ABANICO VETERINARIO E-ISSN 2448-6132 abanicoveterinario@gmail.com
https://abanicoacademico.mx/revistasabanico-version-nueva/index.php/abanico-veterinario

Abanico Veterinario. January-December 2023; 13:1-19. http://dx.doi.org/10.21929/abavet2023.1
Original Article. Received: 26/01/2022. Accepted:20/01/2023. Published: 05/02/2023. Chave: e2022-8.
https://www.youtube.com/watch?v=OvqkS2QHU8A

Preliminary studies of metabolizable energy and apparent ileal amino acid digestibility of jumbo squid (*Dosidicus gigas*) meal in chicken diets

Estudio preliminar de energía metabolizable y digestibilidad íleal aparente de aminoácidos de harina de calamar gigante (*Dosidicus gigas*) en dietas para pollo

Benjamín Fuente-Martínez[1] ID , María Carranco-Jáuregui*[2] ID, Silvia Carrillo-Domínguez[2] ID, Luz Tejeda-Jarero[3], María Calvo-Carrillo[2] ID

[1]Centro de Enseñanza, Investigación y Extensión en Producción Avícola, Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de México, Manuel M. López S/N, Colonia Santiago Zapotitlán, Alcaldía Tláhuac, 13209, Ciudad de México, México. [2]Departamento de Nutrición Animal Dr. Fernando Pérez-Gil Romo, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Vasco de Quiroga No. 15, Col. Belisario Domínguez Sección XVI, Alcaldía Tlalpan 14080, Ciudad de México, México. [3]Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de México. Av. Universidad No. 3000, Universidad Nacional Autónoma de México, CU, Alcaldía Coyoacán, 04510, Ciudad de México. *Author for correspondence: Carranco-Jáuregui Ma. Elena. Vasco de Quiroga No. 15, Col. Belisario Domínguez Sección XVI, Alcaldía Tlalpan 14080, Ciudad de México, México. Correo electrónico: rexprimero@hotmail.com, benjaminfuente@yahoo.com.mx, silvicarrillo3@hotmail.com, lunet_ly@hotmail.com, concepcion_calvo1@hotmail.com

ABSTRACT

The objective was to determine apparent metabolizable energy (AMEn) and apparent ileal digestibility (AID) of amino acids in giant squid (*Dosidicus gigas*) (GSM) meal, as a protein alternative in diets for broilers. Experiment 1: in GSM the AMEn and the AID of its amino acids were determined. Ninety-six chickens were fed a diet with 8 and 16% GSM for 21 days of age. Experiment 2: 72 chickens were fed with 16 and 20% GSM in the diet for 42 days. Productive parameters, carcass yield, skin color and flavor meat were evaluated. Results: AMEn was 3376.15 kcal/kg and AID of amino acids greater than 68%, except histidine (21%) and cysteine (47%). Weight gain and final weight were reduced with 16% GSM ($P < 0.05$) compared to the control at 21 days of age, however at 42 days no differences were detected ($P > 0.05$) between treatments with 16 and 20%, both in the productive parameters and in carcass yield and chicken weight ($P > 0.05$). The taste of the meat was not affected by GSM ($P > 0.05$). It is concluded that GSM is an alternate source of protein in diets for broilers.
Keywords: Squid meal, metabolizable energy, apparent ileal digestibility, broilers.

RESUMEN

El objetivo fue determinar la energía metabolizable aparente (EMAn) y digestibilidad íleal aparente (CDI) de aminoácidos en la harina de calamar gigante (*Dosidicus gigas*) (HCG), como alternativa proteica en dietas para pollos de engorda. Experimento 1: en la HCG se determinó la EMAn y el CDI de sus aminoácidos. A 96 pollos se les proporcionó dieta con 8 y 16% de HCG durante 21 días de edad. Experimento 2: 72 pollos fueron alimentados con 16 y 20% de HCG en la dieta, durante 42 días. Variables productivas, rendimiento de canal, color de piel y sabor fueron evaluados. Resultados: EMAn fue 3376.15 kcal/kg y CDI de los aminoácidos mayor a 68%, excepto histidina (21%) y cisteína (47%). Ganancia de

peso y peso final se redujeron con 16% HCG (P < 0.05) respecto al testigo a los 21 días de edad, sin embargo, a los 42 días no se detectaron diferencias (P > 0.05) entre tratamientos con 16 y 20%, tanto en las variables productivas como en rendimiento de la canal y peso de pollos (P > 0.05). El sabor de la carne no se vio afectado por HCG (P > 0.05). Se concluye que la HCG es una fuente alterna de proteína en dietas para pollos de engorda.
Palabras clave: Harina de calamar, energía metabolizable, digestibilidad íleal aparente, pollos de engorda.

INTRODUCTION

In Mexico, chicken meat production in 2020 was 3.952 million tons, representing two thirds of total meat consumption. Poultry farming constitutes 38.8% of the activities generating protein (meat) for human consumption (31 kg/per capita) (UNA, 2021), and has played an important role in the family, especially during the pandemic, thus contributing to the welfare of the population. On the other hand, the annual production of balanced animal feed in the last 4 years has maintained 4% growth, which covers the demand required to guarantee quality animal protein for Mexicans. The Global Feedstuffs Survey mentions that Mexico, at the end of 2020, climbed to the 5th position worldwide with a production of 38 million MT (Alltech, 2021; CONAFAB, 2021).

Within the total production price, feed is the item with the greatest impact, representing approximately 63% of costs (UNA, 2021). Therefore, industries dedicated to feed formulation are in a constant search for economically viable resources that do not sacrifice diet quality and, therefore, production variables (UNA, 2021). One of these possible resources could be giant squid meal (*Dosidicus gigas*), a cephalopod distributed throughout the American continent from California, United States, to southern Chile. Studies indicate that it has a high protein content, but the information that has been published on this aspect and others is scarce; in fact, it is not known how available this protein is for birds. Worldwide, Mexico is among the leading countries in giant squid (*Dosidicus gigas*) production (2.598 MT), with Sinaloa State being the main producer with 1.600 MT (De la Cruz *et al*., 2011).

