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RESEARCH ARTICLE
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Effect of Kurunga Probiotic on Growth Parameters, the Activity of Intestinal Glycosidases and Peptidases, and Excreta Quality in Quail Conditions

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ABSTRACT

Background: Digestion constitutes one of the most important functions the body.

Aims: This study aimed to evaluate the effect of Kurunga probiotic on growth parameters, the activity of glycosidases and peptidases in small intestinal chyme and mucosa, as well as excreta quality, in the meat-type chicks of Texas White Quail.

Methods: A total of 60 day-old quails were randomly assigned to two groups of 30 birds (15 birds/ cage) from day 1 to day 90. The quails from the experimental group were daily given Kurunga with water (0.01 g per 1 kg of live weight) from day 10 onward after hatching.

Results: Although the body weight and average daily gain of the quails from the control and experimental groups were found to be the same, the addition of the probiotic resulted in lower mortality and increased excreta pH. No significant differences were observed in amylolytic and peptidase activities in the chyme between the control and experimental groups. Maltase and peptidase activities in the intestinal mucosa were found to be 26% higher in the birds from the experimental group. In conclusion, the addition of Kurunga can reduced mortality, higher activity of digestive enzymes in the intestinal mucosa, and alkaline pH shift of excreta.

Conclusion: The addition of Kurunga probiotic when raising quails can promote the growth of normal microflora, an increase in the activity of digestive enzymes in the small intestine.

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INTRODUCTION

Poultry farming constitutes a major economic sector in many countries, including Russia. In particular, quail breeding is an intensively developing and economically viable branch of agriculture, which can provide the population with complete protein (meat and eggs), as well as raw materials to be processed (down, feathers, and excreta). However, the prolonged use of antibiotics can result in drug-resistant bacteria, disturbed normal microflora, and accumulation of antibiotic residues in poultry products (Manafi et al., 2016; Soomro et al., 2019). Therefore, antibiotics have been effectively replaced by probiotics, i. e., live, specially selected strains of microorganisms or specific substances of microbial, plant, or animal origin (Vali, 2009; Sugiharto, 2016; Alagawany et al., 2018; Yadav and Jha, 2019; Popov et al., 2021). Probiotics can increase the number of beneficial microorganisms in the intestines (Vali, 2009; Abou-Kassem et al., 2021), improve feed digestibility and assimilability (Taha et al., 2019;

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Excreta, Intestinal glycosidase and peptidase activities, Growth, Meat-type quail, Probiotic, Technology.

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Soomro et al., 2019), enhance quail growth performance and meat quality (Wang and Gu, 2010; Soomro et al., 2019), as well as contributing to higher productivity (Abou-Kassem et al., 2021). Kurunga is a commercial granular probiotic that is based on a fermented cow's milk mixture containing various microorganisms: lactobacilli and bifidobacteria, acetic and propionic acid microorganisms, lactic streptococci, and milk yeast (Baikal-Biotech LLC, Russia).

Digestion constitutes one of the most important functions providing the body with building and energetic material. Feed digestibility and assimilability largely depend on the activity of digestive enzymes, as well as their activity conditions. The addition of Kurunga to the feed (0.01 g per 1 kg of bird weight) increases the activity of hemoglobinolytic peptidases both in small intestinal chyme and mucosa while stimulating the activity of caseinolytic peptidases only in the chyme, which produces no effect on the gain and survival in young poultry (Skvortsova et al., 2019). Furthermore, the use of Kurunga in the diet (1 g per 1 kg of bird live weight) contributes to the promotion of normal digestive system microflora in the case of diarrheal symptoms, having a positive effect on the production performance of Pharaoh quails (Schmidt, 2012). However, no data are available on the effect of Kurunga on the activity of enzymes providing the initial assimilation stages of protein and carbohydrate feed components in quail.

The paper aimed to study the effect of EM-Kurunga on growth parameters, the activity of intestinal glycosidases and peptidases, as well as excreta quality, in the meat-type chicks of Texas White Quail.

