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Evaluation of the impact of plant extracts in different concentrations on the ecosystem of broilers' intestine

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ABSTRACT

Modern research shows that several plant extracts containing tannins can improve the nutrition and the health status of farm livestock. In view of the fact that there is a certain dependence of the tannin activity on the source, geographical distribution, the assessment of their effects on changing the intestine microbiota is poorly studied. Experimental studies were conducted on 120 heads of 7-day-old broiler chickens (4 groups, n = 30, 4 replications). The control group received the main ration (MR); the first experimental group – MR + extract 1 (1 ml/kg of live weight); the second experimental group – MR + extract 2 (2 ml/kg of live weight); the third experimental group – MR + extract 3 (3 ml/kg of live weight). An extract from Quercus cortex was additionally included in the composition of experimental groups' rations. Sequencing the content of samples from the small and cecal intestine was carried out at the second generation sequenator MiSeq Illumina (the United States). As the concentration of the extract increased, micro-organisms of phylum Bacteroidetes shrunk (I) and then disappear (II, III). The maximum concentration found a decrease of phylum Firmicutes ($p \leq 0.05$) and an increase of Proteobacteria ($p \leq 0.05$). This group also revealed a decline of representatives of class Bacilli ($p \leq 0.05$). In groups I and II, representatives of class Clostridia decreased by 14.6-50.5% in comparison with the control. The ratio of representatives of microorganisms in the intestine depends on the number of tannin-containing substances in the extract, which reveals the need for a more detailed examination of this factor as a source of such substances.

Keywords: *ecosystem, plant extract, microbiota, birds.*

1. INTRODUCTION

Propagation of microbial resistance to the effects of various antibiotics led to the active search for substances liable to weaken or eliminate the effect at breeding and fattening. In this regard, herbal substances and some of their components bear particular interest. Many studies proved that various plant extracts containing tannins can improve the nutrition and the health status of farm livestock including birds [1-4]. Other authors note that the inclusion of tannin-containing compounds in the diet has a positive effect on the quality of broiler's meat [5], improves the health of the intestine and biodiversity of microbiota [6-8], and is effective against several types of intestinal pathogens [9-11]. In view of the fact that there is a certain dependence of the tannin activity on the source, geographical distribution [12], the assessment of their effects on changing the intestine microbiota is poorly studied.

2. MATERIALS AND METHODS

Our research was carried out at the Center for collective use of scientific equipment of the Federal State Scientific Institution "Federal Research Center of Biological Systems and Agro-technologies of the Russian Academy of Sciences" (FSSI FRC BST RAS) on broiler chickens "Smena-8". Experimental studies were conducted on 120 heads of 7-day-old broiler chicks (4 groups, n = 30, 4 replications). The control group received the main ration (MR); the first experimental group – MR + extract 1 (1 ml/kg of live weight); the second experimental group – MR + extract 2 (2 ml/kg of live weight); the third experimental group – MR + extract 3 (3 ml/kg of live weight). An extract from Quercus cortex was additionally included in the composition of the experimental groups' rations. The preparation of the Quercus cortex extract consisted of the following stages: grinding (pharmaceutical form), adding distilled water (1:1), heating in a water bath (30 min), percolation and additional filtration (decalcified filters). The treatment to birds and experimental procedures met the requirements and recommendations of Russian rules (order of the Ministry of the health of the USSR № 755 of 12.08.1977) and "The Guide for the Care and Use of Laboratory Animals" (National Academy Press Washington, D.C. 1996). All efforts were made to minimize animals' suffering and to reduce the number of samples. Decollation of birds under Nembutal ether was held on the 42nd day. Evaluation of microbial biodiversity of the small intestine was carried out on the 42nd day and included: sampling, extraction, purification, measuring concentrations of DNA, carrying out PCR, validation, and normalization of libraries with subsequent sequencing at the platform of the high-performance sequenator of the second generation MiSeq Illumina, the United States. Bioinformatical processing of results was carried out using PEAR software (Pair-End AssembleR PEAR v 0.9.8, April 9, 2015). Statistical processing was accomplished by Statistica 10.0 RU software.

3. RESULTS

The study found that as the concentration of the extract increased, micro-organisms of phylum Bacteroidetes shrunk (I) and then disappear (II, III) in the experimental groups (Tables 1-2).

