Use Of Pomegranate Marc In Composition Of Compound Feed For The Prevention Of Diarrhea Of Farming Poultry

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Abstract

Analysis of non-contagious diseases of poultry has been carried out, and the specific weight of these diseases has been identified, in which diseases of the digestive system, in particular diarrhea, are prevalent. The use of pomegranate processing products (marc) for the preventive treatment of this disease in chickens has been substantiated. The purpose of research was to develop a compound feed formula with pomegranate marc and to study its antidiarrheal properties. Traditional research methods for clinical studies on white outbred rats have been used. Comparative data on the results of the experience of the influence of pomegranate marc in the composition of feed on the effectiveness of the treatment of diarrhea in chickens have been presented. It has been established that the introduction of the studied therapeutic and prophylactic compound feed contributed to a decrease in the weight of the stool and moisture in as opposed to the control; did not have a negative effect on peripheral blood parameters in the intact and control groups, which allowed maintaining the optimal physiological status throughout the entire test period. The authors consider feasible to use pomegranate marc in the production of combined feed for special purposes.

Keywords: Diarrhea of Farming Poultry, Cattle-breeding Sectors, Astringent Action.

INTRODUCTION

Today, to ensure the food safety of the country is the main problem of the agro-industrial complex. The most important sector that can solve this problem in the Republic of Uzbekistan is poultry farming, which is one of the most science-consuming and dynamic sectors of the agro-industrial complex. Farm poultry is characterized by fast reproduction rates, intensive growth, high productivity and viability. Growing and keeping poultry requires less labor and material resources per unit of output than in other cattle-breeding sectors.

Nevertheless, some poultry diseases can lead to rapid death of the livestock population. In the conditions of broiler and egg poultry farming, diseases of non-contagious etiology are widespread, which, according to statistical reporting, in countries with developed industrial poultry farming account for 94.0...98.0% of dead birds, and infectious diseases - less than 6.0% [12].

Diseases of non-contagious etiology, as a rule, arise from the impact of adverse factors, violations of feeding technology, deterioration in the quality of feed and their components, in stressful situations. Unlike infectious diseases, diseases of non-contagious etiology are widespread in all farms without exception, and their number depends on the conditions of feeding, maintenance, and production technology [1; 3; 4].

B.F. Bessarabov and others [5; 6] proposed the following classification of non-contagious diseases of poultry, i.e. diseases of the digestive system, alimentary dystrophy, hypovitaminosis, cannibalism, postembryonic dystrophy, diseases of the genital organs, respiratory diseases, hepatitis, hepatosis (Fig. 1).

From the data presented in Fig. 1 it results that diseases of the digestive system, often accompanied by the development of diarrhea, dominate among diseases of non-contagious etiology.

Among other diseases of non-contagious etiology, nephritis and nephrosis are common, as well as uric acid diathesis (gout), asphyxia, trauma, hypothermia, and murrain during transportation [6].
Timely diagnosis, prevention and treatment of poultry diseases in the conditions of intensive industrial technology of poultry farming are one of the most important factors in productivity growth, on which the profitability of production depends.

At present, research are conducted to create prophylactic biologically active feed supplements from natural plant substances, which obviously have certain medicinal properties in relation to such a pathological condition as chicken diarrhea [7].

Diarrhea (ancient Greek διά-ρροια) is a pathological condition in which the patient has frequent defecation, while the stool becomes watery, has a volume of more than 200 ml and is often accompanied by pain in the navel, urge to defecate and anal incontinence [8].

For the prevention of diarrhea, as well as for the treatment of its minor type, methods traditional veterinary medicine are used. Chickens are prepared with decoctions and infusions from plants, they are given medicinal herbs (fresh in summer or dried in winter). Nettle, yarrow, St. John's wort, coltsfoot, wormwood, meadow clover, etc. are very common. Choosing the right combined feed is also important [9].

Pomegranate peels are most widely used in traditional and folk medicine for diarrhea, because the peel contains the strongest tannins that can stop even severe stool disorder provoked by the use of poor-quality feed, helminthic invasion, etc. [10].

Pomegranate, botanically named Punica granatum L., is a widely consumed fruit originating from a deciduous tree of the genus Punica L., in the loosestrife family (Lythraceae). This fruit derives its name from the Latin words “pomus” and “granum” (apple with grains), which refer to the consumed part of the pomegranate tree [11].

Analysis of the profiling literature [12-16] showed that pomegranate is an important source of beneficial compounds that play a key role in maintaining homeostasis and good health (Fig. 2).

From the aspect of the above, pomegranate marc is of scientific and practical interest as a biologically active additive in the production of compound feed for special purposes, in this case, for the prevention of diarrhea in poultry (chickens).

