



Dietary Feed Additive Microencapsulation Synbiotic on Physical Quality of Broiler Chicken Meat

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ABSTRACT

This research aimed to investigate the microencapsulation of synbiotics that combine probiotics, *Bacillus subtilis*, with prebiotic MOS for poultry diet to influence the physical quality of poultry meat. The material used in this study was 100 one-day-old unsexed broilers Cobb 500. The birds were randomly divided into the following 4 experimental groups, with five replications: The treatments were (T1) basal diet (control) without microencapsulation Synbiotic, T2, T3, and T4 supplementation basal diet with 0.25, 0.50, and 0.75% of microencapsulation synbiotics of *Bacillus subtilis* and MOS, respectively. The variables were physical qualities of poultry meat, including acidity (pH), cooking losses, water holding capacity (WHC), and hardness. The basal diet was formulated to meet completely the nutrient requirements broilers based on NRC (1994) recommendations. The dietary treatments were based on corn grain-soybean meal and balanced with other nutrients. The data were analyzed with one-way ANOVA in a Completely Randomized Design (CRD) using the Statistical Package for Social Science or IBM SPSS Statistics version 26, significant differences among the means were determined by using Duncan's multiple range test at $P < 0.05$. The results showed that the treatment groups did not have a significant effect ($P \text{ value} > 0.05$) on the physical qualities of broiler meat, which are acidity (pH), cooking losses, water holding capacity (WHC), and tenderness. To conclude, supplementation of microencapsulation synbiotic has no negative effects on the physical qualities of the meat reflected in the value of all variables, which are in the normal range of the physical quality of broiler meat.

Introduction

The poultry industry has strengthened its position in the agri-food industry in many countries. The manufacture and consumption of poultry meat has been increasing five times more, which is due to the growing world population, and has become a challenge for the animal production sector worldwide. Forecasts indicate that the size of the human population in 2050 will increase by around 9.3 billion. It means that agricultural production and consumption will be 60% more than today (Krysiak *et al.*, 2021; Capan and Bagdatli, 2022). Tremendous improvement in poultry production has been achieved with enhanced genetic selection, health status, nutrition, and the use of antibiotics as a growth promoter (Al-Shawi *et al.*, 2020).

Antibiotic Growth Promoter (AGP) has been used in poultry production, mainly functioning as a growth promoter and enhancing efficiency. AGP has antimicrobial activity against different targets of microbial pathogens, such as the cell wall, cell membrane, protein synthesis, and DNA replication (Suresh *et al.*, 2018). The overuse of AGP for a long time can trigger the growth of pathogen bacteria resistant to drugs used to cure illnesses caused by infections and high potential for transferring resistance to humans (Al-Khalaifah, 2018; Baéza *et al.*, 2022). AGP can increase foodborne allergies and has negative impacts on the environment (Al-Shawi *et al.*, 2020). Therefore, using AGP was banned to create safe poultry products. In Indonesia, AGP for poultry has been banned through the Indonesian government regulation by the Ministry of

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Agriculture, Republic of Indonesia (PERMENTAN) with a number 14/PERMENTAN/PK.350/5/2017 (Ningsih *et al.*, 2019). One such alternative to replacing antibiotics as growth promoters is the supplementation of synbiotics to the poultry diet.

