Morphological pattern of the livers of different lines of broiler chickens during rearing

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ABSTRACT: The aim of this study was to analyse the microscopic and ultrastructural lesions of the livers of broiler chickens during fattening. Three genetic lines of broiler chickens (Ross 308, Cobb 500, Hubbard F15) were investigated. The liver samples were taken on the 3rd, 10th, 17th, 24th, 31st, and 38th day of life from six healthy broiler chickens from each commercial broiler flock. The dominant microscopic lesions were associated with prolonged hypoxia and bile ductules, including: fatty, vacuolar and parenchymatous degeneration, necrosis of epithelial cells of bile ductules, necrosis of hepatocytes around the proliferating bile ductules, lymphoid cell infiltration around the bile ductules and blood vessels, proliferation of the bile ductules, proliferation of the connective tissue around bile ductules and stimulation of the lymph nodules. Ultrastructural evaluation revealed abnormalities involving mitochondria and rough endoplasmic reticulum. The mitochondria underwent swelling, polymorphism, proliferation and damage. The rough endoplasmic reticulum underwent defragmentation and acinar transformation. The cytoplasm of most hepatocytes showed vacuoles of varying size or lipid droplets and the presence of cytoplasmic myelin-like structures. This study shows that the livers of broiler chickens are the most predisposed to the occurrence of lesions on the 17th, 31st and 38th days of life.

Keywords: broiler chicken; Cobb; Ross; Hubbard; liver; pathomorphology

The growing demand for poultry meat has prompted producers of breeding lines of broiler chickens to seek to optimise breeding through genetic means. Modern broiler lines are expected to grow fast (shorter breeding period), be healthy (characterised by a good metabolism and a high degree of utilisation of nutrients), have a strong skeleton, high survival rate, be resistant to disease, have high adaptive capacity and show steady growth. Currently, broiler chickens reach an average body mass of between 2.2–2.5 kg within 42 days of rearing and consume 1.60–1.80 kg feed per kg of growth, depending on the breeding line. Clear genetic progress can be seen when comparing this data with the results from 10 years ago. At that time, the final body mass of broiler chickens reached 1.95–2.15 kg, the average fattening period was 43–45 days and the average feed consumption per kg of body mass ranged from 2.00–2.10 kg. The current production results are even more impressive when viewed in the long term (Havenstein et al. 2003a,b). In 1957, the average body mass of broilers after 42 days of rearing was 539 g and the average feed consumption per kg of body mass was 2.34 kg. These data shows how much progress has been made in the field of breeding and genetic selection.

Unfortunately, genetic progress has also led to several negative effects. Thus, changes are now found in broiler chickens which not occurred before or were rather uncommon. These include: sudden death syndrome, pulmonary hypertension syndrome, abnormal bone growth, limb disease, the occurrence of diseases in subclinical form (*Clostridium perfringens*, *Escherichia coli*), immunosuppression and susceptibility of birds to mycotoxin intoxication. Subclinical infections are a

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major problem in broiler fattening (Onderka et al. 1990; Lovland and Kaldhusdal 1999) and also result in liver damage (Hutchison and Riddell 1990; Onderka et al. 1990; Sasaki et al. 2003).

The aim of this study was to analyse the morphological pattern during fattening of the livers of broiler chickens which did not show clinical signs of disease. Furthermore, the aim was to determine the most susceptible period for the occurrence of morphological lesions in the liver during fattening and determine whether these changes may accompany disorders occurring in these birds in a subclinical form.

MATERIAL AND METHODS

Broiler chickens of three genetic lines (Cobb 500, Hubbard F15, and Ross 308) were reared in three commercial broiler flocks – each line in a separate house. Rearing parameters were regulated in accordance with the recommendations of the producers of breeding lines. These factors did not differ significantly from each other. The quantitative composition of the feed was slightly modified depending on the farm with reagard to the percentage of total protein in the feed and the amount of metabolisable energy (ME).

All three broiler flocks had the same vaccination program: on the 1st day of life – against Marek's disease, Infectious Bursal Disease (IBD) and Infectious Bronchitis (IB, H-120 strain), on the 16th day – against IB 4/91 strain and the IBD vaccination was repeated on the 18th day of life.

From each genetic line, six broiler chickens were taken randomly for morphological examination on the 3rd, 10th, 17th, 24th, 31st and 38th days of life (n = 36). The birds were weighed and slaughtered following the protocols established by the Local Ethics Committee in Olsztyn, No. 3/N dated 22.01.2008).

