JWPR

Journal of World's Poultry Research

2025, Scienceline Publication

J. World Poult. Res. 15(3): 303-313, 2025

Research Paper

DOI: https://dx.doi.org/10.36380/jwpr.2025.29

PII: S2322455X2400029-15

ISSN: 2322-455X

Circular Valorization of Acid Silage from Invasive *Pterygoplichthys* Species in Hens' Diets: Impacts on Laying Performance and Egg Quality

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Received: July 04, 2025, Revised: August 10, 2025, Accepted: September 05, 2025, Published: September 25, 2025

ABSTRACT

Pterygoplichthys species, an invasive fish, offers a sustainable protein alternative in poultry feed, aligning with circular economy goals and reducing environmental impact. This study aimed to assess the impact of incorporating various levels of acid silage derived from *Pterygoplichthys* species (ASP) into laying hen diets on productive performance and egg quality, as part of a biological waste valorization strategy aligned with circular economy principles. Sixty 35-week-old Rhode Island Red hens (BW 1932.1 ± 10.81 g) were randomly assigned to four isoproteic and isoenergetic dietary treatments containing 0%, 6%, 12%, and 18% ASP over 13 weeks. Productive, egg quality, and economic indicators were recorded weekly. The inclusion of 12% ASP resulted in the highest egg production (82.08%), egg mass (49.32 g/hen/day), and number of eggs per hen per week (5.6), along with improved feed conversion ratio (2.59) and the highest economic efficiency index (17.90%) and profitability, in comparison to the other treatments. Egg quality also improved with ASP, regardless of the inclusion level, particularly in egg weight (60.08 g), shell thickness (0.35 mm), and Haugh units (73.83), compared to the control group. Quadratic regression models identified optimal ASP inclusion levels ranging from 11.2% to 12.3%, depending on the variable analyzed. In conclusion, the inclusion of 12% ASP in laying hen diets represents an effective, profitable, and environmentally responsible nutritional strategy that aligns with the principles of the circular economy and sustainable food production.

Keywords: Acid silage, Circular economy, Egg production, Invasive species

INTRODUCTION

The introduction of exotic fish species into freshwater ecosystems represents an increasing global challenge, with negative impacts on biodiversity and the local economies of fishing communities (Britton et al., 2023). Among exotic fish species, *Pterygoplichthys* species native to South America stands out as one of the most concerning invasive species due to its remarkable adaptability and high reproductive capacity (Hussan et al., 2021). Its proliferation has contributed to the displacement of native species of commercial value, the disruption of trophic chains, and the degradation of aquatic habitats in tropical and subtropical regions of the Americas, Asia, and Africa (Sarkar et al., 2023; Marr et al., 2024). Within this context, the circular economy emerges as a strategic tool to mitigate the ecological impact of invasive species by promoting the utilization of undesirable biomass through its transformation into value-added products (Duan et al., 2022). The use of acid silage techniques to process *Pterygoplichthys* species enables the transformation of a potential environmental contaminant into a valuable resource for animal nutrition (Raa et al., 1982; Raeesi et al., 2023). This type of silage is produced from fishery by-products viscera, scales, heads, and discarded whole fish, and has proven to be a viable alternative to fishmeal due to its high protein value and low production cost (Bianchi et al., 2014; Madage et al., 2015; Raeesi et al., 2023).

Several studies have demonstrated the potential of fish silage as a functional ingredient in animal feeding. The inclusion of fish silage in diets for monogastric animals such as pigs (Parrini et al., 2023), broiler chickens (Garcés et al., 2015; Shabani et al., 2021), and laying hens (Gaviria et al., 2022) has shown improvements in productive parameters, feed efficiency, and product quality, comparable to those achieved with conventional ingredients. Beyond its nutritional benefits, the use of fish silage supports the sustainability of agri-food systems by decreasing reliance on traditional protein feedstuffs such as soybean meal or fishmeal, whose production is linked to significant environmental costs (Islam and Peñarubia, 2021; Libonatti et al., 2023). In poultry production, the use of non-conventional feed ingredients is a key strategy to enhance the resilience of production systems in the face of market fluctuations and ecological constraints (Malenica et al., 2023; Edenakpo et al., 2025). Incorporating alternative ingredients derived from underutilized biomass, such as fish by-products or invasive species, enables the formulation of sustainable diets without compromising productivity, while improving resource efficiency and reducing the environmental impact of the sector (Libonatti et al., 2023). This strategy is consistent with the United Nations Sustainable Development Goals, especially those focused on responsible production, waste minimization, and the protection of aquatic ecosystems (Neale et al., 2025).

Considering the abundance and ecological threat of *Pterygoplichthys* species in aquatic ecosystems worldwide, as well as its suitable nutritional composition, its use as an ingredient in poultry diets represents an innovative and sustainable strategy in response to its uncontrolled expansion (Marr et al., 2024), which has negatively affected biodiversity and small-scale fisheries (Hussan et al., 2021). This invasive species, characterized by its high reproductive potential and ecological adaptability, has demonstrated a remarkable nutritional profile, with more than 50% crude protein content, comparable to that of conventional protein sources such as fishmeal (Hasrianti et al., 2022). Utilizing its biomass through acid silage not only enables the valorization of a problematic biological residue but also contributes to reducing pressure on traditional inputs, in alignment with circular economy principles and environmental sustainability. Accordingly, the objective of this study was to assess the impact of varying inclusion levels of acid silage derived from *Pterygoplichthys* species in laying hen diets on productive performance and egg quality, as a sustainable alternative in balanced feed formulation.

