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Effects of Methionine-Cysteine Amino Acid Supplementations in the Aflatoxin B1 Contaminated Diet on Broiler Production Performance

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ABSTRACT

This research aimed to observe the interaction of methionine-cysteine amino acid supplementation to decrease the effect of aflatoxin B1 (AFB1) on diet against production performance of broiler chicken. A number of 240 mixed sex broiler chickens were treated in 9 treatments by factorial design 3 x 3 with methionine-cysteine amino acid (M+C) (75,100, dan 125%) factors and AFB1 levels (0, 200, dan 400 ppb). Variables observed were: Weight gain, feed consumption, and feed conversion ratio (FCR). The results showed that increased AFB1 content in diet from 0 to 400 ppb increased chicken body weight (P <0.05) in each age group. The high body weight was balanced with high feed consumption along with increased nutrient needs, mainly sulfuric amino acid (M+C) as the precursor of glutathione to eliminate toxic through conjugation reactions. The interaction effect was firstly occurred between M + C and AFB1 treatment (P <0.05). Meanwhile increased supplementation of M + C from 75 to 125% caused decreased feed consumption in each age group of chickens, but increased AFB1 levels further increased feed consumption (P<0.05). The interaction effect between the level of M + C and AFB1 contamination in diets on feed consumption were seen in 21-day-old chickens (P<0.05). FCR was also increased (P <0.05) with the reduction of M + C content in diet at 7 days old. The effect of AFB1 on diet and interaction between M + C and AFB1 on chicken FCR in this study was not significant in all age groups. It can be concluded from the current study that supplying methionine-cystine amino acid with 75, 100 and 125% in AFB1 contaminated diet of 0, 200 and 400 ppb improves the performance of broiler chicken production.

Keyword: Aflatoxin B1, Broiler chicken, Detoxification, Methionine-cysteine

Introduction

Poultry product mainly broiler is the largest contributor of national meat production with 62.56% (Dirjen Peternakan, 2013). The important factor to increase livestock productivity is feed. Corn is one of energy source in feed reaching 60% of total feed ingredient. Tropical climate condition in Indonesia with high temperature and humidity will accelerate the occurrence of reduced feed ingredient quality and growth of mold during storage, especially *Aspergillus flavus* and *A. parasiticus*, producing a secondary metabolite called aflatoxin.

Aflatoxin B1 (AFB1) is the most pathogenic mycotoxin, which is carcinogenic and causes aflatoxicosis, inhibiting the livestock production and leaving residue when consumed by human, therefore impacting the human health quality. The influence of AFB1 is unably underestimated, as it causes loss or damage to both livestock and humans. Based on Indonesian National Standard, the aflatoxin level that can be tolerated tested in feed is 50 ppb.

Approach conducted in this study was supplementing methionine and cysteine amino acid in the diet formulation to reduce the toxic effects inflicted. Methionine and cysteine amino acid are amino acids containing sulphur as glutathione precursor (y-glutamylcysteinglycine), which eliminates toxins through conjugative reactions placed in liver and transforms into nontoxic form that can be secreted.

Materials and Methods

This study was conducted in Leather, Waste and By-Products Technology Laboratory, Faculty of Animal Science UGM on January – Mei, 2015 to contaminate corn ingredient with *Aspergillus flavus* fungi producing aflatoxin. The second study step, namely broiler chicken maintenance was performed in Poultry Science Laboratory, Faculty of Animal Science UGM on October 2015.

Materials

Chicken maintenance. Chickens used were 240 *Lohmann MB 202 platinum* broiler chickens which had been vaccinated from live Newcastle Disease (ND), IB-ND killed, and IBD Transmune. Diets used are presented on Table 1. Cages used were open house cage with 40 cages. Diet composition and nutrient content can be seen on Table 1, while diet proximate analysis can be seen on Table 2.

Methods

Corn contamination. Corns used in this diet were contaminated with *A. flavus* fungi isolates obtained from PAU UGM with FNCC 6122 and FNCC 6109 code, besides from SEAMEO BIOTROP Bogor with BIO 2237 and BIO 2236 code. Four isolates were grown on corn media that was used in diet formulation for 10 – 15 days at 25°C. Aflatoxin activity was determined with Elisa assay method using *Maxsignal® Total Aflatoxin Elisa Kit BIO Scientific*.