Samples of liver for microscopic evaluation were fixed in 10% neutralised formalin and embedded in paraffin blocks. The paraffin sections (5 µm) were stained with haematoxylin, eosin (HE) and frozen sections of livers on the 3rd day of life were also stained with Oil Red O to detect lipids (Bancroft and Gamble 2008). Histologically, changes were graded subjectively as focal, multifocal and diffuse, and the intensity of these lesions was classified as low (mild), medium (moderate) and high (severe).

Liver sections were also fixed for ultrastructural examination for two hours in 2.5% paraformaldehyde and 2% glutaraldehyde in a pH 7.4 phosphate buffer. Samples were post-fixed in 2% osmium tetraoxide in a pH 7.4 phosphate buffer and the material was embedded in Epon 812. Semi-thin sections were obtained from the blocks and were subsequently stained according to the method described by Lewis and Knight (1977) and viewed under an optic microscope in order to establish the appropriate place for preparing ultrathin sections. The ultrastructural analysis was carried out with an Opton 900 PC TEM (Germany).

Due to the relatively small number of groups and deviations from normal distributions of variables, a Kruskal-Wallis non-parametric ANOVA test was performed.

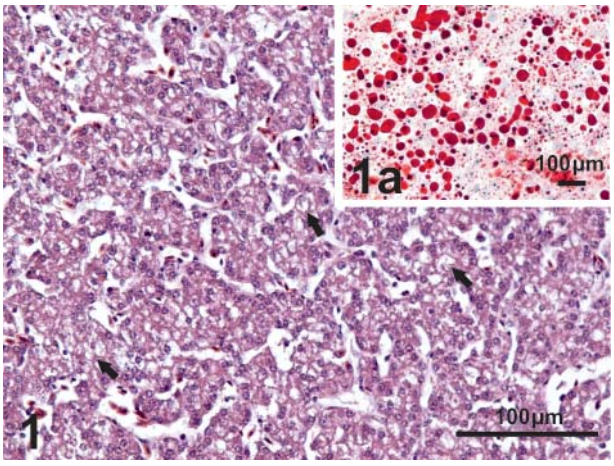

Figure 1. 3rd day (Ross 308): hepatic lipidosis, fatty degeneration (arrows); HE. 1a (box): 3rd day (Cobb 500): lipid vacuoles; Oil Red O

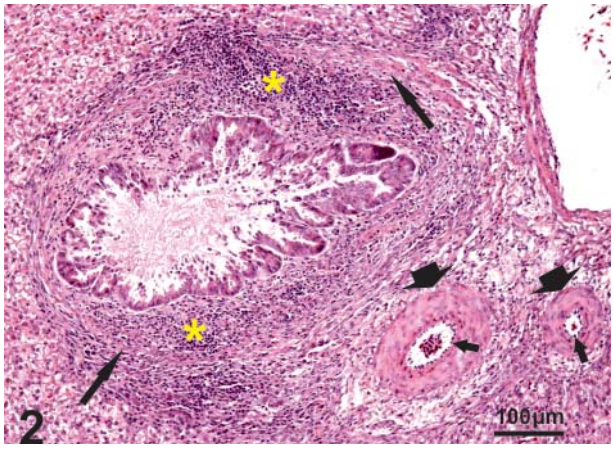

Figure 2. 17th day (Hubbard F15): cholangiohepatitis chronica – infiltration of lymphoid cells (asterisks), proliferation of the connective tissue (long arrows), hypertrophy of the endothelial cells in artery (short arrows), hypertrophy of smooth muscle in artery (arrowheads); HE

Table 1. Morphological lesions in the livers of the broiler chicken Cobb 500 genetic line

