

Effects of Encapsulated Okra (*Abelmoschus Esculentus* L.) on Intestinal Morphology, Microbiological, and Physicochemical Properties of Meat in Broiler Chicken

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Abstract.

*This study investigates the impact of encapsulated okra (*Abelmoschus esculentus* L.) using an amorphous maltodextrin matrix on microbiological parameters, intestinal morphology, and the physicochemical properties of meat in broiler chickens. The study used 200 broiler chickens of the MB-202 Platinum strain, aged 15 days, with an average body weight of 430 ± 0.31 g. The research material consisted of okra powder encapsulated with an amorphous maltodextrin matrix (CMO). The CMO was mixed into the basal feed under the following treatments: MCF-0, control or basal feed; MCF-1, basal feed + 0.5% CMO; MCF-2, basal feed + 1% CMO; and MCF-3, basal feed + 1.5% CMO. The variables measured included microbiological parameters, lactic acid bacteria and coliforms in the duodenum, jejunum, and ileum, intestinal morphology, and physicochemical properties of meat. Results showed that adding CMO supplementation significantly affected ($P < 0.05$) total LAB, coliform counts in the jejunum and ileum, intestinal pH, villus height and crypt depth in the duodenum, jejunum, and ileum, the villus height to crypt depth ratio in all intestinal segments, and abdominal fat. However, CMO supplementation did not significantly affect water holding capacity, tenderness, color, dressing percentage, or meat moisture content. The study concluded that okra encapsulated with maltodextrin can improve the small intestinal profile and selected physicochemical characteristics of meat, while increasing LAB and reducing coliform populations in broiler chickens.*

Keywords: Broiler chicken; encapsulation; intestinal morphology; maltodextrin and okra.

I. INTRODUCTION

Okra (*Abelmoschus esculentus* L.) is a plant that grows in tropical climates and is well known for its high content of bioactive compounds that function as natural antibacterial and antioxidant agents [1]. Bioactive compounds in okra, such as flavonoids, tannins, saponins, alkaloids, and polyphenols, can be used as nutraceutical components mixed into chicken feed as phytochemical substances to support improvements in intestinal profiles while reducing pathogenic bacteria [2]. The research from [3] suggest that the application of okra as a natural feed additive has a positive impact on optimizing the digestive health of broiler chickens. The utilization of phytochemical compounds in chicken feed is associated with improved intestinal mucosal health. Bioactive compounds in herbal plant play an important role as natural feed additives in broiler chicken diets. However, their efficacy easily decreases due to several external factors during processing and digestion. [4] indicate that bioactive compounds from herbal plants are classified as volatile compounds, so appropriate mechanisms are needed to prevent evaporation and degradation, such as the addition of biopolymer materials like starch. Another study by [5] also reported reduced okra bioavailability due to processing, particularly the influence of temperature, as bioactive compounds easily evaporate when exposed to heat.

Therefore, encapsulation techniques can be applied to protect stability and control the release of bioactive compounds. Maltodextrin is used as a coating material for encapsulation because it prevents degradation and enhances the stability of bioactive compounds. According to [6], encapsulation using maltodextrin as a coating agent forms a cohesive, thin, and protective layer around the core material, thereby reducing the degradation of bioactive compounds in herbal plants. This supports the need for a coating mechanism for bioactive components in okra so they can be utilized optimally to improve intestinal profiles

and reduce pathogenic bacteria in the small intestine. This study aims to determine the use of encapsulated okra (*Abelmoschus esculentus* L.) with maltodextrin as a phyto-genic feed additive and its effects on intestinal morphology, microbiological parameters, and physicochemical properties of meat in broiler chickens.

II. METHODS

Ethical approval

The research was conducted in accordance with the regulations on Animal Health under the Indonesian Law on Livestock and Animal Health (UU/18/2009, Article 80).

Microencapsulated okra preparation

Okra (*Abelmoschus esculentus* L.) and maltodextrin were obtained from Semarang, Indonesia. The okra was first cut into small pieces and then ground using a flour machine (Chopper KT-72). A 10% methanol solution was used as the solvent for extraction, with a ratio of 50 g of okra to 500 mL of methanol (10% w/v). The extraction process was performed using the maceration method with three repetitions until a paste-like filtrate was obtained, following the method of [6] with minor modifications. The resulting okra filtrate was then encapsulated using maltodextrin as a coating material at 10% of the sample weight. The mixture of okra filtrate and maltodextrin was dried using an FD-18MTP Labfreez freeze dryer. The final research material consisted of okra powder encapsulated with maltodextrin (CMO).

