



Revista Brasileira de Ciência Avícola

ISSN: 1516-635X

revista@facta.org.br

Fundação APINCO de Ciência e  
Tecnologia Avícolas  
Brasil

Lei, XJ; Park, JH; Hosseindoust, A; Kim, IH  
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Revista Brasileira de Ciência Avícola, vol. 19, núm. 2, abril-junio, 2017, pp. 239-245  
Fundação APINCO de Ciência e Tecnologia Avícolas  
Campinas, Brasil

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#### ■Keywords

Ammonia emission; cassava root meal; corn dried distillers grains with solubles; egg quality; production performance.

## Effects of Cassava (*Manihot esculenta crantz*) Root Meal in Diets Containing Corn Dried Distillers Grains with Solubles on Production Performance, Egg Quality, and Excreta Noxious Gas Emission in Laying Hens

### ABSTRACT

This study was conducted to evaluate effects of cassava (*Manihot esculenta Crantz*) root meal (CRM) in laying hen diets containing corn dried distiller grains with soluble (DDGS) on production performance, egg quality, and excreta noxious gas emission. Two hundred and forty Hy-Line brown laying hens (40 weeks of age) were randomly divided into 1 of 4 dietary treatments (10 replications with 6 hens per replication) for 6 weeks. The dietary treatments were as follows: 1) corn-based diet (CON); 2) diet containing 10% CRM and 8% DDGS (CRM10); 3) diet containing 20% CRM and 8% DDGS (CRM20); 4) diet containing 30% CRM and 8% DDGS (CRM30). The inclusion of 30% CRM in the diet containing 8% DDGS significantly decreased ( $p<0.05$ ) average daily feed intake (ADFI), egg production, and feed conversion ratio. Increasing the level of CRM in laying hen diets did not affect ( $p>0.05$ ) the egg quality with the exception of decreased ( $p<0.05$ ) egg yolk color when 30% of CRM was included in laying hens diet. CRM20 and CRM30 dietary treatments tended to decrease ammonia emission compared with CON dietary treatment ( $p=0.08$ ). In conclusion, the results of the current study demonstrated that CRM may be incorporated to a concentration of 20% in laying hen diets containing 8% DDGS without detrimental effects on production performance and egg quality. Furthermore, the addition of 20% and 30% CRM in laying hen diets containing 8% DDGS tended to reduce the excreta ammonia emission.

### INTRODUCTION

Corn and wheat are the main cereal grains that widely serve as energy sources in poultry diets (Frikha et al., 2009). However, their prices have increased due to the increased demand by the food and ethanol industry (Tyner & Taheripour, 2007). Consequently, the increasing cost of energy sources has resulted in the poultry industry to seek alternative cost-effective ingredients to decrease feed costs (Munyaka et al., 2015). Cassava or tapioca (*Manihot esculenta Crantz*) is an important economic crop grown in tropical and sub-tropical areas including Nigeria, Brazil, and Thailand (Garcia & Dale, 1999). Cassava root meal (CRM), whole cassava meal, cassava leaf meal, cassava peel meal are the main cassava products used in poultry diets (Borin et al., 2006; Obikaonu et al., 2006; Anaeto & Adighibe, 2011; Aderemi et al., 2012). Previous studies demonstrated that cassava can be a possible alternative energy source in diets for laying hens. Aderemi et al. (2012) observed that whole cassava meal could replace 25% of the corn in laying hen diets without negative effect on laying performance and egg shell thickness, yolk weight, albumen weight, yolk color, and Haugh unit. Kyawt et al. (2014) found that inclusion of 17.2% of cassava meal in diets for laying hens had no adverse effect on production performance



and egg shape index, yolk index, yolk weight, Haugh unit, egg shell weight, egg shell strength, and egg shell thickness, whereas yolk color was decreased. Because of the high level of starch (about 60-70%), CRM is a mainly energy ingredient in poultry feed (Garcia & Dale, 1999). Additionally, due to the high content of amylopectin (more than 80 percent), cassava starch is highly digestible when compared with corn (Gomes *et al.*, 2005; Chaunyarong *et al.*, 2009). However, the presence of hydrocyanic acid (HCN), the lack of pigments, and low protein and fat concentrations limit the use of CRM in poultry diets (Garcia & Dale, 1999; Akapo *et al.*, 2014).

