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Encapsulated Fermeherbafit Bioavailability and the Application to Broilers

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Abstract: The research was conducted to evaluate the physical, chemical and biologic as well as the bioavailability of encapsulated fermeherbafit in broiler chickens. Fermeherbafit is a term coined in this study for a mix herbal fermentation. The materials used were: fermeherbafit material consisting of *Curcuma domestica* (turmeric), *C. xanthorrhiza* R. (ginger), *Allium sativum* L. (garlic), *Morinda citrifolia* (noni), *Moringa oleifera* (*Moringa* leaves), brown sugar and lactic acid bacteria (LAB) probiotic. Eighty broiler day-old chicks of strain MB 202 Platinum were reared until the age of 35 d. Research Phase 1 was the evaluation of the encapsulated ingredients consisted of treatments P_1 = 2% alginate:2% chitosan; P_2 = 4% alginate:2% chitosan; P_3 = 2% alginate:4% chitosan; P_4 = 4% alginate:4% chitosan; P_5 = 2% alginate:2% chitosan; P_6 = 4% alginate:2% chitosan; P_7 = 2% alginate:4% chitosan; P_8 = 4% alginate:4% chitosan. Research Phase 2 was the encapsulated fermeherbafit with treatments R_0 = control; R_1 = non-encapsulated fermeherbafit; R_2 = 1.5% encapsulated fermeherbafit; R_3 = 3.0% encapsulated fermeherbafit; R_4 = 4.5% encapsulated fermeherbafit. The results of Phase 1 study indicated that the most well-encapsulated fermeherbafit was treatment R_7 (2% alginate:4% chitosan) regarding the levels of protein, energy, LAB amount. The results of Phase 2 showed that the use of encapsulated fermeherbafit had significant effect ($p < 0.05$) on final weight, carcass weight, carcass percentage and liver weight, but had no effect ($p > 0.05$) on the abdominal fat percentage, intestine weight, bursa Fabricius weight, and the proventriculus weight. It can be concluded that the use of 1:1 of alginate and chitosan may retain fermeherbafit bioavailability, and its application to the chicken could be of up to 4.5%, with final broilers body weight of 1,179.75 ± 27.76 g and carcass weight of 826.7 ± 30.27 g (70.06% ± 1.33%) and liver weight of 22.625 ± 2.55 g.

Key words: Fermentation, herbal, feed, chicken, nutrition, carcass.

1. Introduction

Medicine, antibiotics and other additives are always used in broiler keeping to maintain good health status and increase the growth rate of chicken. The use of antibiotics has adverse effects such as the residues in meat and increasing bacterial resistance. It is necessary to give the efficient and safe feed to chicken by manipulating chicken feed and administering natural feed additives such as fermented herbs by lactic acid bacteria (LAB) called "fermeherbafit". "Fermeherbafit" is a natural feed additive for chickens developed from the previous studies by Iriyanti *et al.* [1] for chickens. The use of herbs as feed will be more beneficial if fermented using LAB which will increase the value of herbal nutrients. The result of the fermentation process will produce many essential compounds such as amino acids, vitamins and probiotics which help the process of digestion and metabolism and increase endurance.

Previous study by Iriyanti *et al.* [2] has showed that the use of herbs in broiler chickens is able to increase feed consumption from 3,213.912 ± 235.531 g/bird to 3,309.378 ± 291.616 g/bird. Additionally, an absolute average growth was observed from 1,528.02 ± 181.03 g/bird to 1,791.82 ± 80.02 g/bird, and the average relative growth was from 0.3648 ± 0.0044 kg/d up to 0.3702 ± 0.0022 kg/d. The results showed that treatment with 6% of herb into feed resulted in 1,791.82 ± 80.02 g/bird absolute growth, 73.551% ±

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1.77% carcass percentage, 251.88 ± 7.97 × 10^2/μL leukocyte level, and 2.33-2.52 million/μL erythrocyte levels. Blood hematocrit average levels ranged from 29.56% to 31.80%.

