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Effect of prolonged feeding of turkeys with a diet containing oxidized fat on morphological lesions of internal organs

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Summary

Microscopic analyses investigated specimens of internal organs and muscles of 20 BUT-9 turkeys fed for 16 weeks with mixtures supplemented with fat of varying peroxide values: as high as 5, 50 100 and 150 mEq O_2/kg. During the 4-week experiment period 2, 3, 4 and 5% fat (rapeseed oil and poultry fat at a proportion of 66:34 oxidized under controlled conditions) was added respectively to the feed.

Supplementing the oxidized fat at PV 5 mEq O_2/kg did not cause any morphological lesions in the internal organs of the turkey hens. However, an increase of up to 50 mEq O_2/kg in the fat peroxide value of their diet resulted in retrogressive changes and disturbances in the blood supply to the liver and cross striated muscles. Fat at PV 100 mEq O_2/kg resulted in a slight intensification of the above-mentioned changes mainly in these organs, however, fat oxidation of 150 mEq O_2/kg led to a visible increase in the type and intensity of lesions as well as the areas in which they occurred (kidneys and alimentary tract). The observed morphological defects were lesions of a damaging and adaptive profile.

Keywords: oxidized fat, turkeys, internal organ pathomorphology

To maximize body growth rate, turkey diet can be supplemented with fat (12). Although it produces body mass increase, it can also bring a risk of some undesirable effects. Fat is one of the unstable components in feed and is easily oxidized during storage (11). The process is based on spontaneous oxygen incorporation by unsaturated fatty acids. The nutritional value of partially oxidized fat can be reduced due to the lower level of unsaturated fatty acids, vitamins (A and E) and fat-dissolvable provitamins (6, 9).

The introduction of a improper compound into poultry diets leads to changes in feed intake and disturbances in animal growth or eggs production (1, 4). It was clearly observed when diets were supplemented with thermoxidized and polymerized sunflower oil (11, 16). Lopez et al. (1995) show, that negative effect of fat on the liver secretion is stronger when oxidative fat changes are significant and lead to the production of polymerized products. There has been less evidence on the consequences of fat oxidation at lower temperatures. Information concerning the nutritional, toxicological and pathomorphological effects of fat oxidized in low temperature are still contradictory. Peroxides found in the feed are probably poorly absorbed by the organism (12, 18). On the other hand, aldehydes and other secondary products of fat oxidation are well absorbed and transported in the organism by lipoproteins and may be toxic to the liver, kidneys and spleen (7).

Taking into account the introduced facts, the aim of the study was to examine the impact of diets containing fats of different degree of oxidation on the development of morphological lesions of some internal organs in turkeys.

Material and methods

Microscopic analyses covered specimens of internal organs and muscles of 20 BUT-9 turkeys fed for 16 weeks with mixtures supplemented with fat of different peroxide value: up to 5, 50, 100 and 150 mEq O_2/kg. The fat supplemented to diet was a blend of rapeseed oil and poultry fat at a proportion of 66 : 34. It was oxidized under controlled conditions as described by Zduńczyk et al. (2000). In consecutive 4-week periods of the experiment, the fat content in the mixtures was increased (2, 3, 4 and 5%) so that the nutritional value of diets met nutritional requirements of turkeys (14). A new batch of mixture was supplemented with fat every week in order to prevent oxidation during storage. Conditions of maintenance and growth effects of more numerous groups of turkeys (56 turkeys each), from which 5 turkeys were selected for microscopic studies, were described by Jankowski et al. (6).

After the slaughter of turkeys, segments of liver, kidneys, heart muscle, medial gluteal muscle, duodenum and ileum were removed for analysis. The samples were fixed in 10% neutralized formalin, subjected to routine histological processing and stained with haematoxylin and eosin (HE). Liver sections were also stained with Sudan III according to the Lillie Ashburn method (in order to determine possible lipid infiltration).

Samples from lobus hepatic sinister medial and medial gluteal muscle were taken immediately after slaughter in order to carry out ultrastructural examination. Sections were fixed in a mixture of 1% paraformaldehyde and 2% glutaraldehyde in a 0.2 M phosphate buffer (pH 7.4) at 4°C for 2 h and after that was post fixation in 2% osmium tetroxide for 1 h. Specimens were embedded in Epon 812. The ultrathin sections were cut on a LKB ultramicrotome and contrasted with uranyl acetate and lead citrate and examined in an Opton 900 PC TEM (FRG).

