The Effect Nanocapsule of Turmeric Extracts in Rations on Nutrient Digestibility of Broiler Chickens

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Abstract. The use of turmeric is restricted by its low solubility in water, therefore it has low bioavailability. This obstacle can be solved by the development of nanoparticle technology to improve drug delivery profile. This study aimed to develop nanoparticle formulation using turmeric extract and industrial chitosan as the matrix and sodium-tripolyphosphate as cross linker, to study its ability to improve feed digestibility. Method used in the formulation of nanoparticle in this study was by ionic gelation followed by oven drying at 50°C. Method used to evaluate the digestibility was total collection. One hundred and twenty broiler chickens with an average body weight of 900 g, were randomly divided into 20 treatments (one treatment was fasted and 19 were treated with the ration plus feed additive), and six replicates were performed on each test. Chickens were fasted one day before and after they were treated with rations. Nutrient levels and the amount of feed consumed and excreta released were weighed to calculate the digestibility of the ration. It was found that the basal ration had dry matter digestibility of 70.48% significantly lower compared to the basal ration plus nanocapsule turmeric extract: NP level at 0.4% for in EE and EA were 73.11 and 75.90%. The results of this study concluded that formulation of nanocapsule using turmeric extract and industrial chitosan as the matrix and sodium tripolyphosphate as cross linker was potential to increase nutrient digestibility, therefore, it can be an alternative for feed additive in broiler chicken diet.

Key words: Digestibility, nanocapsule, turmeric extract, broiler chicken

Abstrak. Penggunaan kunyit/kurkumin terbatas karena kelarutannya yang rendah dalam air sehingga bioavailabilitasnya juga rendah. Masalah ini dapat diatasi dengan teknologi nano yang dikembangkan sebagai alternatif penghantaran obat bagi bahan kimia yang mempunyai bioavailabilitas rendah. Penelitian ini bertujuan untuk mengembangkan formulasi nanokapsul menggunakan ekstrak kunyit dan kitosan industri sebagai matrik atau polimer serta sodium tripolifosfat sebagai *cross linker*, untuk dipelajari kemampuannya dalam meningkatkan kecernaan ransum. Metode yang digunakan dalam formulasi nanokapsul adalah gelasi ionik dilanjutkan pengeringan dengan oven pada suhu 50°C. Sedangkan metode untuk mengevaluasi kecernaan menggunakan total koleksi. Seratus dua puluh ekor ayam broiler dengan bobot badan rata-rata 900 g, dibagi secara acak ke dalam 20 perlakuan (satu perlakuan dipuasakan dan 19 diberi ransum perlakuan dengan penambahan *feed additive*), masing-masing enam ulangan dan satu ekor untuk tiap ulangan. Ayam dipuasakan sehari pada sebelum dan sesudah ayam diperlakukan dengan ransum, kadar nutrien dan jumlah pakan yang dikonsumsi serta ekskreta yang dikeluarkan ditimbang beratnya untuk menghitung kecernaan ransum. Telah ditemukan bahwa kecernaan bahan kering ransum basal (70,48%) nyata lebih kecil dibanding ransum basal yang ditambah nanokapsul ekstrak kunyit: pada NP level 0,4% sebesar 73,11% pada EE dan 75,90% pada EA. Dapat disimpulkan bahwa formulasi nanokapsul menggunakan ekstrak kunyit dan kitosan industri sebagai matrik dan sodiumtripolifosfat sebagai *cross-linker* berpotensi sebagai *feed additive* alternatif untuk meningkatkan kecernaan nutrien pada ransum ayam broiler.

Kata kunci: Kecernaan, nanokapsul, ekstrak kunyit, ayam broiler

Introduction

Antibiotics are widely used to gain higher income in broiler farming as growth promotors and high-fat or high-energy ration. Antibiotics has facilitated the efficient production of poultry, allowing the consumers to purchase at a reasonable price on high quality meat and eggs (Donoghue, 2003). A wide range of antibiotics are used in poultry not only to treat disease but also to maintain health, promote growth and enhance feed efficiency (Gaudin et al., 2004). In particular, broiler chickens are often grown actively with antibiotics to attain maximum weight within a short period of time (Nonga et al., 2009). The uncontrolled and unlimited use of these antibiotics may however lead to the accumulation of undesirable residues in the animals treated and their products (Wachira et al., 2011).

