ISSN 1682-8356
ansinet.org/ijps

INTERNATIONAL JOURNAL OF POULTRY SCIENCE

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International Journal of Poultry Science 13 (1): 31-35, 2014
ISSN 1682-8356

Effect of Nanocapsule Level on Broiler Performance and Fat Deposition

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Abstract: This research investigated the effects of levels turmeric extract nanocapsule or nanoparticle (NP) in ration on broiler performance and fat deposition. Seventy-two Lohmann broilers chicks were randomly divided into 6 treatments with 3 replications, each complied 4 broilers. Six treatments were Basal ration+bacitracin 50 ppm (T0), Basal-Ration/BR (T1), BR+nanocapsule 0.2% (T2), BR+nanocapsule 0.4% (T3), BR+nanocapsule 0.6% (T4), BR+nanocapsule 0.8% (T5). The analyzed variables covered production performances (feed consumption, weight gain, feed conversion ratio (FCR), carcass weight), fats deposition (abdominal fat, subcutaneous fat, EPA, DHA, total cholesterol). The data were subject to one way ANOVA analysis followed by Duncan test in case of significant effect. Results showed that the nanocapsule levels significantly ($p<0.01$) affected abdominal fat, increased meat EPA and DHA. It showed significant ($p<0.05$) effects on subcutaneous fat, total cholesterol of liver and weight gain, but no significant ($p>0.05$) effects on feed consumption, FCR and carcass weight. Accordingly, 0.4% turmeric extract nanocapsule, equal to 50 ppm bacitracin, was a compatible feed additive for broiler chicken ration to improve the meat quality of DHA-enriched and least-subcutaneous fat without negatively affecting performance.

Key words: Nanocapsule, turmeric extract, performance, fat deposition, broiler

INTRODUCTION

The uncontrolled and unlimited use of antibiotic leads to accumulative residue which is harmful for animals and their products (Wachira *et al.*, 2011). Consumed antibiotics residue can cause allergy, poisoning and germ resistance (Kusumaningsih *et al.*, 1996). Heart disease and arteriosclerosis are closely-related to cholesterol intake and saturated fat as the main cause of death (Omojola *et al.*, 2009). Fat or high energy in ration makes cholesterol in the drumstick of broiler reach 87.6 mg/100 g (Daneshyar *et al.*, 2011). The controversy of antibiotic and high energy ration as broiler feed additive has been a dilemma that requires efforts to find the alternative natural feed additive to replace antibiotic and reduce cholesterol. Curcumin extracted from turmeric has biological functions such as antibiotic, antiinflamation, anticoagulant and hipolipidemic; however, its usage for therapy is very limited because of its low solubility, fast metabolism and easy excretion (Anand *et al.*, 2007) or low systemic bioavailability (Bisht *et al.*, 2007). Curcumin antibacterial activities are related to polyphenol which can denaturize protein (Okoro *et al.*, 2010) and destroy bacterial cell membrane (Bhawana *et al.*, 2011). Curcumin is easily degraded in neutral pH solution and light (Goel *et al.*, 2008). In order to maximize curcumin, technology and polymer are needed to transport for effective absorption, as in nanoparticle chitosan which is cross-linked to STPP (Sodium Tripolyphosphate) to become nanocurcumin (Kar *et al.*, 2010). The use of nanocurcumin for animal is considered costly, therefore this research used turmeric extract as the nanoparticles (NP) to substitute antibiotic as feed additive in the ration. The dosage of 160 ppm curcumin can be used as synthetic antibiotic substitute to boost pig's growth (equal to virginiamicin 50 ppm) (Sinaga *et al.*, 2010). Al-Sultan (2003) reported that supplying 0.5% turmeric powder (equal to 165 ppm curcumin) in broiler ration was expected to result in better weight and good ration conversion. Given 400 mg/kg curcumin for 10 weeks, the rats was expected to have lower total cholesterol and LDL cholesterol and to form foam cell more effectively (Fikriah, 2007).

MATERIALS AND METHODS

Experimental design: The research was subject to one-way CRD (Completely randomized design), classifying 72 broilers aged 2-6 weeks into 6 treatments with 3 repetitions, each contained 4 broilers. The six groups were given feed additive namely: BR + bacitracin 50 ppm (T0, controle positive), Basal-Ration/BR (shown in Table 1) + nanocapsule 0.0% (T1, controle), BR + nanocapsule 0.2% (T2), BR + nanocapsule 0.4% (T3), BR+nanocapsule 0.6% (T4), BR+nanocapsule 0.8% (T5). Feed and drinking water were given *ad-libitum* during four weeks.