Materials and experimental design

A total of 200 male broiler chickens of the MB-202 Platinum strain, aged 8 days, with an average body weight of 192.03 ± 1.4 g, were used in this study. The experiment consisted of four treatments, each with five replications, and each replication included 10 birds. Broiler chickens were randomly allocated to 20 floor pens (120 cm \times 120 cm \times 100 cm). Feed and water were provided ad libitum throughout the experiment. The environmental temperature was maintained at $33 \pm 1^\circ\text{C}$ at the start of the experiment and gradually reduced to $23 \pm 1^\circ\text{C}$ between 21 and 35 days of age. Humidity and ventilation were controlled automatically. Basal feed mixed with CMO was provided to the chickens from 8 to 35 days of age in crumble form. The composition and nutrient content of the experimental diets are presented in Table 1. The treatments were as follows: MCF-0, control or basal feed; MCF-1, basal feed + 0.5% CMO; MCF-2, basal feed + 1% CMO; and MCF-3, basal feed + 1.5% CMO.

Table 1. Ingredient and nutrient composition of basal feed

Ingredient (%)	Starter (8-21 d)	Finisher (15-35 d)
Corn	54.50	58.80
Soybean meal	30.40	26.40
Fish meal	6.60	5.40
Monocalcium phosphate	1.70	1.60
Methionine	0.10	0.10
Soybean oil	5.00	6.00
Lysine	0.10	0.10
Limestone	0.90	0.90
Broiler Premix ¹	0.40	0.40
Salt	0.30	0.30
Total of basal feed	100.00	100.00
Nutrient composition		
Metabolizable Energy (kcal/kg) ²	3097.54	3204.21
Crude protein (%)	22.78	19.05
Ether extract (%)	4.27	5.13
Crude fiber (%)	3.73	3.22
Arginine (%)	1.18	1.13
Calcium (%)	0.94	0.90
Phosphorus (%)	0.53	0.51

¹ Providing the following (Each kg broiler premix):240,000 IU Vitamin A; 81,000 IU Vitamin D₃; 300 mg Vitamin B₁; 950 mg Vitamin B₂; 700 mg Vitamin B₆; 800 mg Vitamin B₁₂; 7,500 mg Calcium D pantothenat; 7,000 mg Fe; 5,500 mg Mn; 4,000 mg Zn; 450 mg Cu; 200 mg Vitamin C.

² Calculated value

Sampling and analysis

Microbiological analysis followed the method of [7] with some modifications. Coliform populations were identified as colorless and red colonies after 24 hours of aerobic incubation at 38°C, using MacConkey Agar (MCA). The population of lactic acid bacteria (LAB) was determined after 48 hours of anaerobic incubation at 38°C, using MRS Agar (De Man, Rogosa, and Sharpe). Intestinal pH was measured using an OHAUS ST300 portable pH meter. Intestinal morphology was evaluated based on villus height, crypt depth, and the villus to crypt ratio, following the method described by [8] with some modifications. Intestinal samples were collected immediately after slaughter and fixed in 10% formalin. The samples from the duodenum, jejunum, and ileum were immersed in 200 mL of 90% ethyl alcohol, embedded in paraffin, and cleared using xylene. The paraffin blocks were sectioned at 4 µm using a rotary microtome and stained with hematoxylin and eosin. Morphometric analysis was performed at 4x magnification using a Leica ICC50HD microscope equipped with Leica Application Suite software version 3.4.0. Physicochemical properties of meat were analyzed using six birds at 35 days of age from each treatment, selected randomly to represent all replications. The birds were slaughtered to evaluate meat pH, water holding capacity, tenderness, color, dressing percentage, abdominal fat, and breast fat.

Statistic analysis

The data were analyzed using SAS software version 9.2 under a completely randomized design. Four treatments with five replications were applied for all measured parameters.

III. RESULT AND DISCUSSION

Microbiological

The effects of maltodextrin-encapsulated okra on the broiler intestine (duodenum, jejunum, and ileum) are presented in Table 2. Statistical analysis showed significant effects ($P < 0.05$) on the number of LAB, coliform bacteria, and pH value. Duncan's test indicated that the number of LAB in MCF-2 and MCF-3 increased significantly compared with MCF-0 and MCF-1. Coliform populations in MCF-2 and MCF-3 decreased significantly compared with MCF-0, but did not differ significantly from MCF-1. The pH value decreased significantly in MCF-3 compared with MCF-0, MCF-1, and MCF-2. The antibacterial compounds in okra encapsulated with maltodextrin were able to inhibit pathogenic bacterial populations more effectively as the level of inclusion in the basal diet increased. The strongest inhibitory effect on pathogenic bacteria was observed in the MCF-3 treatment. [9] reported that okra contains antibacterial compounds such as flavonoids, alkaloids, saponins, tannins, and terpenoids.