Type of morphological lesions	Number of lesions in Cobb 500 line broiler chicken livers					
	day of life					
	3	10	17	24	31	38
A – parenchymatous degeneration	0/6	5/6	6/6	6/6	6/6	3/6
B – vacuolar degeneration	6/6	6/6	5/6	6/6	0/6	4/6
C – fatty degeneration	6/6	4/6	3/6	0/6	0/6	4/6
D – necrosis of the epithelium cells of the bile ductules	0/6	0/6	0/6	2/6	0/6	0/6
E – congestion	0/6	5/6	5/6	5/6	4/6	6/6
F – infiltration of lymphoid cell around bile ductules and blood vessels	0/6	3/6	4/6	3/6	3/6	3/6
G – stimulation of the lymph nodules	0/6	0/6	6/6	2/6	3/6	0/6
H – proliferation of the bile ductules	0/6	4/6	6/6	4/6	5/6	6/6
I – proliferation of the connective tissue around the bile ductules	0/6	0/6	2/6	0/6	0/6	2/6
J – hypertrophy of the endothelium cells in arteries	0/6	4/6	6/6	4/6	5/6	2/6
K – hypertrophy of the smooth muscle in arteries	0/6	4/6	6/6	3/6	0/6	3/6

RESULTS

Clinical examination showed that broiler chickens on the 3rd, 10th, 17th, 24th, 31st and 38th days of life were active, had an appropriate appetite and did not show clinical signs of disease. Macroscopic examination showed normal morphology of tissues and organs in most birds. By the third day, all livers were light-brown with a loamy/brittle consistency and the cross-sectional lobular pattern had disappeared.

The results of microscopic evaluation

During rearing, the livers of the examined broiler chickens showed several microscopic lesions (presented in Tables 1–3 and Figures 1–4). The livers of the examined birds mainly showed parenchymatous, vacuolar (Figure 4) and fatty degeneration (Figure 1). These were observed with high intensity and most often vacuolar degeneration throughout the breeding (92 birds) was described along with parenchymatous degeneration from the 10th day (88 chickens). In addi-

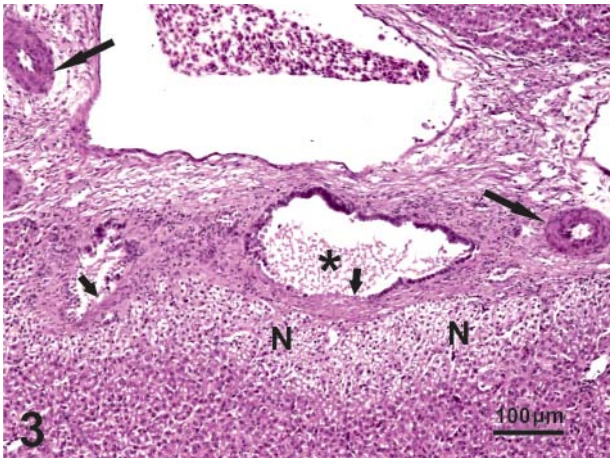

Figure 3. 24th day (Cobb 500): necrosis of epithelium cells in the bile ductules (short arrows), hypertrophy of smooth muscle in arteries (long arrows), coagulative necrosis of hepatocytes around the bile ductules (N), dilatation of the bile ducts (asterisk); HE

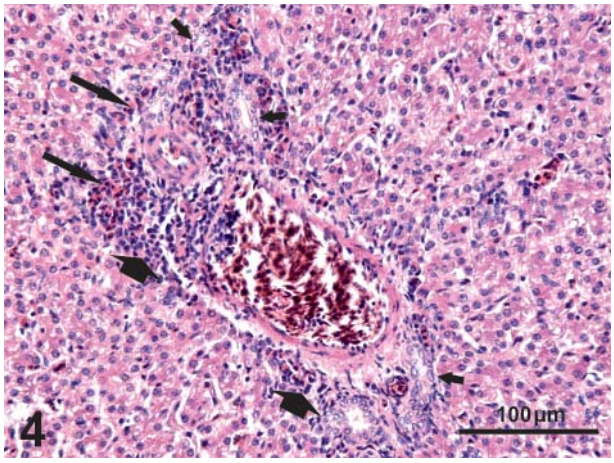

Figure 4. 38th day (Ross 308): proliferation of the bile ductules (short arrows), infiltration of lymphoid cells (arrowheads) and myeloid cells (long arrows) around the portal area, vacuolar degeneration; HE