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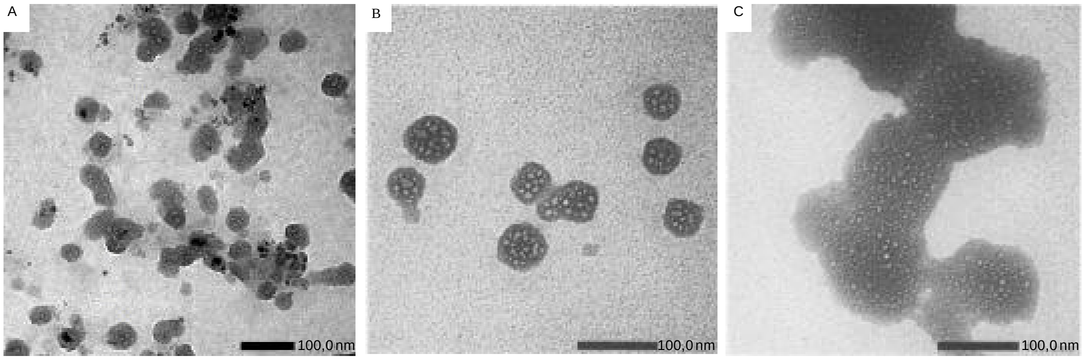

Fig. 1: Transmission electron micrographs of turmeric extract nanocapsule: (A) Turmeric extract concentration 0.02%, (B) Turmeric extract concentration 0.2% and (C) Turmeric extract concentration 2%at magnification 30000

Procedure: Nanoparticles or nanocapsule were made by using bottom-up approach with ionic gelation method. To obtain the feasible formulation for broiler chicken ration, several concentration (A, B and C) were tested and the particle diameters were measured using transmission electron microscope as shown in Fig. 1. Since formula A and B were not feasible, nanocapsule formula C was used and produced, namely 50 g in 1 L solution consisting of 20 g turmeric extract dissolved in 100 mL ethanol, 20 g chitosan dissolved in 800 mL buffer asetat pH 4 and 10 g STPP dissolved in 100 mL aquadest. By using magnetic stirrer, chitosan solution added to turmeric extract solution was homogenized for 20 min, added with STPP solution, then stirred again for 20 min. The suspension was then filtered and oven-dried at 50°C for one night (12 h), then grounded using blender to produce meal the ready use ±40 g NP.

Parameter measured: The variables included feed consumption, body weight gain, Feed Conversion Ratio (FCR), carcass weight, abdominal fat (Fadilah, 2005) and subcutaneous fat measured by Soxhlet method (AOAC, 2006), EPA (eicosapentaenoic acids) and DHA (docosahexaenoic acids) of meat measured using Gas Chromatography (Supadmo, 1997) and total cholesterol (TC) measured using modification CHOD-PAP method (Supadmo, 1997) to test TC of serum, liver and meat.

Data analysis: The data obtained were subject to Analysis of Variance (ANOVA), followed by Duncan test in case of significant effect using SPSS-16.

Table 1: Composition of basal ration (BR)[1]

Ingrediens	Starter (0-3 wks) (%)	Grower (3-6 wks) (%)
Yellow Corn	52.00	52.00
Rice brand	10.00	12.50
Soy Bean Meal 45	21.00	19.50
Fish meal 55	12.00	9.50
Crude Palm Oil	3.70	5.10
Limestone	0.13	0.30
Salt NaCl	0.08	0.20
Masamix[2]	0.44	0.10
L-Lysine HCl	0.35	0.40
DL Metionin	0.30	0.40
Total	100.00	100.00
Nutrient composition		
Crude protein (%)	22.13	20.21
ME (kcal/kg)	3143.99	3201.77
Extract eter (%)	5.3	5.41
Crude fiber (%)	3.14	3.35
Calsium (%)	0.92	0.90
Phosphor available (%)	0.5	0.43
L-Lysin HCl (%)[3]	1.51	1.41
DL-Methionine (%)[3]	1.41	1.35

Description:

[1]Nutrient requirement of broilers (NRC, 1994)

[2]Composition of masamix per kilogram:

vit A 810000 IU	D3 212000 ICU
E 1.8 g	K3 0.18 g
B1 0.112 g	B2 0.288 g
B6 0.3 g	B12 0.0036 g
Co 0.028 g	Cu 0.5 g
Fe 6.0 g	Mn 6 g
Iod 0.1 g	Zn 5 g
Se 0.025 g	DL-Met 212.5 g
L-Lys 31 g	Foleic ac. 0.11 g
Panthotenic ac. 0.54 g	Niacin (vit B3) 2.16 g
Cholin Cl60% 75 g	

[3]Higher of NRC (1994) but non excess of Lys (<3%) and Met (<2%) (Acar *et al.*, 2001)