Flavonoids can disrupt bacterial cell membrane permeability and damage microsomes and lysosomes, resulting from the inhibition of bacterial DNA activity [10]. The use of maltodextrin as an encapsulating material for okra has a positive role in protecting these antibacterial compounds. Maltodextrin functions effectively as a wall or coating material that preserves okra bioactive compounds. As stated by [11], maltodextrin encapsulation helps maintain the stability of bioactive compounds that are susceptible to degradation during processing and storage. In addition, [12] reported that okra can serve as a natural antimicrobial component in chicken rations, providing benefits in preventing diseases associated with pathogenic bacterial exposure. The flavonoid content in maltodextrin-encapsulated okra contributes to an increase in short-chain fatty acids (SCFAs), which leads to the formation of acidic conditions in the small intestine. The composition of SCFAs, such as butyrate, propionate, and acetate, lowers intestinal pH and exerts inhibitory effects on pathogenic bacteria, including coliforms [2].

Table 2. Selected intestinal bacteria in broiler chickens

Parameters	MCF-0	MCF-1	MCF-2	MCF-3	SEM	<i>p</i> value
Duodenum						
LAB (log CFU/gm)	10.08 ^b	10.15 ^b	10.23 ^a	10.61 ^a	0.302	0.042
Coliform (log CFU/gm)	5.21 ^a	5.18 ^{ab}	5.04 ^b	4.91 ^b	0.482	0.039
pH	6.61 ^a	6.57 ^a	6.54 ^{ab}	6.49 ^b	0.205	0.043
Jejunum						
LAB (log CFU/gm)	9.84 ^b	9.93 ^b	10.16 ^a	10.38 ^a	0.385	0.048

Coliform (log CFU/gm)	5.66 ^a	5.52 ^{ab}	5.41 ^b	5.37 ^b	0.451	0.035
pH	6.76 ^a	6.74 ^a	6.70 ^{ab}	6.65 ^b	0.217	0.037
Ileum						
LAB (log CFU/gm)	9.73 ^b	9.87 ^b	10.05 ^a	10.24 ^a	0.451	0.043
Coliform (log CFU/gm)	5.73 ^a	5.66 ^{ab}	5.53 ^b	5.47 ^b	0.322	0.047
pH	6.84 ^a	6.79 ^a	6.75 ^{ab}	6.67 ^b	0.218	0.031

SEM = Standard error of means, LAB = lactic acid bacteria, CFU = colony-forming unit, MCF-0: control/basal feed, MCF-1: basal feed + CMO 0.5%, MCF-2: basal feed + CMO 1%, MCF-3: basal feed + CMO 1.5%, ^{abc}Means in the same row with superscript letters are different at P<0.05

Intestinal morphology

Based on the data in Table 3, broiler basal diets supplemented with maltodextrin-encapsulated okra had significant effects (P<0.05) on intestinal morphology in the duodenum, jejunum, and ileum. Villus height, crypt depth, and the villus height to crypt depth ratio in broilers receiving MCF-3 were significantly higher than in MCF-0, MCF-1, and MCF-2. Treatments MCF-1 and MCF-2 showed significant improvements compared with MCF-0, although their values were lower than those observed in MCF-3. Broilers fed the control diet MCF-0 exhibited the smallest intestinal morphology measurements, which were significantly different (P<0.05) from the other treatments. The addition of CMO to the basal feed at levels ranging from 0.5% to 1.5% was effective in increasing villus height and crypt depth in the intestinal tract of broiler chickens. Villi are mucosal projections that extend into the lumen of the small intestine and function to increase the surface area for nutrient absorption. Each villus is lined by epithelium and contains connective tissue at its core. Villi are composed of simple columnar epithelium [13]. Based on the results of this study, villus height and the villus height to crypt depth ratio in the duodenum were greater than those observed in the jejunum and ileum. This finding is consistent with [14], who reported that duodenal villi are longer than those in the ileum.

Table 3. Intestinal morphology in broiler chickens

Parameters	MCF-0	MCF-1	MCF-2	MCF-3	SEM	p value
Duodenum						
Villi height (µm)	1402 ^c	1584 ^b	1712 ^{ab}	1885 ^a	46.8	0.045
Crypt depth (µm)	302 ^c	328 ^b	349 ^{ab}	366 ^a	22.6	0.037
Villi height to the crypt depth ratio	4.64 ^b	4.83 ^b	4.91 ^{ab}	5.15 ^a	0.35	0.025
Jejunum						
Villi height (µm)	1398 ^c	1469 ^{bc}	1602 ^b	1749 ^a	66.1	0.042
Crypt depth (µm)	298 ^c	311 ^b	336 ^{ab}	358 ^a	19.8	0.046
Villi height to the crypt depth ratio	4.69 ^b	4.72 ^{ab}	4.77 ^{ab}	4.89 ^a	0.25	0.008
Ileum						
Villi height (µm)	1137 ^c	1263 ^b	1302 ^{ab}	1455 ^a	47.6	0.035
Crypt depth (µm)	264 ^c	275 ^b	281 ^{ab}	303 ^a	18.9	0.032
Villi height to the crypt depth ratio	4.31 ^b	4.59 ^{ab}	4.63 ^{ab}	4.80 ^a	0.16	0.032