Squid consumption in Mexico is estimated at 0.53 kg/year/*per capita*, which represents 3.8% of seafood consumption, far below countries such as South Korea, Japan and Spain that consume more than 3.5 kg/year/*per capita*/year, as this is a product with high protein content, fatty acids and low cost (De la Cruz *et al*., 2011). There is another market segment that can give added value to the squid fishery, this is to use the viscera and other parts of the squid or the whole specimen that does not meet the quality characteristics for human consumption for the production of meal for animal consumption, considering that this meal is a source of protein and fatty acids (Calvo *et al*., 2016). Therefore, the objective of this research was to determine the metabolizable energy content and apparent ileal digestibility of amino acids of giant squid (*Dosidicus gigas*) meal to be incorporated as an alternative source of protein in broiler diets.

MATERIAL AND METHODS

The protocol was approved by the Institutional Committee for the Care and Use of Experimental Animals (CICUAE-FMVZ-UNAM) (approval: MC-2017/2-25) (NOM-062-ZOO-1999). Experiments were conducted at the Center for Teaching, Research and Extension in Poultry Production of the FMVZ, UNAM, in Mexico City.

Chemical analysis of giant squid (*Dosidicus gigas*) meal (GSM)

GSM was obtained from whole squid specimens (mantle, fin, tentacles, feather and viscera), caught in Santa Rosalia, Baja California Sur, Mexico. Laboratory determinations were carried out by the techniques described in AOAC (2019); true protein (Tejada, 1992); amino acid profile quantification by HPLC using the AccQ-TAG Waters (1993) method and non-protein nitrogen (NNP) by the formula:

$$\% NNP = \% \text{ N soluble} - \text{N true soluble}$$

Diet preparation and biological assay

Three sorghum plus soybean paste based diets were formulated according to the nutritional requirements of the Ross 308 strain (Aviagen, 2014) for broilers up to 3 weeks of age. Soybean paste was partially substituted with GSM at 8 and 16% using Nutrition for Windows^{TM} software (version 5.0 Pro.) (Table 1).

Ninety-six Ross 308 mixed broilers of 1-day-old were used, distributed in a completely randomized design of 3 treatments with 4 replicates each. Birds were housed in battery cages at a density of 8 birds (4 males and 4 females) per replicate. Feed and water were freely available for 21 days.

Productive variables, GSM metabolizable energy and apparent ileal digestibility (AID) of GSM amino acids

A weekly record of weight gain (g), feed intake (g/bird) and feed conversion ratio (kg:kg) was kept. Excreta were collected on the last 3 days of the experiment, dried (60 °C/24 h) and ground. Both diets and excreta were subjected to gross energy determination by calorimetric pump, Parr 1341 Series, Parr Instrument Company, USA. To calculate the apparent metabolizable energy (AMEn) corrected to zero nitrogen balance, it was considered that if nitrogen is not retained it will appear as uric acid excreted in urine and that 1 g of N excreted as uric acid is equivalent to 8.22, a factor used to make the correction (Potter *et al*., 1960).

Table 1. Dietary composition (kg/t) of experiment 1 for chicks from 1 to 21 days of age

Ingredients	Control Diet (CD)	CD + 8% GSM	CD + 16% GSM
Sorghum	543.929	573.053	605.952
Soybean paste	369.205	317.183	264.413
Squid meal	0.000	29.530	59.100
Vegetable oil	35.307	29.353	22.183
Calcium phosphate	17.605	16.058	14.498
Calcium carbonate	14.744	15.780	16.822
DL-Methionine 99%.	4.248	3.985	3.720
Salt	3.823	3.968	2.242
L-Lysine HCl	3.016	2.062	1.124
Titanium Dioxide	3.000	3.000	3.000
Vitamin and mineral premix[1]	3.000	3.000	3.000
L-Threonine	1.146	1.168	1.192
Coccidiostat[2]	0.500	0.500	0.500
L-Arginine	0.203	1.085	1.980
Antioxidant[3]	0.150	0.150	0.150
Enradin®[4]	0.125	0.125	0.125
Total	1000	1000	1000
Calculated analysis (g/kg)			
Crude Protein	23	23	23
ME (kcal/kg)	3000	3000	3000
Total Methionine	0.738	0.720	0.702
Methionine + Cysteine	1.080	1.080	1.080
Lysine	1.440	1.440	1.440
Threonine	0.970	0.970	0.970
Tryptophan	0.302	0.302	0.302
Arginine	1.520	1.520	1.520
Total Calcium	0.960	0.960	0.960
Available phosphorus	0.480	0.480	0.480
Sodium	0.160	0.160	0.160

[1] Vitamin and mineral premix: Vitamins: A, 4.667 kUI; D3, 1.500 kUI; E, 23.333.5 mg; K3, 1333.275 mg; B1, 1000.04 mg; B2, 3666.4; B6, 1333.32; B12, 8.33 mg; nicotinamide, 26,667 mg; pantothenic acid, 8.333.1 mg; folic acid, 666.4 mg; biotin, 66. 7; choline chloride, 199,999.8 mg; Minerals: Cu, 5000 mg; Fe, 26,666.8 mg; Mn, 41,333.54 mg; I, 400 mg; Zn, 36,666.72 mg; Se, 100 mg; $CaCO_3$, 216 mg; mineral oil, 5 g; vehicle q.s. 1,000 kg. [2] Nicarfeed® (HELM de México, S.A.); nicarbazin at 25%. [3] Feed OX® (Dresens Química S.A. de C.V.); BHA (Butylated Hydroxyanisole), 1.2 %; BHT (Butylated Hydroxytoluene), 9.0 %; Ethoxyquin, 4.8%; chelating agents, 10 %: excipient q.s. 100 %. [4] Enradin® (MSD): 80g/kg enramycin

The following equations were used for these calculations:

$$AMEn\ (kcal\ por\ kg) = EMt - \frac{EMt - EMi}{Inclusion\ ratio}$$

Where:

$$EMt\ or\ EMi = Energy\ d - (Energy\ h + 8.22 \times N\ retained)$$

$$Energy\ d = is\ obtained\ directly\ from\ the\ calorimeric\ unit$$

$$Energy\ h = energy\ per\ mg\ of\ excreta \times \frac{Dietary\ marker}{Feces\ marker}$$

$$N\ retained = N\ per\ mg\ of\ diet - N\ per\ mg\ of\ excreta\ \times \frac{Dietary\ marker}{Feces\ marker}$$

Where: *EMt:* Metabolizable energy per mg of control diet; *EMi:* Metabolizable energy per mg of inclusion diet; *Energy d*: Energy per mg of diet; *Energy h*: Excreta energy per mg of diet; *Retained N*: Retained nitrogen (expressed in mg) per mg of diet.

