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

Noel B. Lumbo¹ and Rommel C. Sulabo¹

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-gluta-mate 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.

	Ingre	edients
Item	Lys-DFB ¹	MSG-DFB ²
DM, %	91.00	91.00
CP (N × 6.25), %	73.00	68.00
SID AA ³ , %		
Lys	6.57	2.20
Thr	3.54	2.66
Met	1.12	0.84
Cys	0.28	0.15
Trp	0.75	0.57
Ile		2.18
Val	2.69	2.88
Arg	2.99	2.85
His	0.97	0.97
Leu	3.94	3.94
Phe	1.92	1.92
Tyr	0.92	0.92
Glu	5.53	15.71
Ca	0	0.10
P, available	0.30	0.30
Analyzed, %		
DM	89.68	93.29
GE, kcal/kg	5,284	5,040
AMEn ⁴ , kcal/kg	3,212	3,775
CP (N × 6.25)	68.74	66.67
Crude fiber	0.05	0.20
Crude fat	6.82	2.19
NDF	1.46	0.34
ADF	0	0
Ash	5.46	7.23

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

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

⁴AMEn = N-corrected apparent metabolizable energy.

				Phase 1			
Items			Lys-DFB			MSG-DFB	
	Control	1%	2%	3%	1%	2%	3%
Ingredient, %							
Corn, yellow	50.928	59.703	59.851	60.008	58.436	57.328	56.220
Soybean meal	39.579	31.537	30.765	29.993	32.475	32.639	32.804
Lys-DFB ¹	I	1.000	2.000	3.000	1	1	ł
MSG-DFB ²	I	ł	1	1	1.000	2.000	3.000
Coconut oil	4.722	2.623	2.331	2.039	2.940	2.965	2.990
L-Lysine HCl	0.224	0.394	0.337	0.280	0.420	0.390	0.359
DL-Methionine	0.356	0.410	0.410	0.400	0.410	0.400	0.390
L-Threonine	0.135	0.207	0.183	0.160	0.204	0.177	0.150
MCP, 21% P	1.268	1.288	1.280	1.272	1.285	1.274	1.262
Limestone	1.534	1.590	1.600	1.611	1.581	1.583	1.585
Salt	0.501	0.496	0.491	0.486	0.496	0.491	0.487
Choline chloride	0.250	0.250	0.250	0.250	0.250	0.250	0.250
Vitamin premix ³	0.130	0.130	0.130	0.130	0.130	0.130	0.130
Mineral premix ⁴	0.100	0.100	0.100	0.100	0.100	0.100	0.100
Antioxidant	0.013	0.013	0.013	0.013	0.013	0.013	0.013
Antimold	0.200	0.200	0.200	0.200	0.200	0.200	0.200
Coccidiostat	0.050	0.050	0.050	0.050	0.050	0.050	0.050
Phytase	0.010	0.010	0.010	0.010	0.010	0.010	0.010
TOTAL	100.000	100.000	100.000	100.000	100.000	100.000	100.000

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Table 2. Ingredient and nutrient compc

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				Phase 1			
Items			Lys-DFB			MSG-DFB	
	Control	1%	2%	3%	1%	2%	3%
Calculated analysis, %							
DM	89.33	88.90	88.87	88.83	88.97	89.01	89.05
AMEn ⁵ , kcal/kg	2,900	2,900	2,900	2,900	2,900	2,900	2,900
$CP(N \times 6.25)$	23.14	21.11	21.42	21.72	21.41	22.02	22.63
SID Lys ⁶	1.37	1.37	1.37	1.37	1.37	1.37	1.37
Ca	0.95	0.95	0.95	0.95	0.95	0.95	0.95
P, available	0.38	0.38	0.38	0.38	0.38	0.38	0.38
^T Lys-DFB: Dried fermentation biomass from L-Lys HCl production (IVP 73L, Intraco Ltd., Antwerp, Belgium).	omass from L-Lys H(Cl production (IVF	73L, Intraco Ltd.,	Antwerp, Belgium)	-		
² MSG-DFB: Dried fermentation biomass from monosodium L-glutamate production (PL68, Intraco Ltd., Antwerp, Belgium).	viomass from monos	odium L-glutamate	Production (PL68)	3, Intraco Ltd., Antw	erp, Belgium).		

