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Response of broiler internal organs to administering *Rhizopus oryzae* and *Lactobacillus casei* based probiotics

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Abstract This study identified the effect of giving probiotics based on *Rhyzopus oryzae* and *Lactobacillus casei* on the internal organs of broilers. The internal organs of broiler parameters were heart weight, liver weight, abdominal fat weight, gizzard weight, intestine length and broiler pancreas weight. The *R. oryzae* used in the research isolated from tempeh yeast and *L. casei* isolated from yakult (fermented milk). The results found significant differences ($p<0.05$) in the weight of heart, liver, abdominal fat, intestine length, and pancreas weight of broilers and there was no significant difference ($p>0.05$) in the weight of broiler gizzards between administration of probiotics and without giving probiotics. The broiler heart weight was increased by administering probiotics was found to reach 10.15g, liver reach 31.32g, pancreas reach 3.96g, decreased abdominal fat reach 28.89g, and increased intestinal length reached 192.48 cm. The findings indicated that giving probiotics based on *R. oryzae* and *L. casei* are provided the positive benefits for the internal organs of broilers.

Keyword: Broiler, Internal organs, Probiotics

Introduction

Broiler productivity experiences various stresses due to intensive production pressures in livestock systems, which adversely affects their performance. Broiler livestock are generally kept in intensive production systems, exposed to various infections, which reduce broiler production performance (Nilawati and Gustian, 2023). According to Kabir (2009), disease prevention and increased broiler productivity in recent years have led to an increase in the use of antibiotics on a large scale in poultry farming. The usefulness of antibiotics themselves has been questioned, because their negative effects include antibiotic resistance in pathogenic bacteria and are dangerous to human health (Kabir, 2009). The use of antibiotics in broilers today has almost been banned throughout the world, including Asia and European Union has also

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banned the use of chemical antibiotics because of their bad effects on human health in the long term (Immarseel *et al.* (2004). The declining performance of broiler production and the challenge of the dangers of using AGP in broiler rations are ongoing problems questioned in circles livestock community. How keep broiler production optimal and there is no danger be focused the consequences for livestock and consumers have been research by many researchers in various parts of the world (Nilawati and Gustian, 2023).

Probiotics have been proven for their potential and benefits in improving broiler performance and have been recommended as a replacement to using growth promoter (AGP) antibiotics in broiler (Jha *et al*., 2020). Growth promoter (AGP) antibiotics play a role in reducing the production and excretion of catabolic media in intestinal inflammatory cells, and in the long term have an impact on reducing the microflora population in the broiler intestine (Kabir, 2009). Probiotics themselves work in the opposite way to antibiotics, namely by increasing growth by modulating the broiler's intestinal environment and increasing the population of beneficial intestinal microbes, suppressing pathogenic microbes, and increasing broiler immunity (Shanmugam, 2022).

Probiotics are live microorganisms originating from bacteria or fungi that are given through broiler feed or drinking water and have benefits in maintaining the balance of microflora in the broiler intestine (Hrnčár *et al*., 2018). Probiotics play a role in preventing the negative effects of microbes which have the potential to become pathogenic and dangerous for broilers, so that broilers can utilize the nutrients from feed more optimally (Dibner and Richards, 2005). Hill *et al.* (2014) explained that the aim of giving probiotics to broiler chickens is to increase broiler immunity (health) and improve broiler performance.

Probiotic microorganisms that can be used for broilers include *Basil, Streptococus, Aspergillus, Bifidobacterium, Enterococcus, Lactobacillus, Saccharomyces,* and *Candida* species which have been reported to have positive benefits for broiler performance and health (Ashayerizadeh *et al.,* 2009), modulating gut microbes and inhibiting pathogens, and improve the microbiological quality of poultry meat (Kabir *et al.,* 2009). Probiotic microorganisms work in broilers by maintaining normal intestinal microbes by suppressing pathogenic microbes, suppressing intestinal pH, playing a role in mucosal adhesion and nutrition, stimulating the intestinal immune system, producing bacteriocins and increasing production of short chain fatty acid (SCFA) (Sarangi *et al*., 2016).

Several researchers have found that *R. oryzae* is suitable for use as a probiotic and has positive benefits for broiler chickens (Yudiarti *et al*., 2014; Hamza and Gunyar, 2022; Sugiharto *et al.,* 2015). Some researchers have also

evaluated the antioxidant activity of *R. oryzae* and found *R. oryzae* is a type of filamentous fungus that has quite large antioxidant activity (Yadav *et al.,* 2013; Schmidt *et al.,* 2014). Then *L. casei* used as a probiotic in broiler chicken rearing to improve the performance and immunity of broiler chickens (Pertiwi *et al.,* 2019). *L. casei* able to inhibit various types of pathogenic bacteria such as *Salmonella* sp*., Vibrio* sp*., Shigella* sp*., Staphylococcus* sp., and *E. coli* (Sunaryanto *et al.,* 2014). Based on this, researchers were investigated *R. oryzae* and *L. casei* as probiotics in broilers and observed how they responded to the broiler's internal organs.