Synbiotics are additives that combine probiotics and prebiotics. Probiotics are a live microbial feed supplement, while prebiotics are nondigestible carbohydrates that stimulate the growth of the host in the digestive system (Sobotik *et al.*, 2021; Song *et al.*, 2022). Dev *et al.* (2020) reported that feed supplementation with a synbiotic of *Lactobacillus acidophilus* (LBA) 10^6 CFU/g and 0.2% mannan-oligosaccharides (MOS) enhanced body weight gain (BWG) 1.9 Kg, compared to control 1.6 Kg, and feed efficiency of broiler chickens 1.61, than control 1.86. The combination of probiotics and prebiotics has a greater effect on broiler chickens than their individual use, as the probiotics' effects can be optimized with prebiotics as a nutrient source, therefore, can improve their protection against pH, oxygen, and temperature. The most common probiotics used are *Bacillus subtilis*, which can improve intestinal barrier integrity, mucosal immunity, digestive enzyme activities, nutrient metabolism, and promote the microbial balance in the digestive tract of poultry (Ningsih *et al.*, 2023; Al-Baadani *et al.*, 2018). Wang *et al.* (2021) explained that using *Bacillus subtilis* feed additive for broiler chickens during the entire growth phase (days 1-63) significantly reduced feed intake to 83.07 g/day, compared to 86.48 g/day in the control group, improved feed efficiency to 2.42, compared to control group 2.55, and decreased mortality rates among the broilers to 4.55%, then control 8.75%. As for prebiotics, the most popular one for poultry is mannan-oligosaccharide (MOS), which can inhibit the colonization of enteric pathogens, enhance immunity, modify microflora fermentation to favor nutrient availability for the host, enhance the brush border mucin barrier, reduce enterocyte turnover rate, and improve the integrity of the gut lining (Dev *et al.*, 2020). The addition of *B. subtilis* DSM 32315 on broiler feed with necrotic enteritis (NE) challenge resulted in a reduction of NE lesions of 0.79, compared to a control group of 1.37, the coccidial index decreased to 5.75, the control group index of 6.85, while the enteritis index decreasing to 28.9 compared to the control group index of 33.0 (Sokale *et al.*, 2019). To protect and improve synbiotics beneficial effects on the poultry digestive tract, microencapsulation technology has been commonly used as one of the effective ways. Microencapsulated synbiotics, which consist of *Enterococcus faecium* (1×10^8 cfu/g), *Lactobacillus plantarum* (1×10^8 cfu/g), *Bacillus subtilis* (1×10^9 cfu/g),

250 U/g β -mannose, and 250 mg/g fructo-oligosaccharide, significantly impacted the average daily gain (ADG) of broilers aged 1-21 days. The treated group showed a higher ADG of 34.0 g/day, compared to 31.7 g/day in the control group. Additionally, the treated broilers exhibited increased *Lactobacillus* counts in the cecum, measuring 7.66 log 10 cfu/g, compared to 6.73 log 10 cfu/g in the control group (Wang *et al.*, 2018).

Encapsulation is a technique to encapsulate or mix a substance into capsules by coating it with another substance or system (Wang *et al.*, 2018). While microencapsulation is used to protect the bioactive component of the microcapsule from adverse effects (moisture, temperature, light, air, etc.) of the current environment, to prevent evaporation loss, to improve stability during handling, transport, and to maintain nutrient content and viability of the microorganism. Microencapsulation has a size of 0.2 to 5 μ m (Öztürk and Temiz, 2018). The effect of various synbiotics supplementation on meat quality in animals, especially poultry, has been reported, but using technology microencapsulation synbiotics for a poultry diet on the physical quality of meat has limited publication. Therefore, this research aimed to determine the effects of the microencapsulation of synbiotics that combine probiotics *Bacillus subtilis* with prebiotic MOS in poultry diet on the physical qualities of poultry meat, including water content, acidity (pH), cooking losses, water holding capacity (WHC), and tenderness.

Materials and Methods

The material used in this study was 100 one-day-old unsexed broilers Cobb 500 (Poultry Breeding Company, PT. Panca Patriot Prima). All the birds were randomly assigned to the following 4 experimental groups, with five replications: (T1) basal diet (control) without microencapsulation of Synbiotic, T2, T3, and T4 supplementation basal diet with 0.25, 0.50, and 0.75% of microencapsulation synbiotics of *Bacillus subtilis* and MOS, respectively. All chickens were inoculated based on the vaccination program of Broilers were weighed and randomly distributed among 20 pens, with five birds for every pen, and were raised for 35 days. The variables were physical quality of poultry meat, which include acidity (pH), cooking losses, water holding capacity (WHC), and hardness. The basal diet (Table 1) was formulated to meet completely the nutrient requirements of broilers based on NRC (1994) recommendations. The dietary treatments were based on corn grain-soybean meal and balanced with other nutrients.