At 21 days of age, chicks were sacrificed by the cervical dislocation method (NOM-033-ZOO-1995). Contents of the ileum (from the portion of the intestine bounded by the vitelline diverticulum to 2 cm before the cecal bifurcation) were extracted (Widyarante & Drew, 2011), oven dried (60°C/36 h), ground and amino acid profile analysis was performed by ion exchange chromatography MME-AA-01 and MME-AA-02 and for tryptophan A-0099-007/11 (INCMNSZ, 2011). 0.3% TiO2 was incorporated into the diets as an indigestible marker and determined in the excreta by spectrophotometry (SP6-500UV spectrophotometer, UK) (Jagger *et al.,* 1992). Once the results of the amino acid profile (of the diets, ileal content and TiO_2) in excreta were obtained, the AID coefficients of the amino acids proposed by Lemme *et al.,* (2004) were calculated:

$$Recovery\ indicator\ (\%) = \frac{Indicator\ excreted}{Indicator\ consumed}\ x\ 100$$

$$DIA\ (\%)\ =\ 100 - \left[\frac{(M\ diet\ x\ AA\ excreta)}{(M\ excreta\ x\ AA\ diet)} x100\right]$$

Where: M diet and M excreta = marker in the diet and excreta, respectively (expressed in dry matter and g/kg); AA diet and AA excreta = amino acids in the diet and excreta, respectively (expressed in dry matter and g/kg). The correction for NNP to the digestibility coefficients was made by subtracting from the proportion of GSM in the diet the percentage of this component.

Statistical analysis

The results obtained for the productive variables were analyzed using a completely randomized design, with a significance level of 95% (Kuehl, 2001). Differences between means were compared by Tukey's test with a $P < 0.05$ using the JMP Ver. 8 program.

Experiment 2

Before formulating the diets, an amino acid profile analysis of the ingredients (soybean paste, corn and GSM) was performed by Near Infrared Spectroscopy (NIR) (AMINONIR® Portable, Germany) (AMINODat, 2016). Three diets based on corn plus soybean paste were formulated according to the nutritional requirements of the Ross 308 strain (Aviagen, 2014) partially replacing soybean paste with GSM (16 and 20%) for 3 phases: initiation, growth and finishing (Table 2). For the formulation, protein, AMEn and digestible amino acid values of GSM obtained from the results of experiment 1 were used, using the Nutrion Windows™ program (Version 5.0 Pro). Seventy-two Ross 308 broilers of 1-day-old (36 females and 36 males) were used, distributed in a completely randomized design in 3 treatments with 4 replicates each. Chicks were housed in battery cages, with a density of 6 birds per replicate (3 males and 3 females). Water and feed were freely available during 42 days of the trial.

Productive variables and carcass yield, *in vivo* skin yellowing and sensory evaluation of meat

Weight gain (g), feed intake (g/bird) and feed conversion ratio (kg:kg) were recorded. At the end of the trial, all animals were slaughtered according to the methods described in the Mexican Official Standard for evaluating hot carcass yield (expressed as a percentage of live weight) (NOM-033-ZOO-1995). The eviscerated carcasses were then frozen (-20°C) for subsequent sensory evaluation. Skin pigment was measured in all chickens on day 42 before slaughter, in the right costal apterylic area with a Konica Minolta Model CR-400®, USA reflectance colorimeter according to the CIELab system, reporting the values of L, a* and b* (Martínez, 1996).

Thirty regular consumers of chicken meat participated in the sensory evaluation. Breast and leg plus thigh were cooked without salt for 45 minutes/85°C, shredded and placed on plates previously identified with random numbers to carry out a taste acceptance test with a 5-point hedonic scale: 1 likes a lot, 2 likes a little, 3 indifferent, 4 dislikes a little and 5 dislikes a lot (Pedrero & Pangborn, 1989).

Table 2. Composition of the diets of experiment 2

	Initiation (1-10 days)			Growth (11-24 days)			Finalization (25-42 days)		
Ingredient	Control diet (CD)	CD+16 % GSM	DT+20% GSM	Control diet (CD)	CD+16% GSM	CD+20% GSM	Control diet (CD)	CD+16% GSM	CD+20% GSM
Corn	618.393	649.891	657.775	666.645	703.552	710.129	702.793	738.321	747.072
Soybean paste	337.695	252.158	230.768	281.338	208.488	190.643	238.480	176.053	160.469
Giant squid meal (GSM)	0.000	54.030	67.539	0.000	45.000	56.270	0.000	38.160	47.70
Calcium carbonate	13.693	15.383	15.805	12.445	13.867	14.220	11.174	12.385	12.688
Calcium phosphate	11.350	8.914	8.305	9.539	7.494	6.986	7.635	5.895	5.460
Vegetable oil	-----	-----	-----	8.777	0.000	0.000	18.090	7.066	4.353
Salt	3.498	2.037	1.672	3.490	2.266	1.962	3.484	2.443	2.183
DL-Methionine 99%	3.530	3.590	3.604	3.212	3.247	3.260	2.878	2.902	2.908
Natural yellow pigment[1]	-----	-----	-----	3.000	3.000	3.000	6.000	6.000	6.000
Mycotoxin sequestering agent[2]	3.000	3.000	3.000	2.000	2.000	2.000	2.000	2.000	2.000
Vitamin and mineral premix[3]	3.000	3.000	3.000	3.000	3.000	3.000	2.400	2.400	2.400
L-yisine 99%	2.452	3.123	3.291	2.564	3.147	3.287	2.252	2.756	2.882
L-Threonine 99%	1.473	1.655	1.700	1.349	1.501	1.539	1.036	1.165	1.197
L-Arginine 99%	0.041	0.828	1.025	0.340	1.013	1.177	0.228	0.806	0.950
L-Tryptophan 99%	0.000	0.093	0.216	0.000	0.124	0.227	0.000	0.098	0.187
Coccidiostat[4]	0.500	0.500	0.500	0.500	0.500	0.500	0.500	0.500	0.500
Antioxidant[5]	0.150	0.150	0.150	0.150	0.150	0.150	0.150	0.150	0.150
Zinc Bacitracin[6]	0.550	0.550	0.550	0.550	0.550	0.550	0.550	0.550	0.550
Phytase[7]	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100
Betaine anhydrous[8]	-----	-----	-----	-----	-----	-----	0.250	0.250	0.250
Choline Chloride 60%	1.000	1.000	1.000	1.000	1.000	1.000	-----	-----	-----
Total	1000	1000	1000	1000	1000	1000	1000	1000	1000
Calculated analysis (%)									
Crude protein	21.421	21.421	21.421	19.000	19.000	19.000	17.161	17.160	17.160
Metabolizable energy (kcal/kg)	3000	3081	3101	3100	3122	3139	3200	3200	3200