MATERIALS AND METHODS

Ethical approval

The procedures for animal care and handling followed the Mexican Official Standard 062-ZOO (NOM-062-ZOO, 1999), which outlines technical specifications for the production, care, and use of laboratory animals, as well as the International Guiding Principles for Biomedical Research Involving Animals (CIOMS, 2012).

Production of acid fish silage

To produce the acid silage from *Pterygoplichthys* species (ASP), whole fish were ground to obtain a homogeneous paste. The fish were collected from the daily catches of local fishermen, who incidentally trapped them in their nets, as this species has become a pest in the region's aquatic ecosystems. The resulting paste was then treated with 85% formic acid (Sigma-Aldrich, Mexico) at a ratio of 2.35 L per 100 kg of fresh fish to promote controlled fermentation (Raa et al., 1982). The resulting mixture was stirred manually once per day for 15 consecutive days under ambient temperature conditions (25-30°C) to facilitate enzymatic activity and the hydrolysis of muscle proteins. To ensure the consistency and reproducibility of the fermentation process, the stirring protocol was standardized. Although no active temperature control was applied, the process was conducted under stable ambient conditions, with temperatures ranging between 25 and 30°C, as verified through daily monitoring. This passive control was sufficient to maintain enzymatic activity and ensure the effectiveness of the fermentation process. Once the pH stabilized between 4.0 and 4.5, indicative of bacterial inhibition and material preservation, the silage was stored in airtight plastic containers until its use in the formulation of experimental diets.

To reduce the moisture content of the silage (64%), an adsorption technique was employed using wheat bran as a drying agent. The liquid silage was blended with wheat bran in specific ratios, determined based on the planned inclusion level for each experimental diet. The resulting mixture was left to dry at ambient temperature in shaded conditions for seven days, allowing gradual moisture reduction without thermal denaturation of the proteins. Once dehydration was complete, the material was processed using a manual blade mill to break down clumps formed during drying and to facilitate its homogeneous incorporation into the rest of the diet ingredients. This methodology for producing and conditioning acid fish silage helped preserve the nutritional quality of the fish

(Table 1), reduce the risk of undesirable microbial growth, and improve the material's physical handling during feed formulation (Raa et al., 1982; Chalamaiah et al., 2012).

Table 1. Nutritional composition and amino acid profile of acid silage from *Pterygoplichthys* species.

Chemical composition	**Value on a dry matter basis**
Metabolizable energy (Mcal/kg)	2.95
Crude protein (%)	51.20
Dry matter (%)	34.33
Calcium (%)	7.75
Total phosphorus (%)	5.36
Essential amino acids (g/100 g of protein)	
Lysine	8.2
Methionine	2.6
Threonine	4.5
Tryptophan	1.2
Valine	5.2
Isoleucine	4.2
Leucine	7.9
Phenylalanine	4.0
Arginine	6.1
Histidine	2.4

Study design

The research was conducted at the poultry facilities of the Faculty of Veterinary Medicine and Animal Science at the Universidad Michoacana de San Nicolás de Hidalgo, located at kilometer 9.5 on the Morelia–Zinapécuaro highway, in the municipality of Tarímbaro, Michoacán, Mexico. A total of 60 dual-purpose Rhode Island Red hens, 35 weeks of age, were used in the study. Although this breed is classified as dual-purpose, the present study focused exclusively on evaluating parameters related to egg production and egg quality. The hens were housed in conventional individual battery cages measuring 40 × 40 × 45 cm (length, width, and height, respectively) and randomly assigned to four dietary treatments in a completely randomized design (n = 15 hens/treatment), to evaluate the effects of increasing levels of ASP in laying hen diets. The hens were housed under conventional open-sided sheds. The temperature ranged from 20 to 30°C, with relative humidity between 50% and 70%. A photoperiod of 15 hours of light per day was maintained, with a minimum light intensity of 20 lux over 80% of the area. Ventilation was natural, ensuring adequate air exchange and thermal comfort. Environmental parameters were monitored daily to ensure consistency throughout the trial.

Before the experimental period, all hens underwent a 7-day adaptation phase to the experimental diets. During this period, the hens were gradually transitioned from the basal diet to the assigned treatment diets by increasing the proportion of the experimental feed daily. This strategy was implemented to minimize feed refusal and ensure physiological adaptation, thereby stabilizing intake patterns before data collection began. The treatments included a control diet (0% ASP inclusion) and three experimental diets containing 6%, 12%, and 18% ASP, respectively, as a partial replacement for conventional protein sources. All diets were formulated to be isoproteic and isoenergetic, and were adjusted to meet the nutritional requirements of laying hens according to NRC (1994) guidelines (Table 2).