Results and discussion

A macroscopic examination of one turkey hen representing group 1 produced the following results: sporadic small hyperaemia in the liver and kidneys. The rest of animals from this group were without macroscopic changes. In birds from groups 2-4 hyperaemia in the liver and kidneys was more visible and was observed in 2 cases in each group. Moreover, there was also parenchymatous degeneration and steatosis of the liver (in one by one bird from groups 2-4) and in 1 bird from group 4 – catarrhal inflammation of the small intestine.

A microscopic examination of the liver sampled from turkey hens representing group 1 indicated small hyperaemia. Its incidence gradually increased as the degree of fat oxidation increased. The parenchymatous degeneration and steatosis simplex were assessed as slight lesions of the liver originating from two turkey hens representing group 2. Instead, all these retrogressive lesions were observed in birds from groups 3 and 4 remarkably more frequently and were more clearly visible (fig. 1, 2). Extravasations, stellate cells proliferation, mononuclear cells infiltrations and

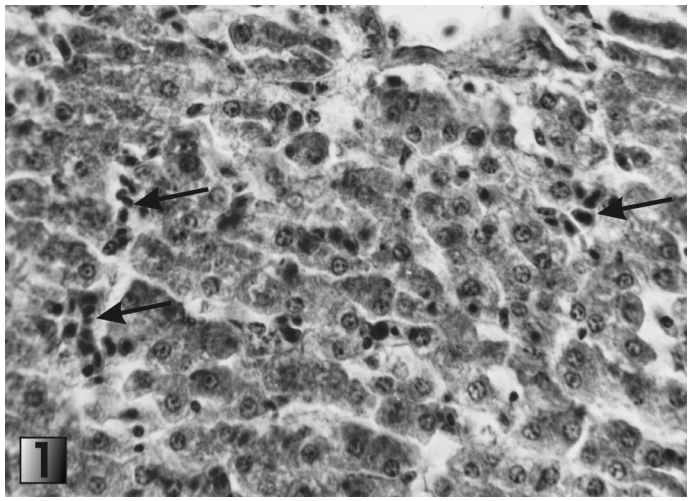

Fig. 1. Liver of a turkey hen from the group 3 – parenchymatous degeneration and proliferation of stellate cells. HE stain., magn. × 250

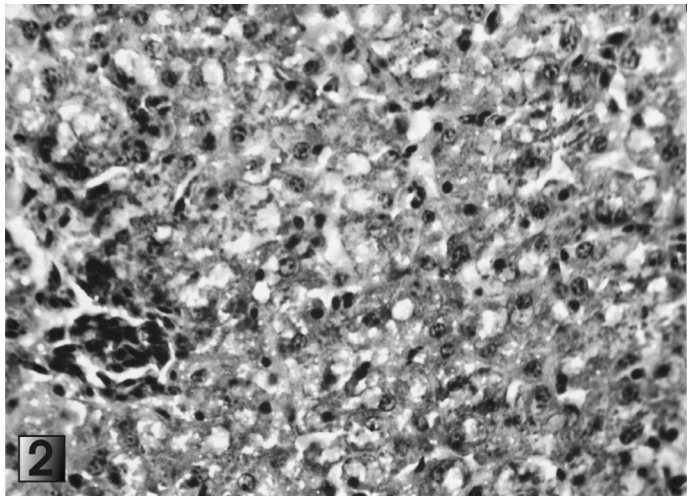

Fig. 2. Liver of a turkey hen from the group 4 – steatosis simplex of hepatocytes (fat droplets – white places), hyperaemia. HE stain., magn. × 250

mitotic figures in hepatocytes nuclei were significant for the birds from groups 3 and 4. Foci of hepatocytes necrosis was found in 3 cases in group 4 of turkeys.

There was also slight parenchymatous degeneration of the tubules epithelium observed in the kidneys of group 3 and 4 birds. In some of birds group 3, these lesions were followed by a slight granular degeneration of the kidneys and hyperaemia. All these defections were more intense in turkey hens fed with a diet containing fat at 150 mEq O_2/kg. In these cases, single tubules of epithelial cells with the markings of necrosis and mononuclear cell infiltration with fibroblasts proliferation were observed.

