Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
DOI: 10.29261/pakvetj/2026.225

RESEARCH ARTICLE

Bacillus velezensis, Inulin and their Synbiotic Combination Improve Productive and Reproductive Performance and Gut Health via Microbiota Modulation in Breeding Geese

Ibrar Muhammad Khan[1§], Nourhan Nassar[1,2§], Deyong She[3], Quanmei Zhang[4], Samiullah Khan[5], Anam Khalid[1], Ni Du[1], Xuezhen Zhu[3] and Zaigui Wang[1*]

[1]School of Life Science, Anhui Agricultural University, Anhui, Hefei 230036, P.R. China; [2]Department of Clinical Pathology, Faculty of Veterinary Medicine, Benha University, Moshtohor 13736, Egypt; [3]Luan Academy of Agricultural Sciences, Agricultural Science and Technology Building, Luan City, Anhui, P.R. China; [4]College of Animal Science, Anhui Science and Technology University, Fengyang, 233100, P.R. China; [5]Guizhou Provincial Key Laboratory for Agricultural Pest Management of the Mountainous Region, Institute of Entomology, Guizhou University; Guiyang, 550025, PR China. [§]These authors contributed equally.
*Corresponding author: wangzaigui2013@163.com

ARTICLE HISTORY (26-457)

Received: April 24, 2026
Revised: August 20, 2026
Accepted: August 22, 2026
Published online: October 08, 2026

Key words:
16S rRNA sequencing
Gut microbiota
Production traits
Short-chain fatty acids
Wanxi White Goose

ABSTRACT

The Wanxi White Goose (*Anser cygnoides*) is an important genetic resource and economic asset valued for its hardiness and local adaptability, but to maximize their health and production capacities, more sustainable and effective management strategies than the traditional feeding methods are required. A total of 180 geese were randomly assigned and divided into six groups and fed, based on dietary inclusion: control feed group, *B. velezensis* level one group, *B. velezensis* level two group, inulin level one group, inulin level two group, and a synbiotic group (e.g., combining *B. velezensis* level one and inulin level one). Geese were humanely slaughtered by trained personnel after the feeding trial, blood, tissues, and cecal content were collected to evaluate the physiological parameters. Synbiotic group showed significantly higher ($P<0.05$) productive and reproductive performance traits, followed by the *B. velezensis* level two group. Reproductive hormone analysis revealed significant ($P<0.05$) increase in prolactin, luteinizing hormone, and follicular stimulating hormone, with the SYN group showing the highest levels. Histomorphometric analysis revealed significant *(*$P<0.05$) differences in intestinal morphology among the treatment groups, including changes in villus height, crypt depth, and other intestinal structural parameters. The gene related to tight junctions, like *Occludin*, *Claudins1* and *TJP1* were upregulated in the duodenum jejunum and ileum and exhibited the upregulated expression trend across the treated groups. The microbiota dynamics and short chain fatty acids production in treated groups showed a higher abundance of the family-level taxa *Bacteroidaceae* and *Prevotellaceae*, while *Peptococcaceae* was more abundant in the control group. These findings demonstrate that combination of *B. velezensis* and inulin positively influences the performance-gut-metabolite axis in Wanxi White breeding geese.

To Cite This Article: Ibrar Muhammad Khan[1§], Nourhan Nassar[1,2§], Deyong She[3], Quanmei Zhang[4], Samiullah Khan[5], Anam Khalid[1], Ni Du[1], Xuezhen Zhu[3] and Zaigui Wang[1*], 2026. *Bacillus velezensis* inulin and their synbiotic combination improve productive and reproductive performance and gut health via microbiota modulation in breeding geese. Pak Vet J, 46(10): 2393-2406. http://dx.doi.org/10.29261/pakvetj/2026.225

INTRODUCTION

The Wanxi White Goose, a prominent native breed with the increasingly valuable characteristics (superior meat quality, and ability to thrive on poor-quality feed), is widely raised in Anhui Province of China (Chen *et al.*, 2018). Being a high-protein and fat source, goose serves as an important industry in food production, both for meats (carcass) and down for feathers for either puffy bedding or apparel (Yang *et al.*, 2024). Although it has these favorable characteristics, but the goose industry has not developed as quickly as chicken and duck due to most of the goose breed are inherently low egg producing poultry species (Akhtar *et al.*, 2021).

