EFFECTS OF ANTIBIOTIC GROWTH PROMOTERS ON BIOCHEMICAL AND HAEMATOLOGICAL PARAMETERS OF BROILER CHICKENS' BLOOD

Ayana Slyamova[1], Nurzhan Sarsembayeva[1], Anda Valdovska[2], Jan Micinski[3], Altay Ussenbayev[1], Assel Paritova[1], Altynbek Mankibayev[1]
[1]Kazakh National Agrarian University, Kazakhstan
[2]Latvia University of Agriculture, Latvia
[3]University of Warmia and Mazury of Olsztyn, Poland
s_ayana_e@mail.ru; Anda.Valdovska@llu.lv; micinsk@uwm.edu.pl

Abstract
This study was designed with the aim to determine the impact of residual antibiotics on haematological and biochemical constituents of broiler chickens' blood. For this, one-day-old chickens were randomly divided into four equal groups with 10 individuals in each group (n = 40). All groups of chickens fed with commercial basal diet; the first group considered as control, fed only with basal ration; the feed of the second, third and fourth groups of chicken were supplemented with amoxystin, tetracycline and chloramphenicol, respectively. Antibiotics were given daily, individually, in sub-therapeutic concentrations: amoxystin at the dosage 10 mg kg^{-1}, tetracycline and chloramphenicol – 20 mg kg^{-1} of the chicken's weight for 41 days. The blood parameters were measured at the end of experiment on the 42nd day. In comparison with the control group, decreasing of leukocytes was observed in the 2nd and 4th groups, and increasing in the 3rd group of broilers. The concentration of erythrocytes and hemoglobin was reduced in the 2nd group and hematocrit was higher in the 3rd and 4th groups ($p \leq 0.01$). The total protein was decreased by 22% in the 2nd group, 16% – in the 3rd and 4th groups as compared to the control group ($p \leq 0.01$) in blood serum. The concentration of glucose was decreased by 45.8, 46.5 and 51.5% in the second, third and fourth groups of treated birds, respectively, compared to those of birds in the control group ($p \leq 0.01$). Based on the results it could be concluded that antibiotics influence the dynamics of haematopoiesis and biochemical indices of broilers.
Key words: Amoxystin, Tetracycline, Chloramphenicol, Broiler, Blood Parameters.

Introduction

Large amounts of antimicrobial agents are widely used in veterinary medicine for the prevention and treatment of diseases caused by microorganisms (Singer *et al*., 2003; Dantas *et al*., 2008), and more controversially, antimicrobial agents used and managed as feed additives or with drinking water for therapeutic, prophylactic purposes (Blasco, Torres, & Pico, 2007; Fabrega *et al*., 2008; Morales-Gutierrez, Barbosa, & Barron, 2015) as well as to improve the ability of animals to convert feed into body weight (Turnidge, 2004; Aarestrup, 2005; da Costa *et al*., 2010).

The theoretical possibility of stimulating action of some microbial agents on the growth of animals was shown in the forties of the XX century. Animal growth acceleration was explained by the presence of stimulants in the culture of used bacteria (Fedorov, 1974). It was found that the daily feed supplement for piglets and chickens contains small portions of definite soil bacterial species associated to the interaction with intestinal microbial population that accelerated growth and increased the weight gain of animals compared with the controls (Dibner & Richards, 2005; Niewold, 2007).

Antibiotic growth promoters are used to destroy or inhibit bacteria and are administered at a low, sub-therapeutic dose (Hao *et al*., 2014). Lee, *et al.* established that antibiotics promote improving the body weight through more efficient digestion of feed in growing animals (Lee *et al*., 2011). These promoters are now recognized in broiler industry as additives to shorten the period for attaining the market weight by stimulating growth: to improve feed efficiency and survivability of broilers (Hossain, Khairunnesa, & Das, 2015).