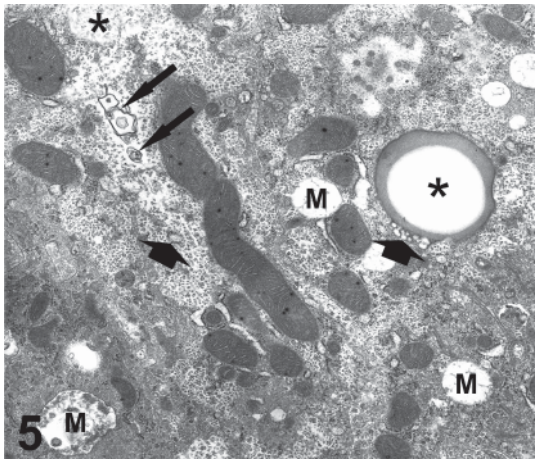

Figure 5. 3rd day (Ross 308): rarefication of cytoplasm, myelin-like structures (long arrows), mitochondrial polymorphism with swollen and damaged mitochondria (M), acinar transformation of the RER (arrowheads) and lipid vacuoles (asterisk); 7500×

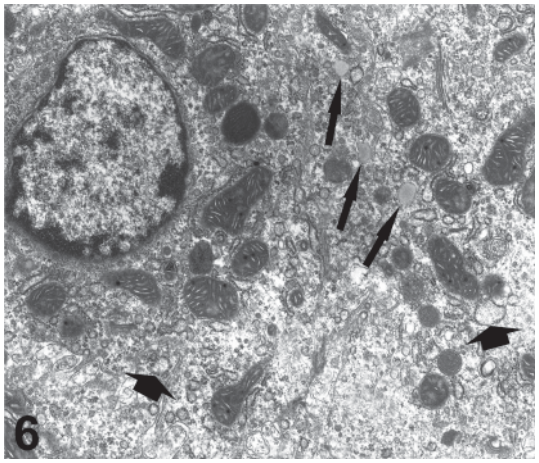

Figure 6. 10th day (Hubbard F15): acinar transformation of RER (arrowheads), lipid vacuoles (long arrows) and rarefication of cytoplasm; 7500×

tion, fatty degeneration was diffuse on the 3rd day, while on the 10th day it was multifocal near blood vessels and the terminal portal venules and focal near the blood vessels and terminal portal venules on the 17th day. Fatty degeneration was multifocal on the 31st and 38th days in 14 chickens. Changes were particularly intense from the 10th day and included bile ductule proliferation (Figure 4), hypertrophy of endothelium cells in arteries and hypertrophy of the smooth muscle in arteries (Figure 2 and 3). Lesions in the bile ductules were recorded together with the proliferation of the connective tissue around bile ductules (lower intensity after the 10th day) (Figure 2), lymphoid cell infiltration around bile ductules and blood vessels (medium intensity after the 10th day) (Figure 2 and 4). These were associated with infiltration of myeloid cells around the proliferating bile ductules (observed focally after the 10th day), with interstitial lymphoid hepatitis diagnosed in seven chickens and outbreaks of coagulative necrosis of hepatocytes around the proliferating bile ductules (occurring from the 10th day). It is noteworthy that multifocal necrosis of epithelial cells in bile ductules after the 17th day (Figure 3) and multifocal stimulation of the lymph nodules (10th day) occurred in 38 chickens.

Table 2. Morphological lesions in the livers of the broiler chicken Hubbard F15 genetic line

Type of morphological lesions	Number of lesions in Hubbard F15 line broiler chicken livers					
	day of life					
	3	10	17	24	31	38
A – parenchymatous degeneration	0/6	6/6	6/6	6/6	6/6	6/6
B – vacuolar degeneration	6/6	6/6	6/6	6/6	3/6	6/6
C – fatty degeneration	6/6	4/6	0/6	2/6	0/6	5/6
D – necrosis of epithelium cells of the bile ductules	0/6	0/6	3/6	0/6	5/6	4/6
E – congestion	0/6	4/6	6/6	6/6	6/6	4/6
F – infiltration of lymphoid cell around bile ductules and blood vessels	0/6	0/6	3/6	5/6	5/6	6/6
G – stimulation of the lymph nodules	0/6	2/6	5/6	0/6	6/6	0/6
H – proliferation of the bile ductules	0/6	6/6	6/6	4/6	6/6	6/6
I – proliferation of the connective tissue around the bile ductules	0/6	0/6	2/6	0/6	4/6	4/6
J – hypertrophy of the endothelium cells in arteries	0/6	4/6	6/6	0/6	5/6	5/6
K – hypertrophy of the smooth muscle in arteries	0/6	0/6	3/6	0/6	3/6	2/6