Coronary heart disease and arteriosclerosis are strongly related to the dietary intake of cholesterol and saturated fatty acids and are among the most important causes of human mortality (Sacks, 2002 cit. Omojola et al., 2009). The used of high fat or high energy in ration of broiler chicken causes cholesterol content of thigh meat as much as 87.6 mg/100 g (Daneshyar et al., 2011) and at local poultry in Indonesia include Native chicken (177.47 mg/100 g), Tegal duck (166.91 mg/100 g) and Muscovy duck (171.94 mg/100g) (Ismoyowati and Widiyastuti, 2003). Controversy over the uses of antibiotics and high-energy rations call for the efforts to find out a feed additive from natural ingredients that has the potential to substitute the function of antibiotics as well as to lower cholesterol. One potential herbal medicine in Indonesia is curcumin, the main active ingredient of turmeric rhizome. Several in vitro and in vivo studies showed that turmeric activities are as: antibacterial, anti-inflammatory, antitoxic, anti-hyperlipidemia, antioxidant and anticancer, but curcumin has low bioavailability (low solubility, poor absorption, high passage rate, high rates of metabolism in the gut cells, and rapid elimination) (Anand et al., 2007). One reason for the low bioavailability of curcumin is that it is water insoluble at acidic or neutral pH, and this causes absorption difficulty (Maiti et al., 2007). This obstacle can be solved by the development of nanoparticle technology to improve drug delivery profile, especially for the less bio-available chemical. Therefore, the application of curcumin needs polymers , such as chitosan that are capable of being carried and absorbed properly by mean of nanotechnology (nanoencapsulation).

This study was aimed to develop nanoparticle formulation using turmeric extract and industrial chitosan as the matrix and sodium tripolyphosphate as cross linker, to study its ability to improve feed digestibility in broiler chickens. Furthermore, it was aimed to show its potential as a feed additive for antibiotics substitute as well as to lower cholesterol content of meat.

Materials and Methods

The nanocapsules with formula 221 were created by ionic gelatinization method, using a ratio of turmeric extract : chitosan : TPP (sodium tripolyphosphate) = 2% : 2% : 1% (w/v). Two kinds of nanocapsules (400-870 nm) were used from: (1). Turmeric extracted and dissolved by ethanol (EE) then added with chitosan dissolved in buffer acetic pH 4 by magnetic stirrer for 20 minutes, and (2). Turmeric extracted and dissolved by aquadest (EA) and chitosan dissolved by citric acid pH 4 by magnetic stirrer for 20 minutes and then both added with TPP was dissolved in aquadest by magnetic stirrer for 20 minutes. Furthermore, the dispersed mixture was precipitated, filtered and oven-dried at 50°C.

A total of 120 broiler chickens weighing approximately 900 g were placed in individual cages equipped with feed and water. Provision of feed additive was done on 20 groups of chickens (1 group fasted and 19 groups feeding treatments shown in Table 2) with 6 replications, each replication used 1 head of chicken. Total collection method was applied in which, the first 10-day adaptation period was followed by total collection. All chickens were fasted but allowed to drink water ad-libitum on the first day of total collection. On the second day, 19 groups of chickens were fed basal rations (Table 1) plus feed additive/nanocapsule turmeric extract and 1 group remained fasting to test the endogenous N. On the third day all chickens were fasted again. Excreta were collected from the second to the third day (completed). On the fourth day all chickens were slaughtered, the ileal/digesta/contents of intestinal ileum were taken. Nutrient contents were analyzed from the samples of feed and excreta or ileum (AOAC, 1995) to determine the effect of addition of nanocapsule feed additives on the digestibility of the ration.

Nutrient digestibility. Samples of feed given (consumed) and excreta were removed, weighed, dried and further ground to pass 1 mm sieve, then swabbed ± 40 g, included in labeled plastic bags and stored at 4°C for nutrient analysis. Nutrient digestibility was calculated by the formula, as follows :

$$Nutrient\ digestibility = \frac{(Nutrient\ input) - (Nutrient\ output)}{Nutrient\ input} x100\%$$

Description: nutrients input is feed intake multiplied by the feed nutrient levels; nutrients output is out excreta multiplied by excreta nutrient levels.