Hyperaemia or small foci of mononuclear cell infiltration in heart muscle were rather sporadic. Nuclei cells proliferation was observed quite often in 4 turkey hens of group 3.

A microscopic examination of the medial gluteal muscle has shown some wavy arrangement of muscle fibers and nuclei proliferation. All these lesions can be described as slight in group 2 turkey hens and more

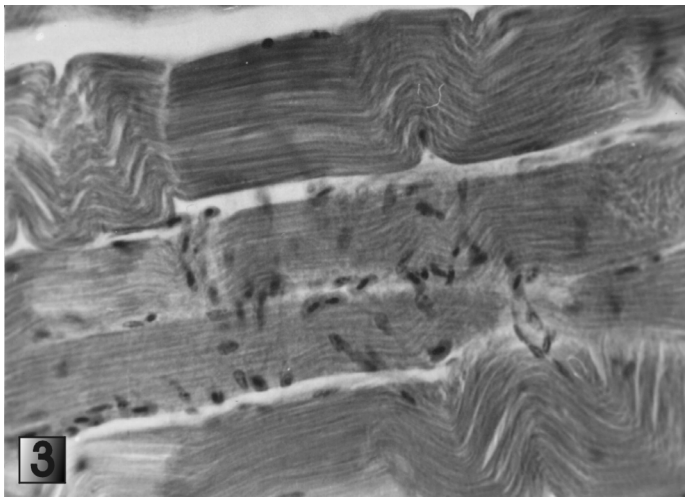

Fig. 3. Medial gluteal muscle of a turkey hen from the group 4 – wavy arrangement of muscle fibers with foci of nuclei multiplication. HE stain., magn. × 500

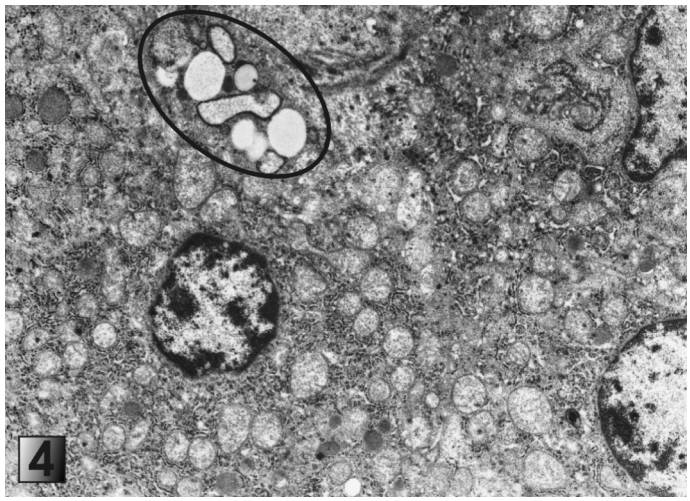

Fig. 4. Liver of a turkey hen from the group 3 – proliferation of mitochondria with rarefaction of their matrix, fat droplets (ellipse). Magn. × 7450

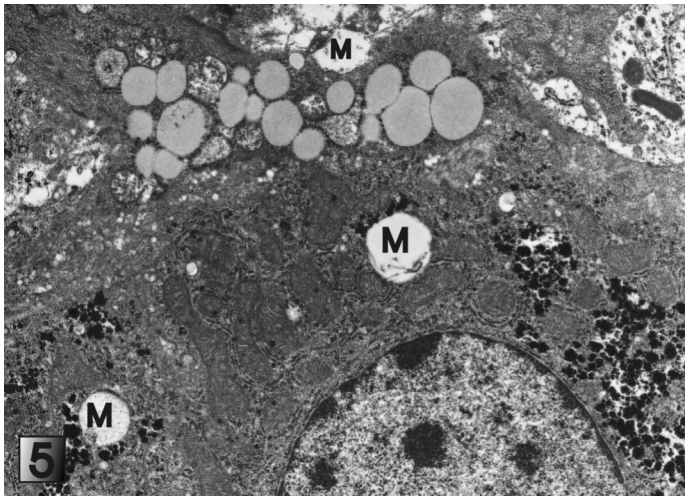

Fig. 5. Liver of a turkey hen from the group 4 – swelling of mitochondria with degradation of crista (M) and presence dense bodies in mitochondria, fat droplets and numerous glycogen granules (black stained). Magn. × 10 100

