In one animal hydropericardium was found with no visible macroscopic changes to the liver. In the T10 group seven animals had enlarged and congested livers and four of them also had hydropericardium.

Thyroid gland. There were no macroscopic changes on the thyroid gland in the control group. In the T5 group four animals had slightly enlarged thyroids and two of them were also congested. The same findings were confirmed in three animals in the T10 group. In one animal the thyroid gland was reduced in size.

Liver, thyroid gland and carcass mass. Liver mass was increased in the T-5 and T-10 groups. The average liver mass in the control group was 46.3 g; in the T-5 group 49.2 g and in the T-10 group 50.7 g.

Thyroid mass did not vary significantly between groups. That is, in the control group the average thyroid mass was 0.25 g; in the T-5 group 0.24 g; and in the T-10 group 0.27 g. The relative mass of carcasses, livers and thyroid glands are given in Table 5.

Table 5. The relative masses of carcass, liver and thyroid gland (average \pm standard error)

Trait	Regimen		
	T-0	T-5	T-10
Carcass, g per 100 g of live body mass	71.53 \pm 4.29	72.28 \pm 2.47	72.21 \pm 4.22
Liver, g per 100 g of live body mass	1.8 \pm 0.06	1.93 \pm 0.01	1.98 \pm 0.07
Thyroid gland, mg per 100 g of live body mass	9.8 \pm 1.12	9.16 \pm 0.86	10.72 \pm 0.86

Histopathological findings

Liver: Histopathological changes in the liver are presented in Table 6. Microscopic examination of the liver revealed mild to severe congestia, edema and occasional hemorrhages in all groups, but they were observed most frequently in the treated groups (T-5 and T-10), (Fig. 1). A significantly higher rate of congestion was found in the T-10 group (χ^2 : 8.75, $P = 0.017$). Parenchymal focal hemorrhages were most prominent in T-10 (six animals), less prominent in T-5 (three animals) and sporadically seen (one animal) in the control group. Degenerative changes (predominantly fibrinoid degeneration) in blood vessels were noted only in treated animals. The highest rate of degeneration of blood vessels was in T10 (8 cases, 80 %), followed by the T-5 group (6 cases, 60 %). The rate of degeneration of blood vessels was significant in the T-10 group (χ^2 : 13.93, $P = 0.001$ (Fig. 2). Vasculitis was registered in one animal in the T-5 group and there was a significantly higher rate of vasculitis in the T-10 group (χ^2 : 11.55, $P = 0.003$). Perivascular mononuclear cells (predominantly lymphocytes and a few plasma cells and histiocytes) and mixtocellular infiltration (heterophiles, lymphocytes, a few plasma cells) were observed in all three groups, but were most evident in the T-5 group. A significantly higher rate of perivascular mixtocellular cell infiltration was found only

Table 6. Histopathological changes to the livers of broiler chickens and their frequency at the end of the experimental feed regimens (42 day old broilers)

Histopathological changes		T-0 (10 samples)	T-5 (10 samples)	T-10 (10 samples)	P-value (Chi-square)
Congestion	Yes	5	9	10	P = 0.017
	No	5	1	0	
Edema	Yes	2	5	4	P = 0.366
	No	8	5	6	
Parenchymal hemorrhage	Yes	1	3	6	P = 0.058
	No	9	7	4	
Degeneration of blood vessels	Yes	0	6	8	P = 0.001
	No	10	4	2	
Vasculitis	Yes	0	1	6	P = 0.003
	No	10	9	4	
Perivascular mononuclear cell infiltration	Yes	6	9	6	P = 0.23
	No	4	1	4	
Perivascular mixto cellular cell infiltration	Yes	3	9	4	P = 0.016
	No	7	1	6	
Interstitial mononuclear cell infiltration	Yes	3	1	0	P = 0.133
	No	7	9	10	
Interstitial mixto cellular cell infiltration	Yes	1	1	0	P = 0.585
	No	9	9	10	
Parenchymal focal mixto cellular cell infiltration	Yes	2	0	3	P = 0.186
	No	8	10	7	
Parenchymatous degeneration	Yes	3	0	4	P = 0.089
	No	7	10	6	
Hydropic degeneration	Yes	1	0	7	P = 0.001
	No	9	10	3	
Prominent perivascular necroses	Yes	1	6	1	P = 0.014
	No	9	4	9	
Atrophy of hepatocyte	Yes	0	2	4	P = 0.082
	No	10	8	6	
Scattered parenchymal fibrosis	Yes	0	0	1	P = 0.356
	No	10	10	9	