Protein digestibility for the experimental diets was based particularly on the ileal digestibility of dietary protein (Lee *et al.,* 2004 modified Julendra, 2010). To determine the amount of crude protein in the excreta, ileal digesta's protein value was multiplied by the dry matter excreta. Differences in crude protein intake to protein wasted in excreta (undigested) was called digestible crude protein (Lee *et al.*, 2004). The formula referring to protein digestibility in Julendra (2010) is as follow :

1. True protein digestibility:

$$CP_{True} = \frac{CP_{fi} - (DM_E xCP_{ile} - DM_{em} xCP_{ilem})}{CP_{fi}} x100\%$$

2. Apparent protein digestibility

$$CP_{Apparent} = \frac{CP_{fi} - (DM_E xCP_{ile})}{CP_{fi}} x100\%$$

Specification formula: CP_{True} = true crude protein digested (%), $CP_{Apparent}$ = apparent crude protein digested (%),CP_{fi} =crude protein feed intake (% DM), DM_E = dry mattter excreta (% DM), CP_{ile} = crude protein ileum (% DM), DM_{em} = dry matter excreta metabolic/chicken fasted (% DM), CP_{ilem} = crude Protein ileum metabolic/ chicken fasted (% DM).

Table 1. Composition and nutrient contents of the basal ration (BR)*

Feed Materials	Percentage (%)
Milled yellow corn	52.00
Rice bran	12.50
Soybean meal	19.50
Fish meal	9.50
Palm oil	5.10
Limestone	0.30
NaCl salt	0.40
Masamix mineral-vitamin **	0.40
L-Lysine HCl	0.20
DL Methionine	0.10
TOTAL	100.00
Nutrient Contents	
Crude protein (%)	20.13
Metabolizable Energy (kcal/kg)	3201.77
Crude fat (%)	5.41
Crude fiber (%)	3.35
Calcium (%)	0.90
Available phosphorus (%)	0.43
Lysine (%)	1.29
Methionine (%)	0.50

* Standard nutrient requirements of broiler chickens 3-6 weeks (NRC, 1994): 20% protein; 1.0% Lys; 0.38% Met; energy 3200 kcal/kg, 0.9% Ca, 0.35% P.av. ** Composition per kilogram of Masamix mineral-vitamin : Ca 32.5%; P 10.0%; Fe 6.0 g; Mn 4 g; Iod 0.075 g; Zn 3.75 g; vit B_{12} 0.5 mg; vit D_3 50000 IU.

The data of nutrient digestibility were analysed by analysis of variance and further significant differences were tested using Least Significant Difference Test, by applying SPSS-16 computer program.

Results and Discussions

Nutrient digestibility of ration plus a nanocapsule of turmeric extract feed additive using chitosan cross linked sodium tripolyphosphate is shown in Table 2. Ration added with chitosan 0.1% (T2) indicated that it lowered fat digestibility compared to Basal ration (T1), because chitosan can bind dietary fat to excreta. This was in line with an opinion that chitosan from shrimp shell can bind fats (FBC) averaging 416.5% for soybean oil, 503% for corn oil, and 400.8% for the sesame oil, FBC value of shrimp shell chitosan origin is higher than the original commercial FBC crab shell chitosan (Sigma Co.)(Sofia et al., 2010). Ration added with 0.1% turmeric extract (T3) improved digestibility of dry matter, organic matter, and protein compared to basal ration (T1), because the turmeric extract contains curcumin that lowers peristaltic intestine that lengthens the digestive enzyme stimulation. This was in line with an opinion that curcumin may increase the secretions of bile, amylase, trypsin and chemotrypsin, and pancreatic lipase activity (Chattopadhyay et al., 2004). In mice and rabbits, curcumin can stimulate an increase in intestine relaxation, resulting in longer food retention in the small intestine and stimulate the secretion of hormones from the brunner glands of the small intestine (Martini, 1998). Curcumin can affect the tone and contraction of the intestine, given at the low and repeated-dose will accelerate contraction of intestinal tone, at high doses it will slow down and even stop the contractions of the small intestine, while at the right dose it will cause spontaneous contraction, i.e. digestibility and absorption of food will increase (Sinaga et al., 2010). The same finding was reported by Bawman (1983) which gives 10% intravenous fluids of Curcuma xanthoriza (meeting buffoonery) in ringer solution intravenously in experimental animals with a speed of 10-20 drops/min, tone and contraction of the small intestine will be slowed. The slower movement of the small intestine makes food moves more slowly so that the absorption of nutrients increases. The same finding was reported by Rao et al. (2003) that curcumin increases spending stimulation of pancreatic enzymes and small intestine and decreases intestinal peristaltic, thereby providing longer time for nutrients absorption of digestion products. Digestibility of dietary dry matter plus NP 0.4% (T 18) of the nanoparticles using extracted turmeric by aquades (EA) and chitosan dissolved by citric acid were significantly ($P<0.05$) better than P13 using extracted turmeric by ethanol (EE) and chitosan dissolved in acetic acid or the other rations. It was assumted that EA nanoparticles solubility in water makes it easier to interact with intestinal cells as well as in bacterial cells. The same finding was reported by Islam et al.(2008) that supplementation of 0.5% citric acid in drinking water of broiler chickens showed positive effect on live weight, feed intake and feed conversion efficiency with no detrimental effect on carcass characteristics when compared to 0 % citric or acetic acid, 0.5% acetic acid and their combinations 0.5% citric acid and 0.5% acetic acid. Ration added with TPP 0.1% (T4) improved digestibility of dry matter, organic matter, and fat but decreased protein digestibility compared to basal ration (T1), this was possible because the TPP had alkaline pH of 9.5 to 9.9 (Bhumkar and Pokharkar, 2006) and was potential to inactivate protease in proventriculus and activate lipase in the intestine. Ration added with nanoparticle (NP) using either ethanol or aquades extracted turmeric (T9–T19) improved digestibility of dry matter, organic matter, protein and fat compared to basal ration (T1). Ration combinations (T5-T8) of the building blocks of nanoparticle, both with and without the encapsulation process showed better digestibility than the basal ration.