Table 1. Basal dietary treatment

Feed Ingredient	Proportion (%)
Corn	55.70
Rice bran	8.00
Soybean meal	31.00
Meat bone meal (MBM)	1.50
Palm oil	1.50
CaCO ₃	1.50
Vitamin Premix	0.3
L-Lysine HCl	0.10
DL-Methionine	0.20
NaCl	0.20
Total	100
Nutrient Content	
Metabolism Energy (KCal)	2917.47
Crude protein (%)	20.44
Crude fiber (%)	3.28
Crude fat (%)	4.88
Calcium (%)	0.76
Phosphorus (%)	0.49

Synbiotic preparation

The first method was the rejuvenation of *Bacillus subtilis* probiotics carried out by streaking 1 needle loop in a zigzag manner on a sterile test tube containing MRS agar slant, then the tube was closed, and incubated at a temperature of 37-39°C for 24 hours, and then a stock culture was obtained. Then, Bacterial Suspensions by dissolving A total of 1 dose of probiotic *Bacillus subtilis* from the stock culture and inoculating in 10 mL of MRS broth then incubating it at 37 °C for 24 hours, and stored in a shaker incubator. Next, the bacterial culture was transferred into 100 mL MRS broth and incubated again for 24 hours at 37°C.

The prebiotic used is the BIO-MOS that comes from the wall cell yeast type *Saccharomyces cerevisiae* and contains Mannan oligosaccharides (MOS) with the composition of at least 30% crude protein, a minimum of 1.4% crude fat, and a maximum of 13% crude fiber. The materials used to make microencapsulation synbiotics were 50 mL of *Bacillus subtilis* suspension, 50 mg BIO-MOS, 2 g of chitosan, 50 mL of acetic acid, and 4% sodium tripolyphosphate. The first stage is making a sodium tripolyphosphate solution by dissolving 4 g of powder in 100 mL of distilled water, then stirring until homogeneous and sterilizing using an autoclave at 121 °C for 15 minutes. Next, make a chitosan solution by dissolving 2 g of chitosan powder with 100 mL of 1% acetic acid, adding STPP and mg of BIO-MOS, then sterilizing using an autoclave at 121°C for 15 minutes, and adding *Bacillus subtilis* bacteria suspension.

Sample collection

The pH value in poultry meat was measured using a portable pH meter (HI9125, HANNA Instruments, Italy), which was calibrated by the 2-point method against standard buffer solutions with pH values of 4.0 and 7.0. Approximately 5 g of the sample was ground and then homogenized with 50 mL of distilled water for 1 minute.

Water holding capacity using 0.3 g of meat placed on 2 glass plates covered with filter paper, pressed with 35 kg of load for 5 minutes, and the forming wet area was calculated, and the total water content of the sample was drained in an oven for 8-24 hours at 105 °C. The formula was $\text{mgH}_2\text{O} = (\text{wet area width (cm}^2\text{)} \times 0.0948) - 8.0$; $\text{free water content} = (\text{mgH}_2\text{O} \times 100\%) / \text{weight}$; $\text{total water content} = \{(\text{sample weight} + \text{pre-baked filter paper}) - (\text{sample weight} + \text{post-baked filter paper})\} / (\text{pre-baked sample} + \text{filter paper weight})$; $\text{percentage of water holding capacity} = \text{total water content} - \text{free water content}$. Cooking loss was determined using a sample of 10 g of meat wrapped in a polyethylene hollow plastic bag tied tightly and placed into a water bath for 30 min at 80 °C. Cooking-loss formula = $\{(\text{pre-cooked weight} - \text{cooked weight}) / \text{pre-cooked weight}\} \times 100\%$ (Soeparno, 2005). Tenderness of meat was measured by the texture analyzer TMS-Pro (Food Technology Corporation is a company registered in Virginia, USA).

Data analysis

The data were analyzed with one-way ANOVA in a Completely Randomized Design (CRD) using the Statistical Package for Social Science (SPSS software) with IBM SPSS Statistics 26 (SPSS GmbH, Munich, Germany). Significant differences among the means

were determined by using Duncan's multiple range test (DMRT) at $P < 0.05$.

Results

The birds in dietary treatments with supplementation of microencapsulation synbiotic

from probiotic *Bacillus subtilis* and prebiotic MOS did not have a significant effect ($P > 0.05$) on the physical quality of broiler meat, which are acidity (pH), cooking losses, water holding capacity (WHC), and tenderness. The results are shown in Table 2.