Although antimicrobial therapy chemicals and their use as growth promoters are of essential importance in maintaining animal health (Bywater, 2004; Prescott, 2008; Persoons *et al*., 2012), the use of antibiotics, along with a positive effect, leads to the emergence and widespread resistance of pathogens to antibiotics. This situation complicates the treatment of animal infectious diseases (Navashin & Fomina, 1982), as well as they adversely affect the immunegenesis and contribute to the sensitization of the human body (Abdullaev, 2006).

As the same classes of antimicrobials are used both in humans and animals, the emergence and spread of antimicrobial resistance in bacteria poses a threat to human health and presents a major financial burden. Moreover, few new antibiotics are being developed to replace those becoming ineffective through resistance (World Health Organization, 2007).

In the last few years, the concern about the use of veterinary drugs such as antibiotics in food-producing animals and their possible negative consequences for the health of consumers has made the control of these

residues in edible animal tissues mandatory at the EU. Maximum residue limits (MRLs) of antibiotics in foodstuffs of animal origin such as multiple animal tissues were established by the Commission Regulation (EC) № 37/2010 (EU Commission, 2010) for safe human consumption.

In 2007, the World Health Organization recommended stopping intensive routine use of antimicrobials for productive animals (Collignon *et al*., 2009), however, antibiotics, except for the prevention and treatment of animals, are still used as growth promoters in the world.

The consequences of the use of antibiotics are very diverse and appear as a failure of individual organs and systems in general. One of the most sensitive systems is the haematopoietic system. The process of haematopoiesis in the body is carried out continuously, and the young dividing cells are very sensitive to the action of drugs (Stolker & Brinkman, 2005).

Toxic effects of drugs on the process of haematopoiesis cause a change of blood parameters. For example, the toxic effect of chloramphenicol on the process of haematopoiesis is manifested by leucopenia. And using of amoxicillin, which is a part of the synthetic antibiotic 'Amoxystin', showed neutropenia and eosinophilia. When tetracycline was applied, there was observed an increase in blood transaminases and alkaline phosphatase (Stolker & Brinkman, 2005).

The aim of this study was to assess the impact of antibiotics used as growth promoters on the haematological and biochemical blood parameters of broiler chickens.

Materials and Methods

The experiment was carried out on 40 broiler chickens of 'Smena-7' cross from 'Allele-Agro' poultry farm's hatchery in the vivarium of the Kazakh National Agrarian University. As objects of research four groups of one-day broiler chickens were randomly formed by 10 birds in each group. The feeding of broilers was performed according to the instructions on the industrial poultry farm scheme 'Allele-Agro' with commercial basal diet. The chickens were allowed to have free access to feed and water.

The first control group of chickens received a basal diet without treatment. Other chicken groups were daily treated with antibiotics individually in aqueous solution, orally in sub-therapeutic concentrations of antibiotics as growth promoters from the first to day 41. So the second group of broilers was medicated with synthetic antibiotic amoxystin at the dosage 10 mg kg^{-1} of the chicken's weight. The third group of broilers was given tetracycline, and the fourth group – chloramphenicol at the dosage 20 mg kg^{-1} of the chicken's weight. Blood in the amount of 9 cm^3 for haematological and 3 cm^3 for biochemical study was collected from the jugular vein of each chicken on the 42nd day of slaughter.

Study of whole blood, stabilized with lithium-heparin, was performed at the Laboratory of Food Safety in the Kazakh Research Veterinary Institute. The following haematological parameters as leukocytes (lymphocytes, monocytes, granulocytes), erythrocytes, hematocrit, hemoglobin concentration, platelet and trombocrit were determined by the haematology analyzer Melet Schloesing MS4-3 with veterinary dial (France). In the separated serum the total protein, triglycerides, alkaline phosphatase (ALP), cholesterol, glucose, urea, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were established by BioChem FC-360 (US) analyzer at the Digestion Physiology Laboratory in the Human and Animal Physiology Institute. The used laboratory equipment has been annually standardized according to the Kazakhstan state rules.

Basic data entry and handling were done using MS Excel 2007. The significance of difference among the groups was determined by one-way analysis of variance (ANOVA) and t-test. Differences were considered significant at $p<0.01$ levels.

The study was approved by the Local Ethical Committee of the Kazakh National Agrarian University, in accordance with the ethical standards of Principles of Animal Care.