Chicken maintenance. Maintenance was performed in the cage of Poultry Science Laboratory, Faculty of Animal Science, UGM. 240 chickens were grouped into 9 different treatments. Each treatment was replicated 5 times containing 6 chickens. Diet treatments were given based on:

T1 = (M+C) 75% + AFB1 0 ppb diet
T2 = (M+C) 100% (normal) + AFB1 0 ppb diet
T3 = (M+C) 125% + AFB1 0 ppb diet
T4 = (M+C) 75% + AFB1 200 ppb diet
T5 = (M+C) 100% (normal) + AFB1 200 ppb diet
T6 = (M+C) 125% + AFB1 200 ppb diet
T7 = (M+C) 75% + AFB1 400 ppb diet
T8 = (M+C) 100% (normal) + AFB1 400 ppb diet
T9 = (M+C) 125% + AFB1 400 ppb diet.

Chickens were maintained until 21 days old. Treatments began from DOC (0-21 days). Diets and water were given *ad libitum*. Cages made from iron shaped as cages/wire were cleaned and fumigated with 4% formalin before used.

Data sampling. Maintenance and diet treatment given were performed from 0-21 days old. The result of growth performance, namely weight gain, feed consumption, and feed conversion ratio (FCR), were taken when chickens aged 7,14, and 21 days.

Data analysis

Design pattern used was factorial design 3x3 with aflatoxin (0, 200, 400 ppb) and M+C (75%, 100%, 125%) factor. Data were processed with Analysis of variance (ANOVA) using *SPSS* software, then continued with Duncan's multiple range test (DMRT).

Result and Discussion

Bodyweight

Statistical analysis result based on factorial pattern on Table 3 shows increased weight gain on broiler chickens ($P<0.05$) along with increased AFB1 level given to all age groups. Bodyweight also increased ($P<0.05$) as M + C

Table 1. Basal dietary nutrient

Feedstuff	Percentage (%)	Percentage (%)
Corn	57.700	57.700
Soybean meal (SBM)	34.690	34.690
Palm olein	2.900	2.900
Dicalcium Phospate	1.350	1.350
Choline	0.005	0.005
DL-Methionine	0.044	0.515
Premix*	0.210	0.210
Salt (NaCl)	0.360	0.360
L-Lysin HCl	0.200	0.200
L-Threonine	0.070	0.070
Bone meal	2.000	2.000
Sand	0.471	0
Total	100	100
Dietary Nutrient Calculation		
ME	3,014.805	3,014.81
CP	21.77	22.04
Ile	0.90	0.90
Lys	1.27	1.27
Met	0.36	0.83
Cys	0.35	0.35
TSAA	0.71	1.179
Thr	0.86	0.87
Trp	0.22	0.22
Valin	0.91	0.91
Ca	0.95	0.95
P avl	0,76	0.76
K	0,87	0.87
Cl	0.3	0.3
Na	0.187	0.188
Zn	85.613	85.73

*Mixed vitamin, cocxydiostat, synthetic amino acid, mineral, and growth promotor. Notes: ME= Metabolic Energy, CP= Crude Protein, Ca= Calcium, K= Potassium, Cl= Chlorine, Na= Sodium, Zn= Zinc.

Tabel 1. Diet proximate analysis

Treatment	M	CL	CP	CF	Ash	Ca	Na	P
T1	8.90	5.95	21.76	2.77	6.06	0.899	0.210	0.74
T3	9.04	6.03	21.94	2.92	6.07	0.939	0.218	0.72
T6	9.04	4.54	21.53	2.88	5.86	0.897	0.221	0.78
T9	9.02	4.51	21.22	2.95	5.99	0.904	0.216	0.75

Notes: CL= Crude lipid, M= Moisture, CP= Crude protein, CF= Crude fiber. Proximate analysis was conducted in Feed Nutrition Laboratory, Faculty of Animal Science, IPB University.

level decreased in feed at 21 days old and occurred an interaction effect between M + C and AFB1 level (P<0.05).