Table 2. Ingredients and estimated nutritional composition of the experimental diets

	Inclusion level of *Pterygoplichthys* species silage (%)			
Ingredient composition (%)	**0 (control)**	**6**	**12**	**18**
Wheat bran	26.0	25.5	25.0	24.5
Ground sorghum	47.0	46.5	46.0	45.5
Soybean meal (44% CP)	19.0	17.5	14.0	10.0
Fish silage (52% CP)	0.0	6.0	12.0	18.0
Calcium carbonate	7.0	6.5	6.0	5.5
Microminerals[+]	1.0	1.0	1.0	1.0
Estimated chemical composition				
Metabolizable energy (Mcal/kg)	2.88	2.87	2.86	2.85
Crude protein (%)	17.6	17.8	17.7	17.5
Calcium (%)	3.7	3.6	3.6	3.5
Total phosphorus (%)	0.85	1.05	1.30	1.50
Lysine (%)	0.82	0.83	0.85	0.86
Methionine (%)	0.31	0.33	0.35	0.37
Threonine (%)	0.65	0.67	0.66	0.64
Tryptophan (%)	0.21	0.22	0.22	0.21

[+]Per kilogram of diet: Vitamin A: 8,000 IU; Vitamin D_3: 2,000 IU; Vitamin E: 50 mg; Vitamin K: 3 mg; Vitamin B_1: 1.5 mg; Vitamin B_2: 4 mg; Vitamin B_6: 0.12 mg; Vitamin B_{12}: 15 mg; Folic acid: 0.6 mg; Pantothenic acid: 10 mg; Niacin: 30 mg; Biotin: 0.1 mg; Choline: 300 mg; Iron: 50 mg; Copper: 10 mg; Zinc: 70 mg; Manganese: 100 mg; Iodine: 1 mg; Selenium: 0.3 mg; Antioxidants: 50 mg.

Dry matter and crude protein levels in the raw materials, experimental diets, and ASP were analyzed following AOAC official methods 934.01 and 976.05 (AOAC, 1990). Gross energy was determined using an adiabatic bomb calorimeter (Model 1281, Parr Instrument Company, Moline, IL, USA). Sample preparation for the quantification of amino acids in the ASP was conducted following the AOAC method 994.12 (AOAC, 1990). This procedure involved hydrolyzing the samples with 6M HCl at 110°C for 24 hours. Methionine determination involved a preliminary oxidation with performic acid, followed by amino acid profiling through reverse-phase high-performance liquid chromatography (HPLC), as outlined by Henderson et al. (2000), using a Hewlett-Packard HPLC system (Model 1100).

Productive performance

The body weight of each hen was monitored weekly throughout the experimental period using a digital scale (Torrey L-EQ®, capacity: 0.001-5.0 kg). Daily feed intake (g) was calculated as the difference between the amount of feed offered and the feed refused, using the same weighing equipment. Weekly weight gain (WWG) was determined using the following formula. $\mathrm{WWG} = \mathrm{F_w} - \mathrm{I_w}$

Where F_w is the final weight and I_w is the initial weight. Feed conversion ratio (FCR) was calculated by dividing the total feed intake (g) by body weight gain.

To assess diet profitability by treatment, the cost per kilogram of eggs produced (Yi) was calculated using a modified version of the equation proposed by Bellaver et al. (1985).

$$\mathrm{Y_i} = \frac{\mathrm{P_i} * \mathrm{Q_i}}{\mathrm{E_i}},$$

Where Yi is the feed cost per kg of eggs produced in the $^{i-}$th treatment (0%, 6%, 12%, and 18% ASP inclusion), Pi is the price per kg of the diet used in the $^{i-}$th treatment, Qi is the total amount of feed consumed by the $^{i-}$th treatment, and Ei is the total kg of eggs produced. Additionally, the economic efficiency index (EEI) was calculated using the following expression.

$$\mathrm{EEI} = \frac{\mathrm{EP_{kg}} \times \mathrm{EC_{\$}}}{\mathrm{FI_{kg}} \times \mathrm{FC_{\$}}}$$

Where EP is total egg production (kg), EC is egg cost ($/kg), FI is feed intake (kg), and FC is feed cost ($/kg).

Egg quality

A total of 13 eggs from each treatment were evaluated. Each egg was considered an individual sample, and the mean values for each treatment were calculated based on the measurements obtained. The whole egg weight (g), as well as the weights of the yolk (g), albumen (g), and shell (g), were recorded. Shell thickness (mm) was also measured. Egg shape index (%) was calculated as the ratio between egg height and width using a digital caliper (model 1114-300a, Georgia, USA). Egg surface area (cm²) was estimated using the following equation.

$$\mathrm{S_a} = 4.835 \times \mathrm{EW}^{0.662}$$

Where Ps is surface area and *EW* is egg weight (g) (Paganelli et al., 1974). Albumen height (mm) was measured using a digital caliper, and the Haugh Unit (HU) was calculated using the following equation.

$$\mathrm{HU} = 100 \times \mathrm{Log}(\mathrm{AH} + 7.7 - 1.7 \times \mathrm{EW}^{0.37})$$

Where AH is albumen height (mm) and EW is egg weight (g; Williams, 1992).