Results and Discussion

Throughout the experiment broiler mortality was not observed. Visual physiological state and behavioral response of broiler chickens of experimental groups did not differ from the control group.

Analysis of haematological parameters showed that under a prolonged exposure to sub-therapeutic concentrations of antibiotics the quantitative and qualitative changes in some blood indices of broilers were observed (Table 1). There was established a marked change in the number of leukocytes towards reduction in the case of amoxystin ($p\leq0.01$) and chloramphenicol ($p\leq0.01$) and an increase in the tetracycline group compared to the control group of broilers. Antibiotics exert a significant impact on leukogram: under the influence of amoxystin and tetracycline ($p\leq0.01$) the relative proportion of granulocytes and lymphocytes increased but monocytes ($p\leq0.01$) decreased. Analogue leukocytosis and limphocytopenia had shown after intramuscular using of therapeutic doses of tetracycline in broiler chickens (Donkova, 2004).

The number of erythrocytes and the amount of hemoglobin were reduced after amoxystin application. In the groups that were given tetracycline ($p\leq0.01$) and chloramphenicol ($p\leq0.01$), an increase of hematocrit

Table 1

Haematological parameters of broilers blood

Parameters		Leukocytes (m/mm^3)	Lymphocytes (%)	Monocytes (%)	Granulocytes (%)	Erythrocytes (m/mm^3)	Hematocrit (%)	Hemoglobin concentration (g/dL)	Platelet (m/mm^3)	Trombocrit (%)
Control, n = 10	M	23.14	41.62	11.49	46.89	2.50	19.98	9.28	573.1	0.53
	SEM	0.34	2.10	0.05	1.06	0.04	0.27	0.09	43.28	0.04
	σ	1.07	6.64	0.17	3.35	0.13	0.87	0.28	136.85	0.14
	C_v	4.61	15.95	1.45	7.15	5.37	4.34	3.00	23.88	25.46
Amoxystin, n = 10	M	18.55*	37.28	8.26*	51.24	2.25	19.34	8.44	475.3	0.40
	SEM	0.87	0.86	0.19	1.64	0.11	0.60	0.27	21.25	0.02
	σ	2.26	2.73	0.61	5.18	0.35	1.90	0.86	67.19	0.06
	C_v	14.88	14.60	7.40	10.11	15.74	9.83	10.18	14.14	15.53
Tetracycline, n = 10	M	24.72	35.71	8.24*	56.05*	2.64	23.52*	9.31	395.3*	0.32*
	SEM	0.55	1.34	0.21	1.57	0.05	0.68	0.19	32.53	0.03
	σ	1.73	4.23	0.67	4.97	0.16	2.15	0.62	102.87	0.10
	C_v	7.00	11.83	8.13	8.87	6.24	9.13	6.61	26.02	32.53
Chloram-phenicol, n = 10	M	19.85*	42.86	11.03	46.15	2.85	23.9*	9.7	432.0*	0.43
	SEM	0.71	0.30	0.33	0.73	0.16	0.54	0.08	10.22	0.01
	σ	2.26	0.95	1.06	2.31	0.51	1.70	0.24	32.31	0.02
	C_v	11.37	2.22	9.60	5.01	17.85	7.08	2.53	7.48	5.18

*Value with common superscript differed significantly from the control group ($p \leq 0.01$).
M – mean, SEM – standard error of sample means, σ – standard deviation, C_v – coefficient of variation.

was observed. A more significant decrease in platelet count and trombocrit was observed in all experimental groups of chickens, than in the control.

These results allow to suggest that a prolonged use of antibiotics in stimulating doses contributed to changes in the blood system and reduced the overall resistance of broiler chickens.

Biochemical study revealed the changes in blood indices of broiler chickens which were given antibiotics (Table 2). An important parameter for the diagnosis of diseases associated with the metabolic disorder is the total protein content. By results of research, it was observed that the amount of total protein in the control group was significantly higher – by 22% – than in the group of broilers which received amoxystin ($p \leq 0.01$), by 16% – in the groups with diet supplemented with tetracycline ($p \leq 0.01$) and chloramphenicol ($p \leq 0.01$). These changes could indicate a protein metabolism disturbance.