Table 2. The physical quality of broiler met with supplementation microencapsulation synbiotic

Parameter	Treatment				P Value
	T0	T1	T2	T3	
Ph	6.06 ± 0.2	6.07 ± 0.09	6.04 ± 0.13	5.98 ± 0.13	0.763 ^{ns}
CL (%)	31.28 ± 3.28	29.28 ± 2.21	28.64 ± 2.75	28.95 ± 2.83	0.459 ^{ns}
WHC (%)	74.91 ± 1.91	76.11 ± 1.6	77.99 ± 1.68	78.11 ± 4.36	0.206 ^{ns}
Hardness (N)	203.49 ± 54.70	156.73 ± 21.58	172.78 ± 39.67	199.2 ± 40.96	0.261 ^{ns}

Note:

ns = Non-significant

pH = Potential Hydrogen

CL = Cooking Loss

WHC = Water Holding Capacity

T0 = Basal diet (control) without microencapsulation of Synbiotic

T2 = Basal diet with supplementation of 0.25% of microencapsulation synbiotics

T3 = Basal diet with supplementation of 0.50% of microencapsulation synbiotics

T4 = Basal diet with supplementation of 0.75% of microencapsulation synbiotics

Discussion

The supplementation of microencapsulation synbiotics composed of *B. subtilis* and MOS did not significantly affect all the physical qualities of broiler chicken ($P > 0.05$). In this study, the pH value was around 5.98 to 6.07. This result is in the normal pH range based on (Negari et al., 2015), stating normal pH for broiler chicken meat was around 6.0. An abnormal decline in pH while the carcass temperature remains high can lead to dark, firm, dry meat and pale, soft, and exudative (PSE) meat defects. The pH value of postmortem broiler meat was influenced by two factors, intrinsic and extrinsic factors; the intrinsic factors were species, type of muscle, and variant of fat, and the extrinsic factors were environmental temperature, treatment of additives, and stress before slaughter. The pH value of poultry meat is also affected by postmortem glycolysis may cause the development of PSE. There are implicit interrelationships between temperature and pH because glycolysis is exothermic, and the effects of pH are severe when a carcass is still near body temperature. Meat with a low pH appears pale. It scatters more light back to the observer than meat with a high pH, which appears dark because it transmits more light into its depth than meat with a low pH (Tashla et al., 2019). In this case, the meat probably exudes water along with water-soluble proteins such as myoglobin, thereby producing a product that has a pale, unsavory appearance. In recent years, researchers have explored the potential

use of probiotics to improve meat color stability and pH. The relationship between probiotic supplementation and postmortem pH decline remains unclear (Al-Shawi et al., 2020).

Water holding capacity (WHC) of meat is influenced by the changes in muscle cellular and extracellular components. The presence of oxygen could also contribute to lipid and protein oxidation, which may cause structural damage to the meat and thus increase water loss after longer storage duration (Aziz et al., 2020). The WHC value from this research is in the normal range of 74.91 to 78.11. The WHC results are higher than the result of Sari and Patriani (2021), who uses supplementation of phytobiotic *Garcinia* extract that had a WHC value of around 66.8-71.4% with an average of 69.30%. WHC was also influenced by pH value, while pH decreased, H⁺ ions increased so the ions were bound to oxygen on water molecules, therefore increasing water holding capacity (Jayasena et al., 2013).

The cooking loss (CL) value determines the weight lost during cooking; the higher the cooking temperature and/or the longer the cooking time, the greater the loss of liquid until it reaches a constant level. Cooking loss is one indicator of nutritional value related to the level of meat juice, and the amount of air that is connected in and between muscle fibers (Sari and Patriani, 2021). The results of the CL value did not differ significantly with supplementation microencapsulation synbiotic *Bacillus subtilis* and MOS in the diet. The value of CL

in this research was around 28.64 to 31.28. The value is in the normal CL range of meat, between 1.5-54.5% with a range of 15-40%. A cooking loss value is affected by some factors, which are Water Holding Capacity (WHC), pH, myofibril contraction status, length of muscle fiber sarcomere, and size of meat. The main factor is meat protein, which can bind water.

Hardness is the maximum peak on the first pressure or the first bite. The units used are kg, g, or N. Meat hardness decreases as a function of the slaughter-deboning interval (Indiarto et al., 2012). The hardness value of broiler meat in this research was also not significant after supplemented with synbiotics consisting of *Bacillus subtilis* and MOS. The hardness value of broiler meat from this research was around 156,73 to 203,49. The higher of hardness value the higher the pressure that is needed, and the quality of meat decreases.

Conclusions

The physical quality of broiler meat includes pH value, cooking loss, water holding capacity, and hardness, was no significant difference with supplementation microencapsulation synbiotic from probiotic *Bacillus subtilis* and prebiotic Mannan oligosaccharides, but the value of all variables is in the normal value of physical quality of broiler meat.

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