³The vitamin premix provided the following quantities of vitamins per kg of diet: Vitamin A, 1.43 MIU/kg; Vitamin D, 0.65 MIU/kg, Vitamin E, 6.5 g/kg; Vitamin K, 390 mg/kg; thiamine, 260 mg/kg; riboflavin, 910 mg/kg; pyridoxine, 390 mg/kg; niacin, 5.2 g/kg; pantothenic acid, 1.95 g/kg; vitamin B12, 1.95 mg/kg; folic acid, 195 mg/kg. ⁴The trace mineral premix provided the following quantities of micro minerals per kg of diet: Fe, 9.2 g/kg; Cu, 750 mg/kg; Zn, 6 g/kg; Mn, 5 g/kg; I, 70 mg/kg; Se, 15 mg/kg.

 5 AMEn = N-corrected apparent metabolizable energy.

⁶SID = Standardized Ileal Digestible Lysine

				Phase 2				
Items			Lys-DFB			MSG-DFB	-	Phase 3
	COULOI	1%	2 %	3%	1%	2%	3%	
Ingredient, %								
Corn, yellow	59.660	61.476	62.807	63.997	61.386	61.807	63.297	59.094
Soybean meal	30.839	28.751	27.000	25.400	28.751	27.600	25.600	30.900
$Lys-DFB^{1}$	1	1.000	2.000	3.000	I	:	1	1
MSG-DFB ²	1	ł	ł	1	1.000	2.000	3.000	1
Coconut oil	5.000	4.240	3.700	3.150	4.280	4.000	3.450	6.000
L-Lysine HCl	0.351	0.340	0.310	0.280	0.390	0.400	0.440	0.160
DL-Methionine	0.377	0.380	0.380	0.390	0.380	0.390	0.400	0.263
L-Threonine	0.185	0.180	0.170	0.150	0.180	0.180	0.180	060.0
MCP, 21% P	1.031	1.060	1.060	1.060	1.060	1.060	1.060	1.270
Limestone	1.584	1.600	1.600	1.600	1.600	1.590	1.600	1.270
Salt	0.350	0.350	0.350	0.350	0.350	0.350	0.350	0.350
Choline chloride	0.120	0.120	0.120	0.120	0.120	0.120	0.120	0.100
Vitamin premix ³	0.130	0.130	0.130	0.130	0.130	0.130	0.130	0.130
Mineral premix ⁴	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100
Antioxidant	0.013	0.013	0.013	0.013	0.013	0.013	0.013	0.013
Antimold	0.200	0.200	0.200	0.200	0.200	0.200	0.200	0.200
Coccidiostat	0.050	0.050	0.050	0.050	0.050	0.050	0.050	0.050
Phytase	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010
TOTAL	100.000	100.000	100.000	100.000	100.000	100.000	100.000	100.000