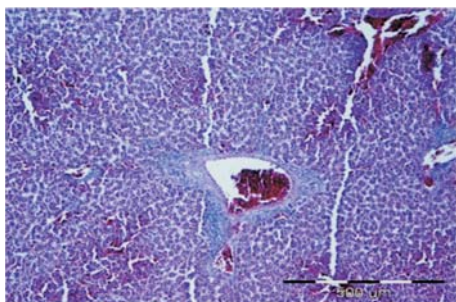


Fig. 1. Congestion, parenchymal hemorrhages, degenerative changes to blood vessels and perivascular necrosis of hepatocyte, perivascular mononuclear cell infiltration. Liver, chicken from the T-10 group. H&E.

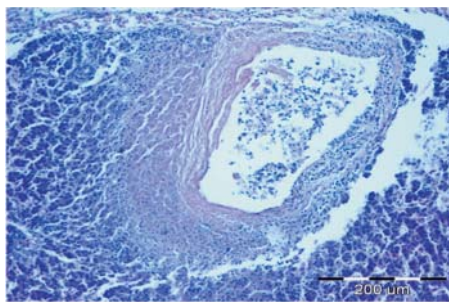


Fig. 2. Fibrinoid degeneration of blood vessels associated with mononuclear cell vasculitis and perivascular necrosis, liver, chicken from the T-10 group. H&E.

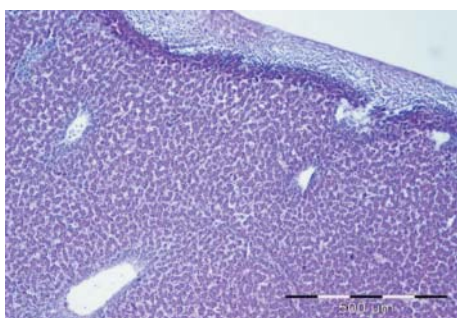


Fig. 3a. Partial fibrosis of capsules. Liver, chicken from the T-5 group. H&E.

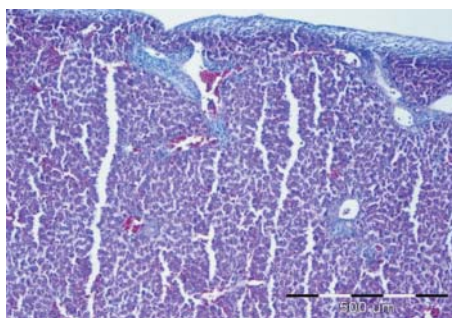


Fig. 3b. Capsular fibrosis and mononuclear perivascular proliferation. Liver, chicken from the T-10 group. H&E.

in the T-5 group ($\chi^2:8.30$, $P = 0.016$). The highest rate of interstitial mononuclear cell infiltration (predominantly lymphocytes and a few plasma cells and histiocytes) was in the T-0 group (3 cases, 30 %), followed by the T-5 group (1 case, 10 %) and it was absent in the T-10 group. Mixtocellular (heterophiles, lymphocytes, a few plasma cells) interstitial infiltration was present sporadically in groups T-0 and T-5. Parenchymal focal disseminated mixtocellular infiltration (heterophiles, lymphocytes, a few plasma cells) and parenchymatous degeneration, characterized by the granular appearance of the hepatocyte cytoplasm, was observed in groups T-0 and T-10 (higher incidence), but the incidence was not significantly high. The highest rate of hydropic degeneration was found in the T-10 group (7 cases, 70 %), followed by the T-0 group (1 case, 10 %) and the

ratio of hydropic degeneration was significant in T-10 (χ^2 :14.66, $P = 0.001$). Six animals (60 %) in T-5, one animal (10 %) in T-0 and T-10 had areas of perivascular necrosis of hepatocytes (Fig. 2). The rate of perivascular necrosis of hepatocytes was significant in the T-5 group (χ^2 :8.52, $P = 0.014$). The highest rate of atrophy of hepatocytes was found in T-10 (4 cases, 40 %), followed by T-5 (2 cases, 20 %). Fibrous proliferation in the portal triad was observed in one animal in T-10 while thickened fibrous capsules were present in three and six animals of T-5 and T-10 respectively (Fig. 3a and 3b).

