Histological evaluation of changing the feeding technology of broiler chickens by introducing a Anethum graveolens-based feed supplement

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Abstract. The paper presents the results of morphological studies of internal organs of broiler chickens when using an Anethum Graveolens-based additive in the diet. In the spring-summer period, an experiment was conducted for three groups of broiler chickens. In addition to feed, the 1st experimental group daily received an alcoholic tincture based on Anethum graveolens; the 2nd experimental group – Anethum graveolens seeds; the 3rd group served as the control. After the end of the experiment, the samples of internal organs were taken for histological studies and stained with hemotoxylin and eosin, picrofuchsin according to Van Gieson, Sudan III, according to Weigert, alcian blue. According to the results of the studies, the Anethum graveolens seeds used in the 2nd experimental group did not have a negative effect on the internal organs of broiler chickens. However, the changes were observed in the 1st experimental group using an alcohol tincture based on Anethum graveolens in the diet. At the same time, the data for broiler chickens of the control group were within normal limits.

1. Introduction

Poultry farming is a branch of agriculture aimed at breeding agricultural poultry, which gives high-quality and valuable meat for human nutrition [1]. Depending on its balance in basic nutrients, the body weight gain of the poultry is 3-5 times higher per unit of consumed food more than that of farm animals. Therefore, it is easier to stimulate the body weight gain of birds than animals.

The maximum productivity and cost reducing are the main tasks that livestock farmers set for themselves. To achieve this, it is possible to fully realize the genetic potential of modern breeds and crosses using only compound feed, balanced not only in proteins, fats and carbohydrates, but also in vitamins, minerals [2] and other additives – enzymes, coccidiostatics, growth stimulants that foster the maximum productivity [3]. Due to the fact that the use of growth stimulants in agriculture is prohibited, many farmers and animal and poultry owners are in the search for an additive that would stimulate the growth or improve an appetite without negatively affecting meat and internal organs [4, 5].

Currently, medicinal plants, decoctions, infusions have been widely used in poultry farming as vitamin feeding and for therapeutic and preventive purposes. As a rule, such plants contribute to the conservation of livestock and allow obtaining high-quality products without harming the human health [6, 7].
The analysis of literature sources made it possible to conclude that there is not enough information on additives based on vegetable feedstock [6] with an exception of carbon enterosorbsents modified with biologically active substances [8] or Urticostim phytocomposition [9].

The purpose of the study is to experimentally prove the positive or negative effect of the Anethum graveolens-based additive on the internal organs of broiler chickens.

2. Materials and methods

Three weeks old broiler chickens were purchased for the experiment. Management, care and feeding of birds were carried out in accordance with GOST 34088-2017 – Guidelines for management and care of laboratory animals. Rules for management and care of agricultural animals. In the spring-summer period, the experiment was conducted for broiler chickens, which were divided into 3 groups. In addition to feed, the 1st (experimental) group daily received an alcoholic tincture based on Anethum graveolens; the 2nd (experimental) group was given Anethum graveolens seeds; the 3rd (control) group – feed mixture only.

For the studies, pieces of liver, duodenum, femoral muscles from each group were taken. The material was fixed in formalin, dehydrated in alcohols of increasing concentration, compacted by pouring it into paraffin. The sections were obtained on sledge and freezing microtomes. The histological agents were studied under a microscope at small (x200) and high magnification (x600), photography was carried out using S-E-YE software. The histological studies included the staining of histological sections with hematoxylin and eosin to identify the general characteristic of muscle tissue; staining with Van Gieson picrofuchsin to identify fibrous connective tissue; staining of histological sections by Sudan III to detect fat; Weigert staining of histological sections with hemotoxylin and eosin to identify the general characteristic of muscle tissue; staining with Van Gieson picrofuchsin to identify fibrous connective tissue; staining of histological sections by Sudan III to detect fat; Weigert staining to detect elastic fibers; alcian blue staining to detect mucus. The works of T.V. Gerunov were used to present the results of the study [10].

3. Research results

The following results were obtained upon the completion of histological studies:

1. Staining of histological sections with hematoxylin and eosin (Table 1).

| Femoral muscles | Group 1 (experimental) | Group 2 (experimental) | Group 3 (control) |
|-----------------|------------------------|------------------------|------------------|
| Duodenum        | Slight increase of goblet cells, expansion (hyperimia) of fibers (vessels), submucosa swelling. | Well-developed muscular layer, goblet cells not enlarged, vascular enlargement, blood content. | The muscular membrane is well developed, which is represented by two layers; mucous membrane – no pathologies found, moderate amount of goblet cells, monocytes are well expressed. |
The results are also shown in Figures 1-3:

**Figure 1.** Femoral muscles stained with hematoxylin and eosin (x600 magnification): (a) – group 1 (experimental), (b) – group 2 (experimental), (c) – group 3 (control) (1 – fibers located lengthwise; 2 – extended layers; 3 – the distance between fibers is wider than in group 1 and 3; 4 – nuclei are clearly visible)

Based on the data in Table 1 and Figure 1 it is possible to make the following conclusions: in group 1 (experimental) the stranding is poorly expressed, inflammatory infiltrates and extended layers (fat), intermuscular edema are possible; in group 2 (experimental) – the stranding is well expressed, the thickness of muscle fibers is expressed more weakly than in the control group; nuclei are visible; cell clusters are not visible, the distance between the fibers is wider; in group 3 (control) – all within normal limits.

