# **EFFECT OF DRIED FERMENTATION BIOMASS FROM L-LYSINE HCL AND MONOSODIUM L-GLUTAMATE PRODUCTION ON GROWTH PERFORMANCE AND SMALL INTESTINAL MORPHOLOGY OF BROILER CHICKENS**

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# **ABSTRACT**

**Dried fermentation biomass (DFB) is a co-product of crystalline amino acid production. An experiment was conducted to determine the effect of two different sources of DFB (Lys-DFB: fermentation biomass from L-lysine HCl production and MSG-DFB: fermentation biomass from monosodium L-glutamate production) on growth performance and small intestinal morphology of broilers. Seven hundred, day-old Cobb 500 broilers were randomly allotted to 7 dietary treatments using a randomized complete block design with 10 replicates per treatment. Phase 1 (d 0 to 10) and phase 2 diets (d 11 to 24) with increasing levels (0, 1, 2 and 3%) of Lys-DFB and MSG-DFB were formulated followed by a common phase 3 diet (d 24 to 35). From d 0 to 24 and the overall period, including Lys-DFB to the diet did not affect growth performance but MSG-DFB resulted in a reduction (linear,** *P***<0.03) in ADG and BW and poorer (linear,** *P***<0.01) F/G. No significant differences in small intestinal morphology were observed among the treatments; however, MSG-DFB resulted in increased (quadratic,** *P***=0.04) incidence of pasty vents. In conclusion, DFB from either L-Lys HCl or monosodium L-glutamate production does not improve growth performance and small intestinal morphology when added to broiler diets.** 

Key words: broilers, dried fermentation biomass, growth performance, intestinal morphology

#### **INTRODUCTION**

Modern broilers have high dietary amino acid needs especially in earlier phases of growth, and to maximize their performance and economic returns, knowledge about amino acid requirements and the effective use of protein ingredients are particularly needed (Beski *et al*., 2015). Greater protein intake may be accomplished by substituting a portion of soybean meal with specialty protein ingredients in young broiler diets. One potential material is dried fermentation biomass (DFB) which is a by-product derived from the production of crystalline amino acids (AA). This is produced after the AA is extracted from a specific strain of bacteria used in the fermentation process with either sucrose or glucose as the

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carbon source (Ikeda, 2003). It is considered waste and is disposed in sewage treatment plants, in landfilling, and composting (Blaesen *et al*., 2007); however, recent research suggests that DFB has potential as a feed ingredient being rich in CP and indispensable AA (Utterback *et al*., 2011; Carpenter *et al*., 2017).

Previous studies in weanling pigs have shown that DFB from the production of L-Lys HCl has greater energy value and concentration of digestible AA than fish meal and soybean meal (Sulabo *et al*., 2013). Monosodium L-glutamate (MSG), which is used primarily in the food industry as a flavor enhancer, is also produced through bacterial fermentation using the same *Corynebacterium* species used in the production of L-Lys HCl (Wijayasekara and Wansapala, 2017). The spent biomass from this industry is very rich in glutamic acid (Glu), which is of critical importance in intestinal metabolism and physiology (Olubodun *et al*., 2015) and may have positive effects on intestinal health of broilers (Porto *et al*., 2015). There is, however, very limited information on the effect of different DFB as a feed ingredient in diets for young broilers. Therefore, the objective of the study was to determine the effect of increasing levels of DFB from L-Lys HCl and MSG production on growth performance and small intestinal morphology in broiler chickens.

## **MATERIALS AND METHODS**

The experimental protocol was reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of the University of the Philippines Los Baños (IACUC approval number IBS-2016-008).

Seven hundred, day-old, straight-run Cobb 500 broiler chicks were purchased from a local hatchery (San Miguel Foods, Inc. Hatchery, Calamba, Laguna, Philippines). On the day of placement (d 0), birds were divided into 70 lots with 10 birds per lot and each lot was weighed. Then using lot weight as the blocking factor, lots were randomly allotted to 1 of 7 experimental treatments following a randomized complete block design and birds in the lot were finally placed into their respective pens. There were 10 replicate pens per treatment. Each pen  $(1 \times 1 \text{ m})$  had slatted floors and was equipped to allow *ad libitum* access to the test diets and water throughout the trial. Each pen was also equipped with a source of heat for the first 2 weeks for brooding. The experiment lasted for 35 d divided into three feeding phases: phase 1, 2 and 3 were from d 0 to 10, d 11 to 24, and d 25 to 35, respectively. Birds were vaccinated against Newcastle disease virus and infectious bursal disease virus. Uniform care and management were provided for the birds throughout the duration of the study.