Liver samples were stained negatively with Sudan III staining in groups T-0 and T-5, and were slightly positive in four animals in group T-10.

Thyroid gland. Histopathological examination showed abnormal secretory activity. The highest rate of medium and large follicles by subjective microscopic assessment was found in T-10 (7 cases, 70 %), followed by T-5 (4 cases, 40 %) (Fig. 4). In T-0 there were follicles of all sizes with equal frequency (6 cases, 66.7 %) followed by T5 (5 cases, 50 %) and T-10 (3 cases, 30 %). The epithelial cells of medium and large follicles were mostly flat to low cuboidal, while in the small follicles the epithelial cells were cuboidal to low columnar. The highest rate of scattered proliferation of follicular epithelial cells was found in the T-5 group (9 cases, 90 %), followed by T-0 (4 cases, 44.4 %) and T-10 (4

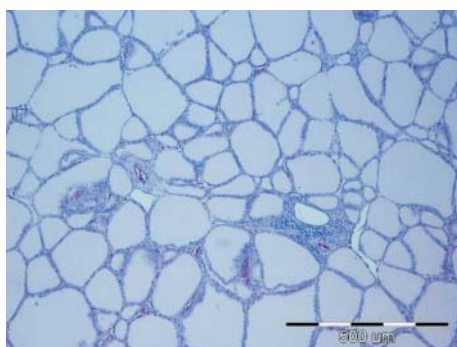


Fig. 4. Dominant large and medium size follicles and interstitial mononuclear thyroiditis. Thyroid gland, chicken from the T-10. H&E.

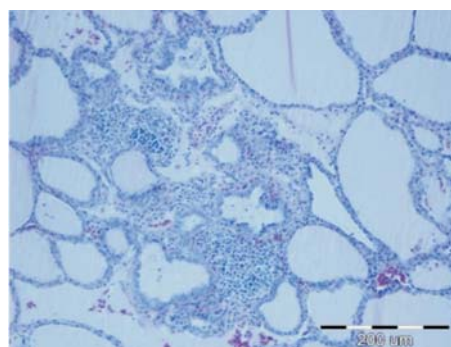


Fig. 5. Focal interstitial mononuclear cell proliferation associated with partial fibrosis and scattered follicular epithelial proliferation. Thyroid gland, chicken from the T-10. H&E.

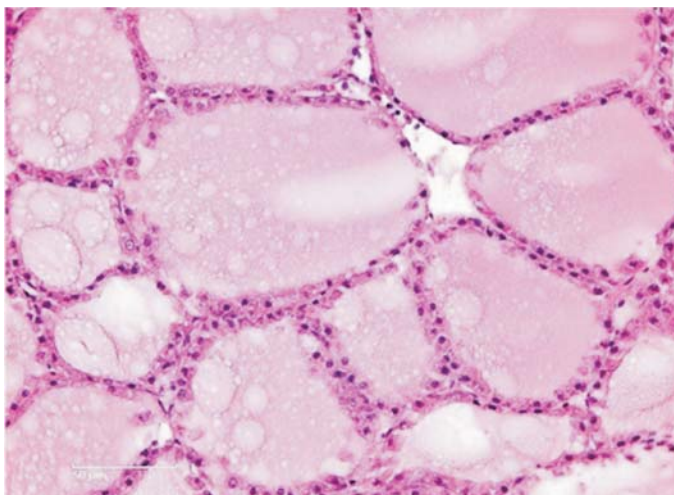


Fig. 6. Vacuolar appearance of colloid. Thyroid gland, chicken from the T-10 group. H&E.

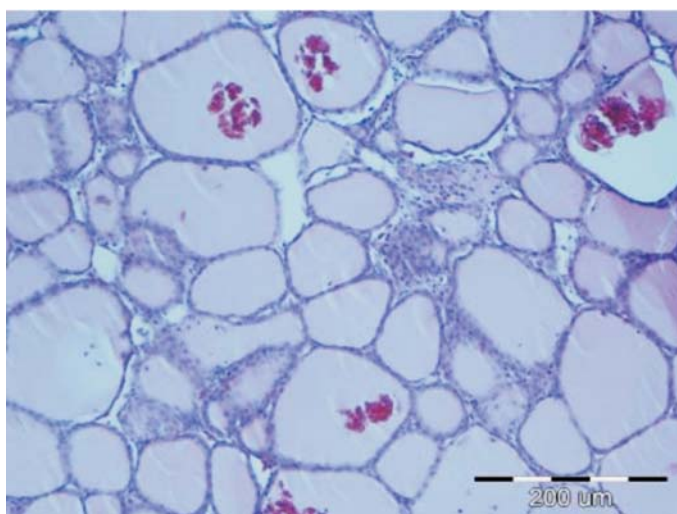


Fig. 7. Colloidal hemorrhages and partial fibrosis. Thyroid gland, chicken from the T-5 group. H&E.