Antibiotics were historically used as a growth promoting agent and for prevention of diseases,

improving feed efficiency, enhancing immune function, and stabilizing gut microbiome in chicken diets (Kim *et al.*, 2024; Liu *et al.*, 2024). However, due to the misuse of antibiotics in poultry feed, problems such as bacterium resistance, multidrug cross-resistance, residual antibiotics in products produced from poultry, remnants in the environment have turned into a serious dilemma (Abdullah *et al.*, 2024; Sana *et al.*, 2025). Consequently, there has been a growing emphasis on developing and applying sustainable, safe, and effective alternatives of antibiotics to enhance poultry production. Prebiotics, probiotics, synbiotics, and postbiotics represent a class of functional compounds with significant potential for enhancing the health and productivity of livestock and aquaculture species (Abd El-Ghany, 2020) including waterfowl (Alqahtani *et al.*, 2024; Magnoli *et al.*, 2024).

Dietary probiotics benefit the host by increasing beneficial gut bacteria, suppressing infections, strengthening the gut barrier, and enhancing nutrient absorption and immunity (Akram *et al.*, 2017). *B. velezensis*, a species of the *Bacillus* genus, stands out for its several benefits, include secreting digestive enzymes, producing secondary metabolites (Khalid *et al.*, 2021; Li *et al.*, 2023a). Supplementation with *B. velezensis* BV-KNF-209, a commercial product, dramatically improves Cobb-500 broiler chicken growth performance by enhancing immunity, digestive enzyme activity, and intestinal SCFA and lactic acid levels (Liu *et al.*, 2024). Inulin supplementation in poultry diets serves to modify the intestinal microbiota by stimulating the rapid growth of beneficial *Bifidobacterium* and *Lactobacillus* strains while inhibiting harmful microorganisms (Li *et al.*, 2025a). The combination of chicory root pulp (inulin) and yeast selenium has been demonstrated to improve egg quality, nutrition, and hen immunity by adjusting gut microbiota and decreasing virulence factors (Wang *et al.*, 2025). Synbiotics, which are probiotic and prebiotic mixtures, aid in the growth and establishment of helpful microorganisms in the digestive tract (Tavaniello *et al.*, 2023). Prebiotics and other synbiotic components especially promote beneficial bacteria, resulting in a healthy gut that protects against pathogens, strengthens the intestinal barrier, and improves nutrient absorption and digestion by producing SCFAs (Khosravi *et al.*, 2025).

The current study aimed to obtain the new dietary inclusion of *B. velezensis* as probiotics, inulin as prebiotics and their combination as synbiotics on performance traits of Wanxi White breeding geese. The following investigated parameters, breeding performance, reproductive hormonal assay, intestinal morphometry, gut barrier functionality, cecal microbial assessment and SCFAs production were potentially affected by this new feeding regime.

MATERIALS AND METHODS

Feed and feeding protocol: All the experimental geese were fed by a complete commercial geese diet (manufactured by the Experimental Base of Lu'an City Academy of Agricultural Sciences, China). The feed and the nutrients composition were formulated to meet the geese nutrient requirements for production as per nutrition expert of Lu'an Academy of Agricultural Sciences (Table 1). Geese were fed twice a day, with leftover feed removed before providing fresh feed.

Table 1: Feed formulation and nutrient profile for Wanxi White Geese.

Feed ingredients	%
Yellow corn	60.1
Soybean meal	17.5
Alfalfa meal	3.50
Rice bran	4.00
Fish meal, 65%	2.50
Calcium carbonate	3.50
Molasses	3.00
Salt	0.30
Dicalcium Phosphate	2.60
Limestone	2.10
Choline Chloride, 50%	0.10
Methionine	0.20
Mineral premix ***	0.20
Vitamin premix **	0.40
Nutrient composition	
ME (kcal/kg)	2800-3000
Crude protein (%)	15.0
Crude fiber	7.00
Calcium (%)	2.96
Phosphorus (%)	0.67

