

Effect of biological silage from *Litopenaeus vannamei* heads on the gut microbial composition and health of laying hens

Efecto del ensilado biológico de cabeza de *Litopenaeus vannamei* en la composición microbiana intestinal y la salud de gallinas ponedoras

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ABSTRACT

The aim of this study was to analyze the microbial composition associated with the intestinal health of laying hens. Over a 4-week period, 30-week-old hens were fed a basal diet containing 16% protein (T0), compared to another diet supplemented with 18% shrimp head (*Litopenaeus vannamei*) biological silage (BS), which had a protein content of 16.76% (T3E). Samples for metagenomic analysis were taken from the jejunal content of the birds using the E.Z.N.A.® Soil DNA Kit (Omega Bio-Tek Inc., USA). A significant increase in beneficial bacteria was observed at the class level, including Bacteroidia and Bacilli; at the family level, Bacteroidaceae and Lactobacillaceae; and at the genus level, *Bacteroides* and *Lactobacillus*. A decrease in harmful bacteria was noted, particularly in the class Erysipelotrichia, family Helicobacteraceae, and genus *Holdemania*, many of which play key roles in intestinal health. The use of the diet with BS promoted an increase in beneficial microorganisms and a reduction in harmful ones, suggesting a favorable modification in the bacterial flora composition, linked to improved intestinal health, making BS a potential functional food.

Key words: Functional food; lactic acid bacteria; hen feeding; bacterial gut metagenomics; intestinal health; *Litopenaeus vannamei*

RESUMEN

El objetivo de este estudio fue analizar la composición microbiana asociada con la salud intestinal de gallinas ponedoras, suplementadas con ensilaje biológico. Durante un período de 4 semanas, se alimentó a gallinas de 30 semanas de edad con una dieta base que contenía 16% de proteína (T0), en comparación con otra dieta suplementada con un 18% de ensilado biológico de cabeza de langostino *Litopenaeus vannamei* (BS), con un contenido proteico de 16,76% (T3E). Las muestras para el análisis metagenómico se obtuvieron del contenido del yeyuno de las aves utilizando el kit E.Z.N.A.® Soil DNA (Omega Bio-Tek Inc., USA). Se observó un aumento significativo de bacterias benéficas de las clases Bacteroidia y Bacilli; de las familias Bacteroidaceae y Lactobacillaceae y de los géneros *Bacteroides* y *Lactobacillus*. Se observó también una disminución de bacterias perjudiciales de la clase Erysipelotrichia, de la familia Helicobacteraceae y del género *Holdemania*, muchas de las cuales juegan un papel clave en la salud intestinal. El uso de la dieta con BS promovió un incremento de microorganismos beneficiosos y una reducción de aquellos perjudiciales, lo que sugiere una modificación favorable en la composición de la flora bacteriana, vinculada a la mejora de la salud intestinal por lo que el BS puede ser considerado un suplemento funcional.

Palabras claves: Ensilaje biológico; flora bacteriana; alimentación de gallinas; metagenómica; salud intestinal; *Litopenaeus vannamei*

INTRODUCTION

Gut health is fundamental for animals to reach their maximum productive potential. The gut microbiota, which is largely shaped by diet, undergoes dynamic changes over time, influencing both gut health and the prevalence of dominant microorganisms [1]. The inclusion of pre-digested feeds containing *Lactobacillus* has been shown to enhance the intestinal microbiota, thereby supporting the overall health and productivity of poultry (*Gallus gallus domesticus*). Studies have highlighted that *Lactobacillus* species, with their fermentative and probiotic properties, are beneficial to animal well-being [2].

Biological silage (BS), a fermentation technique using lactic acid bacteria (LAB), has proven effective in improving productive parameters in monogastric animals [3]. This underscores the importance of investigating the intestinal microbiota in relation to diet to optimize both health and productivity in animals [4, 5].