cases, 40 %) (Fig. 5). The rate of scattered proliferation of follicular epithelial cells was significant in the T-5 group (χ^2 :6.23, P = 0.044).

Table 7. Histopathological changes to the thyroid glands of broiler chickens and their frequency, (%) at the end of the experimental feed regimens (42 day old broilers)

Histopathological changes		T-0 (9 samples)	T-5 (10 samples)	T-10 (10 samples)	P-value (Chi-square)
Equal frequency of occurrence of small-, medium- and large sized follicles	Yes (%)	6 (66.7)	5 (50)	3 (30)	P = 0.277
	No (%)	3 (33.3)	5 (50)	7 (70)	
Predominance of medium and large follicles	Yes (%)	1 (11.1)	4 (40)	7 (70)	P = 0.034
	No (%)	8 (88.9)	6 (60)	3 (30)	
Predominance of small and medium follicles	Yes (%)	1 (11.1)	1 (10)	0 (0)	P = 0.566
	No (%)	8 (88.9)	9 (90)	10 (100)	
Predominance of small and large follicles	Yes (%)	1 (11.1)	1 (10)	0 (0)	P = 0.566
	No (%)	8 (88.9)	9 (90)	10 (100)	
Flat to cuboidal follicular epithelium	Yes (%)	8 (88.9)	7 (70)	10 (100)	P = 0.145
	No (%)	1 (11.1)	3 (30)	0 (0)	
Flat to columnar follicular epithelium	Yes (%)	1 (11.1)	2 (20)	0 (0)	P = 0.339
	No (%)	8 (88.9)	8 (80)	10 (100)	
Cuboidal follicular epithelium	Yes (%)	0 (0)	1 (10)	0 (0)	P = 0.373
	No (%)	9 (100)	9 (90)	10 (100)	
Scattered proliferation of follicular epithelium	Yes (%)	4 (44.4)	9 (90)	4 (40)	P = 0.044
	No (%)	5 (55.6)	1 (10)	6 (60)	
Scattered vacuolisation of follicular epithelium	Yes (%)	4 (44.4)	9 (90)	10 (100)	P = 0.007
	No (%)	5 (55.6)	1 (10)	0 (0)	
Homogenous and eosinophilic colloid	Yes (%)	5 (55.6)	2 (20)	4 (40)	P = 0.277
	No (%)	4 (44.4)	8 (80)	6 (60)	
Vacuoles scattered on periphery or throughout the eosinophilic colloid	Yes (%)	4 (44.4)	8 (80)	6 (60)	P = 0.277
	No (%)	5 (55.6)	2 (20)	4 (40)	
Follicular hemorrhages (erythrocytes incorporated into colloid)	Yes (%)	0 (0)	2 (20)	5 (50)	P = 0.037
	No (%)	9 (100)	8 (80)	5 (50)	
Focal mononuclear cell proliferation in interstitium	Yes (%)	4 (44.4)	7 (70)	2 (20)	P = 0.08
	No (%)	5 (55.6)	3 (30)	8 (80)	
Mild interstitial fibrosis	Yes (%)	2 (22.2)	5 (50)	0 (0)	P = 0.033
	No (%)	7 (77.8)	5 (50)	10 (100)	
Severe interstitial fibrosis	Yes (%)	0 (0)	0 (0)	3 (30)	P = 0.042
	No (%)	9 (100)	10 (100)	7 (70)	

Histopathological changes		T-0 (9 samples)	T-5 (10 samples)	T-10 (10 samples)	P-value (Chi-square)
Foci of parenchymal lymphocytic infiltrate	Yes (%)	0 (0)	1 (10)	6 (60)	P = 0.004
	No (%)	9 (100)	9 (90)	4 (40)	