Note: ***Mineral premix: Cu 15g/kg, Fe 88 g/kg, Zn 55 g/kg, Mn 88 g/kg, Co 0.25 g/kg, I 0.85 g/kg. **Vitamin premix: Vit-A 10,000,000 IU/kg, Vit-D3 2,000,000 IU/kg, Vit-E 20,000 IU/kg, Vit-B1 1 g/kg, Vit-B2 4.8 g/kg, Vit-B6 3 g/kg, Vit-B12 0.01 g/kg, Biotin 0.2 g/kg, Vit-K3 1.5 g/kg, calcium D-pantothenate 10 g/kg, Folic acid 0.5 g/kg, Nicotinic acid 25 g/kg.

Birds and experimental design: A total of 180 Wanxi White mature and healthy breeding geese at one and half year of age were chosen. The geese were randomly divided into six experimental treatment groups, with three replicates per treatment and ten geese per replicate, including two male and eight female geese. The six dietary patterns were as follows: (i) The control group, only basic feed represented by (CK); (ii) addition of *B. velezensis* level one (2.5 x 10^9) CFU/kg of feed, showed by (BV-L1); (iii) addition of *B. velezensis* level two (5 x 10^9) CFU/kg of feed, represented by (BV-L2); (iv) addition of inulin level one 5gm/kg of feed, showed by (INU-L1); (v) addition of inulin level two 10 g/kg of feed represented by (INU-L2); (vi) while the addition of *B. velezensis* level one (2.5 x 10^9) CFU/kg and addition of inulin level one 5 g/kg of feed together and this group denoted by (SYN). *B. velezensis* D1 (isolated from Northeast corn kernels) was used as the source strain. Inulin indicated ≥98% purity, consistent with structure was purchased from Hefei Nuozhuo Biological Technology Co., Ltd. (Hefei, China).

Performance record: The weight, width, and length of each individual egg were measured every day using a digital vernier caliper with a precision of 0.1 mm (Lin *et al.*, 2016). Egg laying rates were measured weekly. To evaluate differences between the early and later phases of the trial, average egg weight, width, length, and laying rate were calculated for two intervals: weeks 1–4 and weeks 5–8 (Sharma *et al.*, 2022).

Breeding performance: Egg fertilization and hatching rates were assessed during the final two weeks of the trial. Ten eggs per replicate, free from obvious defects such as cracks, misshapen, or abnormal shells, were selected for

incubation. These eggs were incubated together under standard conditions using a Renai fully automatic intelligent incubator (Wang *et al.*, 2024).

The calculation methods were adopted as per the (NY/T 823-2020):

1. Goose egg fertility in (%) = (Number of fertile eggs / Number of total eggs set) ×100
2. Hatchability of total number of eggs in (%) = (Number of hatched gosling / Number of total eggs set) × 100
3. Hatchability rate of fertile eggs (%) = (Number of hatched gosling /Number of fertile eggs) × 100

Reproductive hormonal assay: Three female Wanxi White geese were randomly selected from each group, and 3-5 mL of blood was collected from the jugular vein into EDTA-containing vacuum tubes. The blood samples were centrifuged at 4000 rpm, 4°C for 12 minutes, and the resulting plasma was carefully collected and stored at -20°C until analysis. Plasma concentrations of estrogen, prolactin, luteinizing hormone, and follicle-stimulating hormone were quantified using (Beijing North Biotechnology Institute Co., Ltd.) by radio immunoassay, according to the recommendations of the manufacturer. Briefly, for FSH, LH and prolactin, 100 µL of standards or samples and 100 µL of ^{125}I-labeled antibody were added to antibody-coated tubes and incubated at 37°C (FSH: 2 hours; LH: 3 hours). For estrogen, 100 µL of standards or samples and 200 µL of ^{125}I-E_2 were added to E_2-antibody-coated tubes and incubated at 37°C for 1 hour. Radioactivity was counted for 60 seconds per tube using a gamma counter. Hormone concentrations were calculated from standard curves.