Table 2. Nutrient digestibility of the ration plus a feed additive in broiler chickens

TREATMENTS	Dry matter Digestibility	Organic matter Digestibility	Crude Fat Digestibility	True Protein Digestibility	Apparent Protein Digestibility	Crude Fiber Digestibility
	%					
T1. BASAL RATION (BR)	70.48^{b}±0.42	73.04^{c}±0.39	69.70^{d}±0.76	51.74^{b}±1.12	51.05^{b}±1.12	27.42^{def}±2.02
T2. BR + CHITS. 0.1%	69.79^{a}±0.13	72.13^{a}±0.14	42.80^{a}±4.99	52.69^{bc}±0.23	51.98^{bc}±0.22	26.24^{cde}±1.64
T3. BR + TURM. EXT. 0.1%	70.84^{c}±0.15	73.82^{d}±0.23	68.59^{d}±0.27	57.08^{de}±0.57	56.39^{de}±0.58	27.24^{def}±0.50
T4. BR + TPP 0.1%	71.81^{e}±0.14	73.88^{d}±0.20	80.96^{f}±2.38	49.43^{a}±0.55	48.79^{a}±0.56	26.26^{de}±0.20
T5. BR +(TPP+CHITS 0.1%)	72.28^{g}±0.15	74.98^{f}±0.20	79.01^{ef}±2.04	66.35^{h}±0.35	65.69^{h}±0.35	24.98^{bc}±0.53
T6. BR +(TPP+ TURM. EXT. 0.1%)	72.44^{g}±0.07	75.32^{g}±0.19	84.84^{g}±2.63	49.07^{a}±0.51	48.39^{a}±0.552	23.82^{b}±2.18
T7. BR +(CHITS+TURM. EXT. 0,1%)	73.31^{i}±0.15	75.41^{g}±0.22	77.80^{e}±0.33	53.24^{c}±0.28	52.59^{c}±0.28	21.63^{a}±0.08
T8. BR + NON CAPSULATION 0.1% (CHIT+TURM. EXT.+TPP)	71.29^{d}±0.80	72.89^{c}±0.18	65.92^{cd}±3.82	58.01^{e}±0.58	57.31^{e}±0.59	29.04^{g}±0.27
T9. BR + NP D 0.1%	70.81^{c}±0.20	74.34^{e}±0.16	76.49^{e}±0.55	52.49^{bc}±0.26	51.79^{bc}±0.26	25.50^{cde}±0.62
T10. BR + NP EE 0.1%	70.47^{b}±0.12	73.60^{d}±0.16	63.46^{c}±1.07	62.62^{g}±2.26	61.91^{g}±2.30	29.96^{g}±2.15
T11. BR + NPEE 0.2%	71.96^{ef}±0.28	74.37^{e}±0.31	69.76^{d}±0.46	62.71^{g}±0.89	62.02^{g}±0.91	26.61^{def}±0.82
T12. BR + NP EE 0.3%	72.04^{f}±0.05	73.88^{d}±0.15	59.07^{b}±3.95	61.05^{f}±1.52	60.35^{f}±1.55	26.70^{def}±0.24
T13. BR + NP EE 0.4%	73.11^{h}±0.08	75.68^{h}±0.22	78.14^{ef}±0.89	68.55^{i}±0.32	67.83^{i}±0.32	24.96^{b}±1.32
T14. BR + NP EE 0.5%	69.88^{a}±0.03	72.43^{b}±0.06	66.17^{cd}±0.94	68.76^{i}±1.01	68.12^{i}±1.03	26.46^{def}±0.82
T15. BR + NP EA 0.1%	74.21^{j}±0.10	76.51^{j}±0.17	82.79^{fg}±0.44	65.43^{h}±0.26	64.77^{h}±0.26	27.60^{f}±0.73
T16. BR + NP EA 0.2%	73.23^{hi}±0.16	74.87^{f}±0.14	66.83^{d}±2.49	68.68^{i}±0.19	67.99^{i}±0.19	27.88^{fg}±0.37
T17. BR + NP EA 0.3%	74.19^{j}±0.17	76.09^{i}±0.11	77.37^{e}±5.48	56.38^{d}±0.82	55.67^{d}±0.83	26.77^{def}±0.79
T18. BR + NP EA 0.4%	75.90^{l}±0.24	78.52^{l}±0.24	76.05^{e}±2.30	68.48^{i}±1.65	67.79^{i}±1.68	26.20^{cde}±0.60
T19. BR + NP EA 0.5%	74.77^{k}±0.25	77.57^{k}±0.28	77.53^{e}±2.01	63.31^{g}±0.31	62.61^{g}±0.31	27.43^{ef}±0.92