Activity of alkaline phosphatase in the control group was higher than 23.9% in comparison with the group where tetracycline ($p \leq 0.01$) was used and by 12.3% than in the group of chloramphenicol.

The level of carbohydrate metabolism was determined by the glucose content. Blood glucose of chickens in the control group was also significantly higher ($p \leq 0.01$) – by 45.8% – than in the group of amoxystin, by 46.5% – of tetracycline and by 51.5% – in the group of chloramphenicol. The same change processes of gluconeogenesis and decreasing of total protein were described when the broilers were treated by tetracycline (Donkova, 2004).

In assessing the activity of transaminases, it was established that the activity of ALT in the blood of chickens which received antibiotics was significantly reduced: by 30.7% in the group treated with amoxystin, by 34.6% in the tetracycline group and by 37% in the group of chloramphenicol ($p \leq 0.01$). The activity of AST in the group that was given amoxystin was inferior to the control by 14.1%, in the group of tetracycline – by 11.1% and chloramphenicol – by 13.6%. Perhaps it was caused by the major toxic effect mechanism of antibiotics which included their potential to cause lipid peroxidation, which is primarily responsible for toxication and tissue damage (Farombi, 2001).

Table 2

Biochemical parameters of broilers blood serum

Parameters		Total protein (g/l)	Triglycerides (mmol/l)	ALP (U/l)	Cholesterol (mmol/l)	Glucose (mmol/l)	Urea (mmol/l)	ALT (U/l)	AST (U/l)
Control, n = 10	M	35	2.49	1057	3.15	15.05	1.26	12.7	245.3
	SEM	0.77	0.01	33.28	0.12	0.78	0.16	1.07	8.94
	σ	2.45	0.02	105.25	0.37	2.47	0.49	3.37	28.26
	C_v	7.00	0.83	9.96	11.73	16.41	39.10	26.52	11.52
Amoxystin, n = 10	M	27.3*	2.50	1134.3	2.97	8.15*	1.15	8.8*	210.8
	SEM	0.95	0.002	55.25	0.05	0.76	0.14	0.70	4.62
	σ	3.02	0.005	174.71	0.15	2.40	0.45	2.20	14.61
	C_v	11.06	0.19	15.40	5.06	29.46	38.94	25.01	6.93
Tetracycline, n = 10	M	29.3*	2.45	804*	2.95	8.05*	0.54*	8.3*	218
	SEM	1.28	0.02	27.43	0.13	1.77	0.07	0.3	2.35
	σ	4.06	0.05	86.74	0.42	5.60	0.23	0.95	7.42
	C_v	13.84	2.00	10.79	14.10	69.56	42.05	11.43	3.41
Chloramphenicol, n = 10	M	29.5*	2.55*	928.5	3.03	7.3*	1.54	8.0*	212.0
	SEM	0.31	0.02	14.33	0.03	0.22	0.02	0.26	1.11
	σ	0.97	0.07	45.33	0.08	0.70	0.05	0.82	3.50
	C_v	3.29	2.64	4.88	2.77	9.50	3.35	10.21	1.65

*Value with common superscript differed significantly from the control group ($p \leq 0.01$).
M – mean, SEM – standard error of sample means, σ – standard deviation, C_v – coefficient of variation.

In our study, the level of triglycerides, cholesterol and urea in the control and experimental groups of broiler chickens did not deviate from indices of the control group chickens.

The analysis of the data show that all tested antibiotics adversely affect the biochemical parameters of blood, which is apparently connected with the negative impact of these xenobiotics on the liver and other internal organs of broiler chickens.

Conclusion

The use of growth stimulating doses of amoxystin, tetracycline and chloramphenicol during feeding of chicken promotes changes in haematological and biochemical parameters of broiler chickens' blood, which causes decrease of immune status and results in the violation of the haematopoietic system and liver function and, ultimately, affects the overall quality of the slaughter poultry products.

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