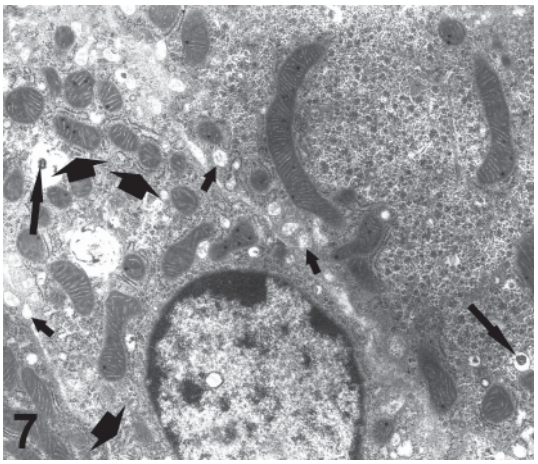

Figure 7. 17th day (Ross 308): rarefication of the cytoplasm, myelin-like structures (long arrows), polymorphic and swollen mitochondria and damage to some of the mitochondria (short arrows) and the defragmentation of the RER (arrowheads); 7500×

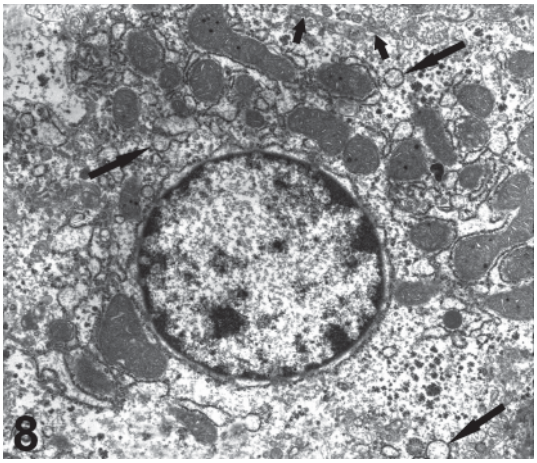

Figure 8. 31st day (Cobb 500): acinar transformation of RER (long arrows), myelin-like structures (short arrows) and rarefication of the cytoplasm; 7500×

A Kruskal-Wallis nonparametric ANOVA test (Table 4; Figure 11) showed that the number of lesions in the form of parenchymatous and vacuolar degeneration was statistically significantly higher than the necrosis of the bile ductule epithelium, hypertrophy of the smooth muscle in arteries as well as the stimulation of the lymph nodules.

Furthermore, the number of animals with necrosis of the bile ductule epithelium cells was statistically significantly lower than those with congestion and proliferation of the bile ductules. In addition, the number of birds with proliferation of the connective tissue around the bile ductules was statistically significantly lower than those with parenchymatous and vacuolar degeneration, congestion and proliferation of the bile ductules. A statistical analysis of the results, using the day of the fattening as a variable (Table 5; Figure 12) showed that the number of broilers with changes on day 3 was statistically significantly lower than the number of chickens on the 17th, 31st and 38th days.

There were no significant statistical differences between the lines, although the largest number of lesions in the liver was found in the Ross 308 line, followed by Hubbard F15 and Cobb 500. Important statistical differences may also emerge with further tests on a larger number of flocks.

Table 3. Morphological lesions in the livers of the broiler chicken Ross 308 genetic line

Type of morphological lesions	Number of lesions in Ross 308 line broiler chicken livers					
	day of life					
	3	10	17	24	31	38
A – parenchymatous degeneration	0/6	6/6	6/6	6/6	6/6	5/6
B – vacuolar degeneration	6/6	6/6	4/6	4/6	6/6	6/6
C – fatty degeneration	6/6	4/6	2/6	0/6	6/6	0/6
D – necrosis of epithelium cells of the bile ductules	0/6	0/6	2/6	2/6	4/6	4/6
E – congestion	4/6	3/6	6/6	6/6	3/6	6/6
F – infiltration of lymphoid cell around bile ductules and blood vessels	0/6	0/6	5/6	5/6	6/6	4/6
G – stimulation of the lymph nodules	0/6	6/6	0/6	2/6	0/6	3/6
H – proliferation of the bile ductules	0/6	2/6	6/6	6/6	6/6	6/6
I – proliferation of the connective tissue around the bile ductules	0/6	2/6	0/6	2/6	2/6	6/6
J – hypertrophy of the endothelium cells in arteries	0/6	2/6	6/6	2/6	0/6	2/6
K – hypertrophy of the smooth muscle in arteries	0/6	4/6	2/6	4/6	2/6	3/6