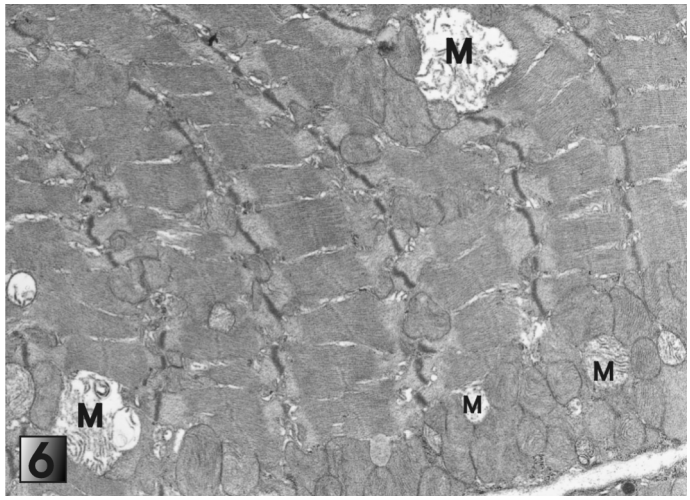

Fig. 6. Medial gluteal muscle of a turkey hen from the group 3 – proliferation of mitochondria longwise the cell membrane of muscle fiber, swelling of mitochondria with degradation of their crista (M). Magn. × 5100

intensive in birds representing both following groups (fig. 3). The hyperaemia was observed in groups 3 and 4 animals. Partial cross striation atrophy and the wavy structure of fibers with contractions and muscle fibers with phagocytic cell infiltration presence, as well as sporadic extravasation and circumscribed steatosis and hyaline degeneration were observed in group 4 birds.

Markings of hyperaemia in duodenum and ileum mucosa in 2 turkey hens from groups 3 and 4 were also found. Moreover, excessive desquamation of epithelium and mononuclear infiltration of the alimentary tract wall appeared in 3 birds from group 4.

Ultrastructural examination of the hepatocytes and muscle fibers revealed the following: mitochondria lesions (proliferation, rarefaction of matrix, swelling, degradation of crista), in the rough and smooth endoplasmic reticulum (dilatation of canals, proliferation, degradation) and fat droplets in groups 2-4 (fig. 4-9). All of these lesions were similar in their intensity in turkey hens from groups 2 and 3, but they were present and evident in most of the cases in the group 4 birds. Hepatocytes mitochondria were relatively frequently proliferated and demonstrated the presence of dense bodies (fig. 4, 5).

The ultrastructural pattern of group 3 and 4 turkey hen muscles showed epithelium vessel swelling and myelin-like structures, while birds from group 4 disclosed perturbations in the course of the Z line, degradation of the myofibrils and disorder of the myofibrils arrangement (fig. 10, 11).

Previous research gave only fragmentary information's on the biological results of excessive fat oxidation in poultry feed. In recent years, it was found that fast growing broiler lines poorly tolerate high fat levels in diet. They are also more prone to developing organism disorders (3, 17). However, according to Jankowski et al. (2000), the main factor that degrades the effects of fattening turkeys with the mixtures containing oxidized fat is the lower feed intake, which consequently decreases body mass growth. It was also