Intestinal histology and morphometry: The gut sections, including the duodenum, jejunum and ileum, were collected and fixed in 4% paraformaldehyde immediately and refer to (Wuhan Service-bio Technology, Wuhan, Hubei, China). For morphometric analysis, samples of duodenum, jejunum and ileum were stained with HE. Using the CaseViewer2.4 scanning and browsing software, select the intestinal tissue for 20X imaging. After imaging is complete, use Image-Pro Plus 6.0 analysis software to standardize measurements in millimeters.

Cecal content collection and 16S rRNA sequencing: Following slaughtering, cecal contents were aseptically collected from geese (n = 6 per diet group). Samples were immediately placed into sterile cryogenic vials, flash-frozen in liquid nitrogen, and stored at -80°C prior to microbial DNA extraction and 16S rRNA seq. Total genomic DNA was extracted from each sample using the CTAB method. The V3–V4 hypervariable regions of the 16S rRNA gene were amplified with the primers 338F (5′-ACTCCTACGGGAGGCAGCA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′). Library preparation, purification, and paired-end sequencing (2 × 250 bp) were performed on an Illumina HiSeq platform by Nanjing Tsingke Biotech Co., Ltd. (China). The fqtrim (v0.94) method was used to filter raw reads in order to produce high-quality clean tags. The Vsearch program (v2.3.4) was used to eliminate chimeric sequences. Alpha and beta diversity were calculated from normalized sequence data to guarantee precision. Normalization of feature abundance, based on the relative abundance of each sample, was performed using the SILVA classifier (release 138). Alpha diversity was assessed in QIIME2 via the following metrics: observed species, Chao1, Good's coverage, Shannon, and Simpson indices. We calculated β-diversity in QIIME2 and created visualizations using R.

Detection and quantification of SCFAs: Prior to analysis, a precisely weighed 20 mg aliquot of cecal content was combined with 1 mL of 0.5% (v/v) phosphoric acid and a steel ball in a 2 mL tube. The mixture was homogenized by grinding, followed by 5 minutes of ultrasonication and 10 minutes of vortexing. Following centrifugation (12,000 rpm, 10 min, 4°C), a 100 µL aliquot of the clarified supernatant was transferred to a clean 1.5 mL microcentrifuge tube. Quantification of short-chain fatty acids (SCFAs) was subsequently performed by Nanjing Tsingke Biotech Co., Ltd. (China) using gas chromatography-tandem mass spectrometry (GC-MS/MS) (Zhao *et al.*, 2006; Bianchi *et al.*, 2011).

Validation of gut barrier function: Using Trizol reagent (Life Technologies, USA), total RNA was extracted from the duodenal, jejunal, and ileal tissues of each dietary group. RNA quality was verified via a Nanodrop N60 Touch spectrophotometer (IMPLEN, Germany). High-quality RNA was converted to cDNA with the QuantiTect Reverse Transcription Kit (Qiagen, Germany), following the instructions. Gene-specific primers for *CLDN1*, *OCLN*, and *TJP1* were designed using Primer Premier 5.0 and produced by Youkang Biotechnology Co., Ltd. (Hefei, China) (Table 2). Gene expression levels were determined using real-time quantitative PCR (qPCR) on a StepOnePlus™ Real-Time PCR System (Applied Biosystems, United States). Each 30 µL reaction consisted of 1.2 µL cDNA, 15 µL FastStart SYBR Green Master Mix (Roche, Germany), 0.6 µL each of forward and reverse primers, and 12.6 µL deionized water. The thermal cycling protocol followed established two-step amplification methods (Khan *et al.*, 2021). The quantification cycle (Cq) values from technical triplicates were normalized against the beta-actin reference gene. Relative gene expression was calculated according to the $2^{-\Delta\Delta Ct}$ method using Microsoft Excel (Huang *et al.*, 2009).

Table 2: Primer sequences used for quantitative real-time PCR analysis of target and reference genes.