Materials and methods

This research was carried out at the UPT Farm of Payakumbuh State Agricultural Polytechnic, Limapuluh kota Regency, West Sumatra Province, Indonesia in January-February 2023. This research used 200 DOC broilers that were given commercial feed and probiotics, the DOC broilers were kept for 28 days and then observed and collected research data.

Probiotics

The used microorganisms in the research are *R. oryzae* from tempeh yeast and *L. casei* from Yakult. *R. Oryzae* in tempe yeast has been identified and reported by previous researchers (Bintari *et al*., 2017; Rizal *et al.,* 2020) and also *L. casei* in yakult (Almeida *et al.*, 2012; Douillard *et al.*, 2013 ; Lin *et al.,* 2017; Hu *et al.,* 2019). The probiotics were prepared by mixing all of ingredients (based on treatment) in a bucket until evenly mixed for 2 minutes, then the ingredients were placed in a jerrycan, which was sealed tightly and stored in a place out of direct sunlight for seven days. During these seven days, the jerrycan was closed and opened every two days to let the gas out and then immediately closed tightly again. On the 8th day, the probiotic was ready to use. Twenty milliliters of one liter of drinking water were provided for all treatments.

Experimental design

The research was carried out experimentally using Completely Randomized Design (CRD). The treatment A was control,without giving probiotics, treatment B was Probiotics with 9 liters of clean water, 1 liter of old coconut water, 0.25 kg brown sugar (dissolved in 1 liter of water), 10 grams of tempeh yeast, and 100 ml of yakult milk, treatment C was Probiotics: 9 liters of clean water, 1 liter of old coconut water, 0.5 kg brown sugar (dissolved in 1

liter of water), 20 grams of tempeh yeast, and 150 ml of yakult milk, and treatment D was Probiotics: 9 liters of clean water, 1 liter of old coconut water, 1 kg of brown sugar (dissolved in 1 liter of water), 30 gr of tempeh yeast, and 200 ml of yakult milk. Each treatment was carried out 5 times, and each replication consisted of 10 broilers. The variables observed included heart weight, abdominal fat weight, intestine length, gizzard weight, liver weight, and broiler pancreas weight.

Data collection

Data collection was carried out after the broilers were 28 days old. Broilers are slaughtered and their internal organs are removed. The weight of each internal organ is then weighed (heart, liver, abdominal fat, gizzard, and pancreas) and its length (intestine) is measured.

Statistical analisys

The data obtained in the form of heart weight, liver weight, abdominal fat weight, gizzard weight, intestine length and pancreas weight were processed using the IBM SPSS v.25 application with analysis of variance, if a significant difference was found at the 5% level, wast carried out with Duncan Multiple Range Test (DMRT).

Results

Effect of administration of based probiotics *Rhyzopus oryzae* and *Lactobacillus casei* on the internal organs of broilers can be seen in Table 1. There were significant differences ($p<0.05$) in the weight of heart, liver, abdominal fat, intestine length, and broiler pancreas weight between those given probiotics and those without probiotics. There was no significant difference ($p>0.05$) in the weight of gizzard broilers.

Table 1. Average weight of broiler internal organs

No	Parameter	A	B	C	D
1	Heart (gr)	8.97^{a}	9.59^{ab}	10.15^{b}	9.83^{b}
2	Liver (gr)	29.43^{a}	30.59^{ab}	31.32^{b}	30.41^{ab}
3	Abdominal fat (gr)	32.03^{a}	29.89^{ab}	28.89^{b}	30.31^{ab}
4	Gizzard (gr)	29.82	32.45	27.66	29.76
5	Intestinal length (cm)	178.22^{a}	182.54^{a}	192.48^{b}	176.88^{a}
6	Pancreas (gr)	3.03^{a}	3.33^{a}	3.96^{b}	3.37^{a}

ABCD-Treatment on research. SEM-Standard error of the mean. a.cMeans within rows with different superscript letters differ significantly at $p<0.05$

Discussion

Giving probiotics was found to have a significant effect ($p<0.05$) on the heart, liver, abdominal fat, intestinal length and pancreas of broilers. In line with previous researchers who also found the same thing that giving probiotics had an effect on the heart and liver (Enyenihi *et al.,* 2023; Seifi *et al.,* 2017), abdominal fat (Capcarova *et al.,* 2010), intestinal length, and broiler pancreas (Enyenihi *et al.,* 2023; Pedroso *et al.,* 2003). The absence of a significant effect on gizzard weight is also in line with the findings of (Shabani *et al.,* 2012; Sojoudi *et al.,* 2012) that giving probiotics did not affect the weight of gizzard broilers. Overall research findings, researchers confirmed the positive effects of administering probiotics on the internal organs of broilers.