Dried distillers grains with solubles (DDGS) is a by-product of the ethanol industry, and has been recognized as a valuable source of energy, protein, water-soluble vitamins, and minerals for poultry (Cromwell *et al.*, 1993). Numerous studies suggested that DDGS can be used as an acceptable feed ingredient in laying hen diets. Lumpkins *et al.* (2005) found feeding laying hens diets containing 15% DDGS had no significant effect on egg weight, egg production, and feed intake. Similarly, Wu-Haan *et al.* (2010) reported that inclusion of 20% of DDGS could reduce  $\text{NH}_3$  emission and had no adverse effect on laying hen performance. To our knowledge, there is no study to investigate the effects of the combination of CRM and DDGS on laying hen production performance and egg quality. The present experiment was conducted to test inclusion of graded levels of CRM in laying hen diets containing 8% of DDGS on production performance, egg quality, and excreta noxious gas emission.

## MATERIALS AND METHODS

The experimental protocols used in the study were approved by the Animal Care and Use Committee of Dankook University.

### Birds and experimental treatment

A total of 240 Hy-Line brown laying hens (40 weeks of age) were raised in a windowless and environmentally controlled room with a temperature of 21°C for 6 weeks. Sixteen hours (05:00 to 21:00 h) of artificial lighting were provided daily. The hens were randomly assigned to 1 of 4 treatments with 10 replications and 6 hens per replication (1 hen/cage). The dietary treatments were as follows: 1) corn-based diet (CON); 2) diet containing 10% CRM and 8% DDGS (CRM 10); 3) diet containing 20% CRM and 8% DDGS (CRM 20); 4) diet containing 30% CRM and 8% DDGS (CRM 30).

The analyzed chemical composition of CRM and DDGS are presented in Table 1. Dietary inclusion of 8% DDGS was modified according to the results of Roberts *et al.* (2007). Experimental diets were formulated to meet or exceed the NRC (1994) recommendations for laying hens (Table 2). The diets were provided in mash form. Hens were allowed *ad libitum* access to feed and water throughout the experimental period.

**Table 1** – Analyzed chemical composition of cassava root meal and corn dried distillers grains with solubles (DDGS).

Item	Cassava root meal	DDGS
Dry matter (%)	88.60	92.00
Gross Energy (kcal/kg)	3,571	4,789
Crude protein (%)	3.10	27.00
Ether extract (%)	0.58	9.00
Ash (%)	6.85	4.62
Calcium (%)	0.53	0.14
Total phosphorus (%)	0.14	0.89
Neutral detergent fiber (%)	18.61	31.55
Acid detergent fiber (%)	10.23	14.26
Hydrocyanic acid, ppm	32.52	-

### Chemical analysis

Cassava root meal and DDGS used in this experiment were analyzed for dry matter (AOAC 2007, method 930.15), crude protein (AOAC 2007, method 984.13), ash (AOAC 2007, method 942.05), calcium (AOAC 2007, method 927.02), phosphorus (AOAC 2007, method 984.27) and ether extract (AOAC 2007, method 920.39). Neutral detergent fiber and acid detergent fiber were analyzed using filter bags and fiber analyzer equipment (Fiber Analyzer, Ankom Technology, Macedon, NY, USA) following a modification of the procedure of Van Soest *et al.* (1991). Gross energy was determined by measuring the heat of combustion in the samples using a bomb calorimeter (Parr 6100; Parr instrument Co., Moline, IL, USA). Hydrocyanic acid in CRM was determined using the method described by Bradbury *et al.* (1999).

### Production performance

Live body weight (BW) was registered at the beginning and end of the experimental period, whereas feed intake was determined once a week. The number and weight of the eggs laid were recorded daily. The egg production was expressed as an average hen-day production.