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

				Phase 2				
Items			Lys-DFB			MSG-DFB		Phase 3
	Control	1%	2%	3%	1%	2%	3%	
Calculated analysis, %	, %							
DM	80.08	88.96	88.87	88.79	88.97	88.94	88.86	89.15
AMEn ⁵ , kcal/kg	3,020	3,020	3,020	3,020	3,020	3,020	3,020	3,090
$CP(N \times 6.25)$	19.98	19.87	19.86	19.91	19.86	20.06	19.98	19.64
SID Lys ⁶	1.25	1.25	1.25	1.25	1.25	1.25	1.25	1.10
Са	0.90	0.91	0.90	0.89	0.91	0.00	0.90	0.82
P, available	0.32	0.33	0.33	0.33	0.33	0.33	0.33	0.37
¹ Lys-DFB: Dried fermentation biomass from L-Lys HCl production (IVP 73L, Intraco Ltd., Antwerp, Belgium). ² MSG-DFB: Dried fermentation biomass from monosodium L-glutamate production (PL68, Intraco Ltd., Antwerp, Belgium). ³ The vitamin premix provided the following quantities of vitamins per kg of diet: Vitamin A, 1.43 MIU/kg; Vitamin D, 0.65 MIU/kg, Vitamin E, 6.5 g/kg; Vitamin K, 390 mg/kg; thiamine, 260 mg/kg; riboflavin, 910 mg/kg; pyridoxine, 390 mg/kg; niacin, 5.2 g/kg; pantothenic acid, 1.95 g/kg; vitamin B12, 1.95 mg/kg; folic acid, 195 mg/kg.	tation biomass fre entation biomass f vided the followin, 260 mg/kg; ribofl.	om L-Lys HCl pr from monosodiur g quantities of vi avin, 910 mg/kg;	oduction (IVP 7 n L-glutamate F tamins per kg of pyridoxine, 390	73L, Intraco Ltd., production (PL68 f diet: Vitamin A, 0 mg/kg; niacin,	Antwerp, Belgiu , Intraco Ltd., Ar 1.43 MIU/kg; Vi 5.2 g/kg; pantoth	um). ttwerp, Belgium) tamin D, 0.65 MI enic acid, 1.95 g/	U/kg, Vitamin E, kg; vitamin B12,	. 6.5 g/kg; Vitami 1.95 mg/kg; foli
*The trace mineral premix provided the following quantities of micro minerals per kg of diet: Fe, 9.2 g/kg; Cu, 750 mg/kg; Zn, 6 g/kg; Mn, 5 g/kg; I, 70 mg/kg; Se, 15 mg/kg. ⁵AMEn = N-corrected apparent metabolizable energy.	ix provided the fol parent metaboliza	llowing quantitie able energy.	s of micro mine	erals per kg of die	et: Fe, 9.2 g/kg; (Ju, 750 mg/kg; Z.	n, 6 g/kg; Mn, 5 ;	g/kg; I, 70 mg/k
⁵ SID = Standardized Ileal Digestible Lysine	l Digestible Lysin	le						

Table 3. Continued...

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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 μ 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 α -level that was used to determine significance among means was $P \leq 0.05$.

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

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Table 4.	

			L	Treatment	t						P-Value		
Item			Lys-DFB			MSG-DFB		SEM	D:21	Lys-DFB	DFB	MSG-DFB	-DFB
	Control	1%	2%	3%	1%	2%	3%		Diet	Linear	Quad	Linear	Quad
BW, g													
d 0	50	51	51	51	51	51	51	0.5	0.520	0.12	0.19	0.180	0.14
d 10	222	220	214	220	213	213	212	6.0	0.360	0.46	0.37	0.090	0.36
d 24	883 ^a	863 ^a	867^{a}	852 ^a	854 ^a	835^{ab}	792 ^b	27.0	0.020	0.28	0.88	0.001	0.68
d 35	$1,624^{a}$	$1,590^{a}$	1,603 ^a	$1,568^{a}$	$1,559^{ab}$	$1,575^{a}$	$1,480^{\mathrm{b}}$	36.0	0.040	0.26	0.98	0.003	0.61
d 0 to 10													
ADG, g	17.00	16.80	16.20	16.80	16.20	16.00	16.00	0.70	0.410	0.580	0.38	0.110	0.33
ADFI, g	21.10	21.60	22.20	22.40	22.20	22.00	22.20	0.50	0.200	0.010	0.76	0.070	0.24
F/G	1.25 ^a	1.29ª	$1.38^{\rm b}$	$1.34^{\rm b}$	1.39^{b}	1.39^{b}	1.39^{b}	0.03	<0.001	<0.001	0.08	<0.001	0.002
d 11 to 24													
ADG, g	46.50^{a}	45.80^{a}	46.60^{a}	45.20^{a}	46.00^{a}	44.50^{ab}	41.00^{b}	1.80	0.050	0.59	0.77	0.003	0.25
ADFI, g	74.10	72.00	72.90	72.00	73.90	72.20	70.10	2.10	0.640	0.46	0.72	0.060	0.56
F/G	1.60^{b}	$1.58^{\rm b}$	1.57^{b}	1.60^{b}	1.61^{b}	$1.63^{\rm b}$	1.73^{a}	0.03	<0.001	1.00	0.22	<0.001	0.05
d 0 to 24													
ADG, g	34.00^{a}	33.70^{a}	33.70 ^a 34.00 ^a	33.40^{a}	33.50^{a}	32.40^{ab}	30.50^{b}	1.20	0.040	0.66	0.86	0.002	0.39
ADFI, g	51.70	50.90	51.80	51.30	52.20	50.80	49.90	1.30	0.730	0.96	0.87	0.130	0.48
F/G	1.53°	1.51°	$1.53^{\rm bc}$	1.55^{bc}	$1.56^{\rm bc}$	1.58^{b}	1.65^{a}	0.02	<0.001	0.29	0.40	<0.001	0.34