MATERIAL AND METHODS

The experiment was performed on 60 one-day-old meat-type chicks of Texas White Quail (Coturnix coturnix). The birds were weighed individually using a VLKT-500M electronic balance having a resolution of 0.1 g to determine the initial body weight, which equaled 9.2 ± 0.13 g. The quails were randomly divided into 2 (control and experimental) groups, each including two replications of fifteen birds having the same weight. Then every fifteen birds were placed in four battery cage (100 × 60 × 64 cm3) from day 1 to day 90. Each battery cage was equipped with two 40 and 60 W incandescent lamps. These lamps were used to maintain the required temperature and photoperiod: during the first 2 weeks – temperature of 32–37°C and lighting provided 24 h/day; during the next 6 weeks – temperature of 32–37°C and lighting provided 13–16 h/day; from week 8 onward – temperature of 20°C and lighting provided 16 h/day.

All the birds were raised under the same hygienic, environmental, and economic conditions. During the first 3 days, the quails were kept on paper bedding, which was subsequently removed. The chicks were fed 6–8 times a day for the first 5 days, 3–4 times during the next 5 days, and twice a day from day 10 onward at 8 a.m. and 6 p.m. The feed was given according to the following scheme: 4 g on days 1–7, 8 g on days 8–18, 14 g on days 15–21, 16 g on days 22–28, 18 g on days 29–35, and 22 g from day 35 onward per bird. In addition, fresh water was supplied throughout the entire experiment. To this end, vacuum drinkers were used, with the water changed twice a day. Both drinkers and feeders were cleaned daily. From day 10 after hatching, the quails from the experimental group were daily given the Kurunga feed concentrate with water at a dose of 0.01 g per 1 kg of live weight; this dosage was calculated drawing on bird weight at the end of each week.

For 90 days, the quails from both groups were given a well-balanced complete dry fodder produced in the form of fine granules (Solnyshko, Agrovit LLC, Balashikha, Moscow Region). The content of the dust fraction amounts to 0.5% of the total feed weight. The moisture content of the fodder (10.62%) complies with the requirements, with no observed foreign impurities or traces of mold. The feed contains only natural ingredients without artificial growth stimulants, hormones, antibiotics, and GMOs: a multi-component mixture of extruded grains (wheat, corn, barley, soy grist, and sunflower cake), whose proportion is not specified by the manufacturer. The nutritional value per 1 kg of the feed is as follows: metabolic energy (2700 kcal), crude fiber (0.62%), crude fat (4.87%), crude protein (18.81%), amino acid lysine (1.18%), methionine + cystine (0.92%), as well as salt (0.15%), calcium (1.2%), and phosphorus (0.6%).

In order to determine the live weight and live weight gain, each bird was weighed at the ages of 1, 20, 30, 42, 66, and 90 days. Chick mortality was recorded daily, with the total mortality calculated at the end of the 90-day experiment.

At the end of the experiment (on day 90), fresh excreta (0.5 g) were collected from each experimental group using a plastic spatula and promptly sent to the Chance Bio laboratory for clinical studies. This laboratory participates in the EQAS program (External Quality Assurance Services, Bio-Rad, USA, member code 9471) and the Federal System of External Quality Assessment of Clinical Laboratory Testing of the Ministry of Health of the Russian Federation (member code 10705).

For studying the activity of digestive enzymes, 90-day-old quails were euthanized by cervical dislocation. Their intestines were removed to measure their entire length and weight; after that, the small intestine was cut off and placed on the glass of an ice bath. This section of the intestine was selected to be studied since it is responsible for the final steps in the hydrolysis of carbohydrate and protein food components. The intestines were delivered to the IBIW laboratory in a special thermal container equipped with cooling elements (−18 °C) within 2 hours. All further operations were carried out at 1–3 °C. First, the small intestine was cut lengthwise to remove chyme with a plastic spatula. Next, the intestinal mucosa was separated from the muscular layer using a special scraper. Then the samples of chyme and mucosa were weighed employing an analytical balance. Finally, in order to determine enzyme activity, the homogenates of intestinal chyme and mucosa were prepared separately using a glass homogenizer and Ringer's solution (6.5 g NaCl, 0.42 g KCl, 0.25 g CaCl2, pH 7.4) at a mass-volume ratio of 1:9.