Methionine + digestible cysteine	0.950	0.950	0.950	0.870	0.870	0.870	0.800	0.800	0.800
Digestible Lysine	1.280	1.280	1.280	1.150	1.150	1.150	1.020	1.020	1.020
Digestible tryptophan	0.860	0.860	0.860	0.770	0.770	0.770	0.680	0.680	0.680
Digestible Arginine	0.240	0.200	0.200	0.209	0.180	0.180	0.185	0.160	0.160
Digestible Valine	1.370	1.370	1.370	1.230	1.230	1.230	1.090	1.090	1.090
Total calcium	0.954	0.910	0.898	0.855	0.818	0.809	0.780	0.749	0.741
Available phosphorus	0.960	0.960	0.960	0.870	0.870	0.870	0.780	0.780	0.780
Sodium	0.480	0.480	0.480	0.435	0.435	0.435	0.390	0.390	0.390
Digestible tryptophan	0.160	0.160	0.160	0.160	0.160	0.160	0.160	0.160	0.160

[1] Avelut®; 15g/g xanthophylls (tagetes erecta). [2] Klinsil® (HELM de México, S.A.): Phosphosilicates 30% (Zeolite clay). [3] Vitamin and mineral premix: Vitamins: A, 4.667 kUI; D3, 1,500 kUI; E, 23.333.5 mg; K3, 1333.275 mg; B1, 1000.04 mg; B2, 3666.4; B6, 1333.32; B12, 8.33 mg; nicotinamide, 26,667 mg; pantothenic acid, 8.333. 1 mg; folic acid, 666.4 mg; biotin, 66.7; choline chloride, 199.999.8 mg; Minerals: Cu, 5000 mg; Fe, 26.666.8 mg; Mn, 41,333.54 mg; I, 400 mg; Zn, 36.666.72 mg; Se, 100 mg; $CaCO_3$, 216 mg; mineral oil, 5 g; vehicle q.s. 1.000 kg. [4] Nicarfeed® (HELM de México, S.A.); nicarbazin 25%. [5] Feed OX® (Dresens Química S.A. de C.V.); BHA (Butylated Hydroxyanisole), 1.2%; BHT (Butylated Hydroxytoluene), 9.0%; Ethoxyquin, 4.8%; chelating agents, 10%: excipient q.s. 100%. [6] Bacitra-Feed 10%; zinc bacitracin, 100g, q.s. 1000g. [7] *Ronozyme Hiphos* M® 6-phytase from *Aspergillus oryzae* 50,000 FYT/g. [8] Betafin, betaine anhydrous 96% food grade

Statistical analysis

The productive variables for the initiation phase were analyzed under a completely randomized design. Due to the correlation that existed between the different phases, the growth and finishing variables were analyzed using a completely randomized design with covariates, where these were from the phase immediately preceding each one (Kuehl, 2001). The yellowing and carcass yield variables were analyzed according to a randomized design with a 3x2 factorial arrangement where the first factor was percentage of GSM inclusion (0, 16 and 20%) and the second factor was sex (female or male) (Kuehl, 2001). Differences between means were analyzed by Tukey's test with a $p < 0.05$ using the JMP Ver. 8 program. Sensory evaluation results were analyzed using Friedman's nonparametric test ($p < 0.05$) (Pedrero & Pangborn, 1989).

RESULTS

Experiment 1

Tables 3 and 4 present the chemical composition, digestibility coefficients (DC), as well as the AMEn of GSM. Crude protein (73.50%), true protein (55.14%) and NNP (18.36%) of the total nitrogenous compounds. AMEn value was 3376.15 kcal/kg. The AID of amino acids was greater than 68%, except for histidine (21%) and cysteine (47%).

Table 3. Chemical composition of giant squid (*Dosidicus gigas*) meal

Component	g/100g
Humidity	6.11
Crude protein[1]	73.50
True protein[1]	55.14
Non-protein nitrogen (NNP)[1]	18.36
Total phosphorus[2]	1.32
Available phosphorus	1.19
Ash	9.65
GE (kcal/kg)	4875.00
AMEn (kcal/kg)	3376.15

[1]Expressed on the basis of flour moisture.
[2]Based on 90% digestibility.
GE= Gross energy
AMEn= Apparent Metabolizable Energy

Table 5 shows that the treatment with 16% GSM in final weight and weight gain presented lower values compared to the control diet and the highest feed conversion ratio ($P < 0.05$). Feed intake was similar for all treatments.