Statistical analysis

All statistical analyses were performed using SAS software version 9.4 (SAS Institute Inc., Cary, NC, USA). Before data analysis, the normality of the distribution and homogeneity of residual variance were assessed using the PROC UNIVARIATE procedure. The Shapiro-Wilk test was used to verify normality, and Bartlett's test was applied to assess variance homogeneity.

All statistical analyses were carried out with SAS version 9.4 (SAS Institute Inc., Cary, North Carolina, USA). Data were analyzed by repeated measures ANOVA using the PROC MIXED procedure (Littell et al., 1998). In the model, the individual hen was considered the experimental unit. The effects of treatment, week, and their interaction were evaluated on feed intake, body weight, weight gain, and egg production. The following statistical model was used in this study.

$$\mathrm{Y_{ijkl}} = \mu + \mathrm{T_i} + \mathrm{G(T)_{j(i)}} + \mathrm{W_k} + \mathrm{T} \times \mathrm{W_{ik}} + \mathrm{e_{ijkl}}$$

Where, Y_{ijkl} is response variable; μ is overall population mean; T is fixed effect of the $^{i-}$ésimo treatment (*i*: 0%, 6%, 12%, and 18% ASP inclusion); $G(T)_{j(i)}$ is random effect of the $^{j-}$ésima hen nested within the $^{i-}$ésimo treatment; W_k is fixed effect of the $^{k-}$ésima week (*k*: 1, 2, 3, ..., 13); $T \times W_{ik}$ is the fixed interaction effect between treatment and week. ε_{ijkl} is a random error associated with each observation, assumed to be normally and independently distributed ($\sim NID = 0, \sigma_e^2$).

Egg quality indicators were analyzed using ANOVA with the PROC GLM procedure. The effects of treatment, week, and their interaction were evaluated. The following statistical model was used.

$$\mathrm{Y_{ijk}} = \mu + \mathrm{T_i} + \mathrm{W_j} + \mathrm{T} \times \mathrm{W_{ij}} + \mathrm{e_{ijk}}$$

Where, Y_{ijkl} is the response variable; μ is the overall population mean; T is the fixed effect of the $^{i-}$ésimo treatment (i = 0%, 6%, 12%, and 18% ASP inclusion); W_j is the fixed effect of the $^{j-}$ésima week (k: 1, 2, 3, ..., 13); $T \times W_{ij}$ is the fixed interaction effect between treatment and week. ε_{ijkl} is a random error associated with each observation, assumed to be normally and independently distributed ($\sim NID = 0, \sigma_e^2$).

Statistical differences among means were evaluated using the least squares means (LsMeans) method, with a significance threshold set at $\alpha \leq 0.05$. Data are reported as LsMeans ± standard error of the mean (SE).

To determine the optimal inclusion level of ASP in the diets of laying hens, a quadratic regression analysis was performed using SAS software (version 9.4; SAS Institute Inc., Cary, NC, USA). The regression was based on six key performance indicators: Weekly feed intake (g/hen), feed conversion ratio, laying rate (%), eggs per hen per week, egg weight (g), and the economic efficiency index. For each dependent variable, a quadratic regression model of the following form was fitted.

$$Y = \beta_0 + \beta_1 X + \beta_2 X + \varepsilon$$

Where Y is the response variable, X is the inclusion level of fish silage in the diet (%); β_0, β_1, β_2 are coefficients to be estimated; ε is a random error term associated with each observation ($\sim$NID = 0, σ^2_e). The model was fitted using the PROC REG procedure, and associations were considered statistically significant at $p < 0.05$. The optimal inclusion level was determined by solving for the vertex of the parabola generated by the quadratic model using the following expression.

$$X_{optimal} = -\frac{\beta_1}{2\beta_2}$$

Where $X_{optimal}$ is the percentage of ASP inclusion that maximizes or minimizes the evaluated variable, depending on the direction of the curve's concavity (β_2).

RESULTS

Productive performance

During the 13-week evaluation period, the inclusion of ASP in laying hen diets significantly influenced ($p < 0.05$) various indicators of productive performance, growth, and economic efficiency (Table 3). Feed intake increased progressively with higher inclusion levels, reaching its highest value ($p < 0.05$) in the 12% ASP treatment (127.67 g/hen/day), in comparison to the other treatments. In contrast, final body weight also showed a significant increase ($p < 0.05$), with the 12% and 18% ASP inclusion treatments resulting in higher weights (1914.5 g and 1904.0 g, respectively) compared to the control group (1819.2 g).

Body weight change was negative across all treatments; however, the 12% ASP treatment exhibited the smallest reduction of body weight ($p < 0.05$), with respect to the other treatments, suggesting better weight maintenance (Table 3). The number of eggs per hen per week showed significant differences ($p < 0.05$), reaching its peak in the 12% ASP group (5.6 eggs), followed by the 18% and 6% groups (5.1 and 5.0 eggs, respectively), while the control group had the lowest value (4.7 eggs; Table 3). The laying rate was highest in the 12% inclusion group (82.08%), showing significant differences ($p < 0.05$) compared to the control group (66.94%). Egg mass was also significantly greater in the 12% ASP treatment (49.32 g/hen/day) compared to the other treatments ($p < 0.05$, Table 3).

