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Bobot dan Panjang Relatif Organ Limfoid Itik Magelang Jantan Yang Mendaptkan Penambahan Eksktrak Kulit Jengkol dan *Bacillus amyloliquefaciens* DALAM AIR MINUM

(*Relative Weight and Length Lymphoid Organs of Male Magelang Ducks Using Jengkol Peel Extract and Bacillus amyloliquefaciens in Drinking Water*)

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ABSTRAK

Penelitian ini bertujuan untuk mengetahui bobot dan panjang relatif organ limfoid itik Magelang jantan yang mendapatkan fitobiotik dari ekstrak kulit jengkol dan probiotik berbasis *Bacillus amyloliquefaciens*. Materi yang digunakan dalam penelitian ini adalah Itik Magelang jantan sebanyak 100 ekor, ekstrak kulit jengkol, isolat *Bacillus amyloliquefaciens*, dan pakan komersial itik. Rancangan percobaan yang digunakan adalah Rancangan Acak Lengkap (RAL) dengan 5 perlakuan dan 4 kali ulangan. Perlakuan yang diterapkan adalah perbedaan persentase ekstrak kulit jengkol + *Bacillus amyloliquefaciens* dengan dosis P0: (tanpa penambahan perlakuan); P1 (0,05% + 0,5%); P2 (0,1% + 1%); P3 (0,15% + 1,5%); dan P4(0,2% +2%) pada air minum. Data dianalisis menggunakan analisis sidik ragam (ANOVA) pada taraf 5%. Data yang berbeda nyata diuji lanjut menggunakan Duncan's Multiple Range Test (DMRT). Variabel yang diamati meliputi bobot dan panjang relatif timus, bursa fabrisius, dan limpa. Hasil penelitian menunjukan bahwa penambahan ekstrak kulit jengkol dan *Bacillus amyloliquefaciens* dengan dosis yang berbeda tidak berpengaruh terhadap bobot dan panjang relatif timus, bursa fabrisius, dan limpa itik Magelang jantan.

Kata kunci: *Bacillus amyloliquefaciens*; ekstrak kulit jengkol; itik magelang jantan; organ limfoid

ABSTRACT

This study aimed to determine the relative weight and length of the lymphoid organs of male Magelang ducks that received phytobiotics from jengkol peel extract and probiotics based on *Bacillus amyloliquefaciens*. The materials used in this study were 100 male Magelang ducks, jengkol peel extract, *Bacillus amyloliquefaciens* isolates, and commercial duck feed. The experimental design was completely randomized (CRD) with five treatments and four replications. The treatment applied was the difference in the percentage of jengkol peel extract + *Bacillus amyloliquefaciens* at a dose of P0: (without additional treatment); P1 (0.05% + 0.5%); P2 (0.1% + 1%); P3 (0.15% + 1.5%); and P4(0.2% +2%) in drinking water. Data were analyzed using analysis of variance (ANOVA) at the 5% level. Significantly different data were further tested using Duncan's Multiple Range Test (DMRT). The variables observed included the relative weight and length of thymus, bursa of Fabricius, and spleen. The results showed that the addition of jengkol peel extract and *Bacillus amyloliquefaciens* at different doses did not affect the relative length and weight of thymus, bursa Fabricius, and spleen of male Magelang ducks.

Keywords: *Bacillus amyloliquefaciens*, jengkol peel extract, lymphoid organs, male Magelang ducks

INTRODUCTION

Magelang ducks are native Indonesian ducks that must be cultivated and preserved to become Indonesia's local genetic resources. Based on the Decree of the Indonesia Minister of Agriculture No.701/Kpts/PD.410/2/2013, Magelang ducks are native Indonesian ducks. Magelang ducks are a local fowl developed by the people of Central Java, especially in the Magelang area and its surroundings. The advantages of pofMagelang ducks are a high adaptation to the environment in the lowlands and highlands and increased meat production and eggs (Setiyawan *et al*., 2015). Magelang ducks have characteristics, namely the color of brown fur with white wing tips, a white ring on the neck 1-2 cm thick, shaped like a necklace, a slender body, a small head, and short legs. Magelang male ducks have tail feathers sticking up (Supriyadi, 2009).