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				Treatment							<i>P</i> -Value		
Item			Lys-DFB			MSG-DFB		SEM		Lys-DFB)FB	MSG-DFB	DFB
	Control	1%	2%	3%	1%	2%	3%		Diet	Linear	Quad	Linear	Quad
d 24 to 35													
ADG, g	63.90	63.90 62.90 62.90	62.90	61.70	60.60	64.00	59.60	59.60 2.20 0.610	0.610	0.450	0.950	0.270	0.78
ADFI, g	128.60	128.60 125.10 127	127.40	125.10	127.20	128.80	121.00	2.40	0.280	0.450	0.820	0.050	0.18
F/G	2.03	2.00	2.04	2.03	2.13	2.02	2.05	0.06	0.570	0.780	0.830	0.810	0.40
d 0 to 35													
ADG, g	43.00	43.00 42.70	42.70	42.00	41.80	41.80	39.10	1.30	090.0	0.470	0.850	0.005	0.39
ADFI, g	74.90	73.60	74.60	73.90	75.10	74.10	71.10	1.60	0.350	0.720	0.850	0.030	0.20
F/G	1.74°	1.73°	$1.75^{\rm bc}$	$1.77^{\rm bc}$	1.80^{ab}	$1.78^{\rm abc}$	1.83^{a}	0.03	0.009	0.310	0.450	0.010	0.76
Viability, %	93	94	91	93	95	92	90	2.30	0.406	0.700	0.780	0.130	0.26
PEI^3	231 ^a	234^{a}	224^{a}	225 ^a	223 ^a	219^{ab}	196^{b}	14.00	0.040	196° 14.00 0.040 0.440	0.890	0.004	0.38
¹ Data are least square means of 10 replicate pens per treatment with 10 birds per replicate pen	tare means of	f 10 replicat	e pens per tr	eatment with	1 10 birds p	er replicate pe	'n.						
² Lys-DFB = biomass from L-lysine HCl production (IVP73L, Intraco Ltd., Antwerp, Belgium); MSG-DFB = biomass from monosodium L-glutamate production (PL68,	ass from L-ly	vsine HCl p	roduction (IV	VP73L, Intr	aco Ltd., Ar	twerp, Belgit	ım); MSG-	DFB = bi	omass fro	n monosodi	um L-gluta	mate produc	tion (PL68,
Intraco Ltd., Antwerp, Belgium). Experimental	verp, Belgium	ı). Experim	ental diets we	ere fed in a	2-phase diet	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	1 from d () to 10 and	l Phase 2 f	rom d 11 to.	24) and fed	l a common.	Phase 3 diet

from d 25 to 35. ³Production Efficiency Index (PEI) = [ADG × % viability]/[F/G × 10]. ^{a-c}Values within a row lacking a common superscript letter are different ($P \le 0.05$).