Gene Accession number	Gene name	Primer sequence (5′–3′)	Product size (bp)
XM_048052460.2	*Occludin*	F: GATTCGGCCTTCCTGGAGAA R: ATCTGCCTCTTGATGTGCGT	74
XM_048057903.2	*Claudin 1*	F: GTCAGCGAAGAGGCAGATGA R: GTGATACACCTCCGCCACAA	86
XM_048060260.2	*Tight junction protein 1*	F: GTCTATGACCCCTGATGGTGAC R: CTGAGACACAGTTTGCTCCAA	92
NM_001310421.1	*Beta-actin*	F: AGCGCAAGTACTCTGTCTGG R: AGGGTGTGGGTGTTGGTAAC	156

Statistical analysis: The experiment followed a completely randomized design with six dietary inclusions (n=3), where each replicate consisted of one pen of 10 geese. Data were analyzed with SPSS 18.0. An initial one-way ANOVA was used to evaluate the overall effects of *B. velezensis* and

inulin supplementation. Pairwise comparisons with Tukey's HSD test were performed following a significant ANOVA result ($P<0.05$) or a statistical trend ($0.05 \leq P<0.10$). For visualization, we created bar and box plots using the ggplot2 library within RStudio (version 1.1.453).

RESULTS

Egg morphometry traits: During the (first and second week), egg length and weight in geese were not significantly affected by supplementation with *B. velezensis*, inulin, or their combinations ($P=0.0713$ and $P = 0.88$, respectively). The (third week) egg measurement data revealed that egg length showed a significant difference across treatments, with a *P*-value of 0.0279, indicating that certain treatments, particularly BV-L2 and INU-L1, positively impacted egg length. The (fourth week) measurement reveals that egg length, weight and width of the treatment groups, especially BV-L2, INU-L1, and SYN, showed noticeably higher values compared to the control (CK). The (fifth week) showed significant differences ($P<0.05$) across the traits, indicating strong effects of the SYN group. Measurements from weeks 6 to 8 showed highly significant differences in the measured traits (Fig.1). For egg length, weight and width the SYN treatment, followed by BV-L2, showed the highest values, suggesting that the combination of *B. velezensis* and Inulin positively influenced the egg morphometry traits.