Table 4. *P*-value for multiple comparisons (bilateral) between the morphological lesions. Kruskal-Wallis test; H (10, n = 198) = 58.10, P = 0.00; abbreviations discribed in Tables 1 to 3

	A	B	C	D	E	F	G	H	I	J	K
A		1.00	0.94	0.00	1.00	1.00	0.03	1.00	0.00	0.99	0.05
B	1.00		0.28	0.00	1.00	0.36	0.01	1.00	0.00	0.29	0.01
C	0.94	0.28		1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
D	0.00	0.00	1.00		0.02	1.00	1.00	0.01	1.00	1.00	1.00
E	1.00	1.00	1.00	0.02		1.00	0.23	1.00	0.03	1.00	0.34
F	1.00	0.36	1.00	1.00	1.00		1.00	1.00	1.00	1.00	1.00
G	0.03	0.01	1.00	1.00	0.23	1.00		0.14	1.00	1.00	1.00
H	1.00	1.00	1.00	0.01	1.00	1.00	0.14		0.01	1.00	0.22
I	0.00	0.00	1.00	1.00	0.03	1.00	1.00	0.01		1.00	1.00
J	0.99	0.29	1.00	1.00	1.00	1.00	1.00	1.00	1.00		1.00
K	0.05	0.01	1.00	1.00	0.34	1.00	1.00	0.22	1.00	1.00	

The results of ultrastructural evaluation

Ultrastructural lesions in hepatocytes are shown in Figures 5–10. The relatively most frequent ultrastructural abnormalities concerned mitochondria and rough endoplasmic reticulum (RER). Mitochondria underwent swelling (Figure 5), polymorphism (Figures 5, 7 and 9), proliferation (Figures 9) and damage (Figure 7). Almost all mitochondria in the examined hepatocytes were observed as dense bodies. Broilers from the Hubbard F15 genetic line showed the greatest intensity of these lesions (Figure 9). Mitochondria changed most frequently in chickens on the 10th, 17th, 24th, 31st and 38th day of life. RER often underwent defragmentation (Figure 7) or acinar transformation (Figures 5, 6 and 9). These lesions were particularly intensified on the 17th and 31st day, especially in chickens from the Ross 308 line. Occasionally, RER was devoid of ribosomes or its channels underwent widening. The cytoplasm of most hepatocytes showed vacuoles of varying size (Figure 9) or lipid droplets (Figures 5, 6 and 10) and the presence of cytoplasmic myelin-like structures (Figures 5, 7, 8 and 10). Necrosis of hepatocytes, as well as necrosis of parts of the hepatocytes were visible (often in chickens of the Ross 308 line), especially in the second half of the breeding period, and was visible

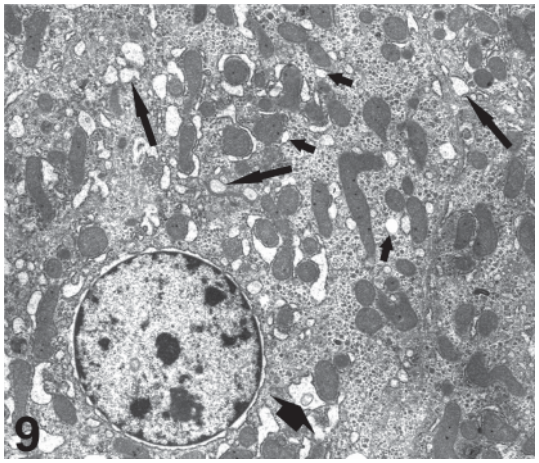

Figure 9. 31st day (Hubbard F15): proliferation and polymorphism of the mitochondria, with numerous dense bodies in the mitochondria, acinar transformation of the RER (long arrows), myelin-like structure (arrowhead) and vacuoles (short arrows); 15 400×