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,

Item Lys-DFB MSG-DFB SEM Lys-DFB MSG-DFB MSG-				Ĩ	Treatment							<i>P</i> -Value		
Outcol 1% 2% 3% 1% 2% 3% 1.710 2.044 1.596 1.773 1.701 1.479 183 0.51 0.72 0.6 VH, μ m 1.719 2.044 1.596 1.773 1.701 1.479 183 0.51 0.72 0.6 VH, μ m 2.84 307 286 3.71 317 4.20 3.77 0.96 0.33 0.27 0.6 VH, μ m 1.278 994 1.258 1.111 1.236 1.079 188 0.27 0.6 0.27 0.6 Jejunum 426 377 279 3.56 0.47 0.19 0.5 Jejunum 426 377 279 3.57 1.079 188 0.92 0.6 0.29 0.6 Jejunum 426 377 279 420 420 403 50 0.47 $0.$	Item			Lys-DFB			1SG-DFE	~	SEM		Lys-l	JFB	MSG-DFB	-DFB
Duodenum VH, μm 1,719 2,044 1,596 1,773 1,701 1,479 183 0.51 0.72 0.0 CD, μm 284 307 286 371 317 420 428 46 0.18 0.27 0.0 VH. μm 1,719 5,066 4.95 5,61 4.20 3.77 0.96 0.33 0.27 0.0 Jejunum 1,278 994 1,258 1,111 1,236 1,079 188 0.92 0.80 0.5 0.0 Jejunum VH. μm 1,278 994 1,258 1,111 1,236 1,079 188 0.92 0.80 0.5 Jejunum VH. CD 3.06 2.62 4.50 3.75 2.94 2.98 0.49 0.5 VH. LD 3.06 2.62 3.15 2.94 2.98 3.37 0.68 0.78 0.95 0.95 VH. μm 1		Control	1%	2%	3%	1%	2%	3%		Diet	Linear	Quad	Linear	Quad
VH, μm 1,719 2,044 1,596 1,769 1,773 1,701 1,479 183 0.51 0.72 0.0 CD, μm 284 307 286 371 317 420 428 46 0.18 0.27 0.0 VH: μm 1,278 994 1,258 1,118 1,111 1,236 1,079 188 0.92 0.80 0.7 CD, μm 1,278 994 1,258 1,118 1,111 1,236 1,079 188 0.92 0.80 0.7 CD, μm 1,278 994 1,258 1,118 1,111 1,236 1,079 188 0.92 0.80 0.7 VH, μm 1,187 279 355 379 420 403 50 0.47 0.19 0.2 VH, μm 1,187 896 895 926 765 833 752 149 0.49 0.26 0.7 Item VH, μm 1,187 896 895 926 765 833 752 149 0.49 0.26 0.7 CD, μm 317 274 272 294 352 391 322 59 0.78 0.79 0.6 VH. μm 1,187 896 895 926 765 833 752 149 0.49 0.26 0.7 VH, μm 1,187 896 895 224 0.5 391 322 59 0.78 0.99 0.7 Item VH: μm 1,187 896 895 224 0.5 833 752 149 0.49 0.26 0.7 H:00 mast stare last square means of 10 replicate pens per treatment with 10 birds per replicate pen. Itys-DFB = biomass from L-lysine HCI production (IVP73L, Intrace Ltd., Antwerp, Belgium); MSG-DFB = biomass from nonosodium L-19 meas from nonosodium L-	Duodenum													
CD, µm284307286371317420428460.180.270.VH:CD 6.47 6.62 5.66 4.95 5.61 4.20 3.77 0.96 0.33 0.22 0.6 JejunumJejunum $1,278$ 994 $1,258$ $1,118$ $1,111$ $1,236$ $1,079$ 188 0.92 0.80 0.6 VH, µm $1,278$ 994 $1,258$ $1,118$ $1,111$ $1,236$ $1,079$ 188 0.92 0.80 0.2 VH.CD 3.06 2.62 4.50 3.15 2.94 2.98 3.37 0.68 0.58 0.49 0.2 VH.CD 3.06 2.62 4.50 3.15 2.94 2.98 3.37 0.68 0.58 0.49 0.2 VH, µm $1,187$ 896 895 926 765 833 752 149 0.49 0.26 0.2 VH, µm $1,187$ 896 895 926 765 833 752 149 0.49 0.26 0.2 VH.CD 3.58 3.52 3.48 3.36 2.21 2.35 2.74 0.59 0.78 0.90 VH.CD 3.58 3.52 2.48 3.36 2.21 2.35 2.74 0.59 0.78 0.97 0.79 VH.CD 3.58 3.52 2.14 2.74 0.59 0.78 0.97 0.79 0.79 0.79 0.79 0.79	VH, μm	1,719	2,044	1,596	1,769	1,773	1,701	1,479	183	0.51	0.72	0.69	0.35	0.46