In terms of feed efficiency, the most favorable feed conversion ratio (FCR) was observed in the group receiving 6% ASP (2.53), whereas the control group exhibited the highest FCR value (i.e., lowest efficiency; $p < 0.05$, Table 3). The economic efficiency index (EEI) increased progressively with higher levels of ASP inclusion, reaching its highest value ($p < 0.05$) in the 12% group (17.90%), while the control group registered the lowest EEI (16.82%; $p < 0.05$, Table 3).

Table 3. Effect of acid fish silage inclusion level on productive performance, growth variables, and economic efficiency in laying hens

	Inclusion level of *Pterygoplichthys* species silage (%)					**p-values**		
Indicator	0 (control)	6	12	18	SEM	T	W*	T × W
Feed intake (g)	105.46	113.81	127.67	116.38	0.116	<.0001	0.3511	0.2268
Initial body weight (g)	1940.2	1937.5	1916.2	1934.0	0.514	0.3275	<.0001	<.0001
Final body weight (g)	1819.2	1865.4	1914.5	1904.0	0.486	0.0046	<.0001	<.0001
Body weight change (g)	-121.2	-68.7	-1.6	-33.3	0.443	<.0001	0.5833	0.9237
Eggs/hen/week	4.7[a]	5.0[ab]	5.6[d]	5.1[b]	0.007	<.0001	<.0001	0.2418
Egg production (%)	66.94[a]	72.72[b]	82.08[c]	73.88[b]	0.108	<.0001	<.0001	0.3324
Egg mass (g/hen/day)	38.09[a]	44.97[b]	49.32[c]	44.37[b]	0.073	<.0001	<.0001	0.0864
FCR	2.73[c]	2.53[a]	2.59[a]	2.63[b]	0.006	<.0001	<.0001	0.0197
EEI (%)	16.82	16. 99	17.90	17.76	0.020	<.0001	0.8723	0.2521

SEM: Standard error of the mean; T: Treatment; W: Week; FCR: Feed conversion ratio; EEI: Economic efficiency index. *13 weeks. [a,b,c] Different superscript letters indicate differences within a row ($p < 0.05$).

Egg quality

Over the 13-week experimental period, dietary inclusion of ASP in laying hen diets led to significant variations in multiple internal and external egg quality traits ($p < 0.05$; Table 4). Egg weight increased significantly with ASP inclusion, rising from 56.93 g in the control group to 61.82 g in the 6% ASP treatment, and remaining around 60 g in the 12% (60.08 g) and 18% (60.05 g) ASP groups ($p < 0.05$, Table 4). A similar trend was observed for shell weight, which was significantly greater in the 6% and 12% ASP groups compared to the control ($p < 0.05$; Table 4). Likewise, eggshell thickness was higher in the 6% ASP group (0.35 mm) than in the control group (0.33 mm; $p < 0.05$). No significant interaction effects between treatment and week were detected for these variables ($p > 0.05$; Table 4). Regarding estimated egg surface area, statistically significant differences were also found ($p < 0.05$), showing a rising trend with increasing ASP inclusion, peaking at 72.70 cm² in the 18% treatment, in comparison to the other treatments (Table 4). Egg shape index increased ($p < 0.05$) in the 12% and 18% ASP groups, rising from 74.03% in the control group to 74.52% (Table 4).

Regarding internal egg quality, albumen weight increased significantly ($p < 0.05$) in the treatments with ASP, particularly in the 6% group (35.69 g) compared to the control (31.03 g; Table 4). Yolk weight was significantly influenced by dietary treatment ($p < 0.05$), with the highest value recorded in the 12% ASP group (15.92 g), and the lowest in the 18% group (15.28 g). The control group showed an intermediate value (15.71 g), differing significantly from the 12% and 18% treatments (Table 4). Albumen height increased significantly with increasing levels of ASP inclusion ($p < 0.05$), from 7.30 mm in the control group to 7.60 mm, 7.80 mm, and 8.00 mm in the 6%, 12%, and 18% ASP groups, respectively (Table 4). In contrast, yolk height differed significantly only in the 18% ASP group, which showed a lower value (16.13 mm) compared to the other treatments ($p < 0.05$; Table 4). No significant treatment per week interaction effects were observed for albumen weight, yolk weight, albumen height, or yolk height ($p > 0.05$).

Haugh unit values were significantly higher ($p < 0.05$) in the ASP treatments, peaking at 73.83 in the 6% group compared to 68.54 in the control group (Table 4). The week of evaluation also had a significant effect ($p < 0.05$), but no treatment × week interaction was detected ($p > 0.05$).

Estimation of optimal inclusion levels of acid silage from *Pterygoplichthys* species

The relationship between the inclusion level of ASP in laying hen diets and productive and economic variables was analyzed using quadratic regression models. Among the evaluated indicators, the economic efficiency index (EEI; percentage profitability) reached its optimal value at an inclusion level of 12.19%. For feed conversion ratio and egg production rate, the estimated optimal inclusion levels were approximately 12.3% and 11.79%, respectively, suggesting a physiological convergence of the positive effects of ASP within a range close to 12% (Table 5).