The highest rate of homogenous and eosinophilic colloid was in the T-0 group (5 cases, 55.6 %), followed by T-10 (4 cases, 40 %) and T-5 (2 cases, 20 %). Conversely, the highest rate of vacuolization in colloid on the periphery or throughout the follicles (Fig. 6) was found in the T-5 group (8 cases, 80 %), followed by T-10 (6 cases, 60 %) and T-0 (4 cases, 44.4 %). A very interesting finding were follicular hemorrhages (Fig. 7). This finding was only observed in treated animals. The highest rate of follicular haemorrhages was in the T-10 group (5 cases, 50 %), followed by the T-5 group (2 cases, 20 %). The rate of follicular haemorrhages was significant in the T-10 group ($\chi^2:6.61$, $P = 0.037$). The highest rate of focal mononuclear cell proliferation (lymphocytes, a few plasma cells and histiocytes) in the interstitium was found in the T-5 group (7 cases, 70 %), followed by the T-0 group (4 cases, 44.4 %) and the T-10 group (2 cases, 20 %). Interstitial proliferation of fibrous tissue was also found (Fig. 5). The highest rate of mild interstitial fibrosis was found in the T-5 group (5 cases, 50 %), followed by T-0 (2 cases, 22.2 %) and the rate of mild interstitial fibrosis was significant in the T-5 group. Severe interstitial fibrosis was found only in the T-10 group, with a significantly high rate ($\chi^2:6.36$, $P = 0.042$). Histopathological changes in thyroid glands are presented in Table 7.

Discussion

The introduction of 5 % and 10 % rapeseed cake, Bristol cultivar "00", with 2.86 $\mu\text{mol/g}$ of GIs to chicken feed did not lead to any negative effect on the final production results of the broilers. This is supported by the mortality rate which is within the limits of tolerance (BEDEKOVIĆ, 2013). These results are in accordance with most literature data (MUSHTAQ et al., 2007; GOPINGER et al. 2014) even though there are references where rapeseed meal showed better production performance of the chicks than soybean meal (MILOŠEVIĆ et al., 2011). MORKUNAS et al. (1998), stated that partial replacement of soybean oil-meal with 5, 7.5 and 10 % of rapeseed cake had no negative effect on the growth of broilers. Also, the results of AHMED et al. (2015) showed that inclusion of canola meal in broiler diets (0-42 d of age) as a substitute for soybean meal, at a level of 5, 10 and 20 %, had no adverse effects on growth performance and carcass traits. BANASZKIEWICZ and BORKOWSKA (2009) stated that rapeseed cake introduced to diets instead of some of the soybean meal had profitable effect on the live body mass of chickens, improved slaughter yield, increased the content of total and breast muscles in carcasses and decreased skin with subcutaneous fat.

On the other hand, the introduction of 5 % and 10 % rapeseed cake resulted in certain liver and thyroid gland lesions. There was a mass increase of the liver in treated animals, consistent with an increase in content of rapeseed cake in feed, which correlates with literature data (TREFNI et al., 1989; WETCHEREK et al., 1990; JANJEČIĆ et al., 2002; TRIPATHI and MISHRA 2007; WOYENGO et al., 2011). Increased liver mass could be attributable to the increased activity of detoxification enzymes as a result of absorption of the gut microbial degradation products of dietary GIs (VANG et al., 2001; TANII et al., 2004; WOYENGO et al., 2011). Findings of degenerative changes to blood vessels, accompanied by vasculitis is in accordance with the reports of YAMASHIRO et al. (1975), MARTLAND et al. (1984), which stated that degenerative changes to blood vessels could be a result of toxic rapeseed products and their metabolites. MILLER et al. (2012) mention that fibrinoid necrosis of blood vessels is frequent in many acute degenerative and inflammatory diseases. Inflammatory vascular changes (mononuclear cell vasculitis) were more frequent in treated animals. The increased incidence of hemorrhage in chickens treated with a higher percentage of rapeseed cake in their feed might be a consequence of vascular lesions. These results are partially in correlation with literature data (YAMASHIRO et al., 1975; MARCH et al., 1978; GOUGH and WEBER, 1978; MARTHLAND et al., 1984; CORNER et al., 1985; RIDDELL, 1987; BHATNAGAR et al., 1980; BEASLEY, 1999; RAMPIN et al., 2005). The above-mentioned authors reported the effects of several different toxic products and their metabolites, primarily glucosinolates and erucic acid, but also the effects of rapeseed on E vitamin and selenium depletion in pigs, which causes frailness of blood vessels with subsequent hemorrhages.