SEM = Standard error of means, MCF-0: control/basal feed, MCF-1: basal feed + CMO 0.5%, MCF-2: basal feed + CMO 1%, MCF-3: basal feed + CMO 1.5%, ^{abc}Means in the same row with superscript letters are different at P<0.05

The highest values of intestinal morphology parameters were observed in the MCF-3 treatment across the duodenum, jejunum, and ileum. An increase in villus height reflects an enhanced capacity of the intestine to absorb nutrients. Changes in villus height were also associated with a reduction in coliform populations when CMO was added at a level of 1.5% (Table 3). The antibacterial compounds in okra that reduced intestinal coliforms likely contributed to increases in villus height and the villus height to crypt depth ratio. Microvilli are present on the surface of villi as cytoplasmic extensions that further enhance absorption efficiency. A larger villus surface area increases the potential for nutrient absorption from the digestive tract [15]. Broiler chickens receiving CMO in the basal diet showed improved villus development in the duodenum, jejunum, and ileum compared with the control group. [16] also reported that longer

intestinal villi improve the efficiency of nutrient absorption through the small intestinal epithelium. Increased villus height and width expand the absorptive surface area and contribute to better intestinal performance [17]. The research from [18] further indicated that the villus height to crypt depth ratio reflects the absorptive capacity of the digestive system.

Physicochemical properties of meat

The physicochemical properties of meat, including pH value, water holding capacity, tenderness, color, dressing percentage, and breast fat, showed no significant differences ($P>0.05$). In contrast, abdominal fat differed significantly ($P<0.05$), as shown in Table 4. Treatments MCF-2 and MCF-3 resulted in the lowest abdominal fat values compared with MCF-0 and MCF-1. The basal diet without the addition of maltodextrin-encapsulated okra (MCF-0) produced the highest abdominal fat value, and this result did not differ significantly from MCF-1.

Table 4. Physicochemical properties of meat

Parameters	MCF-0	MCF-1	MCF-2	MCF-3	SEM	<i>p</i> value
pH	5.83	5.87	5.95	6.01	0.081	0.416
Water holding capacity (cm ² /gm)	18.86	19.41	20.74	21.33	0.117	0.224
Tenderness (cm ² /gm)	10.07	10.42	11.21	11.55	0.074	0.472
Color, optical density	0.174	0.186	0.193	0.207	0.036	0.561
Dressing (%)	70.8	69.1	68.5	67.7	0.725	0.062
Abdominal fat (%)	1.51 ^a	1.27 ^{ab}	1.23 ^b	1.19 ^b	0.068	0.031
Breast fat (%)	5.45	5.41	5.36	5.31	0.784	0.068

SEM = Standard error of means, MCF-0: control/basal feed, MCF-1: basal feed + CMO 0.5%, MCF-2: basal feed + CMO 1%, MCF-3: basal feed + CMO 1.5%, ^{abc}Means in the same row with superscript letters are different at $P<0.05$

Meat quality parameters, including pH value, water holding capacity, tenderness, color, and dressing percentage, showed non-substantial results. This finding is consistent with [2], who reported that bioactive compounds from herbal plants added to feed did not affect several physical components of chicken thigh meat, indicating that the meat quality remains safe for consumption. The research [3] stated that the physical quality of chicken meat is influenced by temperature and other environmental conditions during storage. The basal diet supplemented with maltodextrin-encapsulated okra resulted in a significant reduction in abdominal fat percentage. The addition of CMO at levels of 1–1.5% produced the lowest abdominal fat levels. Broilers in the MCF-2 and MCF-3 treatments effectively inhibited systemic fat accumulation compared with the control group. Non-significant values were observed for breast fat, although a decreasing trend was recorded for this parameter. Flavonoids help reduce fat accumulation in the body by promoting fat burning through lipolysis and limiting the formation of new fat cells through lipogenesis. The flavonoid content in maltodextrin-encapsulated okra can bind to pancreatic lipase, reduce its activity, and decrease dietary fat absorption, thereby contributing to lower abdominal fat in chicken meat.

IV. CONCLUSION

The dietary inclusion of maltodextrin-encapsulated okra at a level of 1.5% in the basal feed improved intestinal growth and morphology and reduced abdominal fat in broiler chickens. This study highlights the importance of further evaluating the appropriate inclusion levels of the investigated supplement to maximize its positive effects on gut tissue and, consequently, the overall health of broiler chickens.

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