In the process of production of pomegranate juice, up to 46.0–48.0% of marc remains, containing a large amount of valuable substances (pectin, tannins, polyphenolic and phenolic compounds, anthocyanides), which have therapeutic, prophylactic, antibacterial and volatile properties [17].
Numerous reviews [18-20] show that pomegranate peels are very effective against diarrhea, as they are rich in tannins, polyphenols, which have strong astringent, antimicrobial and antioxidant properties:

- **Tannins** - astringent action
- **Polyphenols** - antibacterial action
- **Flavonoids** - antioxidant action
- **Catechins** - antioxidant action
- **Ellagic acid** - anti-inflammatory effect
- **Microelements** - enhance immune defense.

Pomegranate peel contains 18.0-20.0% tannins, the content of which is almost 2.5 times higher than the most famous raw material for this component, namely oak bark. The astringent effect of these substances is based on their ability to bind to proteins with the formation of dense albuminates. As a result, a film forms on the surface of the intestinal tissues, intestinal peristalsis gradually slows down, and the consistency of feces is normalized due to the absorption of water from food by tannins. Tannins are also able to form precipitates with alkaloids, glycosides and salts of heavy metals, that is, they are natural antidotes for oral poisoning with these substances. Pomegranate catecholamines are no less effective than traditional antibacterial drugs, they are better absorbed without causing allergic reactions, prevent the proliferation of pathogens of intestinal infections and eliminate minor inflammation. As a result, the effect of medical treatment of diarrhea is significantly improved [18].

Research of fecal culture *in vitro* on the interaction between gut microbiota (Enterobacteriaceae, groups Bacteroides fragilis, Clostridiaceae, Bifidobacterium and Lactobacillus) and polyphenols derived from pomegranate that treatment with pomegranate extract and pomegranate juice (25 and 100 mcg/ml) significantly increased the amount of both Bifidobacterium and Lactobacillus. At the same time, the concentration of the Bacteroides fragilis, Clostridiaceae, and Enterobacteriaceae group decreased depending on the drug concentration [21].

According to the mineral composition, the pomegranate peel does not differ from its juice, however, there are much more minerals in it – about 0.9%. The total amount of P-active substances (catechins, leucoanthocyanins, anthocyanins, flavonols) in the peel reaches 3.63%. Among them, catechins are the most – from 0.82 to 2.12%. The content of vitamin C in the peel varies from 20.7 to 193.1 mg per 100 g. Pomegranate seeds contain cellulose (22.4%), starch (12.6%), nitrogenous substances (3.4%) and ash (1.54%). The fat content in pomegranate seeds exceeds 20%. Pomegranate seed oil is rich in essential unsaturated acids, mainly linoleic (about 67.0%), and in terms of vitamin E content (272 mg per 100 g) it is not inferior to wheat germ oil [22].

Concerning the problem under consideration, it becomes expedient to use pomegranate marc containing a unique complex of natural marc components as part of compound feed for the prevention and treatment of diarrhea and other disorders of the gastrointestinal tract in chickens and other poultry. Therefore, at present, developments on the enrichment of these products with functional substances while maintaining their sensory-adequate traditional characteristics are especially relevant.
METHODS

The purpose of research was development of compound feed formulation with pomegranate marc and research of the therapeutic and prophylactic properties of this product for diarrhea in chickens.

The experimental part of the work was carried out in the laboratories of the Department of Food Technologies of the Bukhara Engineering-Technological Institute and the Department of Sanitary and Epidemiological Surveillance of the Main Medical Department under the Administration of the President of the Republic of Uzbekistan.

Scope of application: recommended for the prevention and treatment of diarrhea in chickens.

Dosage and administration: within 3 days from the first day of diarrhea - 130 - 150 g.

Simultaneously, the composition of the compound feed with the addition of 3.0% of the total amount of raw pomegranate marc (experiment) was stated, which replaced a similar amount of wheat bran in the control variant.

Assessment of the effectiveness of the antidiarrheal activity of therapeutic and prophylactic compound feeds for chickens was carried out in accordance with the requirements of the Methodological recommendations for assessment of the effectiveness of biologically active food supplements, 2004, according to the generally accepted method on the model of diarrhea caused by the introduction of a laxative (we used sodium as a laxative picosulfate, in the form of the “Picolax” oral drops to be consumed internally, “Farmak” PJSC, Ukraine) [23-26].

The experiments were carried out on 30 white outbred rats weighing 110-120 g, followed by division into groups of six animals each. The age of the animals at the beginning of the experiment was 40 days.