The higher incidence of hydropic vacuolar degeneration and hepatocyte necrosis in the treated groups is in accordance with the literature data of YAMASHIRO et al. (1975), HULAN et al. (1982), BEASLEY (1999), and SAIF (2003). They reported that toxic products (erucic acid, glucosinolates and their metabolites) can induce such lesions. Partial capsule fibrosis, along with predominant changes to the blood vessels in the treated groups, is in correlation with reports from RATANASETHKUL et al. (1976). They interpreted this lesion as a result of hydropericardium caused by the effects of erucic acid, although this mechanism remains vague. This finding can also be related to the results of JULIAN (2005) who described changes in the cardiac muscle caused by the damaging effects of rapeseed on connective tissue. Birds are unable to synthesize arginine (KHAJALI and SLOMINSKI, 2012). The arginine content of canola meal is approximately two-thirds of that of soybean meal. Dietary arginine content may not be adequate to fully support production of nitric oxide (NO), a potent vasodilator (IZADINIA et al., 2010). Diminished NO has been implicated in the pathogenesis of pulmonary hypertension and other vascular disorders, including atherosclerosis (SHAUL, 2002). Substitution of canola meal for soybean, in relation to pulmonary hypertension and ascites in broiler chickens, has been documented by IZADINIA et al. (2010). In the study of PAYVASTAGAN et al. (2012), there were no

effects on susceptibility to ascites by substitution of canola meal instead of soymeal, but higher levels of canola meal in the diet probably increased the incidence of chronic heart failure in broiler chickens.

No significant alteration was found in thyroid mass in treated animals, which is different from the reports of WIGHT and SHANNON (1985), KERMANSHAHI and ABBASI POUR (2006), and TARAZ et al. (2006).

Changes to the thyroid gland characterized by an increased number of medium and large follicles in treated animals, and partial proliferation of follicular cells in the T-5 group, suggest the goitrogenic effect of rapeseed. This result is in accordance with the results of ADIBMORADI (2008). He found that the diameter of follicles, epithelial cell number and epithelial cell height in regimen groups (with 10, 15 and 20 % canola rapeseed) were significantly increased compared with the control group. Their results show that although GIs in canola or double-zero rapeseed are very low, canola meal can affect the morphology of thyroid glands in broilers. It is well known that the follicular activity of the thyroid is inversely proportional to the diameter of the follicles (AUGHEY and FRYE, 2001).

The specificity of morphological changes also depends on the amount of GIs hydrolysis products, as well as exposure duration and poultry species. FENWICK and CURTIS (1980) and MAWSON et al. (1993) described stronger goitrogenic effects of glucosinolates in hens and turkeys than in broilers, because of their different life spans and feeding period. Most authors report that the presence of GIs in feed leads to hypothyroidism in poultry (SCHMIDT and REAVILL, 2002; SAIF, 2003). The height of the epithelium was inversely proportional to the diameter of the entire follicle. It should be noted that in our report the size of follicles was determined subjectively. For objective determination the diameter of follicles, the number and height of epithelial cells should be measured under a light microscope, as was done by ADIBMORADI (2008).

For more precise determination of epithelial hyperplasia, morphometry and markers of cellular proliferation should be used, as JELINEK et al. (2003) did for determination of bovine functional thyroid gland activity. BREIT et al. (1998) wrote about the impact of seasonal changes on the size and activity of the thyroid gland (in winter the follicles are larger with greater volume).

The homogeneous eosinophilic appearance of colloids in the control group was to a large extent replaced by colloid vacuolization on the periphery of follicles and in some cases throughout the entire follicle, with erythrocytes present in the colloid (hemorrhages) in treated groups. Haemorrhages in the colloid are in accordance with the results of WIGHT et al. (1986). In our research the haemorrhages were most probably caused by the toxic effects on the blood vessels or by heart injury. A focal mononuclear, predominantly lymphocytic infiltrate was present in all groups, but with a higher rate in

the treated groups. PLESCH et al. (2014), in a study on the tolerance of rapeseed meal in B.U.T. 6 turkeys, described moderate to severe lymphocytic thyroiditis in the control group (feed without rapeseed) and the treated group. Thirty per cent of the mononuclear inflammatory cells were identified as T cells. These lesions resemble Hashimoto's disease in humans. The effect on thyroid function is unknown. Mild hypothyreosis might enhance disposition towards cardiovascular problems. SCHMIDT et al. (2003) reported lymphocytic thyroiditis, with follicular degeneration and proliferation of connective tissue in certain chicken breeds.