BS not only preserves essential nutrients but also promotes the growth of beneficial bacteria and the production of short-chain fatty acids [6]. The industry continuously seeks high-performance LAB to enhance animal nutrition [7], focusing on beneficial bacteria such as Bacteroidetes and Firmicutes, along with other key families important for gut health [8]. Notably, *Lactobacillus brevis* has demonstrated probiotic properties by improving gut health in poultry through lactic acid production [9].

This study aimed to analyze the microbial composition of the digestive tract in laying hens poultry (*Gallus gallus domesticus*), through metagenomic analysis, assessing the impact of a diet supplemented with *Litopenaeus vannamei* (BS) on the gut flora and the increase of beneficial microorganisms.

MATERIALS AND METHODS

The study was conducted at the Universidad Nacional de Tumbes, Peru (latitude 03° 35' 21.1" South, longitude 80° 30' 04.6" West, altitude 5 m.a.s.l.), within the Facultad de Ciencias Agrarias and Facultad de Ciencias de la Salud. A descriptive design was used, involving 20 Hy-Line Brown laying hens (*Gallus gallus domesticus*) aged between 30 and 35 weeks, distributed into two treatments: T0 (diet without biological silage) and T3E (diet with a 12% biological silage in dry matter), with 10 hens per treatment.

All birds received a basal diet containing 16% protein for 30 days (d). The BS was prepared through controlled bacterial fermentation, using 70% *Litopenaeus vannamei* head, 25% activator (cane molasses), and 5% functional ferment containing *Lactobacillus brevis*. The basal diet was prepared by controlled bacterial fermentation [10]. The *Litopenaeus vannamei* head (pre-cooked for 5 min), molasses and functional ferment (activated bacteria in whole milk) were poured in and mixed in a 500 L tank, hermetically sealed until use after 30 days of fermentation [10]. The viability of the BS was assessed through titratable acidity, pH (HANNA HI8424 Portable pH/ORP Meter, Mexico), presence of pathogenic bacteria, and counts of LAB and total mesophiles [11]. Samples of BS (200 g) were taken at 3, 5, 10, 15, and 30 d, with microbiological tests performed on d 15 and 30 [10, 12].

For metagenomic evaluation of the jejunum in hens. The jejunum of birds has a low bacterial load and helps to prevent early imbalances in intestinal health. It is directly influenced by the food, manifesting itself in the integrity of the mucosa and promoting the presence of beneficial microorganisms [13]. Genomic DNA was extracted using the E.Z.N.A.® Soil DNA kit (Omega Bio-Tek Inc., USA) and quantified with a spectrophotometer (EzDrop1000, Blue-Ray Biotech, BRED-1000, Taiwan). DNA was stored at -30°C (Laboratory ultra-freezer, UF V 700, Germany) until further analysis. DNA aliquots were sent to MR DNA (USA) for genetic sequencing (bTEFAP Illumina Miseq (2x300 PE)) of bacterial diversity, using bTEFAP® Illumina technology targeting the 16S rRNA gene. The data were analyzed using Mothur software [12], and processed for Scimago graphs.

RESULTS AND DISCUSSION

The molecular identification of the *Lactobacillus brevis* strain confirmed its role as a key fermentative bacterium in the preparation of the biological silage (BS). This bacterium, naturally found in the digestive tract of chickens, has demonstrated beneficial properties, including antimicrobial activities and probiotic effects in both poultry and humans [14].

Silage viability assessment

The BS maintained a stable pH below 4.6 and an acidity level above 2.5%, indicating that the BS is suitable and safe for use. These parameters stabilized by the fifth day of fermentation and remained consistent until day 30 [11, 15]. Regarding microbiological viability, the BS showed a concentration of more than 10x10⁹ CFU of lactic acid bacteria (LAB), a sufficient level to ensure the physiological benefits of the product. Additionally, the levels of yeast and fungi at 15 and 30 d of fermentation were within acceptable limits for probiotic foods according to Colombian regulations [16], and no pathogens such as *Salmonella* sp. were detected, likely due to the acidic conditions generated by the LAB [10]. The protein content of the BS remained at 23%, lower than the 28% found in fish silage [17].