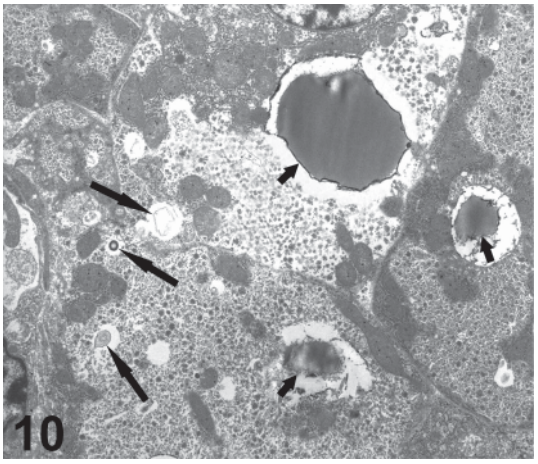

Figure 10. 38th day (Hubbard F15): lipid droplets (short arrows), myelin-like structures (long arrows) and rarefication of the cytoplasm; 3200×

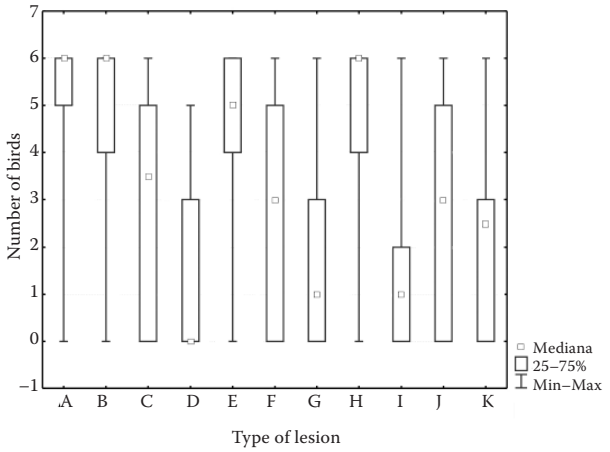

Figure 11. Kruskal-Wallis test. Grouping variable: type of lesion. Abbreviations discribed in Tables 1 to 3

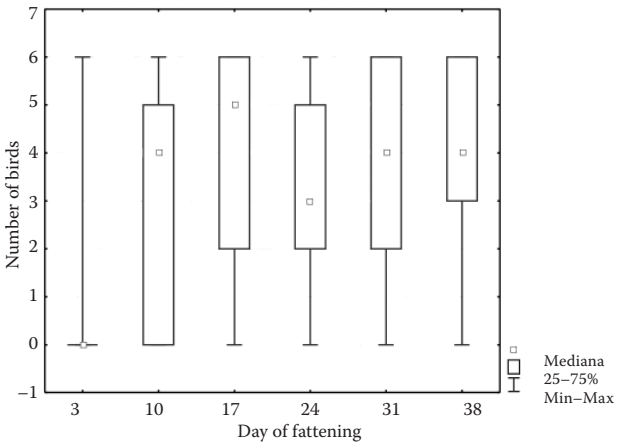

Figure 12. Kruskal-Wallis test. Grouping variable: day of fattening

as damage to mitochondria and defragmentation of the RER with the occurence of myelin-like structures. The presence of macrophages was quite often noted close to necrotic hepatocytes and lysosomes were usually present in the hepatocytes which were partially damaged.

DISCUSSION

All the lesions described in this report were similar to those reported in subclinical *Clostridium perfringens* infection in broiler chickens. In birds with microbiologically confirmed *C. perfringens* infection, Hutchison and Ridell (1990) found massive proliferation of bile ductules, proliferation of connective tissue around ductules, hepatocyte necrosis and massive lymphocyte and heterophil infiltration.

In an experiment on direct *C. perfringens* infection of the bile duct, Onderka et al. (1990) reported lesions similar to Hutchison and Ridell (1990). Sasaki et al. (2003) microbiologically confirmed *C. perfringens* infections in the newly hatched chicks and described the necrosis of hepatocytes (from focal to diffuse), proliferation of the connective tissue and infiltration of heterophils and macrophages. They also noted that necrotic enteritis induced by *C. perfringens* was accompanied by lesions in the liver, which are a major problem in broilers at the age of 2–5 weeks.

Changes such as infiltration of lymphoid and myeloid cells, proliferation of bile ductules and connective tissue around ductules, necrosis of epithelial cell in bile ductules and hepatocytes around ductules are described as cholangiohepatitis (Sasaki et al. 2000; Abdul-Aziz et al. 2008). Particular changes can occur with varying intensity and the cause is usually *C. perfringens* infection. Thus, the changes observed in our study should also be classified as a *cholangiohepatitis chronica*.