### Egg quality parameters



**Table 2** – Ingredient composition and nutrient content of diets.

Item	Dietary treatment <sup>1</sup>			
	CON	CRM10	CRM20	CRM30
Ingredients (%)				
Corn	63.74	45.79	33.20	20.52
CRM <sup>2</sup>	0.00	10.00	20.00	30.00
DDGS <sup>2</sup>	0.00	8.00	8.00	8.00
Soybean meal (46%)	18.89	17.83	19.15	20.02
Rapeseed meal	3.00	3.00	3.00	3.00
Corn gluten meal	3.00	3.00	3.00	3.00
Tallow	0.00	1.54	3.00	4.53
Limestone	8.94	8.9	8.68	8.45
Dicalcium phosphate	1.71	1.22	1.23	1.24
Sodium chloride	0.22	0.22	0.22	0.22
Sodium Bicarbonate	0.10	0.10	0.10	0.10
Methionine	0.10	0.10	0.12	0.14
Threonine	0.00	0.00	0.00	0.30
Tryptophan	0.00	0.00	0.00	0.18
Choline chloride	0.10	0.10	0.10	0.10
Vitamin premix <sup>3</sup>	0.10	0.10	0.10	0.10
Trace mineral premix <sup>4</sup>	0.10	0.10	0.10	0.10
Calculated content				
Metabolizable energy (kcal/kg)	2750	2750	2750	2750
Analyzed composition				
Crude protein(%)	17.05	16.89	17.01	16.93
Lysine(%)	0.80	0.78	0.80	0.79
Methionine + cystine(%)	0.73	0.70	0.68	0.71
Calcium(%)	3.73	3.66	3.71	3.79
Total phosphorus (%)	0.59	0.56	0.55	0.54
Hydrocyanic acid (ppm)	0	4.21	7.41	10.35

<sup>1</sup>The dietary treatments were as follows: 1) corn-based diet (CON); 2) diet containing 10% CRM and 8% DDGS (CRM 10); 3) diet containing 20% CRM and 8% DDGS (CRM 20); 4) diet containing 30% CRM and 8% DDGS (CRM 30).

<sup>2</sup>CRM, cassava root meal; DDGS, corn dried distillers grains with solubles.

<sup>3</sup>Provided per kg of diet: 12,500 IU vitamin A, 2,500 IU vitamin D3, 13 IU vitamin E, 2 mg vitamin K3, 1 mg vitamin B1, 5 mg vitamin B2, 1 mg vitamin B6, 0.04 mg vitamin B12, 0.9 mg folic acid, 55 mg niacin, 14 mg Ca-pantothenate, and 0.1 mg d-biotin.

<sup>4</sup>Provided per kg of diet: Provided per kilogram of diet: 8 mg Mn (as MnO<sub>2</sub>), 60 mg Zn (as ZnSO<sub>4</sub>), 5 mg Cu (as CuSO<sub>4</sub>•5H<sub>2</sub>O), 40 mg Fe (as FeSO<sub>4</sub>•7H<sub>2</sub>O), 0.3 mg Co (as CoSO<sub>4</sub>•5H<sub>2</sub>O), 1.5 mg I (as KI), and 0.15 mg Se (as Na<sub>2</sub>SeO<sub>3</sub>•5H<sub>2</sub>O).

A total of 30 eggs (3 eggs per replication) with the exception of soft and broken eggs were randomly collected at 17:00 h from each treatment on a weekly basis and used to determine the egg quality at 20:00 h the same day. Eggshell breaking strength was evaluated using an eggshell force gauge model II (Robotmation Co., Ltd., Tokyo, Japan); A dial pipe gauge (Ozaki

MFG. Co., Ltd., Tokyo, Japan) was employed for measurements of the egg shell thickness, which was determined on the basis of the average thickness of the rounded end, pointed end, and the middle of the egg, excluding the inner membrane. Egg weight, yolk color, yolk height and Haugh unit were evaluated using an egg multi-tester (Touhoku Rhythm Co. Ltd., Tokyo, Japan).