The maximum concentration found a decrease of phylum *Firmicutes* by 20.6% ($p \leq 0.05$) and an increase of *Proteobacteria* by 19.3-20.3% ($p \leq 0.05$). This group also revealed a decline of representatives of class *Bacillus* ($p \leq 0.05$). In groups I and II, representatives of class *Clostridia* decreased by 14.6-50.5% in comparison with the control.

Earlier studies also demonstrated the positive effect of tannin-containing substances on inhibition of the development of micro-organisms of class Clostridia [13], as well as the great variability in the development of micro-organisms in the small intestine of broilers [14].

Speaking of the cecal intestine, we recorded an increase of phylum *Firmicutes* due to representatives of classes *Clostridia* and *Bacillus* and the absence of representatives of phylum *Bacteroidetes* in the second group. At the same time, we found a high content of representatives of phylum *Bacteroidetes* due to family Rikenellaceae in groups 1 and 3; previous studies [10] showed the opposite effect due to families Ruminococcaceae and Lachnospiraceae.

In addition, [15] observed a higher population of *Lactobacillus* in the cecal intestine of broilers that received extracts from grape. In our case, it was only characterized by the second group.

Table 1. Changes of microbiocenosis in broilers' small intestine, % (n=10).

Group	Taxon			
	phylum	class	family	genus
Control	*Bacteroidetes* (3.19±0.11)	*Bacteroidia* (3.19±0.11)	Rikenellaceae (2.71±0.07)	Alistipes (2.7±0.07)
	Firmicutes (91.7±2.34)	*Bacillus* (27.2±0.56)	*Lactobacillaceae* (26.1±0.54)	*Lactobacillus* (26.1±0.54)
		Clostridia (64±1.66)	*Ruminococcaceae* (19.9±0.61)	*Ruminococcus* (4.13±0.33)
			Clostridiaceae (27.3±0.85)	*Clostridium* (4.5±0.12)
				Faecalibacterium (22.4±0.69)
			Lachnospiraceae (15.2±0.70)	*Blautia* (2.08±0.22)
				Fusicatenibacter (6.4±0.45)
	Proteobacteria (3.03±0.67)	*Gammaproteobacteria* (3.03±0.67)	*Moraxellaceae* (2.55±0.63)	-
Group I	*Bacteroidetes* (2.05±0.40)	-	-	-
	Firmicutes (92.9±3.70)	*Bacillus* (41.0±1.38)	*Lactobacillaceae* (40.6±1.38)	*Lactobacillus* (40.6±1.38)
		Clostridia (49.4±2.20)	*Ruminococcaceae* (19.3±1.01)	*Ruminococcus* (2.95±0.18)
			Clostridiaceae (24.3±0.49)	*Clostridium* (2.87±0.19)
			Lachnospiraceae (5.83±0.68)	*Faecalibacterium* (2.14±0.51)
				Blautia (2.4±0.12)
	Proteobacteria (2.04±0.09)	-	-	-
Group II	*Firmicutes* (95.6±2.50)	*Bacillus* (81.4±1.75)	*Lactobacillaceae* (80.3±1.73)	*Lactobacillus* (78.7±1.72)
		Clostridia (13.5±0.68)	*Ruminococcaceae* (10.2±0.42)	*Subdoligranulum* (5.3±0.37)
			Lachnospiraceae (3.3±0.2)	-
	Actinobacteria (3.9±0.29)	*Actinobacteria* (3.8±0.28)	*Micrococcaceae* (3.8±0.28)	*Rothia* (3.8±0.28)
Group III	*Firmicutes* (72.3±2.60)	*Bacillus* (8.73±0.27)	*Lactobacillaceae* (8.72±0.26)	*Lactobacillus* (7.97±0.24)
		Clostridia (63.6±2.30)	*Ruminococcaceae* (34±1.20)	*Ruminococcus* (15.2±0.66)
				Subdoligranulum (7.74±0.31)
			Oscillospiraceae (3.34±0.18)	*Oscillibacter* (3.34±0.18)
			Clostridiaceae (3.86±0.16)	*Clostridium* (3.86±0.16)
			Lachnospiraceae (22.4±0.53)	*Fusicatenibacter* (2.84±0.20)
	Proteobacteria (22.4±0.42)	*Gammaproteobacteria* (22.2±0.41)	*Enterobacteriaceae* (22.2±0.41)	*Escherichia* (22.1±0.41)

Note: * - microorganisms classified as other not identified taxa were not taken into account

Table 2. Changes of microbiocenosis in broilers' cecal intestine, % (n=10).