A bioactive material as phytochemical compounds in herb is highly sensitive to unfavorable environmental conditions, changes in light, heating, oxygen and pH as well as environmental conditions of the chicken proventriculus and acidic pH of bile. Bioactive material protection could be done with microencapsulation. The materials used as encapsulation agents (encapsulant) are for example carbohydrates, including modified starch and maltodextrin, cellulose derivatives, sap and cyclodextrin, proteins such as whey protein, caseinate and gelatin [3].

Herbs have a low bioavailability due to their curcumin (low solubility, low absorption, quick passing, high rate of metabolism in the intestinal cell, and rapid elimination). Curcumin has a low bioavailability because it is insoluble in acidic or neutral pH; therefore, it is difficult to absorb and require technology to improve absorption.

Fermeherbafit is a probiotic-loaded herb so the cell viability must be maintained to remain alive when reaching the intestines. Therefore, it required efforts to protect the probiotic cells by means of encapsulation techniques [4-8]. Encapsulation materials include carrageenan, resistant starch alginate and chitosan [9].

Chitosan is a natural polymer as the main derivative of chitin. A suitable chitosan depends on the obtained chitin, the solubility of an alkali as well as time spent on the deacetylation. Curcumin and chitosan will be bound by the ionic process (chitosan encapsulating curcumin). Ionic bonds between chitosan and curcumin could be damaged and properties of chitosan are unstable against low pH and protease produced in the stomach, therefore it requires anions ingredients, for example sodium tripolyphosphate (STPP) to stabilize the complex inter-molecular [10]. This study was aimed to investigate the use of encapsulated fermeherbafit as feed additive on broiler performance.

2. Materials and Methods

2.1 Phase 1 of Research

The materials used were fermeherbafit ingredients: 100 g *Curcuma domestica* (turmeric), 100 g of *C. xanthorrhiza* R. (ginger), 25 g *Allium sativum* L. (garlic), 50 g *Morinda citrifolia* (noni), 10 g of *Moringa oleifera* (*Moringa* leaves), 25 g of brown sugar and LAB probiotic. Encapsulated ingredients include alginate and chitosan.

The treatment consists of P_1 = 2% alginate:2% chitosan; P_2 = 4% alginate:2% chitosan; P_3 = 2% alginate:4% chitosan; P_4 = 4% alginate:4% chitosan; P_5 = 2% alginate:2% chitosan; P_6 = 4% alginate:2% chitosan; P_7 = 2% alginate:4% chitosan; P_8 = 4% alginate:4% chitosan. The treatments were allotted in a completely randomized design (CRD) and further followed by honestly significant difference (HSD) test. Variables measured were physical tests including weight, color, crispness, smell and encapsulated fermeherbafit volume. Chemical test included the levels of protein, fat, crude fiber, dry matter (DM), organic matter and energy, and microbiological test included total bacteria and total LAB were performed. The number of colonies in the LAB capsules on each treatment was measured using the total plate count (TPC) according to Fardiaz [11]. A total of 0.5 g capsules LAB was put in 4.5 mL physiological saline 0.85% and divortex for capsule dissolution process, serially diluted (4, 5 and 6 time diluted) and then as much as 0.1 mL was planted on a petri dish containing MRS media agar. The cultures were incubated at room temperature for 2 d. The grown colonies were then calculated as follows: population LAB (CFU/g) = number of colonies × dilution serial.

2.2 Preparing the Encapsulated Fermeherbafit

The microcapsules making was based on modified coacervation method [12, 13]. Modification coacervation method is obtained by the addition of

coacervation in suspension fermeherbafit encapsulation at certain temperature and pH, so the formation of bonds was between molecules in fermeherbafit encapsulation itself or between molecules of the other starch molecule. This would change the liquid characteristic and acid resistance of fermeherbafit encapsulation.

The encapsulated fermeherbafit consisted of 750 g of fermeherbafit, 1,500 mL of aquadest, 30 g of alginate, 60 g of chitosan, 0.75 g of $CaCO_3$ and 37.5 g of casein. The making process was as follows: (1) 1,500 mL of distilled water and 37.5 g of casein were heated in an electric stove, stirred and homogenized with in a mixer; (2) 0.75 g of $CaCO_3$ was added and mixed until homogeneous; (3) 30 g of alginate was added and stirred until homogeneous in a mixer; (4) the compound was let sit for 30 min without stirring or heating; (5) the compound was mixed for 30 min without heating; (6) 4% chitosan solution (60 g of chitosan dissolved in 375 mL of distilled water) was added and stirred with a mixer until homogeneous; (7) 750 g of powder fermeherbafit was added and stirred until homogeneous; (8) the compound was put in a pan and dried in the oven at 40-50 °C for 24 h; (9) after the encapsulated fermeherbafit dry and it was then pulverized in a grinder.