Duck productivity is influenced by several factors, including the condition of the duck's body resistance. Lymphoid organs play a role in maintaining the immune system, including the thymus, bursa Fabricius, and spleen, which are associated with lymphocytes. If the weight of the lymphoid organs decreases, the antibodies produced by lymphocytes become lower (Kusnadi, 2009). Jamilah and Mahfudz (2013) stated that the development of body resistance in the early phase greatly influences the next step. Immunosuppression is possible if the body's resistance in the initial stage does not develop, resulting in stunted growth and even death. Suitable lymphoid organs will work optimally so that the livestock's immunity will be better and the livestock will be healthy. The digestive process in healthy livestock will take place optimally so that nutrient absorption will take place optimally, which will increase livestock productivity. One of the ingredients to promote the growth of poultry livestock to achieve high productivity has long been and is widely used, namely in the form of antibiotics or antibiotic growth promoters (AGP). However, in practice, AGP is not used as it should be. Giving AGP in high doses poses a risk of accumulating residues in livestock and humans. AGP residues can damage the balance of the digestive tract bacterial ecosystem by killing good bacteria. Pathogenic bacteria will also experience resistance if AGP is used for a long time. Research on the use of probiotics and phytobiotics is reported to be able to improve poultry productivity without causing harmful residues. Probiotics and phytobiotics, separately or in combination, are used as natural alternative ingredients to replace AGP, which does not produce residues and impacts poultry and humans. Alternative natural antibiotic innovations are expected to be able to improve growth performance which can increase poultry productivity without causing harm to livestock and humans.

The use of antibiotics as AGP in feed has been banned by most countries in the world. According to the World Organization for Animal Health (OIE), about 51% of OIE member countries have banned the use of antibiotics as growth promoters, about 19% have partially banned them, and only 30% of OIE member countries have not banned the use of antibiotics as growth promoters at all. promoter (Sinurat *et al*., 2017). As of May 2017, in Indonesia, the addition of antibiotics as feed additives has been prohibited and regulated in the Regulation of the Minister of Agriculture of the Republic of Indonesia Number 14 of 2017 in article 16, paragraph 2. Prohibition of the use of drugs in livestock consumed by humans, which have the potential to harm human health as regulated in the PERMENTAN No. 14 of 2017 article 15 (Widyanjaya and Jayawardhita, 2021). The use of antibiotics as feed additives cannot be stopped entirely because it is feared that it will impact the livestock industry. Namely, there will be an increase in mortality in livestock and a decrease in feed efficiency if antibiotics are not replaced by alternative feed additives that are safer or natural. One potential alternative that has begun to be widely researched is the use of feed additives derived from plants as a source of phytogenic compounds (Widyanjaya and Jayawardhita, 2021).

As a tropical country, Indonesia has diverse biological and natural resources. This diversity is very beneficial, especially with the many species of plants that can be used medicinally. Sari (2010) states that the parts of herbal plants that can be used as medicines: are roots, tubers, stems, leaves, shoots, flowers, and fruit. Herbal plants can be used as medicines directly without being processed or processed first. One of the plants that can be taken advantage of is the jengkol plant. Jengkol is one of the plants used by Indonesian people as a traditional medicine because it contains antibacterial compounds (Salni *et al*., 2011). One part of the jengkol plant that can be used as a phytobiotic is the peel. So far, jengkol peel is only considered agricultural waste that has yet to be fully utilized (Pandia and Warman, 2016). The jengkol peel is the outermost part of the jengkol, which is brown and coats the flesh. Jengkol peel can be used as a phytobiotic because jengkol peel contains various phytogenic compounds or compounds.

According to Grashorn (2010), an alternative to safe antibiotics is to use plant products, also known as phytobiotics. According to Hidayat (2015), phytobiotics are natural feed additives derived from plants in the form of herbs with active ingredients that can be used as antibacterials. They can prevent disease, heal, and boost the immune system (Septiana, 2014). Phytobiotics can substitute artificial antibiotics, increasing livestock growth, health, and productivity (Sulistyoningsih, 2014). Phytobiotics in poultry can increase growth and feed