The results mentioned above show that although the content of GIs and erucic acid in the rapeseed cake was very low, changes to the liver and thyroid were very similar to those caused by a higher ratio of GIs and erucic acid. The reason is unknown. WOYENGO, 2011 stated that the toxicity of glucosinolate degradation products varies, depending on their composition. Also, the difference in composition of the basal diets may have resulted in differences in the composition of gut microbes that hydrolyse glucosinolates, and therefore in differences in the severity of toxicity of the glucosinolate degradation products. Therefore, it would be interesting to see the influence of basal diet composition on the effect of dietary inclusion of canola meal on broiler performance, gut microbial composition, and liver and thyroid gland function.

As a final conclusion, it can be said that the introduction of 5 and 10 % rapeseed cake (Bristol-“double low” cultivar) to broiler chickens resulted in liver and thyroid changes. These changes were not severe enough to have a greater impact on production results. The existence of causative factors other than erucic acid and glucosinolates cannot be excluded.

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ARTUKOVIĆ, B., D. BEDEKOVIĆ, J. PINTAR, M. TIŠLJAR, I. KOS, I. ŠIRIĆ, K. SEVERIN, Z. JANJEČIĆ: Patološke promjene u jetri i štitnoj žlijezdi tovnih pilića hranjenih pogačom uljane repice. *Vet. arhiv* 85, 657-676, 2015.

SAŽETAK

SVrha je ovog rada istražiti patološke promjene u jetri i štitnoj žlijezdi tovnih pilića s utvrđivanjem mogućnosti korištenja različitih udjela uljane repice u hrani za piliće. Pokus je obavljen na 360 muških jednodnevnih pilića hibridne linije Cobb koji su podvrgnuti različito hranjeni (T-0 = 0 %; T-5 = 5 %; T-10 = 10 % udjela pogače uljane repice, kultivar Bristol-“00”). Pogača je sadržavala 2,86 μmol/g glukozinolata, a udio eruka kiseline u ukupnoj količini svih masnih kiselina bio je 0,08%. Dodavanje 5 % pogače uljane repice u hranu uzrokovalo je umjereno povećanje mortaliteta pilića, ali je on još uvijek bio u granicama prihvatljivog. Prosječna masa jetre bila je povećana u T-5 i T-10 skupini u usporedbi s kontrolnom skupinom. Štitna žlijezda pilića hranjenih pogačom nije bila značajnije teža u usporedbi s kontrolnom skupinom. Patohistološke promjene na jetri i štitnoj žlijezdi bile su izraženije nego u kontrolnoj skupini. U T-5 skupini bio je značajno visok udio perivaskularne nekroze hepatocita i perivaskularne mikstocelularne stanične infiltracije u jetri. U skupini T-10 u jetri je bio značajno visok udio kongestije, degeneracije krvnih žila, vaskulitisa i hidropične degeneracije. U

štitnoj žlijezdi pilića iz T-5 skupine bio je signifikantno visok udio rasijane proliferacije folikularnih epitelnih stanica i blage fibroze intersticija. Samo u skupini T-10 bila je izražena jača fibroza intersticija sa signifikantno visokim udjelom. U skupini T-100, a nakon toga i u skupini T-5, bio je i najveći udio krvarenja u folikulima. Nejasan je odnos između ovih promjena nastalih pri hranjenju pogačom uljane repice i njihova povezanost s nepoželjnim učincima specifičnim za pogaču uljane repice. Nije isključeno ni postojanje nekih drugih faktora, osim eruka kiseline i glukozinolata, koji su mogli uzrokovati ove promjene. Zaključno, uvođenje 5 i 10 % pogače uljane repice u hranu tovni pilića (kultivar Bristol-“00”) uzrokovalo je promjene na jetri i štitnoj žlijezdi, ali promjene nisu bile takvog inteziteta da su značajnije utjecale na proizvodne rezultate.

Ključne riječi: pogača, uljana repica, tovni pilići, jetra, štitna žlijezda, patološke promjene