Table 4. Amino acid profile of the giant squid (*Dosidicus gigas*) meal

	Amino acids (g/100g sample)				
		With correction by NNP		Without correction by NNP	
	Totals[1]	CD (%)	Digestible (%)	CD (%)	Digestible (%=
Essentials					
Methionine	1.42	89.1	1.27	89.6	1.27
Met + Cis*	2.01	68.2	1.37	69.7	1.40
Lysine	3.87	79.5	3.08	80.4	3.11
Threonine	2.74	73.2	3.08	80.4	3.11
Tryptophan	0.40	ND	ND	ND	ND
Arginine	4.31	82.5	3.56	83.2	3.59
Isoleucine	2.40	76.2	1.83	77.3	1.85
Leucine	4.50	76.2	3.43	77.3	3.48
Valine	2.86	76.7	2.19	77.8	2.23
Histidine	1.58	21.5	0.34	24.8	0.39
Phenylalanine	2.25	78.0	1.75	78.9	1.77
Non-essential					
Cysteine	0.59	47.3	0.28	49.8	0.29
Serine	ND	72.1	ND	73.4	ND
Proline	ND	70.3	ND	71.7	ND
Alanine	ND	73.5	ND	74.8	ND
Glycine	ND	73.8	ND	75.1	ND
Glutamic Acid	ND	79.3	ND	80.2	ND
Aspartic Acid	ND	75.9	ND	77.0	ND
Tyrosine	ND	ND	ND	ND	ND

[1]Expressed on the basis of flour moisture.
*Calculated from the average of the sum of methionine and cysteine coefficients.
NNP= Non protein Nitrogen
CD= Coefficient of digestibility.
ND= Not determined

Table 5. Productive variables in broilers from 1 to 21 days of age, amino acid intake (g/g) and arginine:lysine ratio

Diets	Final weight (g)*	Weight gain (g)	Feed Consumption (g/ave)	Feed conversion ratio (kg:kg)	
Control diet	853 ± 19.34[a]	815 ± 19.81[a]	1164 ± 32.28	1.429 ± 0.058[b]	
CD+8% GSM	830 ± 16.75[ab]	790 ± 17.15[ab]	1166 ± 27.95	1.477 ± 0.050[ab]	
CD+16% GSM	788 ± 16.75[b]	748 ± 17.15[b]	1219 ± 27.95	1.634 ± 0.050[a]	
	Lysine	Methionine + Cysteine	Threonine	Arginine	Arginine:lysine ratio
Control diet	17.57 ± 0.41[a]	11.8 ± 0.14	11.4 ± 0.13	18.27 ± 0.22[a]	1.04
CD+8% GSM	15.80 ± 0.35[b]	12.2 ± 0.17	11.5 ± 0.16	16.20 ± 0.23[b]	1.03
CD+16% GSM	15.15 ± 0.35[b]	12.15 ± 0.41	11.1 ± 0.37	16.80 ± 0.56[ab]	1.11

CD = Control diet. GSM = Giant squid meal. *Mean initial weight: 39g ± 0.5. [a,b] In each column, different literals indicate statistical difference ($P < 0.05$). Mean ± Standard error of the mean; n=12

Experiment 2

Table 6 shows the productive variables; there were no differences in weight gain, feed intake and feed conversion index in the initiation phase. In the growth phase, weight gain was similar in the three treatments, but in feed consumption the inclusion of 20% was lower (P < 0.05) with respect to the control; the conversion index was better in the treatments with GSM (P < 0.05). In the finishing phase, no differences were observed between treatments in any of the variables.

Table 6. Productive variables in broilers fed with different percentages of giant squid (*Dosidicus gigas*) meal

Treatment	Weight gain (g/bird)	Feed consumption (g/bird)	Conversion ratio (kg:kg)
		Initiation (1-10 days)*	
Control diet (CD)	200 ± 5.90	236 ± 4.97	1.181 ± 0.016
CD + 16% GSM	193 ± 5.90	233 ± 4.97	1.203 ± 0.016
CDT + 20% GSM	189 ± 5.90	232 ± 4.97	1.226 ± 0.016
		Growth (11-24 days)	
Control diet (CD)	672 ± 3.40	1089 ± 12.81[a]	1.634 ± 0.016[a]
CD + 16% GSM	680 ± 3.45	1048 ± 12.98[ab]	1.542 ± 0.016[b]
CD + 20% GSM	679 ± 3.45	1031 ± 13.02[b]	1.518 ± 0.016[b]
		Finalization (25-42 days)	
Control diet (CD)	992 ± 38.33	2037 ± 49.41	2.061 ± 0.094
CD + 16% GSM	1051 ± 86.81	2261 ± 111.98	2.148 ± 0.213
CD + 20% GSM	841 ± 46.37	1903 ± 59.79	2.272 ± 0.114
		Whole cycle (42 days)	
Control diet (CD)	1865 ± 53.10	3362 ± 71.63	1.807 ± 0.041
CD + 16% GSM	1792 ± 53.10	3240 ± 71.62	1.809 ± 0.041
CD + 20% GSM	1663 ± 53.10	3121 ± 71.62	1.878 ± 0.041

GSM = Giant squid meal. *Initial mean weight: 39.5g ± 0.7g. [a,b] In each column, different literals indicate statistical difference (P < 0.05). Mean ± Standard error of the mean, n=72

Table 7 shows the performance of the hot carcass at 42 days of age, which did not show an effect of the addition of GSM. Skin color and lightness values (L*) did not show differences; however, the inclusion of 16% GSM showed a lower skin yellowness (b*) and higher redness (a*) with respect to the control (P < 0.05). Females presented higher carcass yield and b* pigmentation (P < 0.05) compared to males by 3.59 b* units.