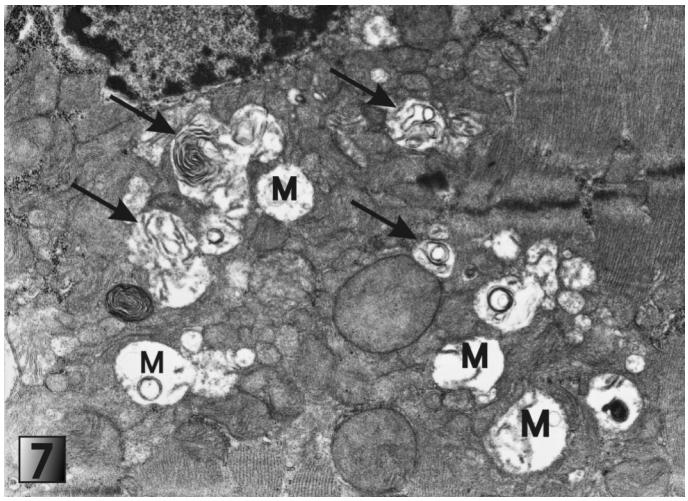

Fig. 7. Medial gluteal muscle of a turkey hen from the group 4 – proliferation of mitochondria close to cell nuclei, swelling of numerous mitochondria with degradation of their crista (M), myelin-like structures in mitochondria (arrows). Magn. × 11 850

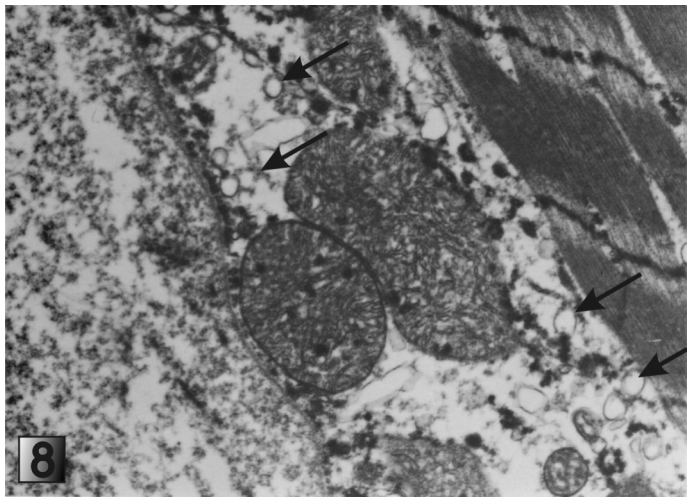

Fig. 8. Medial gluteal muscle of a turkey hen from the group 4 – swelling of mitochondria, dense bodies in mitochondria, necrosis of peripherals parts of myofibrils, dilatation of smooth endoplasmic reticulum (arrows). Magn. × 11 850

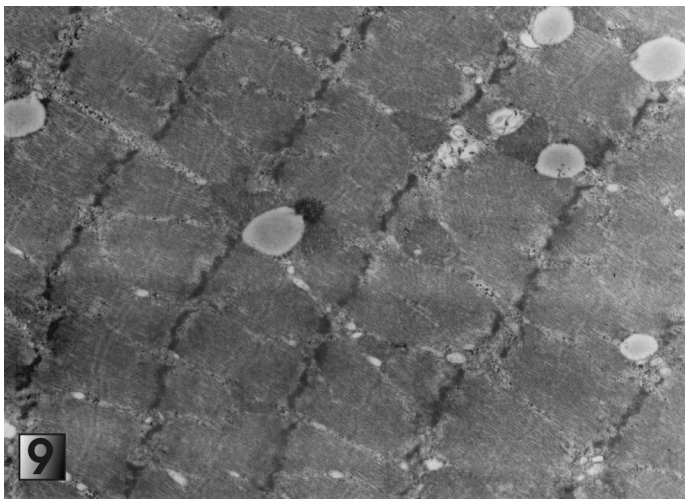

Fig. 9. Medial gluteal muscle of a turkey hen from the group 2 – fat droplets in sarcoplasma. Magn. × 10 100

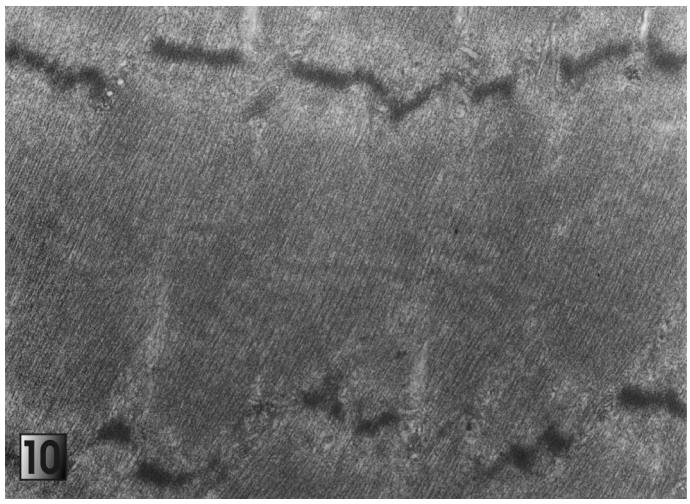

Fig. 10. Medial gluteal muscle of a turkey hen from the group 4 – perturbations in the course of Z line. Magn. × 27 500