efficiency, improve intestinal histomorphology, and function as antimicrobials, thereby increasing the immune system of poultry (Hosseini *et al*., 2016). Phytobiotics are used as feed additives as a substitute for antibiotics because they contain active substances that have been proven to trigger body weight gain and productivity and affect livestock health (Widowati, 2007). The content of active substances from phytobiotics can be helpful as antioxidants that help the body's immune performance, such as bursa fabricius, spleen, and thymus. Physiologically, the older the livestock, the performance of the lymphoid organs will decrease, and their size will decrease. The livestock will experience a decrease in productivity, so to maintain and maintain the performance of the lymphoid organs, it is necessary to intake natural antioxidants from outside the livestock body to trigger the livestock's body resistance so that the performance of the lymphoid organs increases. lymphoid and livestock productivity did not decrease drastically. Indicators of body resistance can be supported by the presence of lymphoid organs such as the bursa of Fabricius, spleen, and thymus (Aughey and Frye, 2001).

Another alternative that can be used as a feed additive is probiotics. Giving probiotics does not have a negative effect because they are natural antibiotics derived from local microbes (Dhama *et al*., 2011). Probiotic microbes have a mechanism that increases livestock productivity by acting on the digestive tract. The workings of probiotic microbes are colonizing the digestive tract, further suppressing pathogenic bacteria growth. Live microbes that are widely used as probiotics are the Bacillus group, one of which is *Bacillus amyloliquefaciens* (Zurmiati *et al*., 2017). These bacterial species include lactic acid bacteria (LAB), which have the performance to suppress the growth of pathogenic bacteria from their production. Probiotics can be given by mixing with feed or drinking water (Riswandi *et al*., 2012).

The use of probiotics and phytobiotics can drive increased productivity of poultry. Providing these alternative materials does not harm livestock and humans, such as residues that can cause resistance to certain diseases (Arifin and Pramono, 2014). The probiotic *Bacillus amyloliquefaciens* can influence the improvement of the microbial ecosystem of the digestive tract to maximize the absorption of feed nutrients, thereby increasing poultry productivity. In addition, the content of active compounds in the form of tannins, saponins, and flavonoids found in jengkol peel can inhibit the performance of pathogenic bacteria. Both of these feed additives have a positive impact on poultry productivity, namely increasing the efficiency of feed and drink consumption, increase in body weight and body immunity, and reduce livestock

mortality, as well as reduce the value of feed conversion. Based on this description, research on probiotic *Bacillus amyloliquefaciens* and phytobiotic jengkol peel extract through drinking water needs to be carried out to determine their effect on productivity seen from the lymphoid organs of male Magelang ducks.

MATERIAL AND METHOD

Time and Place

Research activities have been conducted for 90 days, from July to September 2022. Research activities consist of preparation, feed testing, rearing, and harvesting. The trial consisted of making probiotics and jengkol peel extract, carried out at the Animal Husbandry and Fisheries Technology Laboratory, Faculty of Agriculture, Tidar University. Feed proximate analysis testing activities were conducted at the INTP Laboratory (Nutrition Science and Feed Technology), Bogor Agricultural University. Duck rearing is in the Yogyakarta Agricultural Development Polytechnic poultry house in Magelang, Tejosari Village, Jalan Magelang-Kopeng, Ngablak District.

Materials

The equipment used in the study included: digital scales, a blender, a measuring cup, a filter, a centrifuge, a centrifuge tube, Erlenmeyer, cotton, sterile glass, laminar airflow (LAF), autoclave, tissue, petri dish, bunsen, ose needles, magnetic stirrer, gas compost, basin, funnel, thermohygrometer, refrigerator, individual cages 100 × 100 × 50 cm of 20 plots filled with five ducks in each field, feeders, drinking bowls, hanging scales, research note forms, and stationery. The materials used included: jengkol peel, distilled water, *Bacillus amylolyquefaciens* bacteria, nutrient agar (NA), granulated sugar, fish meal, commercial feed for broiler ducks, and 100 male Magelang ducks aged two months.

Phytobiotic Preparation

The method for making probiotic growth media refers to the method of Anwar et al. (2017). The phytobiotics used in this study were made from jengkol peel extract. Preparing jengkol peel extract consists of several steps: drying, grinding, and mixing with distilled water. Before drying, the jengkol peel is cut into small pieces to maximize it during the drying process. Jengkol peel is included in a paper envelope that has been weighed using an analytical balance. The envelope paper and jengkol peel were considered using a digital scale, and the results were recorded. Jengkol peels drying process using an oven at 60^{o}C for three consecutive days until it reaches a constant weight. Dried jengkol peel is mashed using a blender until smooth. They mixed the soft jengkol peel with distilled water using a ratio of 1:10, namely 100 grams of jengkol peel plus 1000 ml of distilled water and blended until homogeneous. The jengkol peel solution is

filtered using a filter cloth and put into a centrifuge tube.