**Figure 2.** Liver stained with hematoxylin and eosin (x200 magnification): (a) – group 1 (experimental), (b) – group 2 (experimental), (c) – group 3 (control) (1 – central vein is moderately filled with blood; 2 – inflammatory processes are possible; 3 – nuclei and nucleoli are well defined)
Based on the data in Table 1 and Figure 2 it is possible to make the following conclusions: group 1 (experimental) – inflammatory processes are possible; group 2 (experimental) – the central vein is moderately filled with blood, not expanded; nuclei and nucleoli are well expressed; group 3 – all within normal limits.

**Figure 3.** Duodenum stained with hematoxylin and eosin (x200 magnification): (a) – group 1 (experimental), (b) – group 2 (experimental), (c) – group 3 (control) (1 – slight increase of goblet cells; 2 – goblet cells are not enlarged; 3 – moderate amount of goblet cells; 4 – expansion of fibers; 5 – submucosa swelling; 6 – muscular layer)

Based on the data in Table 1 and Figure 3 it is possible to make the following conclusions: group 1 – slight increase of goblet cells, expansion (hyperemia) of fibers (vessels), submucosa swelling; group 2 – well-developed muscular layer, goblet cells are not enlarged, vascular enlargement, blood filling; group 3 – all within normal limits.

2. Staining of histological sections with Van Gieson picrofuchsin (Table 2):

**Table 2.** Results of histological sections staining with Van Gieson picrofuchsin

|                     | Group 1 (experimental)                                                                 | Group 2 (experimental)                                                                 | Group 3 (control)                                                                 |
|---------------------|----------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------|--------------------------------------------------------------------------------|
| Femoral muscles     | Connective tissue interlayers are larger than in the control group, the stranding is well expressed, connective tissue around the vessel | Connective tissue interlayers are larger than in the control group, but narrower than in group 1; the stranding is well expressed, connective tissue around the vessel | Thin layers of connective tissue, the stranding is well expressed, connective tissue around the vessel |
| Liver               | Connective tissue is detected around large blood vessels, in parenchyma – was not found, the capsule stained | Connective tissue is detected around large blood vessels, in parenchyma – was not found | Connective tissue is detected around large blood vessels, in parenchyma – was not found |
| Duodenum            | Connective tissue was found in submucosa, intermuscular tissue, and intestinal lining    | Connective tissue was found in submucosa, intermuscular tissue, and intestinal lining    | Connective tissue was found in submucosa, intermuscular tissue, and intestinal lining |
The results are also shown in Figures 4-6:

Figure 4. Femoral muscles stained according to Van Gieson (x200 magnification): (a) – group 1 (experimental), (b) – group 2 (experimental), (c) – group 3 (control) (1 – connective tissue)

Based on the data in Table 2 and Figure 4 it is possible to make the following conclusions: group 1 (experimental) – connective tissue layers were found to be larger than in the control group, the stranding was well expressed; group 2 (experimental) – connective tissue layers are wider than in the control group, but narrower than in group 1; group 3 – all within normal limits.

Figure 5. Liver stained according to Van Gieson (x600 magnification): (a) – group 1 (experimental), (b) – group 2 (experimental), (c) – group 3 (control) (1 – connective tissue)

Based on the data in Table 2 and Figure 5 it is possible to make the following conclusions: groups 1, 2, 3 – connective tissue was detected around large blood vessels, in parenchyma – was not found.
Based on the data in Table 2 and Figure 6 it is possible to make the following conclusions: groups 1, 2, 3 – connective tissue was found in submucosa, intermuscular tissue and intestinal lining.

3. Staining of histological sections with Sudan III (Table 3):

|                        | Group 1 (experimental)                                                                 | Group 2 (experimental)                                                                 | Group 3 (control)                                                                 |
|------------------------|----------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------|----------------------------------------------------------------------------------|
| Femoral muscles        | Fat layers are smaller than in the 2\textsuperscript{nd} experimental group             | Fat layers on almost the entire surface of the section                                  | Minor fat layers are found                                                         |
| Liver                  | Single dust-like staining was found, individual foci, fat and dystrophy were not found  | There are single drops (vacuoles) in a cell, fat and dystrophy are not found            |                                                                                   |

The results are also shown in Figures 7-8.