The activity of glycosidases was determined as follows. The amylolytic activity reflecting the total activity of starch hydrolyzing enzymes (α-amylase KF 3.2.1.1, glucoamylase KF 3.2.1.3, and maltase EC 3.2.1.20) was estimated according to the increase in hexoses via the modified Nelson method (Ugolev et al., 1969); whereas the maltase activity was assessed via the glucose oxidase method using the Photoglucose clinical biochemistry kit (Impact Ltd., Russia). To this end, the

homogenates were incubated with substrate solutions prepared with the same Ringer's solution. The homogenate and substrate were incubated for 20–30 min at 37°C and pH 7.4 while being continuously stirred. Here, D-glucose (2 mM, chemically pure, Shostka Chemical Reagents) was used as a reference. The enzyme activity was expressed in the micromoles of reaction products formed within 1 min of the incubation of substrate and enzyme-active preparation per 1 g of wet tissue weight, μmol/(g×min). The amylolytic activity was measured using a Lambda 25 UV/VIS spectrophotometer (PerkinElmer, United States) at a wavelength of 670 nm, whereas the maltase activity was measured at 505 nm. The enzyme activity at each point was determined in 3 replications taking into account the amount of glucose in the initial homogenate. The kinetic characteristics of maltose hydrolysis – apparent Michaelis constant (Km) and maximum reaction rate (V) – were determined using the Lineweaver-Burk method, i.e., by constructing a double reciprocal plot showing the dependence of enzyme activity on the substrate concentration for each replication.

The activity of peptidases (primarily trypsin, EC 3.4.21.4) was assessed according to the increase in tyrosine concentration via the modified Anson method using the Folin-Ciocâlteu reagent (Kuz'mina et al., 2021). One percent casein solution prepared with the above-mentioned Ringer's solution was used as the substrate. The homogenates and substrates were incubated at 37ºC for 30 min while being continuously stirred. The color intensity of the samples was measured using a Lambda 25 UV/VIS spectrophotometer (PerkinElmer, United States) at a wavelength of 670 nm. The level of enzymatic activity was estimated according to the increase in reaction products formed within 1 min of the incubation of the substrate and enzyme-active preparation per 1 g of wet tissue mass, μmol/(g×min). The enzyme activity at each point was determined in 3 replications, taking into account the amount of tyrosine in the initial homogenate.

RESULTS

The initial weight of day-old chicks reached 9.12 ± 0.13 g both in the experimental and control groups (Table 1). Over the course of three months, it gradually increased, exceeding the initial value by about 4 times on day 30, by 12 times on day 66, and by 18 times on day 90 of the experiment. However, differences in the live weight of quails from the control and experimental groups were statistically insignificant throughout the observation period (P > 0.05). The average daily gain of the birds also did not differ significantly between groups, amounting to 1.26–3.03 g/day in the control group and 1.60–3.17 g/day in the experimental group in the second and third months. The mortality of chicks from the experimental group receiving probiotic supplementation (0.01 g per 1 kg of body weight) was significantly lower on day 90 of the experiment, reaching 46.7% as compared to 61.5% in the control group (P < 0.05).

By the end of the experiment, the length (57.3±3.4 cm) and weight (3.6±0.4 g) of the entire intestine in quails from the experimental group receiving probiotic were similar to those (65.2±3.9 cm and 3.8±0.5 g) of the quails from the control group (P > 0.05). Furthermore, the amylolytic activity both in the small intestinal chyme and mucosa of the quail from the control and experimental groups exhibited no significant statistical difference, P > 0.05 (Table 2). However, the analysis of variance revealed a higher effect of the probiotic on amylolytic activity in chyme than in mucosa: 17.6 and 0.1%, respectively.