Changes in Gut Microbiota Associated with T3E Treatment

The T3E treatment showed a significant increase in several bacterial classes, families, genera, and species associated with potential benefits for gut health. This effect may be attributed to the use of shrimp head silage, which influences the composition and function of the microbiota.

Predominant classes in both treatments

In both treatments, the class Bacteroidia was dominant, representing 36.93% in T0 and 37.94% in T3E. This class is known for its role in polysaccharide degradation and short-chain fatty acid production, which benefits intestinal health [18], as shown in FIG.1A.

Changes in Bacterial Classes

FIG. 1A shows that the classes Deltaproteobacteria, Bacilli, and Clostridia significantly increased in T3E. Deltaproteobacteria: Increased from 4.09% at T0 to 13.69% in T3E. Their modulation may be related to dietary factors and the presence of pre/probiotics [19].

Bacilli and Clostridia: Increased from 3.86% to 12.46% and from 4.69% to 12.82%, respectively. These classes include bacteria with probiotic roles and involvement in the production of short-chain fatty acids (SCFAs) [20].

Classes with higher abundance in T0

Epsilonproteobacteria was more abundant in T0 (7.34%) than in T3E (3.38%). This bacterial class is associated with intestinal dysbiosis and plays a role in sulfur oxidation, a characteristic activity of this lineage [20], Erysipelotrichia exhibited a significant difference between treatments, showing a higher value in T0 (22.13%) and a markedly lower value in T3E (0.28%). This class is linked to high-fat diets and dyslipidemia issues in humans [21], (FIG.1A).

Changes in Bacterial Families

FIG. 1B illustrates increases in families such as Bacteroidaceae, Lactobacillaceae, Bdellovibrionaceae, and Lachnospiraceae, which are known for their roles in digesting complex carbohydrates, producing SCFAs, and modulating pathogens [22, 23, 24, 25].

Bacterial families with higher presence in the T0 treatment

FIG. 1B also shows that the Sphingobacteriaceae family had a higher abundance in T0 (9.22%) compared to T3E (2.16%). This family is found in the excreta of fish and birds susceptible to enzyme restriction diseases. Erysipelotrichaceae exhibited a notable difference, being significantly more abundant in T0 (22.1%) and almost absent in T3E (0.21%). This family is associated with metabolic disorders and inflammation in humans and rats; its decrease may be related to diets that promote fiber fermentation [26] and is linked to obesity and infections [27, 28].

Changes in Bacterial Genera

FIG. 2A highlights increases in genera such as *Bacteroides*, *Lactobacillus*, and *Vampirovibrio*.

Bacteroides: Increased from 21.4% at T0 to 29.8% in T3E, associated with the degradation of complex polysaccharides and competition with pathogens [29].

Lactobacillus: Increased from 3.8% to 12.03%, playing a key probiotic role in pathogen inhibition and enhancing immune response [30, 31, 32].