RESULTS AND DISCUSSION

Production performance: Table 2 shows that basal ration added with 0.2% NP (T2) produced the best FCR (non-significant difference, p>0.05). NP intake of ≥ 0.2% reduced the growth (non-significant difference, p>0.05) accompanied by the decrease of cholesterol (Table 4) and the intake of ≥ 0.4% NP reduced the feed consumption (non-significant difference, p>0.05). In conclusion, the use of turmeric extract nanocapsule was optimum at 0.2% and maximum at 0.4%. At level > 0.4% NP, ration became poisonous followed by the significant increase of DHA (Table 3). DHA is used by the body to reduce the curcumin effect as anticoagulant (Chattopadhyay *et al.*, 2004). Curcumin increases

Table 2: Effect of additional nanocapsule of turmeric extract in ration on productions performance: feed consumtion, weight gain, feed conversion ratio and carcass weight of broiler chicken

Treatment	Feed consumtion (g) average^{ns}±SEM	Weight gain (g) average*±SEM	Feed conversion ratio average^{ns}±SEM	Carcass weight (g) average^{ns}±SEM
T0	3428.3±47.709	1925.5±86.30^{a}	1.79±0.07	1588.3±86.81
T1	3345.1±45.150	1865.2±69.14^{a}	1.79±0.05	1577.3±20.09
T2	3436.3±78.470	2019.2±81.64^{ab}	1.71±0.11	1591.7±78.09
T3	3522.1±39.621	1834.8±77.43^{a}	1.93±0.07	1518.3±33.46
T4	3415.8±55.570	1830.7±05.72^{a}	1.88±0.14	1456.7±67.88
T5	3377.5±115.472	1890.0±88.49^{a}	1.80±0.12	1490.7±49.26

Description: *Values bearing different superscripts indicate significant difference (p<0.05), ^{ns}superscripts indicate non-significant differences (p>0.05),
T0 (BR+Bacitracin 50 ppm) T1 (BR/Basal ration) T2 (BR+Nanocapsule 0.2%
T3 (BR+Nanocapsule 0.4%) T4 (BR+Nanocapsule 0.6%) T5 (BR+Nanocapsule 0.8%)

Table 3: Effect of additional nanocapsule of turmeric extract in ration on fat deposition: abdominal fat, subcutaneous fat, EPA and DHA meat broiler chicken (%)

Treatment	Abdominal fat average**±SEM	Subcutaneous fat average*±SEM	EPA (C20:5n3) average**±SE	DHA (C22:6n3) average**±SE
T0	1.54±0.15^{a}	55.21±2.82^{ab}	1.27±0.28^{a}	1.67±0.07^{ab}
T1	1.50±0.12^{ab}	65.60±10.86^{bc}	1.86±0.20^{ab}	1.50±0.04^{a}
T2	1.92±0.10^{c}	53.05±8.43^{ab}	1.28±0.03^{a}	1.63±0.07^{ab}
T3	1.90±0.10^{c}	43.89±0.83^{a}	1.38±0.18^{a}	2.08±0.00^{c}
T4	1.86±0.12^{bc}	52.11±3.59^{ab}	1.96±0.17^{ab}	2.32±0.04^{cd}
T5	1.88±0.06^{bc}	57.06±3.71^{ab}	2.19±0.16^{b}	2.45±0.15^{d}

Description: *Values bearing different superscripts indicate significant difference p<0.05), **Values bearing different superscripts in the same column indicate significant differences (p<0.01)
T0 (BR+Bacitracin 50 ppm) T1 (BR/Basal ration) T2 (BR+Nanocapsule 0.2%
T3 (BR+Nanocapsule 0.4%) T4 (BR+Nanocapsule 0.6%) T5 (BR+Nanocapsule 0.8%)

Table 4: Effect of additional nanocapsule of turmeric extract in ration on total cholesterol content of serum, liver and meat broiler chicken (mg/dL)

Perlakuan	TC serum average^{ns}±SEM p = 0.875	TC liver average*±SEM p = 0.01	TC meat average^{ns}±SEM p = 0.093
T0	108.83±18.83	387.27±60.00^{cd}	13.33±5.05
T1	103.92±10.26	265.46±18.19^{bc}	20.70±5.44
T2	98.82±19.19	294.55±10.91^{bcd}	31.92±8.04
T3	115.30±7.80	223.64±1.82^{b}	21.72±5.82
T4	110.59±16.47	169.09±121.82^{b}	15.72±14.83
T5	101.18±13.21	61.882±25.46^{a}	12.04±3.24

Description: *Values bearing different superscripts indicate significant difference p<0.05), ns superscripts in the same column indicate non-significant differences (p>0.05)
T0 (BR+Bacitracin 50 ppm) T1 (BR/Basal ration) T2 (BR+Nanocapsule 0.2%
T3 (BR+Nanocapsule 0.4%) T4 (BR+Nanocapsule 0.6%) T5 (BR+Nanocapsule 0.8%)

bleeding and denaturates protein (Okoro *et al*., 2010) and it destroys bacteria cell membrane (Bhawana *et al*., 2011). It is in accordance with the previous findings that low level curcumin serves as antioxidant, but excessive use will turn it into pro-oxydant (Lopez-Lazaro, 2008). The findings are in line with Hosseini-Vashan *et al*. (2012) that 0.8% turmeric powder intake (equal to 0.4% NP) had no effect on feed consumption.