To do this, the animals of the test group and the comparison group were once administered drugs:

1. intact group (intact) - animals without manipulations
2. control group (control) - animals received distilled water orally, in a volume of 1 ml/100 g;
3. experimental group - the animals received a preparation of therapeutic and prophylactic compound feed for chickens orally, at a dose of 1000 mg/kg, which amounted to 1.0 ml/100 g;

During research, no death of rats of all three groups was noted. The general condition of the animals was satisfactory. In appearance, coat quality, behavior and growth rate, the animals of the experimental group did not differ from the animals of the intact and control groups.

Diarrhea was simulated by intragastric administration of sodium pico sulfate through a metal probe, in the form of a 0.75% suspension, at a dose of 113 mg/kg, in a volume of 1.5 ml/100 g. Then, the animals were placed in individual cages for 24 h, where stool was collected. Stool weight was determined gravimetrically, considering the total weight of the stool. The criterion for evaluating pharmacological activity was a decrease in stool weight and moisture, compared with the control.

Biochemical analysis of the blood were performed on a semi-automatic biochemical analyzer “CYAN Smart” with software (Cypress Diagnostics, Belgium) according to standard methods: total protein (TP), alkaline phosphatase (AP), activity of aspartate aminotransferase (ACT) and alanine aminotransferase (ALT) in animal venous blood serum – reagent kits of Cypress Diagnostics, Belgium, hemocrit was determined on a hematocrit centrifuge Cypress Diagnostics, Belgium, and detailed analysis of peripheral blood was determined in a Goryaev chamber.

Experimental research was carried out on small laboratory animals (white rats and mice, guinea pigs) in accordance with the current regulatory and methodological base.

The results were processed by the method of variation statistics according to Student's criterion at p=0.05. The tables show the arithmetic mean values (M), the corresponding standard errors of the mean value (m), and the criterion (M ± m).

Experimental tests were carried out in compliance with the rules adopted by the European Convenction for the protection of vertebrate animals for experimental or other scientific purposes (ETS No. 123. Strasbourg, 18.03.1986).

RESULTS AND DISCUSSION

Because of research of the antidiarrheal activity of therapeutic and prophylactic compound feeds for chickens, it was established that when animals are fed with the studied feed at an approximate dose of 1000 mg/kg, a significant antidiarrheal effect is observed from the 2nd day of feeding, that is, there is a decrease in the total mass of stool and moisture, a decrease in the total mass stool is statistically significant (Table 1).

Table 1. The results of research of the antidiarrheal activity of the therapeutic and prophylactic compound feed on the 3rd day of observation, (M ± m)

<table>
<thead>
<tr>
<th>Group</th>
<th>Researched indicator</th>
<th>Total mass of feces, mg</th>
<th>Moisture, mg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
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</tbody>
</table>

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Assessment of the effect of antidiarrheal compound feed on the live weight of the animals observed in the experiment was also carried out. It was established that the weight of the animals did not increase in the intact and control groups, while when feeding animals with antidiarrheal compound feed, the live weight of the experimental animals significantly tended to increase (Table 2).

Table 2. The increase in live weight of experimental animals observed in the experiment after 3 days of the experiment

<table>
<thead>
<tr>
<th>Group</th>
<th>Live weight of experimental animals</th>
<th>Initial, g</th>
<th>Final (after 3 days), g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact</td>
<td>162.33±8.29</td>
<td>162.33±7.55</td>
<td></td>
</tr>
<tr>
<td>Control (regular food)</td>
<td>155.67±17.73</td>
<td>155.66±10.78</td>
<td></td>
</tr>
<tr>
<td>Compound feed(1000 mg/kg)</td>
<td>175.33±7.62</td>
<td>184.33±19.26</td>
<td></td>
</tr>
</tbody>
</table>

During assessment of the effect of the studied antidiarrheal compound feed on maintaining metabolism in the body, we studied the hematological and biochemical parameters of the blood of experimental animals treated with drugs, in comparison before and after the end of the experimental administration at the recommended dose (Tables 3 and 4).

Table 3. The influence of the studied therapeutic and prophylactic compound feed on the 3rd day of observation on the hematological parameters of the blood of the animals observed

<table>
<thead>
<tr>
<th>Group</th>
<th>Observation period</th>
<th>Hematological indicators</th>
<th>Biochemical indicators</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>hemoglobin concentration, g/l</td>
<td>leukocytes, 10^9/l</td>
</tr>
<tr>
<td>Intact</td>
<td>before the introduction</td>
<td>136.5±2.1</td>
<td>14.60±0.3</td>
</tr>
<tr>
<td></td>
<td>at the end</td>
<td>134.8±3.8</td>
<td>14.58±0.66</td>
</tr>
<tr>
<td>Control</td>
<td>before the introduction</td>
<td>136.8±2.3</td>
<td>14.61±0.39</td>
</tr>
<tr>
<td></td>
<td>at the end</td>
<td>137.6±5.3</td>
<td>14.57±0.60</td>
</tr>
<tr>
<td>Experimental</td>
<td>before the introduction</td>
<td>131.8±4.2</td>
<td>14.65±0.53</td>
</tr>
<tr>
<td></td>
<td>at the end</td>
<td>142.4±2.4</td>
<td>14.58±0.59</td>
</tr>
</tbody>
</table>

Results of hematological analyzes showed that the introduction of the studied therapeutic and prophylactic compound feed did not have a negative effect on peripheral blood parameters in comparison with the intact and control groups, which allow maintaining the optimal physiological status throughout the entire test period.