In the chicks from the studied groups, the peptidase activity in the chyme was also similar (P > 0.05); however, in the intestinal mucosa, it was 26% higher in the birds receiving the probiotic as compared to the control group, with a 7 times higher factor

Table 1: Dynamics of live weight and gain in quail chicks, g

Age, days	*Parameter*	*Control group*	*Experimental group*
1	Live weight	9.2±0.12	9.2±0.13
20	Live weight Average daily gain	20.9±1.1 0.88	22.8±1.00 1.14
30	Live weight Average daily gain	32.5±2.1 0.80	37.2±0.19 1.20
42	Live weight Average daily gain	53.5±3.3 1.26	58.7±4.4 1.60
66	Live weight Average daily gain	113.4±4.9 3.18	116.4±7.6 3.17
90	Live weight Average daily gain	166.0±12.2 1.75	168.4±6.8 2.38

Table 2: Effect of EM-Kurunga on the activity of glycosidases and peptidases in the small intestinal chyme and mucosa of quails

Parameters	*Variants*	*Control*	*Probiotic, 0.01 g/kg*	*Factor contribution, %*
Amylolytic activity, μmol/(g×min)	Chyme Intestinal mucosa	113.0±2.54 351.1±45.53	143.2±32.55 343.9±57.84	17.6 0.1
Activity of peptidases, μmol/(g×min)	Chyme Intestinal mucosa	6.78±0.12 4.50±0.30	6.16±1.30 5.68±0.39*	5.4 37.0*
Maltase activity, μmol/(g×min)	Intestinal mucosa	166.9±7.65	210.1±9.92**	54.2**
Km of maltase, μmol/l	Intestinal mucosa	12.90±1.63	18.75±1.13*	46.6*
Vmax of maltase, μmol/(g×min)	Intestinal mucosa	176.2±27.51	271.9±9.29**	52.1**

* – P < 0.05; ** – P < 0.01

contribution ($P < 0.05$). The activity of membrane maltase in the intestinal mucosa of the quails from the experimental group also exceeded that of the quails from the control group by 26% ($P < 0.01$), with a factor contribution of 54.2%. In the quails receiving the probiotic, the determined kinetic characteristics of maltase revealed 54% higher Vmax values ($P < 0.01$), whereas Km values were found to be greater by 45% ($P < 0.05$). Such changes in Vmax are consistent with the variations in maltase activity, while higher Km values reflect the decrease in the affinity of the enzyme for the substrate in the birds that were given Kurunga with water for three months.

The mechanisms underlying the effect of Kurunga on the activity of intestinal enzymes require special consideration. It is possible to promote the activity of maltase and peptidases in mucosa both by increasing the enzyme synthesis rate and by synthesizing enzymes having new properties. However, a direct effect of probiotic components on intestinal enzymes or their indirect effect associated with changed activity conditions cannot be ruled out. Therefore, in order to reach a definitive conclusion about the effect of Kurunga on the growth performance of quail and digestive enzyme activity, it is necessary to conduct additional studies using several concentrations of the probiotic, as well as introducing it into the diet of quail in different ways.

A clinical analysis of excreta collected from 90-day-old quails revealed no significant differences in the following parameters between the control and experimental groups: brown color, strong smell, loose consistency, as well as the presence of stercobilin, mucus, and indigestible plant fibers. The quail excreta from both the control and experimental groups exhibited no extracellular and intracellular starch, fats, fatty acids, soaps, cellular elements, unchanged bilirubin, blood, connective-tissue fibers, as well as half-digested and undigested muscle fibers. Only a decrease in excreta acidity in the experimental quail group (pH 7.5) was noted as compared to the control group (pH 6.5), $P<0.05$. The identified pH range of 6.5–7.5 is favorable for promoting normal microflora in the large intestine and cecum of birds, which may prevent the development of pathogenic and undesirable bacteria (Sugiharto, 2016; Premavalli et al., 2018), thus protecting the birds from intestinal diseases.