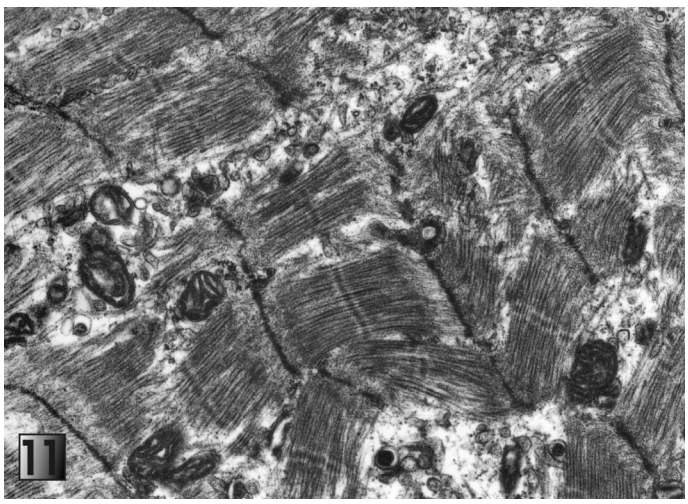

Fig. 11. Medial gluteal muscle of a turkey hen from the group 4 – disorder of myofilaments arrangement, foci of myofibrils degradation. Magn. × 11 850

found that such nutrition influences their blood morphology and liver enzymes activity to a very low degree (9). These results suggest that the turkeys may have adapted to the long-term supplementation of lipid peroxides to their diets. These results were documented by the presented ultrastructural examination of the liver and skeletal muscle, where was proliferation of the smooth endoplasmic reticulum.

The contribution of feed oxidized fats to the genesis of metabolic changes in domestic birds was also found. It was determined that they may cause hepatocyte (10) and liver secretion dysfunctions (11). They also changed lipid metabolism (15, 16) and reduced vitamin E level in blood serum and in liver (9).

All of these changes cause morphological defects which are effects of feeding turkeys with a diet containing high levels of oxidized fat. Moreover, the research has shown retrogressive lesions, especially in

livers from turkey hens fed with a diet containing oxidized fat at PV 200 mEq O_2/kg during 16 weeks. It suggests that in a reduced body mass, growth – apart from a lower feed intake (6) – is also affected by morphological lesions.

However, in the presented here investigations many adaptive-like changes (mitochondria and nuclei proliferation, lesions in endoplasmic reticulum) were observed in the turkey hen organs. The low mortality of turkeys from particular experimental groups is also very interesting. During the 16-week period, the following falls were noted: group 1 – 3.33%, group 2 – 1.67%, group 3 – 0.00% and group 4 – 5.00%.

To interpret the results, it must be considered that they concern an experiment carried out under optimum rearing conditions, thus there may be some risk that more intensive morphological lesions in the internal organs will appear in turkeys reared in large farms. It can be shown by the fact that birds fed with feed containing lipid peroxide have a decreased immunity level (8). Moreover, birds from new, fast-growing lines are less tolerant of high fat level diets and are more prone to metabolic disorders (17). All of these facts and the morphological examination results should increase interest in the influence of dietary fat and especially the products of oxidized (heated) fat on poultry health. The inspection of feed fat quality is also very important (8, 12, 21).

Conclusions

1. Fat at PV 5 mEq O_2/kg added to growing turkey diets fed during 16 weeks did not cause morphological lesions in the internal organs of the birds.

2. The long-term addition of fat at Lea value of 50 mEq O_2/kg to the turkey diet caused morphological lesions in the liver and cross striated muscles.

3. There was a slight increase in morphological lesions along the increase of the PV up to 100 mEq O_2/kg, but they intensified with oxidized fat of 150 mEq O_2/kg added to the feed. The observed morphological defects were damaging and adaptive profile.

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