VH:CD 6.47 6.62 5.66 4.95 5.61 4.20 3.77 0.96 0.33 0.22 0.0 Jejunun UH, µm $1,278$ 994 $1,258$ $1,111$ $1,236$ $1,079$ 188 0.92 0.80 0.7 VH, µm $1,278$ 994 $1,258$ $1,111$ $1,236$ $1,079$ 188 0.92 0.80 0.7 CD, µm 426 377 279 355 379 420 403 50 0.47 0.19 0.2 VH:CD 3.06 2.62 4.50 3.15 2.94 2.98 3.37 0.68 0.28 0.49 0.2 VH, µm $1,187$ 896 895 926 765 833 752 149 0.49 0.26 0.2 VH.cD 3.17 274 272 2.94 352 391 322 59 0.78 0.79 0.2 VH:CD 3.58 3.52 3.48 3.36 2.211 2.35 2.74 0.59 0.78 0.92 0.92 VH:CD 3.58 3.52 3.48 3.36 2.211 2.35 2.74 0.59 0.79 0.97 0.79 Pasty 22.22 21.11 15.0 24.0 14.1 21.4 30.0 4.2 0.179 0.97 0.79 Pasty $veets, veets, veets, veets$ 22.22 21.11 15.0 24.0 14.1 21.4 30.0 4.2 0.1	CD, μm	284	307	286	371	317	420	428	46	0.18	0.27	0.52	0.02	0.08
JejunumJejunumUH, μm 1,2789941,2581,1181,1111,2361,0791880.920.800.7VH, μm 1,278377279355379420403500.470.190.5VH:CD3.062.624.503.152.942.983.370.680.580.490.5VH, μm 1,1878968959267658337521490.490.260.5NH, μm 1,1878968959267658337521490.790.6NH, μm 1,1878968959267658337521490.790.6NH, μm 1,1878968959267658337521490.790.6NH, μm 1,1878968959267658337521490.790.79Oth, μm 317274272294352391322590.780.790.6Pasty2222222222200.790.790.79Pasty222222222204000Pasty2222222220000000000<	VH:CD	6.47	6.62	5.66	4.95	5.61	4.20	3.77	0.96	0.33	0.22	0.66	0.04	0.83
VH, μ m1,2789941,2581,1181,1111,2361,0791880.920.800.20CD, μ m426377279355379420403500.470.190.20VH:CD3.062.624.503.152.942.983.370.680.580.490.20IleumNH:CD3.062.624.503.152.942.983.370.680.790.26VH, μ m1,1878968959267658337521490.790.260.2VH, μ m3172742722.94352391322590.780.790.2VH:CD3.583.523.483.362.2112.352.740.590.780.790.2VH:CD3.583.523.483.362.2112.132.140.000.780.790.2Pasty vents, ϕ_3^3 22.2221.1115.024.014.121.430.04.20.120.970.79Data are least square means of 10 replicate pens per treatment with 10 birds per replicate pens.12.1430.04.20.120.970.790.790.79Use DFB = biomass from L-lysine HCI production (IVP73L, Intrace Ltd., Antwerp, Belgium); MSG-DFB = biomass from monosodium L0.770.790.720.790.770.790.790.790.790.790.790.790.790.7	Jejunum													
CD, μ m426377279355379420403500.470.190.2VH:CD3.062.624.503.152.942.983.370.680.580.490.2 Ileum VH, μ m1,1878968959267658337521490.490.260.2VH, μ m1,1878968959267658337521490.490.260.2VH: μ m317274272294352391322590.780.790.2VH:CD3.583.523.483.362.2112.140.590.480.800.2Pasty vents, ϕ_{3} 22.22.14115.024.014.121.430.04.20.120.970.5Data are least square means of 10 replicate pens21.115.024.014.121.430.04.20.120.970.5Data are least square means of 10 replicate pensPens per treatment with 10 birds per replicate pen.A.20.120.970.77Lys-DFB = biomass from L-lysine HCI production (IVP71L, Intrace Ltd., Antwerp, Belgium); MSG-DFB = biomass from monosodium L-	VH, μm	1,278	994	1,258	1,118	1,111	1,236	1,079	188	0.92	0.80	0.71	0.58	0.98
VH:CD 3.06 2.62 4.50 3.15 2.94 2.98 3.37 0.68 0.58 0.49 0.1 IleumN H, μ m $1,187$ 896 895 926 765 833 752 149 0.49 0.26 0.2 VH, μ m 317 274 272 294 352 391 322 59 0.78 0.79 0.2 CD, μ m 317 274 272 294 352 391 322 59 0.78 0.79 0.2 VH:CD 3.58 3.52 3.48 3.36 2.211 2.35 2.74 0.59 0.48 0.80 0.5 Pasty vents, $\%^3$ 22.2 21.11 15.0 24.0 14.1 21.4 30.0 4.2 0.12 0.97 0.5 Data are least square means of 10 replicate pens per treatment with 10 birds per replicate pen. $MSG-DFB = biomass from L-bysine HCI production (IVP731, Intrace Ltd., Antwerp, Belgium); MSG-DFB = biomass from monosodium L$	CD, μm	426	377	279	355	379	420	403	50	0.47	0.19	0.24	0.91	0.77