Multiply Bacteria

The bacterial culture medium was 10 g of nutrient agar (NA) dissolved in an Erlenmeyer containing 500 ml of distilled water. The media is homogenized and heated using a magnetic stirrer. The Erlemeyer hole is closed using cotton, coated with gauze, and then sterilized in an autoclave at 121^{o}C with a pressure of 1 atm for 15 minutes. All tools used for bacterial propagation are fixed using an autoclave with a temperature of 121^{o}C with a force of 1 atm for 15 minutes. Petri dishes and needle loops to be resolved are wrapped in paper and plastic. All equipment used for bacterial inoculation is put into a laminar airflow (LAFairflowas been sterilized by spraying alcohol. The NA solution that has been sterile is poured into a petri dish until it runs out and is allowed to harden a little. Transfer bacterial culture from isolate tube by scratching (Streak Plate Method). The ose needle is heated over the bunsen and cooled, and held in the right hand while the left hand has the petri dish. The cooled ose is used to scratch the surface of the agar media in a petri dish. Before opening, the mouth of the petri dish is heated with Bunsen as an under the etching process. As much as one ose of bacteria is taken from the pure culture in the isolate tube and streaked (zigzag) on the surface of the agar media starting at one end. The method of etching is on the surface of the agar media. The loop is ignited and cooled for each stroke in another quadrant. The petri dish is then wrapped using plastic and paper to minimize contamination. Identity is given to the packaged petri dish in the form of the name of the bacteria, the date of manufacture, and the group's name. The petri dish was then incubated in an incubator for 24 hours at 39^{o}C upside down, after which growth was observed.

Making Probiotic Growth Media

The method for making probiotic growth media refers to the method of Manin et al. (2007). The growth of probiotics requires media in the form of fish meal solution. The ingredients used to make fish meal solution are fish meal and granulated sugar with a ratio of 1:1. Fifty grams of granulated sugar mixed with 50 grams of fish meal in 1 liter of distilled water. The three ingredients are mixed until homogeneous or no precipitate forms. The solution was then heated and stirred to a temperature of ±60^{o}C. After that, the solution was filtered using a filter cloth to separate it from the fish meal residue. The fish meal solution, after being filtered, is put into a glass bottle and covered using cotton wrapped in gauze, then put into the autoclave to be sterilized at 121^{o}C with a pressure of 1 atm for 15 minutes. The glass bottles were cooled in the LAF for 15 minutes. After cooling, it is stored in the refrigerator, or it can be inoculated with *Bacillus amyloliquefaciens*.

Preparation of *Bacillus amyloliquefaciens* inoculum

The fish meal solution, after being cooled, is then given a pure culture of *Bacillus amyloliquefaciens* bacteria. The way to mix it is that as much as 10 ml of fish paste solution is put into a petri dish that contains pure cultures of *Bacillus amyloliquefaciens*. The Petri dish is shaken or scratched slowly to separate from the media and put into a 1,000 liter Erlenmeyer flask containing a fish meal solution. After that, incubation was carried out in an incubator for 1218 hours at 38-40^{o}C.

Cage Preparation

Making the cage starts with measurements to determine the need for raw materials. The cage to be used is 36 m in size. Before installing the partition, the cage is washed and cleaned. In addition, all equipment, such as feed bowls, drinking bowls, and mats, is washed clean. The cage partitions are made based on measurements. Each partition measuring 100 × 100 × 50 cm is 20 partitions. After installing the partitions, lime, and husks were sprinkled on the cage floor for each section. The following process is the cage biosecurity stage, spraying disinfectants to avoid dirt and insects—sprayed disinfectant by covering the entire cage area using plastic. The disinfectant comes from the Destan brand, as much as 60 ml mixed with 10 liters of water. Cages are sterilized for three days to destroy bacteria and insects.