Based on the data in Table 3 and Figure 7 it is possible to make the following conclusions: group 1 (experimental) – fat layers were found to be less than in the 2\textsuperscript{nd} experimental group; group 2 (experimental) – fat layers on almost the entire surface of the section; group 3 (control) – minor fat layers.

Based on the data in Table 3 and Figure 8 it is possible to make the following conclusions: group 1 (experimental) – single dust-like staining was found, individual foci, fat and dystrophy were not found; group 2 (experimental) – single drops (vacuoles) in a cell, fat and dystrophy were not found; group 3 (control) – fat and dystrophy were not found.
Figure 7. Femoral muscles stained with Sudan III (x200 magnification): (a) – group 1 (experimental), (b) – group 2 (experimental), (c) – group 3 (control) (1 – fat)

Figure 8. Liver stained with Sudan III (x600 magnification): (a) – group 1 (experimental), (b) – group 2 (experimental), (c) – group 3 (control) (1 – fat)
4. Staining of histological sections according to Weigert (Table 4):

**Table 4.** Results of histological sections staining according to Weigert

|                  | Group 1 (experimental)                                                                 | Group 2 (experimental)                                                                 | Group 3 (control)                                                                 |
|------------------|----------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------|----------------------------------------------------------------------------------|
| Femoral muscles  | No elastic fibers found in intermuscular spaces, found around blood vessels             | No elastic fibers found in intermuscular spaces, found around blood vessels             | No elastic fibers found in intermuscular spaces, found around blood vessels       |
| Duodenum         | Elastic fibers found in the outer coat                                                  | The amount of elastic fibers is less in large serous coat vessels                       | In most cases the elastic fibers are found in the outer (serous) coat; elastic    |
|                  |                                                                                        |                                                                                        | fibers have ink-violet color                                                     |

The results are also shown in Figures 9-10:

**Figure 9.** Femoral muscles stained according to Weigert (x600 magnification): (a) – group 1 (experimental), (b) – group 2 (experimental), (c) – group 3 (control) (1 – elastic fibers)

Based on the data in Table 4 and Figure 9 it is possible to make the following conclusions: groups 1, 2, 3 – elastic fibers in intermuscular spaces were not found, they were found around blood vessels.

Based on the data in Table 4 and Figure 10 it is possible to make the following conclusions: group 1 (experimental) and group 3 (control) – elastic fibers were found in the outer coat; group 2 (experimental) – the amount of elastic fibers is less than in groups 1 and 3.
Figure 10. Duodenum stained according to Weigert (x600 magnification): (a) – group 1 (experimental), (b) – group 2 (experimental), (c) – group 3 (control) (1 – elastic fibers)

5. Staining of sections with alcian blue (Table 5):

|                | Group 1 (experimental)                                                                 | Group 2 (experimental)                                                                 | Group 3 (control)                                                                 |
|----------------|----------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------|----------------------------------------------------------------------------------|
| Duodenum       | In goblet cells the accumulation of mucus is smaller than in group 3, but larger than in | In goblet cells the accumulation of mucus is smaller than in groups 1 and 3          |                                                                                  |
|                | group 2                                                                                 |                                                                                        |                                                                                  |

The results are also shown in Figure 11:

Figure 11. Duodenum stained with alcian blue (x600 magnification): (a) – group 1 (experimental), (b) – group 2 (experimental), (c) – group 3 (control) (1 – mucus)
Based on the data in Table 5 and Figure 11 it is possible to make the following conclusions: group 1 – more mucus accumulation in goblet cells than in group 3, but less than in group 2; group 2 – less mucus accumulation than in groups 1 and 3; group 3 – mucus accumulation in goblet cells.

4. Conclusion
After completing the studies, it was concluded that the Anethum graveolens-based feed supplement causes some changes in the internal organs of broiler chickens of experimental groups. Group 1 – small layers of connective tissue were found in the muscles, connective tissue around large blood vessels of the liver, connective tissue in submucosa, intermuscular tissue and in intestinal lining; minor fat layers in the muscles, a single dust-like staining in the liver, individual foci, fat and dystrophy were not found; elastic fibers in muscles were found around blood vessels, in the intestine – the outer coat; a slight accumulation of mucus was found in the intestine. Group 2 – connective tissue layers in the muscles are wider than in the control group, but narrower than in group 1, the connective tissue around the large blood vessels of the liver, the connective tissue in submucosa, intermuscular tissue and in intestinal lining; fat layers on almost the entire surface of the muscle section, single drops (vacuoles) in liver cells, fat and dystrophy were not found; elastic fibers are found around muscle blood vessels, the amount of elastic fibers in the intestine is less than in groups 1 and 3; mucus accumulation in the intestine is less than in groups 1 and 3. Group 3 – all within normal limits.

From all of the above, it can be concluded that Anethum graveolens seeds do not adversely affect the internal organs of broiler chickens, alcohol that is added in the tincture causes changes.

The research was carried out using the equipment of the Agrarian and Technological Research collective use center of Omsk State Agrarian University.

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