Ileum 1,187 896 895 926 765 833 752 149 0.49 0.26 0.3 VH, µm 1,187 896 895 926 765 833 752 149 0.49 0.26 0.3 CD, µm 317 274 272 294 352 391 322 59 0.78 0.79 0.3 VH:CD 3.58 3.52 3.48 3.36 2.21 2.35 2.74 0.59 0.48 0.80 0.6 Pasty vents, % ³ 22.2 21.1 15.0 24.0 14.1 21.4 30.0 4.2 0.12 0.97 0.5 Data are least square means of 10 replicate pens per treatment with 10 birds per replicate pen. 14.1 21.4 30.0 4.2 0.12 0.97 0.5 Lys-DFB = biomass from L-lysine HCI production (IVP731, Intrace Ltd., Antwerp, Belgium); MSG-DFB = biomass from monosodium L 14.1 21.4 30.0 4.2 0.12 0.97 0.5	VH:CD	3.06	2.62	4.50	3.15	2.94	2.98	3.37	0.68	0.58	0.49	0.51	0.76	0.71
VH, μ m1,1878968959267658337521490.490.260.3CD, μ m317274272294352391322590.780.790.3VH:CD3.583.523.483.362.212.352.740.590.480.800.9Pasty vents, $\frac{96^3}{20^3}$ 22.221.115.024.014.121.430.04.20.120.970.2Data are least square means of 10 replicate pens per treatment with 10 birds per replicate pen.21.430.04.20.120.970.2Lys-DFB = biomass from L-lysine HCI production (IVP731, Intrace Ltd., Antwerp, Belgium); MSG-DFB = biomass from monosodium L	lleum													
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VH:CD 3.58 3.52 3.48 3.36 2.21 2.35 2.74 0.59 0.48 0.90 0.9 Pasty vents, %63 22.2 21.1 15.0 24.0 14.1 21.4 30.0 4.2 0.12 0.97 0.2 Data are least square means of 10 replicate pens per treatment with 10 birds per replicate pen. Lys-DFB = biomass from L-lysine HCl production (IVP731, Intraco Ltd., Antwerp, Belgium); MSG-DFB = biomass from monosodium L	CD, μm	317	274	272	294	352	391	322	59	0.78	0.79	0.59	0.85	0.39
Pasty vents, %322.221.115.024.014.121.430.04.20.120.970.1Vents, %3Data are least square means of 10 replicate pens per treatment with 10 birds per replicate pen.Data are least square means of 10 replicate pens per treatment with 10 birds per replicate pen.MSG-DFB = biomass from L-lysine HCl production (IVP731, Intraco Ltd., Antwerp, Belgium); MSG-DFB = biomass from monosodium L-	VH:CD	3.58	3.52		3.36	2.21	2.35	2.74	0.59	0.48	0.80	0.96	0.39	0.16
Data are least square means of 10 replicate pens per treatment with 10 birds per replicate pen. Lys-DFB = biomass from L-lysine HCl production (IVP73L, Intraco Ltd., Antwerp, Belgium); MSG-DFB = biomass from monosodium L	Pasty vents, % ³	22.2	21.1	15.0	24.0	14.1	21.4	30.0	4.2	0.12	0.97	0.22	0.10	0.04
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	Data are least so Lys-DFB = bion ntraco Ltd., Ant	Juare means o mass from L-l werp, Belgiur VH - willie b	f 10 replicat ysine HCl p n). Experim	te pens per production (lental diets v	treatment w IVP73L, In were fed in	ith 10 birds traco Ltd., / a 2-phase di	per replicat Antwerp, Be et series (Pl	e pen. slgium); MS 1ase 1 from	G-DFB = 1 d 0 to 10 ar	biomass frc rd Phase 2	im monosodi from d 11 to	um L-gluta 24) and fed	mate produc a common]	ttion (PL6) Phase 3 di