Table 7. ***In vivo*** **skin yellowing and percent carcass yield of broilers fed different percentages of giant squid (*Dosidicus gigas*) meal for 42 days**

Treatments	Average Post-Breakfast Weight (kg)	Percentage of carcass yield	Skin yellowing Units		
			b*	a*	L*
Control diet (CD)	1.788 ± 0.070	73.49 ± 0.44	22.35 ± 0.83[a]	1.19 ± 0.46[b]	70.31 ± 0.57
CD + 16% GSM	1.751 ± 0.071	72.88 ± 0.47	19.42 ± 0.83[b]	2.85 ± 0.46[a]	69.64 ± 0.57
CD + 20% GSM	1.651 ± 0.069	72.08 ± 0.42	20.58 ± 0.80[ab]	2.66 ± 0.45[ab]	69.26 ± 0.55
Sex					
Male	1.802 ± 0.060	72.29 ± 0.38[b]	18.99 ± 0.70[b]	2.47 ± 0.39	69.54 ± 0.48
Female	1.659 ± 0.054	73.35 ± 0.34[a]	22.58 ± 0.64[a]	2.00 ± 0.35	69.94 ± 0.44
Probability					
Treatment	0.38	0.23	0.050	0.02	0.51
Sex	0.32	0.04	0.0004	0.38	0.54
Treatment*Sex	0.54	0.52	0.08	0.23	0.32

GSM = Giant squid meal. [b*]: yellowing; [a*]: reddening; [L*]: lightness. [a,b] In each column, different literals indicate statistical difference (P<0.05). Mean ± Standard error of the mean; n=72

DISCUSSION

Since NNP (nucleic acids, urea, and amides) is not useful in the feeding of monogastrics, since they require preformed amino acids, its determination is important to avoid an overestimation of the protein of the ingredient (Caravaca *et al.*, 2003). No data on NNP and true protein content were found in other investigations with squid meal; however, (Ezquerra *et al*., 2007) mention that of the total nitrogenous compounds (including protein) found in several squid species, the non-nitrogenous elements (trimethylamine oxide and other amines, free amino acids and octopine, arginine, glycine, betaine, alanine and nucleotides) represent 37%, although these authors do not specify whether the values were in fresh squid or meal, being similar to that reported by Maza *et al* (2003), who mention that the NNP content is 39. 5% in fresh mantle of squid *Dosidicus gigas*. These figures are higher than the percentage obtained in this research (18.36%) for the same species.

The AMEn was 6.6% higher than that reported by Remigio (Remigio, 2006) (3151 kcal/kg) using viscera meal (68.75% CP) of the same species. Compared to other ingredients, it exceeds a fish meal (3037 kcal/kg and 60.3% CP) by 11% and soybean meal (2346 kcal/kg and 48% CP) by 44%, but becomes similar to yellow corn (3340 kcal/kg) and sorghum (3263 kcal/kg) (AMINODat, 2016). Variations between results could be related to the amount of total protein in GSM, as amino acid skeletons can be converted to carbohydrate derivatives, which increase the energy value of the ingredients (Leeson & Summers, 2001).

Digestibility coefficients at 21 days of age with diets formulated based on total amino acids showed similar behaviors to those reported by Carranco (2020) in laying hens, where there were no differences for feed intake, but in both works a higher feed conversion was detected. When analyzing the actual consumption of amino acids over the 21 days of the trial, it was observed that the highest consumption of lysine and arginine was for the control. However, when calculating the arginine:lysine ratio, it was found that the highest ratio was for the 16% inclusion (1:11) and Leeson & Summers (2001) report a normal ratio of 1:1 to 1.05:1, so the higher amount of arginine may have caused a lower absorption of lysine. Since lysine is almost totally destined for muscle formation (Campos *et al*., 2008), these observations become factors to be considered to explain the behavior of the final weight variable, since the 65g difference between the control and 16% GSM treatments contributed to the 8.2% drop in the weight gain variable in the latter treatment.

The productive variables of the complete cycle (42 days) agree with what was reported by Morales *et al* (2022) that included 1.67, 3.34 and 5.01% of GSM based on a formulation with total amino acids and that at the end of the cycle (49 days) found no differences for the same variables. The average yield was 72.82% similar to that suggested for this strain (71.57%) (Aviagen, 2014). Females had a higher yield than males, which is consistent with data from other authors, where females had higher proportions of breast and subcutaneous fat than males, but males outperformed males in proportion to leg and thigh Rondelli *et al*., 2003; López *et al*., 2011).

Since pigment deposition can be affected by decreased feed intake, the total consumption of yellow xanthophylls per treatment was calculated, however, considering the pigment added and the amounts of corn (lutein and zeaxanthin) used, as GSM inclusion was increased during the three study phases, these intakes did not agree with the behavior presented in b*, as the control diet consumed 273. 64 mg; with 16% GSM 297.72 mg and with 20% GSM 259.05 mg, data similar to that reported by Muñoz *et al* (2012) who observed b* units above those recorded in males, this could be explained because females have a greater capacity to deposit fat tissue. It is worth mentioning that the behavior observed in the treatment with 20% GSM inclusion could be due to the effect of the female because of the interaction tendency with sex. Toyes (2016) enriched with astaxanthin, 0.05 mg/100 g chicken egg, because he determined total carotenoid amounts of 11.5 mg/100 g in dried squid viscera meal of which 5. 4 mg/100 g were astaxanthin, this in turn differs with that reported by Ezquerra *et al* (2007) and Aubourg *et al* (2016) who in their studies on squid (*Dosidicus gigas*) skin extract ruled out the presence of carotenoids and melanins in the skin, but did find compounds of the omochrome type (used by animals for camouflage), also lipophilic, of which not enough information is available.

Sensory evaluation is an important point for consumers, since they can give their point of view on a food(s) by rating attributes such as taste, odor, texture, color, among others.

However, both flavor and aroma are main attributes that consumers consider for the acceptance of a food (Sánchez & Albarracín, 2010).

It is important to mention, in this case, that in the production, processing and cooking of chicken meat, the sensory attributes tend to develop chemical changes of sugars, amino acids, thermal oxidation of lipids and degradation of thiamine. But there are also other factors that contribute to these sensory changes such as bird age, strain, feed, environmental conditions, rearing system, blanching temperature, cooling, labeling and storage (Alltech, 2021). (Padilla, 2010).

When using unconventional seafood sources, there is concern about the fishy flavor attribute that develops in chicken meat. One example is a study in which herring meal was supplemented with 4, 8 and 12% herring meal in broiler diets, where these small amounts exerted an effect on flavor. Meat from birds fed 8% herring meal had unpleasant fishy, rancid, non-fresh flavors, which after cooking were less apparent, but 24 hours after refrigeration and re-evaluation, the fishy flavors increased (Poste, 1990). In this research with cooked meat, none of the judges reported unpleasant tastes or odors (fishy), data that agree with those reported by Morales *et al* (2022) when using squid meal in broilers.