Feeding

The feed is commercial for broiler ducks > 22 days old. The results of the proximate analysis of feed carried out at the INTP IPB Laboratory are presented in Table 1. The feeding of livestock is adjusted to the nutritional needs of the ducks in the grower phase. Feeding during the rearing process refers to Sinurat (2000). The amount of feed can change according to environmental conditions affecting livestock consumption needs. Data on the amount of feeding is presented in Table 2.

Table 1. Feed proximate analysis

Kandungan Pakan	Besaran	Nilai
Water content	%	9,94
Ash	%	7,35
Crude Fat	%	8,77
Crude Protein	%	18,365
Crude Fiber	%	5,995
Metanolizable Energy (ME)	Cal/g	3121,6

Table 2. Amount of feed given

Phase	Age	FeedPakan (g/bird/dayi)
Grower	8-9 weeks	130
	9-15 weeks	145
	15-18 weeks	150

Drinking

Drinking water is an essential need besides feed. Consumption of drinking significantly affects the consumption of animal feed, especially poultry. The provision of drinking water for this study was a mixture of water with probiotic and phytobiotic solutions. Drinking water is provided ad libitum.

Data Collection

Lymphoid organ data collection was done by duck dissection. The surgery was carried out after the ducks were reared for 30 days. Before surgery, the feed and drinking containers were removed from the cage. All ducks in each cage plot were weighed for body weight and then sorted from the largest to the slightest body weight data in each cage plot. Ducks with average body weight (mean) were selected for dissection. Slaughter of ducks is carried out using the halal method by cutting three canals in the neck, namely the respiratory tract (trachea), blood vessels (carotid arteries and jugular veins), and food channels (oesophagus) in the necks of slaughtered animals (Musa *et al*., 2022). Ducks were dissected from the neck to the cloaca. All parts of the lymphoid organs were removed from the duck's body. Each lymphoid organ is cleaned with running water, measured, weighed, and recorded.

Data Design and Analysis

The study was conducted using a completely randomized design (CRD) of 5 drinking water treatments, each with four replications, so there were 20 experimental research units. Each repetition consisted of 5 ducks. The treatment in this study was P0: control treatment without the addition of jengkol peel extract and probiotics, P1: treatment with the addition of 0.05% jengkol peel extract and 0.5% probiotics, P2: treatment with the addition of 0.1% jengkol peel extract and 1% probiotics, P3: treatment with the addition of 0.15% jengkol peel extract and 1.5% probiotics, and P4: treatment with the addition of 0.2% jengkol peel extract and 2% probiotics. The data obtained from this study were analyzed using variance (ANOVA) with a significance level of 5%. If any data obtained differed between treatments, a further Duncan's Multiple Range Test (DMRT) test was performed.

RESULTS AND DISCUSSION

The research results showed (Table 3) that the addition of phytobiotics from jengkol peel extract and probiotic *Bacillus amyloliquefaciens* given to drinking water had no significant effect (P>0.05) on the weight and relative length of the thymus, bursa fabrisius, and spleen of male Magelang ducks. This is presumably because the tannin content in the jengkol peel extract is not considered a foreign body by the animal's body. This statement by Ermawati (2019) states that the weight of lymphoid organs, in general, can be affected by infectious agents, nutrient content, age, and ambient temperature. The feed provides nutrients for the growth and development of primary (bursa fabricius and thymus) and secondary (spleen) lymphoid organs (Ullah *et al*., 2012). Aldiyanti et al. (2022) added that one factor that affects the weight gain and size of the lymphoid organs is heat stress or the stressful conditions of livestock. Widiyanti et al. (2019) stated that lymphoid

organs are related to the H/L ratio because lymphoid organs, especially the spleen, take antigens from the blood that bind to lymphocytes. If the spleen's size increases, it accommodates more antigens, resulting in free blood lymphocytes, and the H/L ratio increases. According to Zulfa et al. (2019), the addition of weight and relative length of lymphoid organs occurs when cattle are exposed to heat stress and infection with foreign bodies. Merryana et al. (2007) added that enlargement of the lymphoid organs occurs when cattle are infected with bacteria because one of the functions of the lymphoid organs, especially the spleen, is to play a role in the body's immune mechanism as a producer of lymphocytes. Bacterial infections or diseases in poultry cause the size of the lymphoid organs to enlarge because they have to produce large quantities of lymphocytes to defend the body (Tizard, 1988). However, in this study, the livestock did not experience heat stress and stress, so the performance of the spleen was not disturbed, which resulted in no effect on spleen weight.