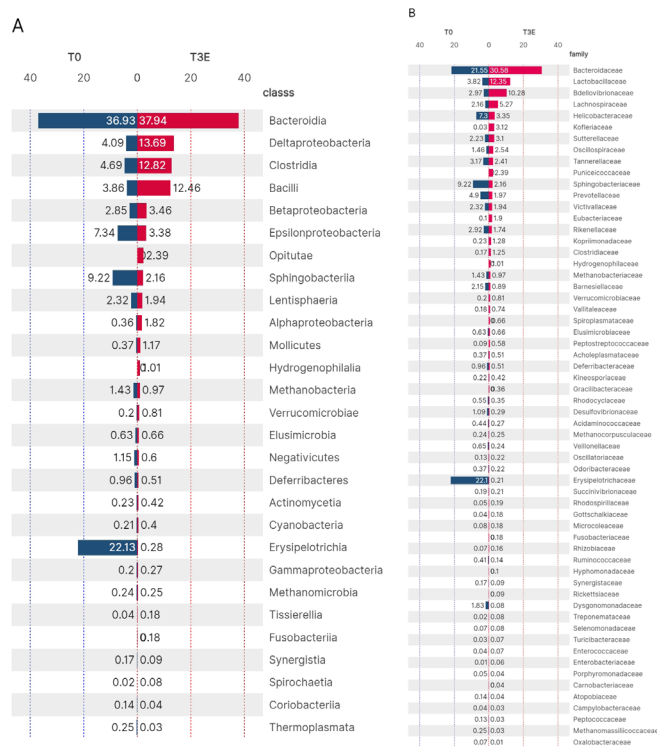


FIGURE 1. Changes in bacterial abundance in the jejunum of hens fed with biological silage (T3E) and without BS (T0) according to class (A) and family (B)

Significant decreases in bacterial genus in T3E

Helicobacter decreased from 7.26 in T0 to 3.26% in T3E, which is beneficial since some species, such as *Helicobacter pullorum*, are pathogenic and can cause digestive issues in birds [33], *Mucilagibacter* decreased from 9.17 in T0 to 2.11% in T3E. Although its role in intestinal health is unclear, it has been associated with diets containing olive oils, the presence of leptin in rats, and an increase in metabolic issues linked to high-fructose diets [34]. *Prevotella* declined from 3.45 in T0 to 0.21% in T3E. This genus is associated with metabolic problems caused by mycotoxins in feed, and with corn and soybean diets, which are rich in fibrous carbohydrates [35, 36]. Genera such as *Eubacterium*, *Alistipes*, and *Parabacteroides* show slight decreases but remain important for intestinal metabolism and the production of short-chain fatty acids (SCFAs). *Eubacterium* produces butyrate, essential for intestinal epithelial health and possessing anti-inflammatory properties, contributing to fiber breakdown and intestinal barrier integrity [37]. *Holdemania* decreased dramatically from 21.96% in T0 to 0.16% in T3E, suggesting that silage is not favorable for this genus. It is associated with inflammation and dysregulation in bile acid metabolism, affecting the intestine's ability to efficiently process nutrients [38]. *Proteobacteria* include genera such as *Methanobrevibacter* and *Desulfovibrio*, which also show decreases. *Methanobrevibacter* helps stabilize the intestinal environment by utilizing hydrogen, preventing gas accumulation and improving digestion [39] (FIG. 2A).

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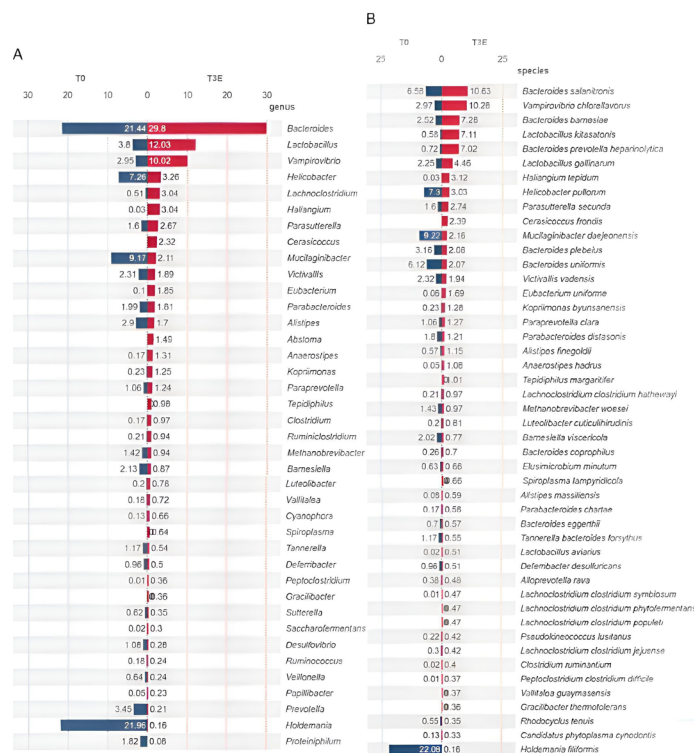


