

Results of the Study the Content of Amprolium in Meat, Eggs, Organs and Tissues in Chickens

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Abstract: The article presents the results of experimental studies to determine the content of the residual amount of the drug (substances) of amprolium in meat, eggs, organs and tissues of chickens that received the drug with feed in various dosages. At the same time, it was found that after the use of the drug with the substance of amprolium, even in the recommended doses, there are restrictions on poultry products for human consumption.

Keywords: Substance, amprolium, chickens, poultry, meat, egg, white mouse, chemical, analytical method, БЭЖХ, УФ detection, dosage.

Relevance. Currently, poultry farming is a very important branch of agriculture all over the world, and the products obtained from birds are an easily accessible, easily digestible and useful food for humans. In order to obtain highly profitable poultry products, veterinary-sanitary and chemical-therapeutic measures aimed at preventing bird diseases or improving the health of farms are constantly carried out in poultry farms. At the same time, specialists of the veterinary service must strictly observe precautionary measures so that residual toxic doses of chemical pharmaceutical preparations together with poultry products as food do not end up on the human table. Food security of the population is the most urgent task of any country in the world today.

Object and methods of research. (*The article is presented in the form of a Report*). The research was carried out in the vivarium department of the manufacturer of veterinary pharmaceuticals on white mice, broiler birds and laying hens, as well as in the chemical analysis and toxicology laboratory of the Institute of Chemistry of Plant Substances of the Academy of Sciences of the Republic of Uzbekistan. The residual amounts of amprolium in meat, eggs and tissues were determined analytically using БЭЖХ with УФ detection. The studies were conducted in accordance with the requirements: GLP (Good Laboratory Practice); NOEL (no-observed-effect level; D_{max} – the maximum harmless daily dose for animals); ADI (acceptable daily intake; DCD – permissible daily dose for a person) and MRL (maximum residue limits; MDY – the maximum permissible level of the substance in food and animal feed).

Amprolium is a hydrochloride of 1 - [(4-amino-2-propyl-5-pyrimidinyl) methyl] - 2-methylpyridine chloride. It is a coccidiostatic agent used for the treatment and prevention of coccidiosis in chickens, including laying hens and turkeys. For treatment, it is used with drinking water at a concentration of 120 to 240 mg/l or with feed at a concentration of 125 mg/kg for 5-7 days. To prevent re-infection, it is used with drinking water at a concentration of 60 mg/l for 1-2 weeks. The maximum dose depends on the age, and varies from 25 to 73 mg/kg of body weight per day for chickens and from 14 to 60 mg/kg of body weight per day for turkeys. In accordance with Directive 70/524/EEC, amprolium is allowed as a feed additive; for adult poultry in a concentration of 62.5 to 125 mg/kg of ready-made feed, as well as in combination with etopobate (in a ratio of 25: 1.6 parts) for chickens, broilers and guinea fowls in a concentration of 66.5 to 133 mg/kg of ready-made feed; authorization prohibits the use of the substance when the egg-laying age is reached and at least 3 days before slaughter.

Research results. Amprolium is an analog of thiamine (vitamin B1) and acts as a competitive antagonist in the biochemical mechanisms of thiamine transport. The coefficient of the chemical agent at admission (K_{iapp}) for amprolium was 1150 mg/l on chicken intestinal cells. The chemical agent for the blood-brain system in white mice was similar to the chemical agent of thiamine. With insufficient intake of thiamine from food, amprolium can cause a decrease in body weight and the concentration of thiamine in tissues, which presumably indicates a selective inhibition of thiamine intake. This effect was observed in white mice receiving daily from 2 to 8 mg of amprolium along with 10 and 20 mcg of thiamine, respectively (the normal intake of thiamine is 40mcg).

Amprolium was added to the feed for white leghorn chickens and broiler chickens, starting from 3 days of age and up to 3 weeks of age. No undesirable effects were observed at doses up to and including 500 mg/kg of feed. At a dose of 800 mg/kg of feed, a decrease in weight gain was observed. A dose of 1000 mg/kg/day caused polyneuritis and death. Undesirable effects were eliminated by adding thiamine in an amount of 100 and 1000 mg/kg of feed. In the second study, no adverse effects were observed in chickens aged from 1 to 8 weeks when consuming feed with an amprolium content of up to 700 mg/kg of feed; at a higher content, polyneuritis and increased mortality were observed. No histopathological changes in organs and tissues were found in White Cross roosters aged from 3 days to 9 weeks who received food with an amprolium content of 100 mg/kg of feed. Feeding 68-week-old laying hens of the white leghorn breed of amprolium in an amount of 250 mg/kg of feed did not have an effect on egg production and hatchability (hatching) of chickens from eggs; hatching was slightly reduced when the content of amprolium was 500 mg/kg of feed.