Description: Values bearing different superscript on the same column differ significantly (P<0.05). CHITS. is chitosan, TURM.EXT. is turmeric extracted with ethanol 96%, TPP is sodium tri poly phosphate, NON CAPSULATION is chitosan plus turmeric extract plus TPP added in ration without encapsulation process, NP D is powder of nanoparticle formula D have concentration turmeric extracted ethanol 0.2% w/v with encapsulation process by ionic gelation, NP EE is powder of nanoparticle formula E have concentration turmeric extracted ethanol 2% w/v with encapsulation process by ionic gelation. NP EA is powder of nanoparticle formula E have concentration turmeric extracted Aquades with encapsulation process by ionic gelation.

Increasing ration digestibility of coupled nanoparticles (turmeric extract was encapsulated with chitosan crosslinked with STTP) relative to that of control was expected, because curcumin which in the chitosan-STTP nanocapsule became more available to the chicken (Figure 1) which indicated that the digestibility of the ration plus 0.1% turmeric extract (T3) was significantly smaller (P<0.01) than the other interventions T8, T14 and T19 (there were without and with the addition of chitosan encapsulation process). This was due to the nature of chitosan that can open tight junction (Sailaja et al., 2010) making it easier for the chicken intestinum to absorb curcumin, therefore, it can be digested more readily, and finally, improve the nutrient digestibility of feed. Beside in the nanocapsule, chitosan was protected by TPP by ionic bond, thus it was protected from degradation in the presence of acidic pH and proteases in the proventriculus stomach (Aranaz et al., 2009). On the other hand, free curcumin that was not encapsulated was more easily degraded in the small intestine at neutral to alkaline pH conditions (Tonnesen and Karlsen, 1985 cit. Fajria, 2009).

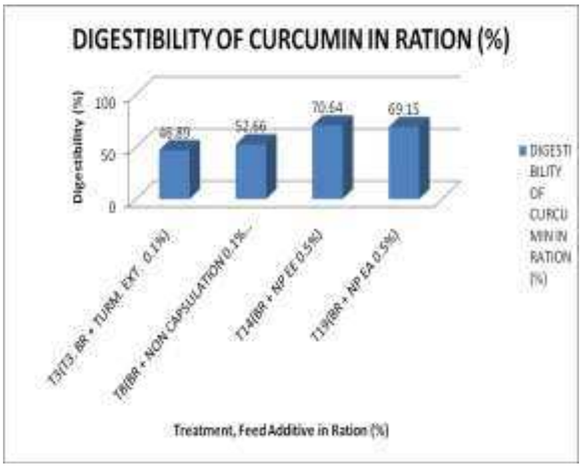

Figure 1. Digestibility of curcumin in ration (%)

Conclusions

Nanocapsule of turmeric extract with chitosan as matrix and technical sodium tripolyphosphate as cross linker has been successfully created, giving new hope as an alternative feed additive as a replacement for antibiotic growth promoters and as a lowering agent for meat cholesterol. It is concluded that the addition of nanocapsule turmeric extract to ration improves nutrient digestibility; dry matter, organic matter, crude fat, and protein.

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