### Excreta noxious gas emission

On the last two days of the experiment, fresh excreta samples were collected from each replication (6 hens per replication) and then mixed well for each replication. For the analysis of noxious gas emission, excreta samples (300 g) obtained from each replication were stored in 2.6-L sealed plastic boxes. Each box had a small hole in the middle of one side of the wall that was sealed by adhesive plaster. After being sealed in the boxes, samples were allowed to ferment for a period of 7 days at room temperature (25°C). After fermentation, the adhesive plaster was then punctured and gas produced from excreta was sampled using a gas sampling pump (model GV-100S, Gastec Co., Kanagawa, Japan) approximately 2.0 cm above the samples at a rate of 100 mL/min. Concentrations of ammonia (No. 3La, detector tube; Gastec Co., Kanagawa, Japan), hydrogen sulfide (No. 4LK, detector tube; Gastec Co., Kanagawa, Japan), and total mercaptan (No. 70L, detector tube; Gastec Co., Kanagawa, Japan) were measured.

### Statistical Analysis

All experimental data were analyzed using the GLM Procedure as a randomized complete block design (SASInst. Inc., Cary, NC). Differences among treatment means were determined using the Tukey's range test. A probability level of  $p \leq 0.05$  was considered significant, whereas  $0.05 < p < 0.10$  was considered a tendency.

## RESULTS

### Laying performance

The addition of up to 30% CRM in diet containing 8% DDGS had no significant effect ( $p > 0.05$ ) on BW, egg weight, and feed conversion ratio in laying hens between 41 to 46 weeks of age (Table 3). However, CRM30 dietary treatment significantly decreased ( $p < 0.05$ ) the average daily feed intake (ADFI) and egg production compared with CON, 10%, and 20% dietary treatments.



**Table 3** – Effects of cassava root meal (CRM) in diets containing corn dried distillers grains with solubles (DDGS) on production performance<sup>1</sup>

Item	Dietary treatment <sup>2</sup>				SEM <sup>3</sup>	p-value
	CON	CRM10	CRM20	CRM30		
Initial live body weight (g)	1,780	1,734	1,884	1,780	87.88	0.71
Final live body weight (g)	1,888	1,866	1,789	1,914	61.16	0.89
Egg production (%)	93.16 <sup>a</sup>	92.48 <sup>a</sup>	93.37 <sup>a</sup>	88.22 <sup>b</sup>	0.96	<0.05
Egg weight (g)	65.97	64.56	64.09	65.18	1.28	0.38
Feed consumption (g/hen per d)	126 <sup>a</sup>	122 <sup>ab</sup>	119 <sup>ab</sup>	114 <sup>b</sup>	3.71	<0.05
FCR <sup>4</sup>	2.05	2.04	1.99	1.98	0.23	0.27

<sup>1</sup>Each mean represents 10 replications with 6 hens per replication.

<sup>2</sup>The dietary treatments were as follows: 1) corn-based diet (CON); 2) diet containing 10% CRM and 8% DDGS (CRM 10); 3) diet containing 20% CRM and 8% DDGS (CRM 20); 4) diet containing 30% CRM and 8% DDGS (CRM 30).

<sup>3</sup>SEM, standard error of mean.

<sup>4</sup>FCR, feed conversion ratio (g of feed consumed/g of egg produced).

<sup>a,b</sup>Means in the same row with different superscripts differ ( $p < 0.05$ ).

### Egg quality

Increasing the level of CRM in laying hens diets did not significantly affect ( $p > 0.05$ ) the eggshell breaking strength, egg shell thickness, yolk height, and Haugh unit. However, compared with CON, CRM10, and CRM20 dietary treatments, a decrease ( $p < 0.05$ ) in yolk color was observed in CRM 30 dietary treatment (Table 4).

### Noxious gas emission

In CRM20 and CRM30 dietary treatments, ammonia emission tended to be decreased compared with CON dietary treatment ( $p = 0.08$ , figure 1). Additionally, there were no significant differences in total mercaptans and hydrogen sulfide emissions among dietary treatments ( $p > 0.05$ ).