FIGURE 2. Changes in bacterial abundance in the jejunum of hens fed with biological silage (T3E) and without BS (T0) according to genus (A) and species (B)

Changes in Bacterial Species

FIG. 2B shows variations in specific species: *Bacteroides salanitronis* and *Lactobacillus kitasatonis* significantly increased, promoting a healthy gut microbiota [40, 41]. *Helicobacter pullorum* and *Holdemanella fallorum* decreased, which could indicate a more favorable intestinal environment in T3E [42]. New species such as *Cerasicoccus frondis* and *Haliangium tepidum* emerged, possibly promoted by the silage, with potential probiotic and antimicrobial properties [43]. *Victivallis vadensis* decreased from 2.32% to 1.94%, suggesting a reduction in favorable feeding conditions in T3E, related to the digestion of diets high in soluble and insoluble fiber [44]. Other species were found in lower proportions, such as *Peptoclostridium clostridium difficile* (T0: 0.01 to T3E: 0.37%), which can cause severe intestinal infections, and *Shigella sonnei* (T0: 0.011 to T3E: 0.038%), which is pathogenic [45].

Decreasing species, such as *Bacteroides uniformis*, showed a decline from 6.12 to 2.07%, associated with high-fiber diets in humans and chickens [46]. Although generally beneficial for carbohydrate fermentation [47], *Vampirovibrio chlorellavorus* increased significantly in T3E (10.28%) compared to T0 (2.97%), suggesting that its presence is related to marine-derived silage [32]. *Clostridium ruminantium* remained constant, indicating that silage does not negatively affect their functions. These species are important for fiber fermentation and cholesterol conversion [48].

Beneficial species for intestinal health

Most lactobacilli enhance intestinal health by producing bacteriostatic compounds and lactic acid, which lower intestinal pH and inhibit pathogenic bacteria [49]. Similar studies have observed an increase in the abundance and diversity of *Lactobacillus* flora in the jejunum and cecum of chickens, such as *L. agilis* and *L. salivarius*, promoting a stable intestinal microbiota. These lactobacilli improve nutrient absorption and intestinal architecture, contributing to better growth performance and immune response. They play key protective roles by lowering environmental pH through lactic acid production [42]. *Bifidobacteria* species also enhance daily egg production in chickens when combined with *Lactobacillus casei*, indicating better overall health and productivity, positively influencing ileal and cecal microbiota [50]. Other species identified include *Bacteroides*, considered part of the normal intestinal biome in chickens, establishing a balanced microbial community. A higher abundance in the gut may be beneficial, as seen with *Lactobacillus salivarius* supplementation [42]. Collectively, these probiotics improve intestinal health by optimizing microbial balance, nutrient absorption, and immune response, leading to better growth performance and productivity in chickens [51].

Considering the factors inherent to biological silage of shrimp heads, this seems to have a considerable impact on the composition of the bacterial community. Some species show a significant increase in their abundance, which could be related to anaerobic conditions and changes in pH [4]. Identifying changes in the microbiota can help predict and prevent potential microbiological risks in silage feed [52].

CONCLUSION

The biological silage of shrimp heads significantly influences the bacterial composition of the digestive tract in chickens, increasing the abundance of beneficial bacteria such as *Lactobacillus* and *Bifidobacterium*. This fosters a balanced and healthy intestinal environment while reducing the presence of harmful species. Identifying these changes in the microbiota is crucial for optimizing the quality of the silage, benefiting both animal nutrition and sustainability in animal feed production by utilizing marine by-products.

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Conflict of Interests

The authors declare that there is no conflict of interest.

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