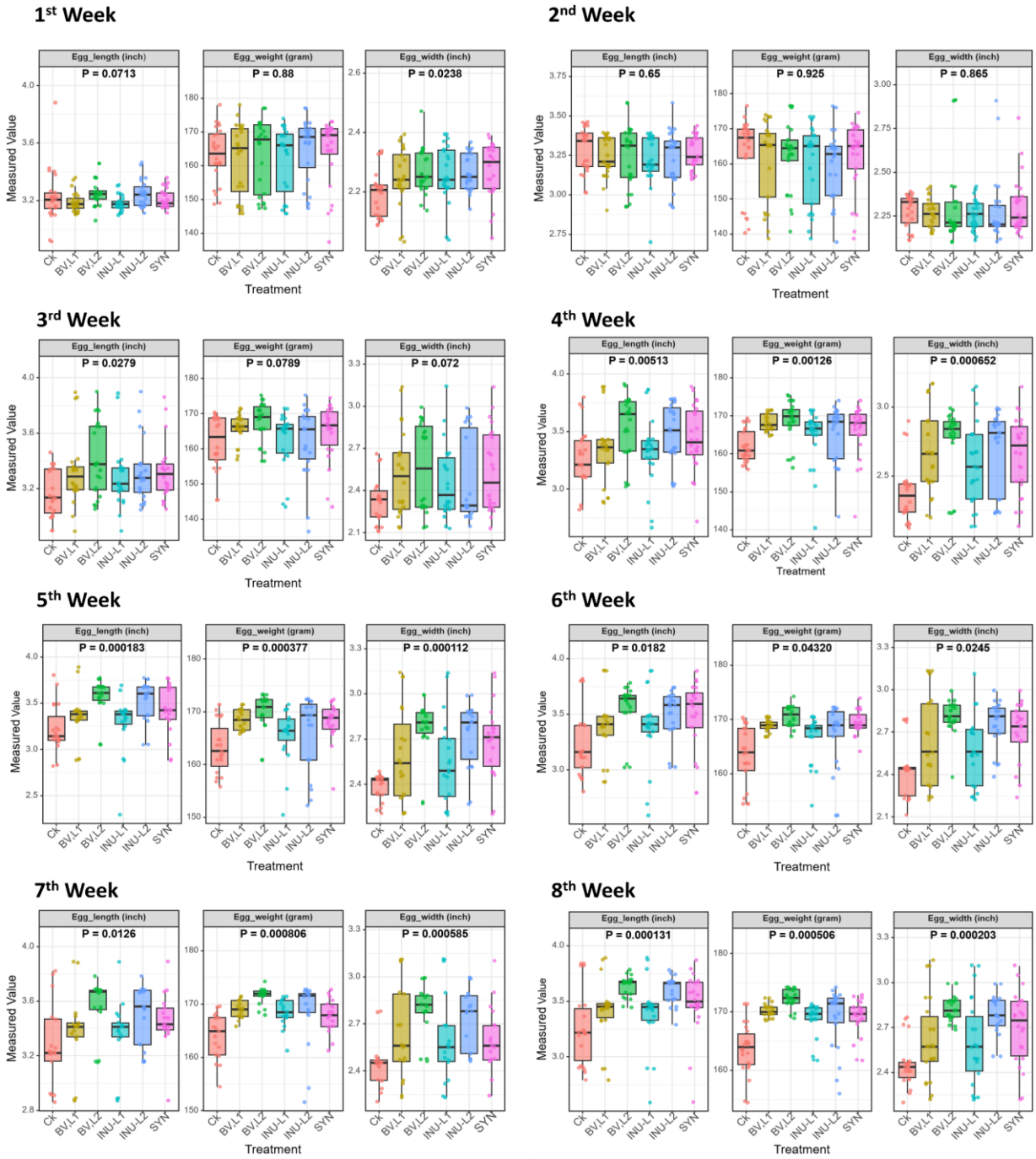


Fig. 1: The results from 1st to 8th week confirm that *B. velezensis*, Inulin, and particularly their synbiotic (SYN) combination significantly improve egg morphometry traits, including length, weight, and width. Ck, control feed; BV-L1, addition of *B. velezensis* level one; BV- L2, addition of *B. velezensis* level two; INU-L1, addition of inulin level one; INU-L2, addition of inulin level two; SYN, Combination of BV-L1 and INU-L1. In each whisker, the mentioned *P*-value signifies the comparison among treatment groups within that week for the egg morphometry trait.

Productive and reproductive indexes: Egg laying rate improved significantly (P<0.05) in both the SYN and BV-L2 treatments, particularly from weeks 5 to 8, with SYN achieving the highest rates. Mortality was recorded in the INU-L1 group during weeks 1–4 and in the SYN group during weeks 5–8, with significant differences among treatments observed during both periods (P<0.05). However, the mortality pattern did not exhibit a dose-dependent relationship, and no clinical signs of treatment-related adverse effects were observed in any group throughout the study. Fertilization rates were significantly higher in the SYN group for both batches (P<0.05), reaching 93.33% in the second batch. The SYN group also exhibited significantly higher hatching rates (P<0.05), peaking in the second batch, which corroborates the treatment's positive effect on overall reproductive outcomes (Table 3).

Reproductive hormonal profile of breeding geese: The prolactin hormone levels (µIU/mL) in different treatment groups, showed significant differences, where the SYN group having the highest (P<0.05) levels (110 µIU/mL) (Fig.2A). The luteinizing hormone (LH) levels across the treatment groups were measured, where the box plots of SYN and BV-L2 group indicated significant differences (P<0.05) (Fig.2B). Estrogen was also measured and no significant differences (*P*>0.05) were found among the treatments (Fig.2C). The measured Follicle-Stimulating Hormone (FSH) levels results showed significant variations, with the BV-L2 and SYN groups having (approximately 3.0 µIU/mL) compared to the other groups (Fig.2D). These findings suggested that BV-L2 and the SYN treatment may have a significant impact on reproductive hormone, and potentially influencing reproductive phase development in geese.