DISCUSSION

The obtained data are consistent with the study into the effect of Kurunga on live weight gain and mortality of Hisex white egg cross chickens (Skvortsova et al., 2019). These findings indicate that while the body weight of quails in the experimental group remained similar to that in the control group, the probiotic supplementation had a positive impact on poultry health, reducing mortality. Similar results were obtained when a mixture of Protexin probiotics was introduced into the diet of meat-type Japanese quail (Coturnix coturnix japonica) at a dose of 0.1 g per 1 kg of feed (Soomro et al., 2019). The introduction of a probiotic mixture combining aerobic (Bacillus toyonensis) and anaerobic (Bifidobacterium bifidum) bacterial strains into the diet of Japanese quails at a dose of 5×108 and 6×108 CFU per 1 ml of the diet, respectively, led to a weight increase in 21- and 42-day-old chicks (Abou-Kassem et al., 2021). No significant differences were observed in mortality between the control and experimental groups. It was found that probiotic supplementation of bird feed increases the number of beneficial microorganisms in the digestive tract (Taha et al., 2019), improves intestinal health by positively affecting its morphological and physiological characteristics (Coskun et al., 2017), which results in better digestion and assimilation of feed components (Nalle et al., 2021). In some cases, it contributes to weight gain and higher meat quality (Premavalli et al., 2018; De Abou-Kassem et al., 2021), whereas in others, it improves poultry health without significantly affecting carcass parameters (Soomro et al., 2019).

Pancreatic enzymes (α-amylase, as well as trypsin entering the intestine in its inactive form as trypsinogen to be then activated by enterokinase) occur in the intestinal cavity as part of chyme, as well as being adsorbed on the epithelium of intestinal mucosa. However, since maltase activity is closely related to the brush border of enterocytes, it is usually not observed in the chyme. Higher digestive enzyme activity accelerates the initial assimilation of the main food components, as well as contributing to their faster digestion. Exogenous enzymes are widely used in poultry farming to enhance growth, product quality, and feed efficiency (Acamovic, 2001; Sugiharto, 2016; Yuan et al., 2017; Alagawany et al., 2018; Jasek et al., 2018; Cho et al., 2020, Giacobbo et al., 2021; Liu et al., 2021; Nalle et al., 2021). Primarily, this is associated with an insufficient number of enzymes in poultry intestines, specifically hydrolases involved in the breakdown of complex carbohydrates. The introduction of exogenous enzymes (non-starch polysaccharide enzyme and acid protease) in the feed of broiler chickens (Ross-308) on days 1 to 42 after hatching, in fact, has a significant effect on growth performance and digestive function, as well as the synthesis and secretion of endogenous pancreatic enzymes. (Yuan et al., 2017). Exoenzyme supplementation increases average daily gain, average daily feed intake, and the apparent digestibility of crude protein. The activity of pancreatic amylase and lipase is found to be significantly lower in birds from the control group. Changes in the activity and mRNA expression level of pancreatic trypsin are dependent on the amount of exogenous enzymes (Yuan et al., 2017). With the addition of α-amylase and ß-xylanase to corn-soy diets, improved growth performance and starch digestibility in the jejunum and ileum of broiler chickens are observed, with their effectiveness being independent of present microbial phytase (Yuan et al., 2017). The introduction of proteases into the diet of broiler chickens enhances the activity of pancreatic enzymes (Yuan et al., 2017), positively affecting the immune system of chicks (Sugiharto and Ranjitka, 2019). Ross 308 broiler chickens fed a multiprotease-supplemented amino acid-deficient diet (150 or 300 g per ton) for 35 days after hatching exhibit compensated and/or improved growth performance commensurate with the increased digestibility in the ileum (Cho et al., 2020). The introduction of multicarbohydrase containing α-galactosidase and xylanase into the diet increases the assimilability and digestibility of amino acids (except for crude protein) in the ileum of broiler chickens (Jasek et al., 2018). The results of introducing xylanase, amylase, and protease (alone or in combination) into the corn feed of male broiler chickens (Ross 308) suggests a synergistic effect between exogenous enzymes on broiler productivity and nutrient assimilability (Amerah et al., 2017). Due to the addition of enzymes, a relative