Group	Taxon			
	phylum	class	family	genus
Control	*Bacteroidetes* (16.7±0.75)	*Bacteroidia* (16.7±0.75)	Rikenellaceae (14.6±0.61)	Alistipes (14.6±0.61)
			Bacteroidaceae (2.1±0.08)	*Bacteroides* (2.1±0.08)
	Firmicutes (73.8±2.68)	*Bacillus* (2.68±0.13)	*Lactobacillaceae* (2.67±0.12)	*Lactobacillus* (2.67±0.12)
		Clostridia (71.0±2.5)	*Ruminococcaceae* (11.1±0.47)	-
			Clostridiaceae (20.8±0.84)	*Clostridium* (17.5±0.59)
				Faecalibacterium (3.1±0.23)
			Lachnospiraceae (3.88±0.14)	*Blautia* (2.19±0.05)
			Et al.(35.2±0.11)	-
	Proteobacteria (3.22±0.13)	*Deltaproteobacteria* (2.32±0.09)	*Desulfovibrionaceae* (2.32±0.09)	-
Group I	*Bacteroidetes* (66.2±3.20)	*Bacteroidia* (66.2±3.20)	Rikenellaceae (56.2±2.44)	Alistipes (56.2±2.44)
			Bacteroidaceae (9.95±0.56)	*Bacteroides* (9.95±0.56)
	Firmicutes (20.5±0.92)	*Clostridia* (19.9±0.88)	*Ruminococcaceae* (8.85±0.51)	-
			Clostridiaceae (2.85±0.12)	*Faecalibacterium* (2.85±0.12)
			Lachnospiraceae (2.19±0.16)	-
Group	*Actinobacteria* (2.1±0.14)	*Actinobacteria* (2.1±0.14)	*Micrococcaceae* (2.1±0.14)	*Rothia* (2.1±0.14)
	Proteobacteria (2.59±0.36)	*Gammaproteobacteria* (2.59±0.36)	-	-

Group	Taxon			
	phylum	class	family	genus
II	*Firmicutes* (93.7±3.78)	*Clostridia* (75.1±2.69)	*Ruminococcaceae* (24.5±0.87)	*Subdoligranulum* (2.64±0.09)
				Ruminococcus (8.24±0.35)
			Clostridiaceae (22.7±0.94)	*Faecalibacterium* (13.4±0.53)
				Clostridium (7.17±0.28)
				Butyricicoccus (2.17±0.10)
			Lachnospiraceae (24.4±0.76)	*Blautia* (3.77±0.14)
				Coprococcus (2.56±0.21)
				Fusicatenibacter (7.65±0.36)
		Bacillus (18.6±0.77)	*Lactobacillaceae* (17.7±0.72)	*Lactobacillus* (17.7±0.72)
Group III	*Proteobacteria* (2.09±0.24)	*Gammaproteobacteria* (2.01±0.24)	-	-
	Firmicutes (49.9±2.47)	*Clostridia* (46.8±2.41)	*Ruminococcaceae* (18.6±0.83)	*Subdoligranulum* (4.59±0.17)
				Ruminococcus (2.28±0.31)
			Clostridiaceae (20.4±0.98)	*Clostridium* (2.39±0.21)
				Faecalibacterium (17±0.72)
			Lachnospiraceae (2.85±0.18)	-
		Bacillus (2.2±0.27)	*Lactobacillaceae* (2.2±0.27)	*Lactobacillus* (2.09±0.23)
	Bacteroidetes (35.2±1.21)	*Bacteroidia* (35.1±1.21)	*Rikenellaceae (30.7±1.12)*	*Alistipes (30.7±1.12)*
			Bacteroidaceae (4.36±0.08)	*Bacteroides* (4.36±0.08)

Note: * - microorganisms classified as other not identified taxa were not taken into account

4. CONCLUSIONS

Applying the extract from Quercus cortex in rations with various ratios of tannin-containing substances has a significant influence on the microbiota of the small and cecal intestine. The changes affect such phyla as Firmicutes and Bacteroidetes, families Bacilli and Clostridia, thus concerning such body's metabolic processes as the resorption of energy and the degradation of proteins and polysaccharides. The ratio of representatives of microorganisms in the intestine depends on the number of tannin-containing substances in the extract, which reveals the need for a more detailed examination of this factor as a source of such substances.

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