The role of probiotics in increasing the weight of broiler internal organs is associated with the role of probiotics in improving the performance of digestive organs and broiler immunity. Shanmugam (2022) said that the effect of probiotics is clearly visible in increasing organ weight. The role of probiotics in broiler digestion has been described previously by Prosekova *et al.* (2021) that intestinal structure reached its maximum value in birds receiving probiotic supplements. This increases epithelial surface absorption and parietal digestion in broiler digestive organs. The presence of a large number of mucus-secreting goblet cells ensures better movement of feed through the intestines (Prosekova *et al.,* 2021). In this case, probiotic microorganisms contribute to better development of the villous layer and crypts and mucous membranes in general (Prosekova *et al.,* 2021). This improves intestinal performance in digesting nutrients from feed due to better development of the villi and crypt layers. Alagawany *et al.* (2018) explained that the size of the absorption surface and the increase in metabolic processes in epithelial cells in various parts of the small intestine are thought to be related to the height of the villi and crypts.

Probiotic microorganisms play a role in increasing broiler immunity by inhibiting the development of pathogenic bacteria in broilers. Probiotic microorganisms play a role in inhibiting the expression of pathogenic bacteria, so that these bacteria cannot colonize and threaten the health of broilers (Cheng *et al.,* 2014). The mechanism of pathogen inhibition involves activation of the immune system, inhibiting pathogenic microbes from obtaining available nutrients, and antimicrobial activity of probiotics by secreting compounds that inhibit or suppress pathogens for intestinal epithelial adhesion receptors (Yang *et al.,* 2009). Probiotics can play a role in regulating broiler immunity mechanisms in intestinal cells to induce the secretion of antimicrobial peptides with the aim of controlling pathogens and reducing negative effects on broiler

health (Cazorla *et al.,* 2018). The peptide produced destroys pathogenic bacteria by forming pores in the membrane (Diamond *et al.,* 2009), so this compound has a vital role in the immune response of broilers by recruiting neutrophils to the site of infection.

Lactobacillus casei species is a micro-aerophilic gram-positive bacterium that can maintain the natural balance in the broiler intestine while maintaining microbial stability in the broiler intestine (Chen *et al*., 2017). This type of microorganism can also play a role in reducing the fat content in the chicken's body. This was also conveyed by Fesseha *et al*., (2021) that *Lactobacillus* reduces fat content in the body and blood and increase poultry efficiency. Role *Lactobacillus casei* in increasing broiler immunity has also been explained, that Lactobacillus administered orally interacts with the intestinal epithelium and regulates the gene expression of various cytokines and thereby activates broiler immunity (Li *et al.,* 2014), *Lactobacillus* also plays a role in improving intestinal immunology, where this has a positive impact on intestinal stability (Durand and Durand, 2010).

Rhyzopus oryzae is also a type of microorganism that has positive benefits for broiler digestion and immunity. Several previous researchers also confirmed that *Rhyzopus oryzae* has probiotic properties that are beneficial for broilers (Hamza and Gunyar, 2022; Sugiharto *et al.,* 2015; Yudiarti *et al.,* 2014). *Rhizopus oryzae* are proteolytic and lipolytic which can produce extracellular enzymes such as amylase, protease, and lipase (Pratiwi *et al.,* 2014), where this extracellular enzyme plays a role in increasing the digestibility and absorption of feed in the intestine. *Rhizopus oryzae* was also found to be able to balance the microbial population in the intestine, thus having a positive impact on the development of broiler viscera (Sugiharto, 2016). *Rhyzopus oryzae* also has antioxidant activity that can increase broiler immunity, which makes this type of fungus very good for use as a probiotic for broilers (Schmidt *et al.,* 2014).

The positive role of probiotics in general and the role of probiotic microorganisms (*Lactobacillus casei* and *Rhyzopus oryzae*) has positive benefits on the digestive performance and immunity of broilers so that it can increase the weight of internal organs (increase heart weight, increase liver weight, reduce abdominal fat, increase intestinal length, and increase pancreas weight) of broilers.

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