CONCLUSIONS

It is concluded that GSM can be an alternative source of protein in broiler diets, but more studies are needed.

CITED LITERATURE

ALLTECH. 2021. (Animal Nutrition, Animal Feed Supplements, Animal Health). Encuesta global sobre alimentos balanceados de Alltech 2021. http://es.alltech.com/encuesta-global-sobre-alimento-balanceado-de-alltech

ASSOCIATION of Official Analytical Chemists. 2019. *Official methods of analysis of AOAC International.* 21st Edition. Arlington, VA, USA. ISBN 0-935584-77-3.
https://www.aoac.org/official-methods-of-analysis-21st-edition-2019/

AMINODat 5.0. Evonik Nutrition and Care GmbH. 2016. Ver 1.02. Animal Nutrition Business Line. www.evonik.com/animal-nutrition

AVIAGEN 2014. Broiler Ross 308: Especificaciones de nutrición. http://es.aviagen.com/assets/Tech_Center/BB_Foreign_Language_Docs/Spanish_Tech Docs/Ross308BroilerNutritionSpecs2014-ES.pdf

AUBOURG SP, Torres-Arreola W, Trigo M y Ezquerra-Brauer JM. 2016. Partial characterization of jumbo squid skin pigment extract and its antioxidant potential in a marine oil system. *European Journal of Lipid Science and Technology*. 118: 1293–1304. ISSN: 1438-9312. https://doi.org/10.1002/ejlt.201500356

CALVO MC, Carranco JME, Salinas CA, Carrillo DS. 2016. Composición química de harina de calamar gigante *Dosidicus gigas*. *Archivos Latinoamericanos de Nutrición*. 66(1): 74-81. ISSN: 0004-0622. https://www.alanrevista.org

CAMPOS A, Salguero S, Albino L, Rostagno H. 2008. Aminoácidos en la nutrición de pollos de engorde: Proteína ideal. III CLANA -Congresso do Colégio Latino - Americano de Nutrição Animal. Cancún, México del 18 al 21 de noviembre. https://www.researchgate.net/publication/268339668.

CARAVACA RFP, Castel GJM, Guzmán GJL, Delgado PM, Mena GY, Alcalde AMJ, González RP. 2003. Bases de la producción animal. Ed. Editorial Universidad de Córdoba, Sevilla, España. Pp. 512. Primera edición. ISBN 84-472-0764-1. https://dialnet.unirioja.es/servlet/libro?codigo=320482

CARRANCO-JÁUREGUI María, Fuente-Martínez Benjamín, Ramírez-Poblano Miriam, Calvo-Carrillo María, Ávila-González Ernesto. 2020. Inclusión de harina de calamar gigante *Dosidicus gigas* como fuente de proteína en dietas para gallinas ponedoras. *Abanico Veterinario*. Enero-Diciembre 2020; 10:1-12.
http://dx.doi.org/10.21929/abavet2020.14

CONAFAB. 2021. Innovación, clave para enfrentar los retos de la industria alimentaria. Consejo Nacional de Fabricantes de Alimentos Balanceados y de la Nutrición Animal, A.C. http://conafab.org/newsletter-septiembre-2021-5

DE LA CRUZ GF, Beltrán-Morales LF, Zavala CA, Cisneros-Mata M, Aragon-Noriega E, Avilés G. 2011. Análisis socioeconómico de la pesquería de calamar gigante en Guaymas, Sonora. *Economía, Sociedad y Territorio*. (11): 645-666. Versión On-line ISSN 2448-6183 versión impresa ISSN 1405-8421.
https://cibnor.repositorioinstitucional.mx/jspui/bitstream/1001/811/1/De%20la%20Cruz%20Gonz%C3%A1lez-FJ.pdf

DOTTAVIO A, Di Masso, R. 2011. Mejoramiento avícola para sistemas productivos semi-intensivos que preservan el bienestar animal. *Journal of Basic & Applied Genetics*. 21(12):1-10. ISSN: 1852-6233.
http://ppct.caicyt.gov.ar/index.php/bag/article/view/44/159.

EZQUERRA BJM, Díaz AC y Fenucci JL. 2007. "Harina de calamar" En: García GT, Villarreal CH, Fenucci JL. Eds. Manual de ingredientes proteicos y aditivos empleados en la formulación de alimentos balanceados para camarones peneidos. Editorial Universitaria de Mar del Plata, Argentina. Pp. 41-55. ISBN: 978-987–1371-02-0 https://www.cibnor.mx/images/stories/biohelis/pdfs/MANUAL_INGREDIENTES_PROTEICOS.pdf

INCMNSZ. (Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán). 2011. Determinación de perfil de aminoácidos por Métodos internos MME-AA-01, MME-AA-02 y MME-AA-03. No. de Acreditación: A-0099-007/11. Dirección de Nutrición, Departamento de Ciencia y Tecnología de los Alimentos. Ciudad de México, México. https://www.incmnsz.mx

JAGGER S, Wiseman J, Cole DJA, Craigon J. 1992. Evaluation of inert markers for the determination of ileal and faecal apparent digestibility values in the pig. *British Journal of Nutrition*. 68(3):729-739. ISSN: 0007-1145 (Impreso), 1475-2662 (En línea). https://doi.org/10.1079/BJN19920129

KUEHL R. 2000. Diseño de experimentos: principios estadísticos de diseño y análisis de investigaciones. Segunda Edición. Estados Unidos,Thomson Learning. ISBN 970-686-048-7. https://www.academia.edu/20386268/Dise%C3%B1o_Robert_Kuehl

LEESON S y Summers JD. 2001. Scott's Nutrition of the Chicken. 4th Ed. Pp. 68. Canada, University Books. ISBN 978-0969560043. https://www.iberlibro.com/buscar-libro/titulo/scotts-nutrition-chicken/autor/leeson-summers/