Increased AFB1 content from 0 to 400 ppb resulted significant chicken weight gain on each age group. This happened as the amount of feed consumption also increased along with elevated AFB1 in diet. High body weight balanced with high feed consumption may be due to increased nutrient requirements, mainly sulphuric amino acid (M + C) as glutathione precursor to eliminate toxic through conjugative reactions. Glutathione levels in cells are limited, therefore the protection against toxic is also limited (Donatus, 2001) and requires more M + C of intake from feed to increase the level of glutathione in the body. The result of body weight was very different from the existing theories of previous studies. Previous studies reported that aflatoxin negatively affected the performance of broiler chickens, i.e decreasing consumption, growth rate, and productivity characterized by low weight gain, reproductive disorder in the form of decreased egg weight and stress resistant, reduced pigment on carcasses, abnormality, and death (Bahri and Maryam, 2004; Yunus *et al*., 2006; Pasha *et al*., 2007).

The interaction effect between M + C and AFB1 against the body weight were observed seen in 21 days old chicken. The highest body weight were found on T7, T9, and T8 treatment with 400 ppb AFB1 and 75, 125, and 100% M + C respectively, while the lowest was found on T2 (100% M + C and 0 ppb AFB1). High body weight on Table 4 is followed with high feed consumption on Table 5.

Amount 200 and 400 ppb AFB1 was still tolerated by the livestock, therefore aflatoxicosis symptoms were unclearly seen, likely the same with Kermanshahi *et al*. (2009) and Yunianta (2013) who provided AFB1 500 and 1,000 ppb exposure as well as Utami (2009) who gave the highest AFB1 exposure with 1,500 ppb level that causesd the livestock to experience aflatoxicosis. Livestock in this study had begun to adapt well as likely reported by Prasetyo and Susanti (2013) using local ducks. Duck is the most sensitive livestock to AFB1, but this livestock productivity was still optimal after AFB1 150 ppb exposure. This study indicates that chickens begin to adapt by increasing the feed consumption to induce M + C intake, therefore utilized as glutathione precursor to aid the toxic elimination through conjugative reactions.

Feed consumption

The results of statistical analysis with factorial pattern on Table 4 showed decreased feed consumption (P<0.05) along with higher level M + C given, either at 7, 14, or 21 days old. Increased feed consumption was occurred (P<0.05) as AFB1 level increased in diet on all age groups. The interaction effect between M + C and AFB1 level only appeared at 21-day old chickens (P<0.05).

Increased M + C from 75 to 125% generally resulted in significant decrease of feed consumption on each age group of chickens. This was due to sulphuric amino acids (M + C) requirement in feed could increase as the livestock lack of sulphuric amino acids supply, therefore the feed consumption increased to fulfill the content of sulfuric amino acids (Aftab and Ashraf, 2009), thereby 75% M + C level had higher feed consumption than 125% M + C consumption,

Table 3. Broiler chicken weight gain on 7, 14, dan 21 days old (g)

AFB1	M+C 75%	100%	125%	Average
7 days old				
0 ppb	139.4±7.4	128.9±9.3	138.9±3.5	135.9±7.9^{m}
200 ppb	141.1±3.8	145.6±6.7	146.1±6.1	144.4±5.7^{l}
400 ppb	152.7±9.2	152.3±2.2	151.2±12.2	152.0±8.5^{k}
Average	145.1±9.3	142.3±11.9	145.4±9.2	
14 days old				
0 ppb	372.6±8.6	336.0±23.0	361.7±6.5	357.2±20.1^{l}
200 ppb	462.9±96.7	431.4±20.2	413.7±24.5	434.3±55.6^{k}
400 ppb	458.5±8.8	446.3±16.3	458.8±29.6	455.1±19.7^{k}
Average	433.4±64.5	404.5±54.1	411.4±46.1	
21 days old				
0 ppb	718.2±68.8^{xy}	513.2±37.1^{z}	584.8±77.6^{yz}	603.8±103.8^{m}
200 ppb	829.8±70.5^{wx}	849.0±69.9^{wx}	741.1±69.6^{x}	801.6±81.3^{l}
400 ppb	938.5±30.0^{w}	914.7±16.8^{w}	923.3±36.9^{w}	926.3±29.3^{k}
Average	837.3±108.3^{a}	759.0±188.4^{b}	749.8±154.9^{b}	

^{a,b,c} Different superscript in the same row and age indicate significant differences (P<0.05) against M+C level.
^{k,l,m} Different superscript in the same column and age indicate significant differences (P<0.05) against AFB1 level.
^{w,x,y,z} Different superscript in the same age indicate significant differences interaction (P<0.05) between M+C and AFB1 level.