2.3 Phase 2 of Research

A total of 80 day old chick (DOC) broiler strain MB 202 Platinum were reared until the age of 35 d. Feed materials used were corn, bran, soybean meal, fish meal, lime, premix, oil, DL-methionine and L-lysine-HCl. The ration was formulated in isoprotein and iso calorie, starter feed with 23% protein content and 3,100 kcal/kg metabolic energy (ME); finisher feed contained 20% protein and 2,900 kcal/kg ME. The treatments were R_0 = control; R_1 = non-encapsulated fermeherbafit; R_2 = 1.5% encapsulated fermeherbafit; R_3 = 3.0% encapsulated fermeherbafit; R_4 = 4.5% encapsulated fermeherbafit. The treatments were allotted in CRD and a post-hoc test of test HSD was performed. Broiler performances including weight end, weight and percentage carcass, weight heart, fat abdomen, weight guts, bursa of Fabricius weight, long guts and the weight of proventriculus was measured in the study.

3. Results and Discussion

3.1 Phase 1 of Research

The results of the bioavailability encapsulated fermeherbafit are shown in Tables 1 and 2.

Table 1 shows that the encapsulated fermeherbafit material with alginate, chitosan and a combination of alginate and chitosan resulted in nearly the same physical conditions; therefore, these materials were potential for coating material bioactive in the fermeherbafit. Coating material protects the bioactive material from unfavorable environment along the digestive tract to optimize the encapsulated fermeherbafit.

According to Lian *et al.* [14], the crack may facilitate the escape of heat from the particles after drying, causing less heat damage (heat injury) against microorganisms trapped inside it.

Mosilhey [15] reported that encapsulation materials were evidenced to extend cell protective properties against bile salt solution. To survive and grow in the gastrointestinal tract, LAB as probiotic bacterial cultures must be able to pass through a variety of unfavorable environmental conditions, especially on the upper intestinal tract where the bile is secreted into the intestine.

The content of nutrients of encapsulated fermeherbafit is shown in Table 2.

Table 2 showed that the results of the obtained LAB in encapsulated are as much as 191 × 10 log6. This number still meets the requirements of the number of probiotic culture starter in which it should have a viability or the number of active cells ≥ 6 × 10 log6 colony/mL. The total average population of encapsulated probiotics conducted by Sultana *et al.* [16] by using alginate and glycerol as crioprotection

Table 1 Physical profile of encapsulated fermeherbafit.

Treatments	Weight (g)	Color	Crispness	Smell	Volume (mL/g)
P_1	21.945	Brown	Crispy	Less fragrant	0.5
P_2	23.684	Dark brown	Less crispy	Less fragrant	0.6
P_3	22.568	Brown	Crispy	Less fragrant	0.8
P_4	23.611	Dark brown	Crispy	Less fragrant	0.8
P_5	45.612	Yellowish brown	Crispy	Fragrant	0.9
P_6	45.887	Yellowish brown	Crispy less	Fragrance	1
P_7	45.173	Brown	Less crispy	Less fragrant	0.7
P_8	37.751	Brown	Crispy	Fragrant	0.8

P_1 = 2% alginate:2% chitosan; P_2 = 4% alginate:2% chitosan; P_3 = 2% alginate:4% chitosan; P_4 = 4% alginate:4% chitosan; P_5 = 2% alginate:2% chitosan; P_6 = 4% alginate:2% chitosan; P_7 = 2% alginate:4% chitosan; P_8 = 4% alginate:4% chitosan.

Table 2 The content of nutrients of encapsulated fermeherbafit.