A total of 7 experimental diets were formulated fed on a 2-phase diet series (Tables 1, 2 and 3) followed by a common phase 3 diet (Table 3). For phase 1 and 2, the first diet was a corn-soybean meal diet that served as the control. The next 3 diets were corn-soybean meal diets with the dried fermentation biomass from L-Lys production (IVP73L, Intraco Ltd., Antwerp, Belgium; Lys-DFB) added at 1, 2, and 3%, respectively. The last 3 diets were corn-soybean meal diets with the dried fermentation biomass from monosodium L-glutamate production (PL68, Intraco Ltd., Antwerp, Belgium; MSG-DFB) added at 1, 2, and 3%, respectively. All experimental diets were formulated to meet or exceed Cobb 500 nutrient specifications and to be isocaloric and balanced to ideal protein using crystalline amino acids. There were no antibiotics included in the diet. All experimental diets were in meal form.



Table 1. Nutrient composition (as-fed basis) of dried fermentation biomass (DFB) sources.

1 Lys-DFB = biomass from L-lysine HCl production (IVP73L, Intraco Ltd., Antwerp, Belgium) 2 MSG-DFB = biomass from monosodium L-glutamate production (PL68, Intraco Ltd., Antwerp, Belgium). 3 SID = Standardized Ileal Digestible Amino Acid

4 AMEn = N-corrected apparent metabolizable energy.



Table 2. Ingredient and nutrient composition of Phase 1 diets (as-fed basis). Table 2. Ingredient and nutrient composition of Phase 1 diets (as-fed basis).





5AMEn = N-corrected apparent metabolizable energy.

 $^6$ SID = Standardized Ileal Digestible Lysine 6SID = Standardized Ileal Digestible Lysine



Table 3. Ingredient and nutrient composition of Phase 2 and Phase 3 diets (as-fed basis). Table 3. Ingredient and nutrient composition of Phase 2 and Phase 3 diets (as-fed basis).



6SID = Standardized Ileal Digestible Lysine

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Birds and feed leftovers were weighed at the end of each phase to calculate for ADG, ADFI and F/G. For mortalities and broilers that were removed from the study for any reason were weighed and recorded. This was accompanied with notes on the suspected cause of death or reason for removal. Percent viability for each replicate was calculated by dividing the number of birds left at the end of the experiment over the initial number of birds multiplied by 100. Finally, production efficiency index (PEI) was calculated using the following equation: PEI= [daily BW gain  $\times$  % viability] / [F/G  $\times$  10].

The incidence of pasty vent on a bird basis was recorded at d 24 by visual examination, based on the presence or absence of sticky feces in the vent area. Percent incidence was calculated by dividing the number of birds with pasty vents to the total number of birds in the pen multiplied by 100.

At d 24, three birds were randomly sampled from each treatment and were killed by cervical dislocation. Immediately after killing, the whole length of the small intestine was separated. Sections of the duodenum (from gizzard to the end of the pancreatic loop), jejunum (segment between the pancreatic loop and Meckel's diverticulum) and ileum (segment between Meckel's diverticulum and ileocecal junction) were removed. Afterward, segments about 2 cm in length were cut from the midpoint of the duodenal, jejunal and ileal samples. The removed segments were then washed with sterilized physiological saline solution to remove any adherent intestinal content and were fixed in 10% buffered formalin for histological measurements.

After dehydration with formalin, the collected samples were subjected to an ethanol series before being cleaned in xylene to dissolve the alcohol and embedded in paraffin. Three intestinal samples  $(5 \mu m)$  thick) were placed on glass slides and stained with hematoxylin and eosin (H and E). An optical microscope (Olympus BX41, Olympus, Tokyo, Japan) was used to measure the micrographs. Ten villi and crypts were measured in each sample. The magnification used for the villi and crypts were 5 and 10, respectively. Morphometric measurements were villus height, which is the length from the tip of the villus to the crypt and crypt depth measured from the base of the villi to the submucosa. The ratio of villus height: crypt depth were also calculated.