Table 4. Cumulative feed consumption during the study (g)

AFB1	M+C			
	75 %	100 %	125 %	Average
7 days old				
0 ppb	211.4±14.9	179.3±11.3	182.5±13.9	190.4±19.1^{l}
200 ppb	215.6±29.2	209.4±12.7	198.0±14.6	206.9±19.6^{k}
400 ppb	212.8±10.3	224.0±28.0	204.8±16.7	213.1±19.0^{k}
Average	213.2±17.5^{a}	204.2±25.9^{ab}	195.1±17.0^{b}	
14 days old				
0 ppb	398.4±87.0	330.7±28.2	331.0±48.6	351.6±62.7^{m}
200 ppb	438.2±10.3	425.2±29.6	401.7±35.5	420.2±30.4^{l}
400 ppb	471.5±46.5	458.6±15.7	436.0±39.2	455.1±38.0^{k}
Average	438.8±60.2^{a}	404.8±61.0^{ab}	389.6±59.3^{b}	
21 days old				
0 ppb	926.5±225.2^{y}	505.20±37.3^{z}	555.82±171.2^{z}	654.3±242.8^{m}
200 ppb	942.0 ±71.2^{y}	1001.7±222.5^{xy}	798.81±82.4^{yz}	905.3±155.5^{l}
400 ppb	127.7±47.4^{x}	1283.1±20.3^{x}	1212.0±113.3^{x}	1255.8±76.7^{k}
Average	1066.3±212.0^{a}	930.02±356.1^{b}	855.56±304.3^{b}	

^{a,b,c} Different superscript in the same row and age indicate significant differences (P<0.05) against M+C level.

^{k,l,m} Different superscript in the same column and age indicate significant differences (P<0.05) against AFB1 level.

^{w,x,y,z} Different superscript in the same age indicate significant differences interaction (P<0.05) between M+C and AFB1 level.

according to feed consumption data at 7, 14, and 21 days old on Table 5. Ukachukwu *et al*. (2007) explained that the methionine deficiency in feed will lead to increased feed consumption, reduced feed efficiency, stunted growth, fewer feathers, and lipid carcass proportion.

Data on Table 5 also indicates increased feed consumption in chickens on any age group as increased AFB1 level given in feed (P<0.05). This occurred due to increased AFB1 exposure made the chickens require more of M + C amino acids intake to aid eliminate toxic AFB1 through conjugative reactions. The elimination capability of AFB1 depends on M + C in diet, therefore increased AFB1 exposure also elevates the feed consumption. Aftab and Asharf (2009) stated that the need for sulphuric amino acids (M + C) in feed will increase when livestock is deficient from feed intake or requires more sulphuric amino acids, therefore causing increased feed consumption to fulfill the content of sulphuric amino acids.

The interaction effect between M + C and AFB1 in diet on feed consumption was occurred on 21 day old chickens (P<0.05). The highest feed consumptions were obtained from T8, T7 and T9 treatments, namely 100.75, and 125% M + C and 400 ppb AFB1. The lowest feed consumptions were found on T2 and T3 treatments, namely 0 ppb AFB1 with 100 and 125% M + C respectively. Results on Table 4 indicates that AFB1 in feed increases feed consumption rate, whether containing high (125%) or low (75%) M + C. This means that higher level of AFB1 will require more M + C supply, causing the chickens to consume more feed. Interactions appeared on 21 days old due to AFB1 and M + C diets were consumed for longer period. AFB1 detoxification takes place in the liver; Liver was unable to detoxify AFB1 from the body during the time, thereby requiring M + C supply from diet as glutathione precursor to conduct conjugative reactions. Increased consumption signifies how M + C will be maximized to eliminate AFB1. Increased feed consumption resulted increased M + C content. Methionine and cysteine as glutathione precursors (Cofey *et al*., 1989; Yunianta, 2013) are able to aid AFB1 elimination through conjugative reactions, thereby reducing the effect of aflatoxicosis, then avoiding declined body weight on chickens consuming 400 ppb AFB1 as presented on Table 6. Glutathione conjugation of AFB1 takes place in liver, which is converted into a non-toxic form that can be secreted.