Treatments	The content of BAL	Water (%)	Dry matter (DM) (%)	DM (%)				
	(× 10 log6)			Protein	Fat	Fiber	Ash	Nitrogen free extract
P_1	41	15.15	84.85	10.44	2.04	7.16	6.49	73.87
P_2	61	15.05	84.95	10.06	1.11	7.58	6.86	74.40
P_3	67.31	14.72	85.28	12.77	3.09	8.58	8.24	81
P_4	281	14.70	85.30	11.37	1.52	10.48	7.28	69.36
P_5	86.88	13.12	120	10.19	3.32	9.56	7.42	69.51
P_6	68.21	13.72	86.28	9.59	3.56	10.87	7.78	132
P_7	191	15.27	84.73	10.45	2.60	6.25	6.74	73.96
P_8	141	13.88	86.12	10.50	2.42	6.69	8.19	72.21

P_1 = 2% alginate:2% chitosan; P_2 = 4% alginate:2% chitosan; P_3 = 2% alginate:4% chitosan; P_4 = 4% alginate:4% chitosan; P_5 = 2% alginate:2% chitosan; P_6 = 4% alginate:2% chitosan; P_7 = 2% alginate:4% chitosan; P_8 = 4% alginate:4% chitosan.

was $\geq 1.0 \times 10^7$ CFU/g. In addition, the population of probiotic bacteria isolates at 4 °C storage refrigerator temperature conforms with the standard of FAO/WHO that the standards for the population of bacteria that must be present in the starter culture is about 10^6-10^7 CFU/g.

Research by Zamora *et al.* [17] states that the LAB isolated from blood using the method of drying spray dried (800-1,700 °C) decreased viability by 50% compared to the method of freeze-dried (-15 °C to 15 °C). It is presumably because sodium alginate and carrageenan have different gelatinization abilities. Gelatinization in sodium alginate mixed with a polymer material $CaCl_2$ was stronger than carrageenan in protecting the LAB. The affinity of divalent cations Ca^{2+} in sodium alginate is faster and stronger than carrageenan in gel formation. Anal and Stevens [18] reported that capsules made of sodium alginate and $CaCl_2$ polymer would form a stronger alginate sodium ions. Lee *et al.* [19] reported that the capsulation of *Lactobacillus bulgaricus* KFRI 673 using a combination of freeze-dried and alginate and chitosan can increase the number of colonies of LAB. Ivanova *et al.* [20] reported that the bacteria capsulation process of *Enterococcus faecium* 2000 by using calcium alginate could improve the inhibition by 50% compared with non-encapsulation. The encapsulated materials are bifidobacteria and *Lactobacillus* encapsulation with alginate-starch [16], *L. casei* with alginate-flour and wheat pollard [21], bifidobacteria with whey protein [22], *Lactobacillus* spp. with calcium alginate [23].

Pacifico *et al.* [24] stated that sensitive components such as microorganisms can be encapsulated to improve the viability and shelf life. Encapsulation is a technique of coating a material to protect from environmental conditions. Bacteria encapsulation would protect the microbes from unfavorable

environmental influences, such as heat and chemicals. Encapsulation method may include spray-drying coat made of hot Arabic gum and with maltodextrin in 2% concentrations or crosslink method without heating [25, 26].

Materials that can be used for encapsulation are: polysaccharides and proteins such as starch, alginate, Arabic gum, gelatin, carrageenan, albumin and casein.

Each material has different characters and does not necessarily fit with the core material to be encapsulated; therefore, encapsulation materials should be carefully selected [27].

Encapsulated fermeherbafit nutrient content is feasible and potential as feed additive/feed supplement for poultry since the protein content of this study is 9.59%-12.77%.

Treatment R_4 (alginate 4%:4% chitosan) is the best for encapsulated fermeherbafit because it resulted in the highest LAB content (281 × 10 log6) and protein content (11.37%).

Encapsulation occurs naturally when bacterial cells grow and produce exo-polysaccharides. The microbial cells are entrapped within their secretions that act as protective structure or a capsule, reducing the permeability of the material through the capsule and the cells are therefore less exposed to adverse environmental factors. The viability of probiotic bacteria in a product at the point of consumption is an essential consideration for their-efficacy, as they have to survive during the processing and shelf life of food and supplements, and as they transit through high acidic conditions of the stomach and enzymes and bile salts in the small intestine [28].