**Table 4** – Effects of cassava root meal (CRM) in diets containing corn dried distillers grains with solubles (DDGS) on egg quality<sup>1</sup>

Item	Dietary treatment <sup>2</sup>				SEM <sup>3</sup>	p-value
	CON	CRM10	CRM20	CRM30		
Eggshell breaking strength (kg/cm <sup>2</sup> )	3.87	3.68	3.93	3.80	0.16	0.16
Eggshell thickness (mm)	0.39	0.38	0.39	0.38	0.01	0.33
Yolk color	7.65 <sup>a</sup>	7.66 <sup>a</sup>	7.04 <sup>a</sup>	6.17 <sup>b</sup>	0.20	<0.05
Yolk Height (mm)	16.08	16.21	16.60	16.12	0.33	0.61
Haugh unit	82.45	83.30	87.10	81.96	1.32	0.67

<sup>1</sup>Each mean represents 30 randomly collected eggs per treatment on a weekly basis.

<sup>2</sup>The dietary treatments were as follows: 1) corn-based diet (CON); 2) diet containing 10% CRM and 8% DDGS (CRM 10); 3) diet containing 20% CRM and 8% DDGS (CRM 20); 4) diet containing 30% CRM and 8% DDGS (CRM 30).

<sup>3</sup>SEM, standard error of mean.

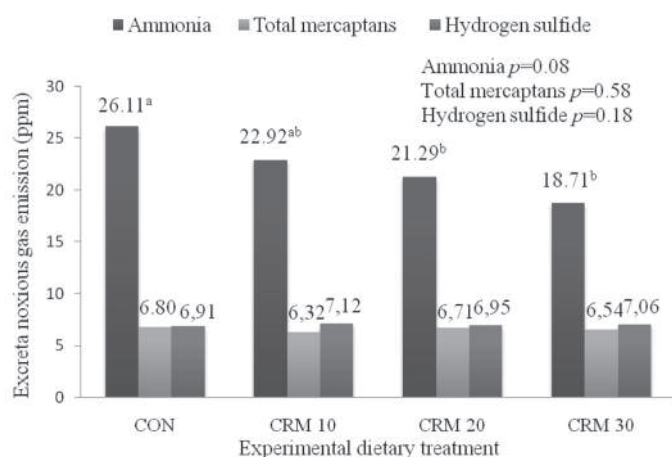
<sup>a,b</sup>Means in the same row with different superscripts differ ( $p < 0.05$ ).

## DISCUSSION

Previous studies have reported that cassava products can be used to replace corn in laying hens diets without negative effects of laying performance (Chauynarong *et al.*, 2009). Enriquez & Ross (1972) indicated that the addition of up to 50% of CRM in laying hens diets did not affect egg production, average egg weight, and BW. Enyenihi *et al.* (2009) observed that inclusion of 25% cassava tuber meal did not affect egg production, average egg weight, BW, and ADFI. In our study, inclusion of CRM in the diets containing 8% DDGS did not affect the BW, and egg

weight, but egg production and ADFI were decreased when the CRM was included at the level of 30%. These results are generally consistent with those of Anaeto & Adighibe (2011), who reported that dietary inclusion of 11.13% and 22.25% CRM had no adverse effect on production performance, whereas feed intake and egg production were reduced by diets containing 33.38% and 44.50% of CRM. Cassava starch is highly digestible due to soft-starch and containing more than 80% amylopectin, but deficient protein, fat and other nutrients (Okigbo, 1980; Gomes *et al.*, 2005). Khajarern & Khajarern (1992) indicated that cassava products can be successfully used to replace





**Figure 1** – Effects of cassava root meal (CRM) in diets containing corn dried distillers grains with solubles (DDGS) on excreta noxious gas emission. Values with different superscripts tended to be different ( $0.05 < p < 0.10$ ). The dietary treatments were as follows: 1) corn-based diet (CON); 2) diet containing 10% CRM and 8% DDGS (CRM 10); 3) diet containing 20% CRM and 8% DDGS (CRM 20); 4) diet containing 30% CRM and 8% DDGS (CRM 30).

corn to be satisfactory for egg production if the diet is nutritionally balanced. The presence of HCN limits the utilization of cassava in animal diets (Oke, 1978). The HCN is toxic and negatively affect the palatability of cassava (Régner *et al.*, 2010). In the present study, the concentration of HCN in CRM30 dietary treatment was 10.35 ppm. Therefore, the depressed ADFI and reduced egg production of laying hens fed diets containing 30% CRM could be probably due to the presence of HCN.