Figure 1 shows the fitted quadratic regression curves for each of the evaluated indicators. In all cases, the curves exhibit an initial increase followed by a stabilization or slight decrease, reflecting the nonlinear response of productive and economic variables to increasing levels of ASP inclusion. The inflection points of the curves indicate the estimated levels of maximum response for each variable.

Table 4. Effect of acid fish silage inclusion level on internal and external egg quality parameters in laying hens

Egg quality	Inclusion level of *Pterygoplichthys* species silage (%)				SEM	p-values		
	0 (control)	6	12	18		T	W*	T × W
Egg weight (g)	56.93^{a}	61.82^{c}	60.08^{b}	60.05^{b}	0.055	<.0001	<.0001	0.5654
Shell weight (g)	4.6^{ab}	5.0^{bc}	5.0^{bc}	4.8^{b}	0.005	<.0001	0.7361	0.4170
Shell thickness (mm)	0.33^{a}	0.35^{b}	0.34^{ab}	0.34^{ab}	0.001	<.0001	0.7409	0.5302
Egg surface area (cm²)	68.8^{a}	71.65^{b}	71.54^{b}	72.70^{c}	0.043	<.0001	0.225	0.132
Shape index (%)	74.03^{a}	74.09^{a}	74.52^{b}	74.40^{b}	0.032	<.0001	0.5252	0.0914
Albumen weight (g)	31.03^{a}	35.69^{d}	33.70^{c}	33.29^{b}	0.047	<.0001	0.5850	0.1780
Albumen height (mm)	7.30^{a}	7.60^{b}	7.80^{c}	8.00^{d}	0.001	<.0001	0.3753	0.1391
Yolk weight (g)	15.71^{b}	15.79^{bc}	15.92^{c}	15.28^{a}	0.013	<.0001	0.0239	0.2742
Yolk height (mm)	15.00^{a}	15.01^{a}	14.98^{a}	16.02^{b}	0.018	<.0001	0.4667	0.8243
Haugh units	68.54^{a}	73.83^{c}	71.35^{b}	70.20^{b}	0.062	<.0001	<.0001	0.1897

SEM: Standard error of the mean; T: Treatment; S: Week. *13 weeks. ^{a,b,c} Different superscript letters indicate differences within a row ($p < 0.05$).

Table 5. Regression estimates and optimal inclusion levels of acid fish silage on productive performance and economic efficiency variables in Rhode Island Red hens

	Regression estimates*			Critical point		
Indicator	**Intercept**	**β1**	**β2**	**OI (%)**	**OV**	**R^2**
Feed intake (g)	104.2965 (<.0001)	2.9294 (<.0001)	-0.1206 (<.0001)	12.14	122.08	0.830
FCR	3.4296 (<.0001)	-0.1205 (<.0001)	0.0049 (0.0040)	12.30	2.69	0.942
Egg production rate (%)	64.2053 (<.0001)	2.1861 (0.0010)	-0.0927 (0.0018)	11.79	77.09	0.799
Eggs/hen/week	4.5558 (<.0001)	0.1239 (<.0001)	-0.0055 (<.0001)	11.25	5.25	0.626
Egg weight (g)	55.6823 (<.0001)	0.7521 (0.0190)	-0.0336 (0.0321)	11.18	59.89	0.781
EEI	16.86681 (<.0001)	5.34776 (0.0013)	0.0017 (0.05012)	12.19	17.91	0.653

FCR: Feed conversion ratio; EEI: Economic efficiency index; OI: Optimal inclusion level of silage; OV: Optimal value of the indicator. *Estimator (p-value)