Chitosan is a polycationic polysaccharide or is an amino polysaccharide. Chitosan is a fiber-like substance and a homopolymer of β-(1→4)-linked N-acetyl-D-glucosamine.

This component has not shown a reasonable efficiency for increasing cell viability by encapsulation, and it is preferably used as a coat but not as a capsule [29].

Alginates formed a firmer gel with excellent mechanical stability and demonstrated the natural release of the encapsulated bacteria. Chitosan, a water-soluble polymer (pH < 6) has been used in microencapsulate [28].

In fact, encapsulation of probiotic bacteria with alginate and a chitosan coating protects in simulated gastro intestinal (GI) conditions; therefore, it is immediately delivering viable bacterial cells to the colon [30]. Also, Shah and Ravula [31] reported that the encapsulated probiotic bacteria survived in acidic conditions (at pH 2.5) in calcium alginate beads; however, the organisms were released into the presence of bile. Cationic chitosan can form gels with non-toxic multivalent anionic counterions such as

Table 3 The performance of broiler chickens with encapsulated fermeherbafit in ration.

	R_0	R_1	R_2	R_3	R_4
Final weight (g)*	1,025 ± 71.96[a]	1,032 ± 106.18[a]	1,087 ± 27.14[a]	1,063 ± 41.61[a]	1,179 ± 27.76[b]
Carcass (g)*	739.03 ± 46.44[a]	741.33 ± 83.85[a]	768.68 ± 13.99[b]	740 ± 46.31[a]	826.7 ± 30.27[b]
Carcass (%)*	67.42 ± 4.91[a]	71.79 ± 0.92[b]	70.73 ± 0.68[b]	69.54 ± 2.08[a]	70.06 ± 1.33[b]
Liver weight (g)*	18.75 ± 2.84[a]	17.5 ± 2.28[a]	21.78 ± 1.021[a]	21.025 ± 1.80[b]	22.625 ± 2.55[b]
Abdomen fat (%)[ns]	1.94 ± 0.8	1.55 ± 0.61	1.03 ± 0.31	1.89 ± 0.94	1.04 ± 0.41
Intestinal weight (%)[ns]	3.03 ± 0.41	2.61 ± 0.22	2.51 ± 0.16	2.75 ± 0.31	2.92 ± 0.20
Bursa of Fabricius (g)[ns]	2.23 ± 0.35	2.78 ± 0.79	2.35 ± 0.60	2.05 ± 0.66	2.18 ± 1.17
Length of intestine (cm)[ns]	135.75 ± 17.15	137.75 ± 17.15	132.25 ± 12.28	139.75 ± 11.0	139.5 ± 13.08
Proventriculus weight (g)[ns]	4.4 ± 0.63	3.65 ± 0.51	4.15 ± 0.26	3.9 ± 0.53	4.85 ± 0.84

R_0 = control; R_1 = non-encapsulated fermeherbafit; R_2 = 1.5% encapsulated fermeherbafit; R_3 = 3.0% encapsulated fermeherbafit; R_4 = 4.5% encapsulated fermeherbafit.

*significant differences ($p < 0.05$); [ns]non-significant differences ($p > 0.05$); [a, b]the same letters present the non-significant difference at 95% confidential level.

polyphosphate and sodium alginate [18, 32]. The alginate-chitosan-carboxymethyl chitosan microcapsules extended the survival of *L. casei* after drying [33].

3.2 Phase 2 of Research

The results of the broiler performance by providing more encapsulated Fermeherbafit are shown in Table 3. The final weight, carcass weight and liver weight percentage in R_4 (4.5% encapsulated fermeherbafit) showed a more significant effect ($p < 0.05$) than the other treatments because it contains bioactive material that could enhance the growth (final weight). Noni contains bioactive materials such as anthraquinone, acubin and alizarin, xeronine and precursor xeronine. Proxeronine is converted into xeronine in the intestine by the pro-xeronase enzyme and then to be absorbed by the body's cells to activate the inactive protein [34]. Scopoletin capable of binding serotonin is the chemical that narrows the blood vessels, so that blood pressure increases [35]. Coumarin and fatty acids in noni is effective to expand blood vessels and eliminate active oxygen superoxide [36]. Noni as a medicinal plant contains beneficial, synergic active substances such as anti-stress [33], anti-bacterial [37] and anti-cancer properties [38, 39].