Samples of the DFB and all experimental diets were collected and properly labeled for subsequent analyses. The DFB samples were analyzed in triplicates for DM (method 930.15; AOAC, 2007), CP (method 990.03; AOAC, 2007), ether extract (method 920.39; AOAC, 2007), crude fiber (method 978.10; AOAC, 2007), ash (method 942.05; AOAC, 2007), ADF (method 973.18; AOAC, 2007), and NDF (Holst, 1973).

Homogeneity of variances and outliers were tested using the UNIVARIATE procedure of SAS (SAS Inst. Inc., Cary, NC). Data were analyzed using the MIXED procedure of SAS with pen as the experimental unit. The model included diet as the fixed effect and block as the random effect. Orthogonal polynomial contrasts were performed to determine linear and quadratic effects of DFB level in the diet. Least square means were calculated for each independent variable and the  $\alpha$ -level that was used to determine significance among means was *P*≤0.05.

#### **RESULTS**

From d 0 to 10, birds fed increasing levels of Lys-DFB resulted in an increase (linear, *P*=0.01) in ADFI but did not affect ADG and d 10 BW (Table 4). As a result, birds







Intraco Ltd., Antwerp, Belgium). Experimental diets were fed in a 2-phase diet series (Phase 1 from d 0 to 10 and Phase 2 from d 11 to 24) and fed a common Phase 3 diet from d 25 to 35.<br>
Production Efficiency Index (PEI) = [ADG × % viability]/[F/G × 10].<br>
<sup>a-</sup>Values within a row lacking a common superscript letter are different (*P*≤0.05). i, ą. from d 25 to 35.

3Production Efficiency Index (PEI) = [ADG × % viability]/[F/G × 10].

a-cValues within a row lacking a common superscript letter are different (*P*≤0.05).

Table 4. Continued... Table 4. Continued...

fed diets with 2 and 3% Lys-DFB had poorer (linear, *P*<0.001) F/G compared with those fed the diet with 1% Lys-DFB and the control diet. In contrast, birds fed the diets with MSG-DFB did not affect ADG, ADFI and d 10 BW but resulted in poorer (quadratic,  $P=0.002$ ) F/G compared with those fed the control diet. From d 11 to 24, including Lys-DFB in the diet did not influence ADG, ADFI, F/G and d 24 BW of the birds; however, including 3% MSG-DFB in the diet resulted in lower (linear,  $P<0.003$ ) ADG and d 24 BW compared with the rest of the treatments except for those fed 2% MSG-DFB. It also had the poorest (quadratic, *P*=0.05) F/G among the treatments.

From d 0 to 24, Lys-DFB did not affect the growth performance of the birds but increasing levels of MSG-DFB in the diets resulted in a reduction (linear, *P*=0.002) in ADG and poorer (linear, *P*<0.001) F/G. As expected, from d 24 to 35, there were no significant differences in ADG, ADFI, and F/G among the treatments as birds were fed the same diet. Overall (d 0 to 35), including Lys-DFB in phase 1 and 2 diets did not affect growth performance and PEI of the birds. However, including MSG-DFB in phases 1 and 2 diets resulted in a decrease (linear, *P*<0.03) in ADG and ADFI and poorer (linear, *P*=0.01) F/G which reduced (linear, *P*=0.004) PEI. Birds fed the diets with 3% MSG-DFB in phase 1 and 2 diets had poorer ( $P=0.009$ ) F/G compared with those fed the control diet. Likewise, birds fed the diets with 3% MSG-DFB had the least  $(P=0.04)$  d 35 BW and PEI among the treatments. Percent viability did not significantly differ among the treatments.

No significant differences were observed in the villus height, crypt depth and villous height: crypt depth ratio in the three segments of the small intestine (Table 5). Inclusion of Lys-DFB in phase 1 and phase 2 diets did not affect the incidence of pasty vents, but including MSG-DFB resulted in increased (quadratic, *P*=0.04) incidence.

#### **DISCUSSION**

The microbial production of AA has gained significant attention in recent years after the discovery of AA-producing bacteria (Kinoshita *et al*., 2004). The global demand for synthetic AA such as L-Lys, DL-Met, L-Thr and L-Val has tremendously increased because of their extensive use in the feed, food, and pharmaceutical industries (Research and Markets, 2020), and the production of the spent biomass from this industry will, therefore, also increase. Likewise, worldwide production of monosodium L-glutamate has been expanding especially in Asia (Tonouchi and Ito, 2017). Therefore, it is important to determine the potential of DFB as an animal feed ingredient.