Broiler fats deposition (abdominal fat, subcutaneous fat, EPA and DHA meat): The research showed that by adding basal ration (T1, lisin and metionin-rich) the least percentage of abdominal fat was significantly (p<0.05) produced compared to T3 and T4. In T0 the percentage of abdominal fat was 1.54%, smaller than that of basal ration added with topmix (with 42 ppm bacitracin) producing 1.75% abdominal fat (Wahyurinaningsih, 2001). Previous researchers stated that the use of turmeric powder reduced the abdominal fat (Al-Sultan, 2003; Zainali *et al*., 2009). Ration added with 0,4% nanoparticle (T3) was capable of significantly reducing subcutaneous fat (p<0.05) compared to basal ration (T1). It is also in line with (Ejaz *et al*., 2009) that curcumin was able to inhibit the expression of PPARγ and C/EBPα as the main transcription factor in adipogenesis and lipogenesis in adipos subcutaneous.

Table 3 shows that the supplementation of turmeric extract nanocapsule of >0.4% was significant (p<0.05) in increasing DHA, indicating curcumin's ability to transform AA (arachidonic acid) metabolism into EPA and DHA of the broiler and its functions as anti-inflammatory. With the existence of curcumin, both ciclooxigenase and lipoxigenase are blocked, so that the conversion of AA into PGE2 and LTB4 proinflammation are strongly blocked. Because AA is strongly blocked by curcumin and the enzyme that catalizes AA and EPA is the same namely delta-5-desaturase, it smoothens the EPA. Due to the fact that linolenic acid (LNA) has multiple bonds, more enzymes operating in the same path tend to work faster in LNA Smith and Borgeat (1985) cit. Rusmana *et al*. (2008), so it can increase DHA synthesis.

At the dosage of NP $\leq$ 2%, curcumin contributed to body weight gain as the primary product and at the dosage of NP > 2% the body weight gained would decrease (non-significant difference, $p>0.05$) but followed the increase of EPA and DHA (significant difference, $p<0.05$). The increase of EPA and DHA was useful to balance the side effect of curcumin as anti-coagulant which could cause bleeding due to the fragility of the endothelial wall or the destruction of cell membrane caused by decreasing liver, blood and meat cholesterol (Table 4). The decrease of cholesterol would hamper the function of cell membrane. The findings were in accordance with Sukandar *et al.* (2008) that 100 mg/kg of turmeric extract and garlic can increase the bleeding time and hamper platelet aggregation.

All treatments of turmeric extract nanocapsule (Table 4) did not show significant differences ($p>0.05$) in the decrease of blood serum and meat cholesterol, but there was a significant difference ($p<0.05$) in the decrease of liver cholesterol. THC (tetra hidro curcumin, a derivative of curcumin) at the level of 80 mg/kg used for hypercholesterol rabbit feed can reduce HMG CoA reductase activity (Prasanth *et al.*, 2012) while the intake of curucumin along with the supplement of lisin-metionin tend to increase the insulin which can increase the activities of HMG CoAreductase (Ness and Chambers, 2000). The existence of curcumin and insulin at the same time causes the neutral effect on synthetic cholesterol. It is in accordance with Srinivan (2005) who stated that 0.5% curcumin intake in mouse feed stimulated the production of bile acid (62%). It is also in line with Feng *et al.* (2010) and Zhao and Dahlman-Wright (2010) who reported that curcumin reduces mRNA encoding plasma NPC1L1 of synthetic protein that transports from cholesterol in cell membrane so that it can hamper the cholesterol uptake in enterocytes. In T3, there was a significant ($p<0.05$) decrease in subcutaneous fat compared to other treatments due to body cholesterol withdrawal to the liver in order to synthesize the bile and eliminate through excreta so that the deposit in the subcutaneous decreased.

Conclusion: The turmeric extract nanocapsule could be used as feed additive for broiler chicken ration at level 0.4% (equal to 50 ppm bacitracin used). It could improve the meat quality of DHA-enriched and least-subcutaneous fat without negatively affecting performance.

ACKNOWLEDGMENTS

The authors are grateful to the Directorate of Research and Community Service, the Directorate General of Higher Education, Ministry of Education and Culture of the Republic of Indonesia for financial support through BPPS scholarships and research grants "multidisciplinary UGM".

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