Moringa leaves (*M. oleifera*) have active compounds as antimicrobial agent such as saponins, tannins, flavonoids, alkaloids and terpenoids obtained from the extraction process [40-44], and other phenolic compounds with antimicrobial activity [45-47]. The antimicrobial active ingredients have a mechanism of destroying the bacterial cell membranes by increasing the permeability of the bacterial cell wall so the bacteria will lyse [48].

Flavonoids are phenolic compounds that exhibits antimicrobial properties by forming complex compounds of extracellular proteins that interfere with the integrity of the membrane and the cell wall. An antimicrobial agent affect the synthesis of protein and DNA synthesis and damage the integrity of the membrane and cell wall of bacteria that would interfere with the permeability of the cell [49]. Turmeric contains active substances of curcumin and many were reported to inhibit the growth of pathogenic bacteria such as *Clostridium botulinum*, *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhimurium* [50, 51]. *Curcuma* contains active substances xanthorrhizol and curcumin that could inhibit the growth of fungi such as *Candida* species and *Aspergillus* [52], bacteria *E. coli* and *Shigella dysentriae* [53].

Biological activities that include antioxidant turmeric broad spectrum, antibacterial, and hypocholesteremia exhibit the nature cholagogue (emetic bile); therefore, it could improve the absorption of vitamins A, D, E and K. Garlic contains allicin as a natural antibiotic that could eradicate microbes. Antibacterial substance could lyse toxin attached to the intestinal wall so that nutrients are better absorbed because antibiotics act as the growth promoter.

The herb also contains essential oils and curcumin which increases the performance of digestion tract, stimulates bile wall to secrete bile, and stimulate the release of pancreatic sap containing amylase, lipase, and protease to improve the digestion carbohydrates, fats and proteins as feed ingredients [54, 55].

Table 3 shows that the percentage of abdominal fat, weight intestine, bursa Fabricius, proventriculus and intestine length was not affected ($p > 0.05$). Accordingly, encapsulated fermeferbafit up to 4.5% was suitable to incorporate in chicken feed.

Turmeric and curcumin have cholagogue (emetic bile) activity can increase production and secretion of bile salt, when entered into the duodenum and fat excretion with feces [56]. Herbs especially ginger can metabolize body fat and increase endurance because it contains bioactive substances that can improve the work of hormonal system, especially the metabolism of carbohydrates and metabolize fat herbal ingredients contain crude fiber, high-functioning dissolve fat in the body chicken so that the percentage of abdominal fat decreased [21].

Colon's weight and length and proventriculus' weight are affected by crude fiber feed; the higher the crude fiber, the heavier the intestine. The crude fiber of encapsulated fermeherbafit by 10.48% did not affect the work of the intestine. Hermana *et al.* [57] found that leaves as an antibiotic did not affect the weight and length of broiler intestines.

Bursa Fabricius is a lymphoid organ that was strongly affected by hormone corticosterone. This organ affects immunity. The use of encapsulated fermeherbafit with various bioactive ingredients did not affect the immune system of broiler chickens. Lymphocytes diminish when chicken is under stressful conditions [58, 59].

The immune system in birds is closely connected with the function of the lymphoid organs such as bursa Fabricius. Stock Fabricius serves as the cell maturation of the chicken antibody-forming system capable of destroying the antigens that enter the body [58]. The immune system starts to develop during the embryonic phase and continues during the first week after hatching [60]. The development of broilers endurance in the initial phase of growth is very influential on the next phase; when the starter is not well developed, the immunosuppression suffers and may lead to weak growth even death. Feed supplies the nutrients for growth and development of the primary lymphoid organs (bursa Fabricius and thymus) and secondary (spleen, mucosa-associated lymphoid tissue, gland lymph). The immune system particularly the mucosal immune system requires feed to proliferate.

4. Conclusions

The use of 1:1 alginate and chitosan may retain fermeherbafit bioavailability encapsulation, and supplementation of fermeherbafit encapsulation up to 4.5% in chicken could be increase for final weight by 15% and carcass weight by 20%.

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