A study conducted on 5 generations of mice. In each generation, 20 males mated with 20 females, at the age of 8 weeks, amprolium was fed in the amount of 500 and 100 mg/kg of feed, as well as in the amount of 25,000 mg/kg of feed together with 1000 mg/kg of thiamine feed, such a content in the feed approximately corresponds to 200, 1000 and 5000 mg of amprolium per kg of body weight per day and 200 mg of thiamine per kg of body weight per day, respectively. It was noted that at 1000 mg/kg, maternal mortality occurred and the number of mice that became pregnant decreased. There was also a decrease in the number of litters in the group receiving 5000 mg/kg of amprolium and 200 mg/kg of thiamine. At the same time, there were no adverse maternal effects or effects on fertility at a dose of 200 mg/kg.

Although detailed information on reproductive studies in mice is not available, studies on the teratogenicity of amprolium have been conducted in rats and rabbits that comply with the available guidelines for conducting such studies and GLP (Good Laboratory Practice) requirements. There were no undesirable maternal or fetal effects up to and including 200 mg/kg/day, which can be taken as a general NOEL (no-observed-effect level) level for reproductive effects.

In one of the studies (conducted in accordance with the GLP requirements), chickens consumed feed containing ^{14}C – amprolium 125 mg/kg of feed for 18 hours to 8 days. The residual concentrations found in the muscle, liver and kidney samples were different. The tissues during the medicalization process contained radioactivity equivalent to 0.03 to 1.93 mg of amprolium. After a waiting period of 2 days, residual amounts were detected only in the muscle tissue. There was no evidence of bioaccumulation, any preferred separation and differentiation of distribution across tissues as a result of the different location of the radioactive label (picoline or pyrimidine ring). Since the total residual amount and marker compounds of residual amounts were not studied simultaneously in the same individuals, various dosage regimens were used in radiometric studies, as well as amprolium without a radioactive label was used in studies, then it is not possible to estimate the quantitative ratio of marker compounds to the total number of residual metabolites.

Studies that meet the requirements of GLP (Good Laboratory Practice) were conducted on laying

hens and broilers. The birds were given ^{14}C – amprolium orally for 21 days. The use of the drug was planned in such a way that it would simulate the use of commercial drugs dissolved with drinking water at a concentration of 240 mg/l for 7 days, followed by a lower concentration of 60 mg/l for 14 days. Groups of 6 laying hens and 6 broilers were slaughtered after 6, 12, 24, 36 hours and 7 days after receiving the last dose. In laying hens after 6 hours, the average values of residual amounts were 701, 1160, 63 and 94 mcg/kg in the liver, kidneys, meat and skin with subcutaneous fat. 12 hours after the last application of the drug, the average values of residual amounts were 406, 401, 38 and 90 mcg/kg, respectively. After 24 hours, the residual amounts in all meat samples and in one (out of 6) skin samples with subcutaneous fat were 2 times lower than the background detection level (28 and 38 micrograms/kg for meat and skin with subcutaneous fat, respectively). In the liver, the average residual amounts after 24 hours were 173 mcg/kg and decreased to 116mcg/kg after 36 hours. In the kidneys, the average residual amounts after 24 hours were 139 mcg/kg and decreased to 85 mcg/kg after 36 hours. The residual amounts in broilers decreased in a similar way. 6 hours after the last application of the drug, the average values of residual amounts in the liver, kidneys, meat and skin with subcutaneous fat were 519, 765, 46 and 137 mcg/kg, respectively. After 24 hours, the average values of residual amounts in the liver, kidneys, meat and skin with subcutaneous fat were 191, 120, 45 mcg/kg, respectively, and the residual amounts in all meat samples were less than twice the detection limit (28 mcg/kg for meat).

In the same study, eggs were collected from laying hens. The contents of some eggs obtained on the same day were mixed, and other eggs were analyzed separately. The highest residual amounts were found in eggs collected on the 7th and 8th days of receiving the drug, corresponding to the end of the period of feeding the drug in a high dose. For eggs collected on the 7th day of the experiment, the residual amounts in the eggs ranged from 390 to 1530 micrograms of equivalent radioactivity/kg with an average value of 733 micrograms of equivalent radioactivity/kg. For eggs collected on the last day of the experiment (21 days), the residual amounts in the eggs ranged from 107 to 434 micrograms of equivalent radioactivity/kg with an average value of 271 micrograms of equivalent radioactivity/kg. 6 days after discontinuation of treatment, the residual amounts in all eggs were less than twice the detection limit (34 mcg/kg).