LEMME A, Ravindran V y Bryden WL. 2004. Ileal digestibility of amino acids in feed ingredients for broilers. *World's Poultry Science Association*. 60(4):423–38. ISSN 0043-9339 (Impreso) ISSN 1743-4777 (En línea). https://doi.org/10.1079/WPS200426

LÓPEZ KP, Schilling MW y Corzo A. 2011. Broiler genetic strain and sex effects on meat characteristics. *Poultry Science*. 90(5):1105-1111. ISSN 0032-5791. https://doi.org/10.3382/ps.2010-01154

MARTÍNEZ GAI. 1996. Evaluación de los valores de pigmentación de la piel en la pechuga de pollo de engorda comparándola con otras regiones anatómicas medidas con un colorímetro de reflectancia. Tesis de Licenciatura. Universidad Nacional Autónoma de México. Ciudad de México, México. http://www.sidalc.net/cgi-bin/wxis.exe/?IsisScript=UACHBC.xis&method=post&formato=2&cantidad=1&expresion=mfn=085727

MAZA S, Rosales M, Castro R. 2003. Efecto de un proceso de lixiviación ácida salina sobre la calidad del surimi de *Dosidicus gigas* "pota". *Boletín de Investigación del Instituto Tecnológico Pesquero del Perú*. 5:81-88. ISSN 1023-7070.
http://repositorio.itp.gob.pe/handle/ITP/103

MORALES BJ, Carranco JM, Téllez IG, Sandoval MA, González AM y Carrillo DS. 2022. Gigant squid (*Dosidicus gigas*) meal in chicken diets to enrich meat with n-3 fatty acids. *Animals*. 12:2210. ISSN: 2076-2615. https://doi.org/10.3390/ani12172210

MUÑOZ-DÍAZ JI, Fuente-Martínez B, Hernández-Velasco X y Ávila-González E. 2012. Skin pigmentation in broiler chickens fed various levels of metabolizable energy and xanthophylls from *Tagetes erecta*. *Journal Applied Poultry Research*. 21(4): 88–796. ISSN 1056-6171 https://doi.org/10.3382/japr.2011-00507

NORMA Oficial Mexicana NOM-062-ZOO-1999. Especificaciones técnicas para la producción, cuidado y uso de los animales de laboratorio. Publicada en el Diario Oficial de la Federación del 18 de junio de 2001.
https://www.gob.mx/senasica/documentos/nom-062-zoo-1999

NORMA Oficial Mexicana NOM-033-ZOO-1995. Sacrificio humanitario de los animales domésticos y silvestres. Publicada en el Diario Oficial de la Federación del 16 de julio de 1997. México.
https://www.dof.gob.mx/nota_detalle.php?codigo=5376424&fecha=18/12/2014

PEDRERO DL, Pangborn RM. 1989. Evaluación sensorial de los alimentos. 1ª. ed. Métodos analíticos. Editorial Alhambra Mexicana, S.A. de C.V. México. Pp. 251. ISBN: 9684440936 9789684440937.
http://www.sidalc.net/cgi-bin/wxis.exe/?IsisScript=UACHBC.xis&method=post&formato=2&cantidad=1&expresion=mfn=006913

POSTE LM. 1990. A sensory perspective of effect of feeds on flavor in meats: poultry meats. *Journal of Animal Science*. 68:4414-4420. ISSN: 1525-3163.
https://doi:10.2527/1990.68124414x
POTTER LM, Matterson LD, Arnold WJ, Pudelkiewicz WJ y Singsen EP. 1960. Studies in evaluating energy content of feeds for the chick: I. The evaluation of the metabolizable

energy and productive energy of alpha cellulose. *Poultry Science*. 39:1166-1178. ISSN: 0032-5791. https://doi.org/10.3382/ps.0391166

REMIGIO ERI. 2006. Energía metabolizable de la harina del subproducto de calamar gigante y su evaluación productiva en reemplazo de la harina de pescado prime en dietas para pollos de carne. Tesis posgrado, Universidad Nacional Agraria La Molina, Lima, Perú.
http://www.sidalc.net/cgi-bin/wxis.exe/?IsisScript=tesispe.xis&method=post&formato=2&cantidad=1&expresion=mfn=001414

RONDELLI S, Martinez O y García PT. 2003. Sex effect on productive parameters, carcass and body fat composition of two commercial broilers lines. *Revista Brasileira de Ciência Avícola*. 5(3):169-173. ISSN 1516-635X.
https://www.redalyc.org/articulo.oa?id=179713981002

SÁNCHEZ IC, Albarracín W. 2010. Análisis sensorial en carne. *Revista Colombiana de Ciencias Pecuarias.* 23:227-239. ISSN 0120-0690.
https://www.redalyc.org/pdf/2950/295023450012.pdf

TEJADA de HI. 1992. Control de Calidad y Análisis de Alimentos para Animales. Sistema de Educación Continua en Producción Animal, A.C. México. Pp. 397. http://www.sidalc.net/cgi-bin/wxis.exe/?IsisScript=libroan.xis&method=post&formato=2&cantidad=1&expresion=mfn=018059

TOYES VE, Calderón BAM, Duran EY, Palacios E y Civera CR. 2016. Marine co-product meals as a substitute of fishmeal in diets for white shrimp *Litopenaeus vannamei* improve growth, feed intake and muscle HUFA composition. *Aquaculture Research*. 48(7):3782–3800. ISSN: en línea: 1365-2109. https://doi.org/10.1111/are.13205

UNA (Unión Nacional de Avicultores). 2021. Compendio de indicadores económicos del sector avícola 2021. Editorial UNA. CDMX, México. https://www.una.org.mx

WIDYARATNE GP, Drew MD. 2011. Effects of protein level and digestibility on the growth and carcass characteristics of broiler chickens. *Poultry Science*. 90(3):595–603. ISSN: 0032-5791. https://doi.org/10.3382/ps.2010-01098

WATERS (Water AccQ-Tag Chemistry Package). 1993. Manual Number WAT052874. Millipore Corporation, Milford, MA, USA. www.waters.com

Errata Erratum

https://abanicoacademico.mx/revistasabanico-version-nueva/index.php/abanico-veterinario/errata