The administration of phytobiotics from jengkol peel extract and the probiotic *Bacillus amyloliquefaciens,* which was given, had no significant effect, also presumably due to the non-infection of livestock by pathogenic bacteria. This statement is supported by Zulfa et al. (2019) stated that changes in the weight and size of lymphoid organs are possible due to bacterial infection. The infection spreads to the primary lymphoid organs. Its main target is to destroy B lymphocyte cells and some T lymphocytes so that the lymphoid organs work hard to produce antibodies for the body's resistance.

Table 3. Relative weight and length lymphoid organs of male Magelang ducks using jengkol peel extract and *Bacillus amyloliquefaciens* in drinking water

Variables		Treatments[1]				
		P0	P1	P2	P3	P4
Weight	Thymus[ns]	7.35±0.122	7.15±0,104	7.58±0.206	7.28±0.084	7.18±0.087
	Bursa Fabricius[ns]	1.25±0.013	1.24±0.010	1.26±0.036	1.25±0.037	1.27±0.028
	Spleen[ns]	1.54±0.016	1.52±0.014	1.53±0.012	1.53±0.011	1.52±0.008
Length	Thymus[ns]	8.47±0.013	8.38±0.166	8.35±0.179	8.47±0.025	8.50±0.120
	Bursa Fabricius[ns]	3.39±0.036	3.50±0.195	3.55±0.144	3.50±0.122	3.35±0.206
	Spleen[ns]	0.16±0.028	0.16±0.028	0.17±0.040	0.16±0.085	0.16±0.025

[1]P0: control treatment without the addition of jengkol peel extract and probiotics, P1: treatment with the addition of 0.05% jengkol peel extract and 0.5% probiotics, P2: treatment with the addition of 0.1% jengkol peel extract and 1% probiotics, P3: treatment with the addition of 0.15% jengkol peel extract and 1.5% probiotics, and P4: treatment with the addition of 0.2% jengkol peel extract and 2% probiotics

[ns]Non-significantly different

This causes the follicles (in the bursa of Fabricius) to experience depletion, which affects the weight and size of the lymphoid organs to shrink. Jamin (2012) states that lymphoid organs that work hard to form antibodies for the body's resistance over time will experience depletion and shrinkage of lymphoid follicles so that the weight of the lymphoid organs, especially the fabric bursa, decreases. Rokhmana et al. (2013) added that the more often the lymphoid organs form antibodies, the depletion and shrinkage of lymphoid follicles will be followed by a decrease in the number of lymphocytes so that the antibodies produced are lower.

The administration of phytobiotics from jengkol peel extract and the probiotic *Bacillus amyloliquefaciens* given had no significant effect. It can also be suspected because of flavonoids from the given phytobiotics, which can function as antioxidants and boost immunity. This statement is supported by Rokhmana et al. (2013), who that the role of phytobiotics containing flavonoids can act as antioxidants. Harmanto (2004) added that flavonoids function as antioxidants, boost the immune system, improve blood circulation throughout the body and prevent blockages in blood vessels. Apriliyani et al. (2013) also added that in terms of the weight and size of the bursa of Fabricius that did not change, it was thought to be caused by the bursa Fabricius not working hyperactively in producing antibodies because the role of antibodies was assisted by the active substances contained in the given phytobiotics. Kusnadi (2009) added that the size and weight of the bursa fabricius are relatively constant but will increase with increasing absolute weight or the age of livestock, so livestock is resistant to disease and other conditions such as heat stress. The presence of stress can reduce the importance of the bursa fabricius and thymus because it is influenced by the hormone corticosterone (Kusnadi, 2009), resulting in the depletion or shrinkage of lymphoid follicles.

CONCLUSION

Based on the research that has been done, it can be concluded that the administration of a combination of phytobiotics from jengkol peel extract with probiotic *Bacillus amyloliquefaciens* is safe for consumption because it does not affect the weight and length of the lymphoid organs of male Magelang ducks.

RECOMMENDATION

Further research is suggested to increase the number of ducks used and the length of maintenance; it is necessary to measure the content of bioactive substances in drinking water and test the effectiveness of probiotics and phytobiotics before being given.

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