Feed consumption in this study was higher than the standard commercial feed consumption based on Aviagen (2014), namely 169 g at 7 days old, 361 g at 14 days old, and 675 g at 21 days old. This study was also not in accordance with the existing theory, whether AFB1 in feed is able to disrupt feed consumption, affecting the livestock production. Smith *et al*. (1994), Nahm (1995), and Yunus *et al*. (2006) stated that the effects of AFB1 on poultry are reduced feed consumption, slow growth, liver carcinoma, immunosuppressive, and death.

Feed conversion ratio (FCR)

The analysis result of factorial pattern on Table 5 showed increased FCR (P<0.05) along with lower M + C content in diet at 7 days old. The effect of AFB1 supplementation on feed and interaction effect between M + C and AFB1 against FCR chickens in this study were insignificant (P<0.05) at all age groups.

The results showed that significant increased FCR as decreased M + C rate was found at 7 days old. High feed conversion shows high feed consumption, but low meat production. This can be caused as low M + C content can increase feed consumption, unaccompanied by increased body weight during chicken growth period. Aftab and Asharf (2009) mentioned that low content of sulphuric amino acids (M + C) in feed can increase feed consumption required for SAA content fulfillment, but causing low body weight increase, resulting high FCR. Deaner and Bessei (2003), Hoehler *et al*. (2005), and Yong *et al*. (2012), reported that improper use of methionine can lose the weight of broiler chicken, increase feed conversion value (FCR), and induce mortality rate. Excess M + C imbalanced with the

Table 5. Feed conversion ratio (FCR) on 7, 14, and 21 days old

AFB1	M+C			
	75%	100%	125%	Average
7 days old				
0 ppb	1.52±0.18	1.39±0.14	1.31±0.13	1.40±0.16
200 ppb	1.52±0.19	1.44±0.03	1.36±0.13	1.43±0.14
400 ppb	1.39±0.03	1.47±0.19	1.37±0.18	1.41±0.14
Average	1.47±0.15[a]	1.43±0.13[ab]	1.35±0.14[b]	
14 days old				
0 ppb	1.70±0.32	1.59±0.04	1.49±0.23	1.59±0.23
200 ppb	1.44±0.35	1.49±0.04	1.50±0.13	1.48±0.19
400 ppb	1.54±0.14	1.56±0.13	1.43±0.19	1.51±0.16
Average	1.56±0.27	1.55±0.09	1.47±0.18	
21 days old				
0 ppb	2.67±0.41	2.85±0.08	2.51±0.30	2.66±0.31
200 ppb	2.62±0.35	2.43±0.53	2.50±0.47	2.52±0.43
400 ppb	2.67±0.22	2.74±0.12	2.61±0.21	2.67±0.19
Average)	2.65±0.30	2.67±0.34	2.54±0.32	

[a,b,c] Different superscript in the same row and age indicate significant differences (P<0.05) against M+C level

use of sulphuric amino acids in the toxic conjugation, mainly AFB1, can decrease body weight and enlarge FCR value.

The effects of AFB1 and interaction between M + C against FCR of chickens in this study were insignificant in all age groups. This shows that all age groups produce the same bodyweight as the amount of feed consumed, however, FCR in this research was relatively high compared to other studies. Wijaya (2015) stated that FCR chicken with M + C treatments in feed have excellent FCR, i.e 1.026 at 21 days old, while Yunianta (2013) obtained FCR value of 1.14 – 1.17 at the same age after AFB1 and methionine treatments. High FCR shows the feed consumed is more than the weight gain produced. FCR is an indicator of the livestock efficiency to transform the feed into meat production.

Conclusions

This study concludes that the supplementation of methionine-cysteine amino acid with 75, 100, and 125% in AFB1 contaminated diet of 0, 200, and 400 ppb on broiler chicken indicates that the chickens were adaptive by elevating feed consumption to improve the production performance due to toxic exposure. Statistical test results showed that the interaction between methionine-cysteine amino acid supplementation with different aflatoxin B1 level (P<0.05) was significantly different against weight gain and feed consumption at 21 days old. AFB1 exposures in feed increases the feed consumption as a result of requiring more methionine-cysteine amino acid in the feed, thereby increasing the body weight, but does not improve FCR value.

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