improvement in energy digestibility is greater in the ileum than that measured in the excreta. The addition of amylase, xylanase, and protease to the feed of broiler chickens has a positive impact on intestinal morphology, changes the diversity and composition of the cecal microbiota, as well as resulting in increased productivity and nutrient assimilability on day 21 of the experiment (Giacobbo et al., 2021). With the introduction of the combined preparation of α-galactosidase and xylanase in the diet of Arbor Acres broiler chickens on day 42, an increase in the assimilability of nutrients and amino acids in the ileum, a trend toward the higher activity of endogenous enzymes (amylase, trypsin, and sucrases), and a statistically significant increase in maltase activity (on day 21) in duodenal chyme are observed, as well as a decrease in chyme viscosity in the jejunum (Liu et al., 2021). Bacillus coagulans NJ0516 probiotic added to the commercial basal diets of Arbor Acres broilers stimulates amylase and protease activity without affecting the duodenal lipase activity (Wang and Gu, 2010).

The activity of caseinolytic and hemoglobinolytic peptidases in the chyme and mucosa of both the small and large intestines, as well as the live weight of meat-type broiler chickens of Hisex white egg cross, increase with the addition of dry sapropel in the amount of 3% of the daily feed weight (Kuz'mina et al., 2021). Furthermore, the authors identified higher peptidase activity in the large intestinal mucosa as compared to the small intestinal mucosa, considering this as an adaptation that contributes to better protein digestion in animals having relatively short intestines. On the other hand, it is assumed that pancreatic juice enzymes have no time to be adsorbed on the brush border of the anterior intestine due to increased intestinal peristalsis (Kuz'mina et al., 2021). It is worth noting that the digestive tract of animals exhibits two different types of bacterial populations, with the first freely occurring in the intestinal lumen and the second existing in close association with the intestinal epithelium (Vali, 2009). In addition, the quantitative and qualitative microbiota composition varies in different parts of the intestine (Yadav and Jha, 2019; Popov et al., 2021). Since the activity of intestinal mucosa enzymes reflects both the activity of host enzymes and intestinal microbiota, the relatively high activity of peptidases in the large intestine may likely be attributable to the significant contribution of microflora enzymes to the total enzyme activity. Moreover, feed additives such as fermented soy or cottonseed meal can affect the activity of digestive enzymes (amylase, protease, trypsin, and lipase) in the broiler intestine, as well as causing changes in the structure of the intestinal microbiome (Pan and Yu, 2014). In all likelihood, these diets stimulate certain bacteria, such as Bifidobacterium and Lactobacillus, which can promote digestive enzyme activity while inhibiting other bacteria (e.g., Escherichia coli) that can either suppress digestive enzyme secretion by damaging mucosal microvilli or secrete a proteolytic enzyme to break down digestive enzymes (Pan and Yu, 2014).

The addition of Kurunga probiotic to water (0.01 g per 1 kg of quail live weight) for 90 days after hatching does not significantly affect the weight of birds while reducing mortality. Lower excreta acidity observed in the quails from the experimental group can promote the growth of normal microflora in the large intestine, which along with an increase in the activity of digestive enzymes in the small intestine can have a positive effect on the processing and subsequent assimilation of protein hydrolysis products and feed disaccharides. Therefore, when raising quails, the addition of Kurunga to water can be recommended to achieve reduced mortality, higher activity of digestive enzymes in the intestinal mucosa, and alkaline pH shift of excreta.

Confirmation

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