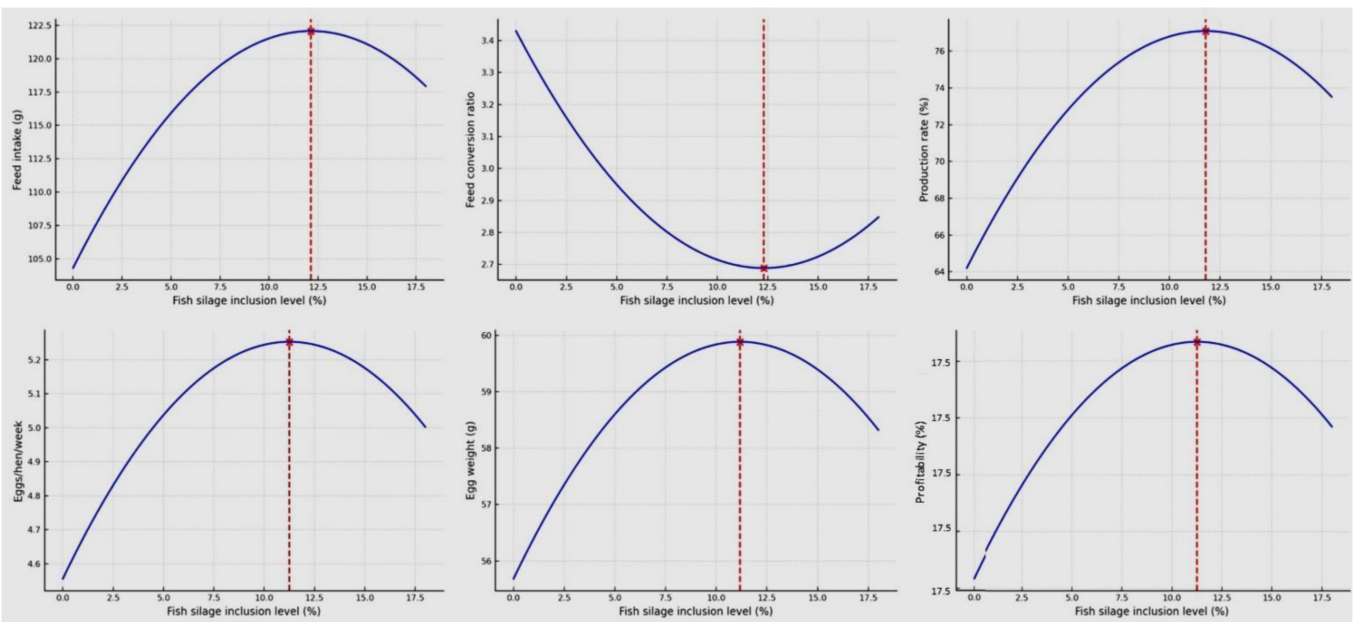

Figure 1. Regression curves for productive and economic indicators as a function of fish silage inclusion level in Rhode Island Red hens

DISCUSSION

The inclusion of ASP in laying hen diets was evaluated as a protein alternative within a circular economy framework, aiming to reduce dependence on conventional ingredients such as soybean meal and enhance production sustainability. The results demonstrated that inclusion levels between 12% and 13% led to significant improvements in productive, economic, and egg quality indicators, suggesting efficient utilization of this by-product.

From a productive standpoint, a significant increase was observed in the number of eggs per hen, egg mass, and laying rate in the treatments that included ASP, particularly in the 12% group. This effect could be associated with enhanced bio-accessibility of key essential amino acids, including lysine, leucine, and valine, provided by the ASP (Chalamaiah et al., 2012). These amino acids play a central role in protein synthesis for reproductive tissues, yolk formation, and ovarian follicle development processes that are critical for sustained egg laying and egg quality in hens (Ji et al., 2014; Macelline et al., 2021).

Acid silage, being subjected to controlled hydrolysis during the fermentation process with formic acid, produces a shorter peptide fraction that is highly digestible (Mayta-Apaza et al., 2022). This characteristic facilitates the transcellular transport of peptides and amino acids across the intestinal epithelium via specific transporters such as PepT1, thereby optimizing absorption and reducing metabolic competition among nutrients (Cruz-Casas et al., 2021). This enhanced digestive efficiency may partly explain the improved protein utilization in diets containing acid silage, particularly in adult hens, whose intestinal enzymatic activity and absorptive capacity may be moderately diminished due to age. Additionally, bioactive peptides derived from fermented fish have been reported to possess immunomodulatory and antioxidant properties, which help maintain intestinal integrity and reduce oxidative stress, an important factor associated with productivity decline during prolonged laying periods (Alizadeh-Ghamsari et al., 2023; Chaklader et al., 2023). In this context, improved gut health may lead to more efficient energy redistribution toward reproductive functions, such as yolk production and egg formation. The nutritional behavior of silage in the gastrointestinal tract of hens also includes modulation of gastric pH, which enhances the activity of proteolytic enzymes such as pepsin, thereby improving the solubilization of structural proteins and the release of amino acids in the proventriculus and gizzard (Olukosi and Dono, 2014). The modulation of pH, combined with a reduction in pathogenic microorganisms due to the acidity of the silage, contributes to a more stable and metabolically efficient intestinal environment (Olukosi and Dono, 2014; Alizadeh-Ghamsari et al., 2023).

The slight reduction in body weight observed during the experimental period is consistent with physiological adaptations that occur in laying hens as they transition from peak to post-peak production (Khatibi et al., 2021). During this stage, metabolic priorities shift toward maintaining egg production efficiency rather than supporting further somatic growth or weight gain. This reallocation of nutrients, along with the natural aging process, may contribute to a gradual loss of body mass (Noetzold and Zuidhof, 2025). Importantly, the observed change was not related to dietary restriction or health issues, as hens had *ad libitum* access to feed, exhibited normal behavior, and remained clinically healthy throughout the study.

Egg quality also showed positive responses in hens fed with ASP. Shell thickness was greater in the silage-supplemented treatments, which may be attributed to improved intestinal absorption of minerals, particularly calcium, phosphorus, and zinc elements essential for the mineralization of the eggshell's organic matrix (Li et al., 2017). This improvement could be linked to the acidic environment generated by residual formic acid in the silage, which has been associated with increased solubility and availability of calcium in the digestive tract of poultry (Guinotte et al., 1995; Gordon and Roland, 1997; Ricke et al., 2020). Eggshell formation relies on the activity of calcium channels and transport pumps regulated by calcitriol, the active form of vitamin D_3. The expression and efficiency of these mechanisms are influenced by the bioavailability of dietary minerals (Nys and Guyot, 2011). Therefore, the improved eggshell structure observed in hens supplemented with ASP may be explained by enhanced calcium availability, promoted by the acidifying effect of the silage and the reduction in mineral competition.