Recently, a few studies were conducted to evaluate the nutritional value of DFB from the production of L-Lys HCl (Sulabo *et al*., 2013), L-Thr (Almeida *et al*., 2014; Oliveira *et al*., 2020) and L-Val (Oliveira *et al*., 2020) and showed a greater concentration of digestible AA than soybean meal when fed to pigs. There is, however, no previous study conducted in broilers with the Lys-DFB and MSG-DFB used in the present experiment. Results indicate that when diets were formulated to be isocaloric and balanced to ideal protein, both DFB sources had a negative effect on F/G. The Lys-DFB, however, only significantly affected F/G in phase 1 and became more acceptable in the later phase. Whereas for the MSG-DFB, growth performance of the birds was negatively affected especially when 3% was added to the diets. These results indicate that the feeding value of Lys-DFB was greater than MSG-DFB when fed to young broilers.

The negative effect of MSG-DFB on growth rate was a result of reduced feed intake,



Table 5. Effect of dried fermentation biomass from L-Lys HCl and monosodium L-glutamate production on gut morphology and incidence Table 5. Effect of dried fermentation biomass from L-Lys HCl and monosodium L-glutamate production on gut morphology and incidence of pasty vents in broilers<sup>1,2</sup>.

from d 25 to 35. VH = villus height;  $CD$  = crypt depth.

3Percent incidence of pasty vents was calculated by dividing the number of birds with a pasty vent to the total number of birds in the pen multiplied by 100.

which suggests that there may be palatability issues with greater inclusion of the material. It is possible that the bacteria species may have toxic or an anti-nutritional effect even after the drying and processing of the fermentation biomass (Kiessling and Askbrandt, 1993). Another possible reason for the poorer F/G may be nutrient imbalances, which may be related to the accuracy of the AA digestibility values used for DFB in diet formulation. It is possible that the AA digestibility of both Lys-DFB and MSG-DFB were lower than published values (Sulabo *et al*., 2013) used in diet formulation, which may have reduced the AA supplied by the diet and negatively affected growth and F/G of the birds. It is expected that AA digestibility of DFB may vary between the type of synthetic AA produced, the bacterial species used in fermentation, the production plants, and the type of drying process used to dry the fermentation broth. Future research may be conducted to determine the standardized ileal digestibility of AA in both Lys-DFB and MSG-DFB when fed to broilers.

It was hypothesized that feeding MSG-DFB, which contains nearly twice the concentration of Glu than soybean meal, may positively influence small intestinal morphology. Glutamic acid is a dispensable amino acid that is involved in the production of energy required for cell turnover in the intestine (Burrin and Stoll, 2009). However, MSG-DFB did not improve the intestinal morphology of broilers which is in contrast with the results of a previous study that added 1% L-Glu in the diet (Porto *et al*., 2015). The estimated amount of Glu supplied by MSG-DFB in the diets was only between 0.16 to 0.47% Glu, which may help explain the difference in responses. Porto *et al*. (2015) also observed that the effect of supplemental Glu was affected by environmental temperature, where intestinal measures were improved when birds were raised under constant heat stress (33-37°C) but there were no improvements in those raised under thermoneutral conditions. Birds in the current study were grown under standard conditions where there is normal fluctuation of ambient temperature within the day, and this may mute the response from Glu supplied by MSG-DFB.

The incidence of pasty vents especially in young broilers is used as an indicator of the degree of nutrient utilization particularly of dietary fat and protein (Roy *et al*., 2010) as well as the prevalence of healthy conditions in the gut (De Cesare *et al*., 2017). The increase in the incidence of pasty vents in birds fed MSG-DFB may suggest poorer protein and fat digestibility compared with those fed the untreated controls. Increased supply of undigested fat and proteins in the ceca may have major impacts on animal health and production performance (Roy *et al*., 2010; Apajalahti and Vienola, 2016). This may help explain the reduced BW and F/G observed in birds fed the diet with 3% MSG-DFB.

In conclusion, when diets are formulated to be isocaloric and balanced for ideal protein, the inclusion of DFB from L-Lys HCl and MSG production up to 3% of the diet do not improve growth performance and small intestinal morphology of broiler chickens. Future research may be conducted to determine the standardized ileal digestibility of AA in different DFB sources fed to broilers and to determine factors that affect the degree of variation and nutritional quality of DFB as a feed ingredient in broiler diets.

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