Research on the effect of therapeutic and prophylactic compound feed on biochemical blood parameters allowed establishing an increase in total protein in experimental animals by an average of 5.0%, which indicated the activation of protein metabolism processes and an improvement in the energy supply of biochemical processes occurring in the body.

Table 4. The influence of the studied compound feed on biochemical parameters of the blood of animals observed in the experiment

<table>
<thead>
<tr>
<th>Group</th>
<th>Statistic indicators</th>
<th>Observation period, week</th>
<th>Biochemical indicators</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>AIT, E/l</td>
</tr>
<tr>
<td>Intact</td>
<td>M±m</td>
<td>1</td>
<td>56.1±3.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>56.1±3.1</td>
</tr>
<tr>
<td>Control</td>
<td>M±m</td>
<td>1</td>
<td>52.0±3.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>58.8±2.4</td>
</tr>
<tr>
<td>Experimental</td>
<td>M±m</td>
<td>1</td>
<td>56.1±3.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>52.0±3.7</td>
</tr>
</tbody>
</table>
Indicators of liver enzymes in all experimental groups were in the limits of the physiological norm, which confirms the possibility of using therapeutic and prophylactic compound feed to maintain the physiological status of the animals in an optimal state.

In order to determine the neurotrophic effects of therapeutic and prophylactic compound feed, we evaluated changes in the behavioral reactions of experimental animals that received the studied compound feed in the OF (open field) testing daily at the same time of day (from 9.00 to 11.00) after 3 days of feeding with compound feed after stool normalization.

Testing in the OF is widely used in various experimental research, and it allows a relatively quick assessment of the overall physiological response of the animal to the action of the studied drugs and feeds. This reaction includes elements of motor, tentatively exploratory and emotional behavior.

In the research, the OF test zone was an area 80x90 cm in size, divided into 20 squares and bounded by a barrier. During the experiment, carried out in a darkened soundproof room, the field was uniform illuminated by a 100 W incandescent lamp, which was located at a height of 1 m from the center.

The rat was placed in the middle of the area, and the following indicators were recorded for 2 minutes: horizontal motor activity, vertical motor activity, and the frequency of defecation. According to modern concepts, the intensity of animal motor activity is a genetically programmed species trait and affects the formation and manifestation of the most important morphologic-functional features in species and individuals, including the nature of metabolism and the degree of development of a number of physiological systems.

Generally accepted that the motor reactions of an animal under OF conditions characterize its locomotor and exploratory activity, and the frequency of defecation acts mainly reflects the emotional reactivity of the animal.

During the first testing in the OF, the indicators of both HDA - horizontal motor activity - (9.60 ± 0.30) and VMA - vertical motor activity - (2.06 ± 0.60) in intact and control rats had an unreliable tendency to increase compared to those in the experimental groups. Orientation-exploratory reactions were realized, as a rule, in the first seconds of testing. In the experimental groups, a slight increase in the intensity of the horizontal and vertical components of motor activity was observed. The number of acts of defecation in experimental animals practically did not reveal any specific dynamics during the entire experiment. This indicator did not show significant differences in the intergroup comparison of animals.

At the end of the experiment, the rats of the intact group and the control group, who received therapeutic and prophylactic antidiarrheal compound feed, were euthanized in a gentle way (by introducing into deep ether anesthesia). The condition of the internal organs was assessed visually during autopsy, which confirmed the absence of edema, impaired blood supply, or tissue hemorrhages. There were no significant differences between the experimental and intact groups.

CONCLUSION

Thereby, the use of natural food additives from plant materials in the production of combined feed for the preventive treatment of diarrhea in chickens is a promising and relevant scientific area of research and is of practical significance. This determines the implementation of the priority directions of the state development strategy in the field of providing the population with basic food products and food safety. Most food manufacturers do not sufficiently use secondary raw materials (waste) from the processing industries of the food industry, the use of which in the production of combined feed for the animals will reduce the cost of production and make it more affordable even for socially vulnerable segments of the population.

Received experimental results allow concluding that the studied composition of the combined feed for poultry has a significant antidiarrheal activity and can be positioned as a therapeutic-preventive combined feed for the designated purpose.

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