Studies that meet the requirements of GLP were conducted on broilers. The bird was given ^{14}C – amprolium for 21 days. The use of the drug was planned in such a way that it would simulate the use of commercial drugs dissolved with drinking water at a concentration of 240 mg/l for 7 days, followed by a lower concentration of 60 mg/l for 14 days. Groups of 3 roosters and 3 chickens were slaughtered after 6, 12, 24, 36 hours and 7 days after receiving the last dose. The average total residual amounts in the liver decreased from 544 micrograms of equivalent radioactivity/kg (after 6 hours) up to 260 micrograms of equivalent radioactivity/kg (after 12 hours) and 188 micrograms of equivalent radioactivity/kg (after 24 hours).during the same period, the total averaged residual amounts in the kidneys decreased from 496 micrograms of equivalent radioactivity/kg to 219 micrograms of equivalent radioactivity/kg and to 118 micrograms of equivalent radioactivity/kg. In a study conducted not in accordance with the GLP requirements, chickens aged from 1 day to 8 weeks (10 heads for each time period) were on a diet containing 150 and 250 mg of amprolium per kg of feed. After slaughter, using the fluorimetric thiochromic method (having a detection rate of 73-79%/ of the total number of metabolites/), residual amounts were determined in samples of meat, kidneys, skin together with subcutaneous fat (detection limit of 10 mcg/kg) and liver (detection limit of 20 mcg/kg). Without a waiting period and with a 2-day period of pre-slaughter exposure after feeding the feed with an amprolium content of 250 mg/kg, the residual concentrations were respectively: in meat – 90 mcg/kg and less than 10 mcg/kg, in the liver – 410 mg/kg and less than 20 mg/kg, in the kidneys – 380 mg/kg and 40 mg/kg, in the skin together with subcutaneous fat – 420 mg/kg and 20 mg/kg. At the same time, after feeding the feed with an amprolium content of 150 mg/kg of feed, the residual amounts and residual concentrations were respectively: in meat – 90 mcg/kg and less than

10 mcg/kg, in the liver – 420 mg/kg and less than 20 mg/kg, in the kidneys – 350 mg/kg and less than 10 mg/kg, in the skin together with subcutaneous fat – 160 mg/kg and less than 10 mg/kg. The reliability of these results is questionable due to the fact that small amounts of amprolium were detected in the control samples and the results had to be recalculated in such a way as to prevent the estimation of this error value.

A study in which adult laying hens consumed feed containing 150 and 250 mg/kg of feed. The residual amounts were determined by gas chromatography in eggs (2 eggs from each of 4 laying hens) and edible tissues from 3-5 individuals, so that after 3 days the residual concentrations were 30-60 mcg/kg in the liver and less than 10 mcg/kg in the kidneys. In accordance with the requirements of the GLP, a study was conducted on broilers; birds were given amprolium dissolved in water at a dose of 240 mg/l for 7 days, followed by amprolium at a lower dose of 60 mg/l for 14 days. 6 broilers were slaughtered after 0, 1, 2, 4 and 7 days after the termination of treatment. The residual amounts in the tissues were studied using an analytical method based on BЭЖХ with УФ detection. For the liver, the limits of quantitative and qualitative determination were 100 and 40 mcg/kg, respectively. In the first group of broilers slaughtered immediately after treatment, the residual amounts of amprolium in the liver ranged from 178 to 330 mcg/kg (on average 250 mcg/kg). The residual amounts in most liver samples taken at later time intervals were below the detection limits. In the study, the chickens also consumed feed containing from 0.6 to 2000 mg of amprolium per kg of feed for various periods of time, ranging from 21 days to 24 weeks. The results showed a linear relationship between the content of amprolium in the feed and its concentration in the egg yolk. Feeding feed with an amprolium content of 250 mg/kg gave about 400 mcg/kg in egg yolk in the absence of a pre-slaughter aging period. At a lower concentration of 125 mg/kg of feed, the concentration of amprolium in the yolk was approximately 200 mcg/kg in the absence of a period of pre-slaughter exposure. Based on the study of 89 samples, the coefficients between the consumption of amprolium and the concentrations in egg yolk were calculated using regression analysis.

In accordance with the requirements of the GLP, a study was conducted on 16 laying hens. The birds were given amprolium dissolved in water at a dose of 240 mg/l for 7 days, followed by amprolium at a lower dose of 60 mg/l for 14 days. Eggs were collected from all birds that were slaughtered 7 days after the end of treatment. The residual amounts of amprolium in eggs and tissues were determined analytically using BЭЖХ with УФ detection. The residual amounts of amprolium in all kidney samples were below the limit of the possibility of quantitative determination (200 mcg/kg) and in most samples were generally below the limit of qualitative detection (40 mcg/kg). Residual amounts in the liver ranged from levels below the detection limit (40 mcg/kg) to 135 mcg/kg. The highest residual amounts in eggs were found on the 7th day of the use of amprolium, corresponding to the end of the period of the use of amprolium at a higher dose. For eggs collected on the 7th day of treatment, amprolium levels ranged from levels below the limit of quantification (500 mcg/kg) to 854 mcg/kg. On day 21 (the last day of use), the residual amounts in all eggs were below the level that allows quantitative measurements.

Conclusion:

1. For the treatment of coccidiosis in chickens and turkeys, the drug containing amprolium is changed strictly according to the instructions for use of the manufacturer's enterprise.
2. It is not allowed to use the drug containing amprolium to repair young chickens after 16 weeks of age. It is forbidden to use amprolium to laying hens, because the drug is excreted with eggs.
3. The slaughter of poultry for meat is allowed 5 days after the termination of the use of the drug. Meat obtained from forcibly killed birds, before the expiration of the specified period, is used for feeding carnivorous animals or for the production of meat and bone meal.

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