Haugh units also increased in the treatments supplemented with ASP. This parameter primarily depends on the viscosity and density of the albumen, which can deteriorate under conditions of oxidative stress, chronic inflammation, or low protein efficiency (Williams, 1992; Obianwuna et al., 2022). The presence of antioxidant peptides derived from fish collagen and muscle proteins may help reduce systemic oxidative damage, preserving the three-dimensional structure of ovalbumen and other functional egg components (Walayat et al., 2022). Additionally, fish fermentation generates short peptides with high digestibility, which can be rapidly absorbed and utilized by the liver for the synthesis of plasma and storage proteins such as ovalbumen and ovo-transferrin, which are subsequently secreted into the egg albumen (Obianwuna et al., 2022). This efficient metabolic pathway supports the stability of the protein gel in the egg white, resulting in higher Haugh unit values. In contrast, yolk pigmentation did not differ among treatments, which can be attributed to the low carotenoid content of the silage. Unlike ingredients such as yellow corn, marigold, or alfalfa, which are rich in lutein and zeaxanthin, acid silage lacks these compounds and therefore does not alter yolk coloration (Karadas et al., 2006). With respect to the higher values of albumen weight and Haugh units observed at the 6% ASP inclusion level, these outcomes may be attributed to the greater egg weight recorded in this group, supporting a positive correlation between albumen mass and structural integrity (Chang et al., 2024). In contrast, at higher inclusion levels of ASP (12-18%), the decline in these parameters could be partially explained by reduced albumen weight and potentially lower protein quality possibly due to amino acid imbalances, elevated non-protein nitrogen content, or decreased nutrient digestibility which may have limited albumen height and Haugh unit expression despite continued silage inclusion (Alagawany et al., 2020).

From a productive efficiency perspective, both the economic efficiency index (EEI) and percentage profitability reached their highest values in the treatment with 12% inclusion of ASP. The use of quadratic regression models allowed this level to be identified as the point of maximum economic return, indicating a favorable balance between feed cost and hen productivity. This finding aligns with the report by Boumans et al. (2022), who stated that the use of animal-derived by-products can reduce feed formulation costs without compromising zootechnical performance. The improvement in EEI observed in this study may be attributed to the relatively low cost of the silage (6.00/kg USD), combined with its high protein density and digestive functionality, which supports the maintenance or even enhancement of feed conversion efficiency and egg production. Moreover, the quadratic regression analysis revealed that the optimal inclusion level of ASP (12%) aligned closely with the inflection points of key productive and economic indicators, including egg production (11.79%), feed conversion ratio (12.3%), and the economic efficiency index (12.19%). This convergence suggests that, at this level, the diet achieves a biologically efficient balance between nutrient utilization and productive output, which is further reflected in the improved cost-effectiveness of egg production.

Additionally, from an environmental and sustainability perspective, the use of ASP as a feed ingredient represents an innovative strategy for the valorization of an invasive species, contributing to its population control while simultaneously reducing pressure on conventional protein sources, such as soybean meal or wild-caught fish. This approach not only offers zootechnical and economic benefits but also aligns with the principles of the circular economy promoted by the Bianchi et al. (2014) and the United Nations Sustainable Development Goals (Neale et al., 2025), particularly those related to the responsible use of natural resources, sustainable food production, and the mitigation of environmental impacts on aquatic ecosystems.

CONCLUSION

The use of acid silage produced from *Pterygoplichthys* species represents a nutritionally viable and functional alternative in laying hen nutrition. Isoproteic and isoenergetic diets does not compromise productive performance or egg quality and provides benefits in terms of feed efficiency and profitability. These attributes support its potential role as a sustainable input aligned with the principles of the circular economy and environmentally responsible production systems. A 12% inclusion level provides optimal performance across multiple zootechnical and economic parameters, including egg production, egg mass, feed conversion ratio, economic efficiency index, and profit margin. Future research could explore its interaction with functional additives, its long-term effects on reproductive health, or its integration into sustainable production certification systems.

DECLARATIONS

Acknowledgements

The authors would like to express sincere gratitude to the Coordination of Scientific Research at the Universidad Michoacana de San Nicolás de Hidalgo, México.

Authors' contributions

All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by Aureliano Juárez and Juan Carlos Cuellara. The first draft of the manuscript was written by Aureliano Juárez, Guillermo Salasa, Ernestina Gutiérrez, Gerardo Ordaz, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Funding

The present study was financially supported by the Coordination of Scientific Research at the Universidad Michoacana de San Nicolás de Hidalgo, México.

Availability of data and materials

The datasets generated for the current study are available from the corresponding author upon reasonable request.

Competing interests

The authors declare that they have no conflicts of interest.

Ethical considerations

Ethical issues, including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy, have been checked by all the authors.

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