The examined livers also demonstrated vascular remodelling, with hypertrophy and hyperplasia of endothelial cells in arteries (especially from the 10^{th} day of life) and hypertrophy and hyperplasia of the smooth muscle in arteries (intensively after the 10^{th} day). In addition, hypertrophy and hyperplasia of the smooth muscle were observed in the wall of terminal portal venules in the liver, but with lower intensity. All these processes should be interpreted as a vascular remodelling. Confirmation

Table 5. *P*-values for multiple comparisons (bilateral) between days. Kruskal-Wallis test; H (5, n = 198) = 26.08, P = 0.00

	3	10	17	24	31	38
3		0.08	0.00	0.06	0.01	0.00
10	0.08		1.00	1.00	1.00	1.00
17	0.00	1.00		1.00	1.00	1.00
24	0.06	1.00	1.00		1.00	1.00
31	0.01	1.00	1.00	1.00		1.00
38	0.00	1.00	1.00	1.00	1.00	

of this hypothesis can be found in the reports of Julian (2007), which describe pulmonary vascular remodelling in pulmonary hypertension syndrome and showed that blood vessels in other organs may also undergo these changes. It was also stated in this report that the reason for these phenomena is the reaction of the endothelium and smooth muscle to changing blood pressure and blood flow.

A significant group of lesions found in the liver were regressive changes, not associated with subclinical forms of *C. perfringens* infection. *Steatosis degenerativa* on day 3 accompanied *steatosis simplex* of the liver, which is a physiological phenomenon (Cullen 2007). The cause of degenerative changes in fast-growing broiler chickens is a prolonged state of hypoxaemia leading to hypoxia (Olkowski et al. 2005). Under conditions of continuous and high demand for oxygen and nutrients, the liver tissue may respond with regressive lesions (parenchymatous, vacuolar and fatty degeneration, and necrosis of hepatocytes) (Madej et al. 2007). Prolonged hypoxia causes swelling of the mitochondria, Golgi apparatus and endoplasmic reticulum canals. Water penetrates these structures and they become larger and acinar (parenchymatous and vacuolar degeneration). Under conditions of hypoxia, fatty hepatocytes develop rapidly and the process is described as *infiltratio adiposa degenerativa hypoxaemica* (Madej et al. 2007). On the 3rd day, hepatocyte damage due to lipid accumulation in 13 chicks was also observed and the damage was sometimes accompanied by infiltration of lymphoid cells. Madej et al. (2007) reported that lipids could be used in the combustion process, if the damaging agent has been removed. The current results confirmed this view. In the first days of rearing fatty degeneration occured, which was accompanied by *steatosis simplex*. In the following days, fatty degeneration had a less extensive character and occurred multifocally near blood vessels and terminal portal venules. The presence of fatty degeneration in older birds on the 31st and 38th day of life was also noteworthy. It is suspected that the reason could be poorly balanced feed. An inappropriate ratio of metabolic energy and protein, as well as an excessive concentration of energy in the feed may result in the accumulation of lipid vacuoles in hepatocytes and ultrastructural damage (Cullen 2007; Madej et al. 2007).

In analysing the liver lesions reported in the present study, mycotoxin contamination (aflatoxin B1, fumonisin) should also be taken into consideration. Mollenhauer et al. (1989) described the experimental intoxication of broilers aflatoxin and found fatty degeneration, enlarged bile ductules, lymphoid infiltration and necrosis of hepatocytes. In the course of aflatoxin intoxication observed in the liver, Ortatatli et al. (2005) observed focal necrosis of hepatocytes, inflammatory cell infiltration, vacuolar and fatty degeneration, proliferation of bile ductules and proliferation of the connective tissue around blood vessels. The authors concluded that no hepatocyte necrosis was observed around proliferating bile ductules or ductules becoming necrotic (as in the current study); therefore, the presence of mycotoxins in the feed seems to be doubtful.

In summary, the greatest threat to health in the livers of the examined chickens involved changes usually associated with subclinical *C. perfringens* infection. Lack of microbiological examination of the examined livers does not allow us to be absolutely certain regarding the occurrence of this infection. However, our data are supported by other reports in which microbiological studies were performed where similar results were obtained. In addition, prolonged hypoxia of hepatocytes resulted in regressive lesions of liver tissue throughout the fattening period. The statistical analysis revealed that the livers of broiler chickens are the most predisposed to the occurrence of morphological lesions on the 17th, 31st and 38th days.

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