Table 5. Effect of dried fermentation biomass from L-Lys HCl and monosodium L-glutamate production on gut morphology and incidence

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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.

REFERENCES

Almeida FN, Sulabo RC and Stein HH. 2014. Amino acid digestibility and concentration of digestible and metabolizable energy in a threonine biomass product fed to weanling pigs. *J Anim Sci* 92:4540–4546.

- AOAC (Association of Official Analytical Chemists). 2007. *Official Methods of Analysis*.18th ed. Howitz W and Latimer Jr. GW, ed. Gaithersburg, MD: AOAC Int.
- Apajalahti J and Vienola K. 2016. Interaction between chicken intestinal microbiota and protein digestion. *Anim Feed Sci Technol* 221:323-330.
- Beski SSM, Swick RA and Iji PA. 2015. Specialized protein products in broiler chicken nutrition: A review. *Anim Nutr* 1:47-53.
- Blaesen M, Friehs K and Flaschel E. 2007. Recycling of bacterial biomass in a process of l-threonine production by means of a recombinant strain of *Escherichia coli*. J Biotechnol 132:431–437.
- Burrin DG and Stoll B. 2009. Metabolic fate and function of dietary glutamate in the gut. *Am J Clin Nutr* 1:850-856.
- Carpenter AJ, Binversie E, Ruiz-Moreno M and Stern MD. 2017. Effect of dried fermentation biomass on microbial fermentation in continuous culture and *in vitro* intestinal digestibility. *Anim Feed Sci Technol* 230:47-58.
- De Cesare A, Sirri F, Manfreda G, Moniaci P, Giardini A and Zampiga M. 2017. Effect of dietary supplementation with *Lactobacillus acidophilus* D2/CSL (CECT 4529) on caecum microbiome and productive performance in broiler chickens. *PLoS ONE* 12:e0176309.
- Holst DO. 1973. Holst filtration apparatus for Van Soest detergent fiber analysis. J Assoc of Anal Chem 56:1352–1356.
- Ikeda M. 2003. Amino acid production processes. Adv Biochem Eng Biotechnol 79:1-35.
- Kiessling A and Askbrandt S. 1993. Nutritive value of two bacterial strains of single-cell protein for rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* 109:119–130.
- Kinoshita S, Udaka S and Shimono M. 2004. Studies on the amino acid fermentation. Part 1. Production of L-glutamic acid by various microorganisms. *J Gen Appl Microbiol* 50:331-43.
- Olubodun J, Zulkifli I, Hair-Bejo M, Kasim A and Soleimani AF. 2015. Physiological response of glutamine and glutamic acid supplemented broiler chickens to heat stress. *Europ Poult Sci* 79:1-12.
- Porto ML, Givisiez PEN, Saraiva EP, Costa FGP, Moreira Filho ALB, Andrade MFS, Brandão PA and Guerra RR. 2015. Glutamic acid improves body weight gain and intestinal morphology of broiler chickens submitted to heat stress. *Braz J Poult Sci* 17:355-362.
- Research and Markets. 2020. Amino Acids Market: Global Industry Trends, Share, Size, Growth, Opportunity and Forecast 2019-2024. Retrieved on 8 May 2020 from https://www.researchandmarkets.com/reports.
- Roy A, Haldar S, Mondal S and Ghosh TK. 2010. Effects of supplemental exogenous emulsifier on performance, nutrient metabolism, and serum lipid profile in broiler chickens. *Vet Med Int* 2010:1-9.
- Oliveira MSF, Espinosa CD, Berrocoso JD, Rojas OJ, Htoo JK and Stein HH. 2020. Concentration of digestible and metabolizable energy in L-threonine and L-valine biomass products fed to weanling pigs. *Anim Feed Sci Technol* 263:114463.
- Sulabo RC, Mathai JK, Usry JL, Ratliff BW, Mckilligan DM, Moline JD, Xu G and Stein HH. 2013. Nutritional value of dried fermentation biomass, hydrolyzed porcine intestinal mucosa products, and fish meal fed to weanling pigs. *J Anim Sci* 91:2802–2811.

- Tonouchi N and Ito H. 2017. Present global situation of amino acids in industry. *Adv* Biochem Eng Biotechnol 123:3-14.
- Utterback P, Jimenez E, Block S, Less J and Parsons C. 2011. Threonine biomass as a source of amino acids for poultry. *Poult Sci* 90 (E-Suppl. 1).
- Wijayasekara K and Wansapala J. 2017. Uses, effects and properties of monosodium glutamate (MSG) on food and nutrition. *Int J Food Sci Nutr* 2:132-143.