In this study, no deleterious effects on egg quality were detected in laying hens fed with incremental levels of CRM with the exception of the egg yolk color. Similar results were reported in previous studies. Enriquez & Ross (1972) reported that there was no negative effect on shell thickness due to the addition of CRM at the levels of 10%, 25%, and 50%. However, the yolk color was reduced when fed diets containing 25% and 50% of CRM. Aina & Fanimo (1997) demonstrated shell thickness, Haugh unit, and shell thickness were not affected by the dietary supplementation of 52.3% cassava. Sapatrattananan *et al.* (2005) indicated that feeding cassava or corn basal diets had similar egg shell thickness and Haugh unit, whereas the yolk color score was lower in cassava diet. Aderemi *et al.* (2012) observed that the inclusion of whole cassava meal did not affect shell thickness and yolk weight, but yolk color was decreased as the whole cassava meal inclusion increased beyond 12.5%. Additionally, the inclusion of DDGS in laying hens diets had no negative effects on egg quality but increased yolk color (Roberson *et al.*, 2005; Świątkiewicz & Koreleski, 2006; Cheon *et al.*, 2008). The color of egg yolk largely depends on the

pigments including carotenes and xanthophylls from diets as laying hens cannot synthesize the pigments (Sun *et al.*, 2013). The xanthophylls in corn is a main contributor of yolk pigmentation (Lumpkins *et al.*, 2005). In this study, the dietary treatment with CRM decreased the yolk color and can be attributable to the CRM being fed largely at the expense of corn. DDGS is of corn origin and provides more xanthophylls than corn (Masa'deh *et al.*, 2011). Therefore, the provision of DDGS may compensate for the loss of xanthophylls when the corn was replaced by CRM.

The noxious gas emissions from chicken manure not only impair the health of poultry and poultry stockmen but also raise environmental pollution (Zhang *et al.*, 2013; Zhang *et al.*, 2014). Therefore, excreta ammonia, hydrogen sulfide, and total mercaptans emissions were detected to evaluate the effect of combination of different levels of CRM and 8% DDGS on excreta noxious gas emission in laying hens. In a previous study, Roberts *et al.* (2007) found that inclusion of 10% DDGS in laying hens diets decreased manure ammonia emission. Wu-Haan *et al.* (2010) demonstrated that feeding diet containing 20% DDGS lowered ammonia and hydrogen sulfide emissions. Khempaka *et al.* (2016) reported that feeding 5, 10, 15, 20 and 25% of dried cassava pulp showed no effect on cecal ammonia concentration. In the current study, the inclusion of different levels of CRM and 8% DDGS had no effect on excreta hydrogen sulfide and total mercaptans emissions, whereas CRM 20 and CRM 30 dietary treatments tended to decrease ammonia emission compared with CON. The concentration of ammonia in feces can be reduced by the supplementation of non-starch polysaccharides (NSP) in the diets (Wang *et al.*, 2009). The concentration of NSP in DDGS is 2 to 3 times than corn (Moran *et al.*, 2016). Additionally, Promthong *et al.* (2005) indicated that cassava has higher NSP as compared with corn. The presence of NSP in the diets promotes carbohydrate-fermenting bacteria such as *Bifidobacteria* and *Lactobacillus* in the hindgut, resulting in enhanced nitrogen absorption, and thereby reducing excreta ammonia emission (Mroz *et al.*, 2000; Ferket *et al.*, 2002; Hansen *et al.*, 2007; Jeong *et al.*, 2015). Therefore, the decreased trend of ammonia emission may be due to the increased level of NSP by the addition of CRM and DDGS.

## CONCLUSIONS

It can be concluded that, the results of this study demonstrated that CRM may be used up to 20% in laying hens diet containing 8% DDGS without harmful



effects on production performance, and egg quality. The addition of 20% or 30% CRM meal and 8% DDGS tended to reduce excreta ammonia emission.

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