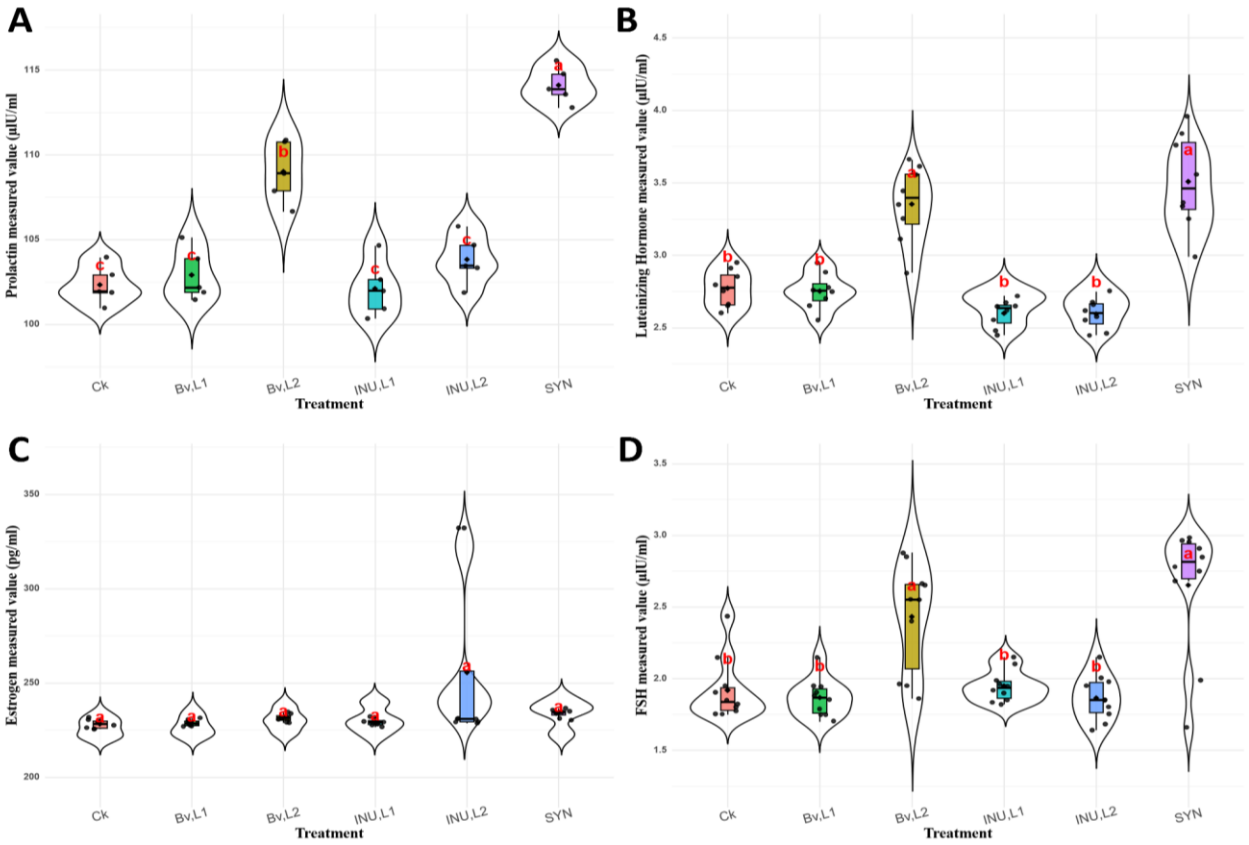


Fig. 2: Potential of the synbiotic combination (SYN) to influence hormonal regulation in Wanxi White Geese. (A) prolactin hormone levels in different dietary treatment groups. (B) luteinizing hormone (LH) activity across different treatment groups of geese. (C) estrogen level in blood plasma of geese fed different levels of *B. velezensis* and Inulin. (D) Follicle-Stimulating Hormone (FSH) levels. Different superscripts letters above the violin plots show significant difference among the treated groups within the same panel (*P* <0.05), whereas groups sharing the same letter are not significantly different (P≥0.05).

Table 3: Productive and reproductive performance of control and treated Wanxi White geese.

		Basal, *B. velezensis* and inulin inclusion Feed Groups						*P*-values		
Performance Indexes (%)	Time	Ck	BV-L1	BV-L2	INU-L1	INU-L2	SYN	ANOVA	Linear	Quadratic
Egg laying Rate	1st to 4th weeks	14.48^{a}± 0.45	14.08^{a}± 0.45	15.47^{b}± 0.40	14.29^{a}± 0.45	14.08^{a}± 0.45	15.87^{b}± 0.52	0.015	0.045	0.385
	5th to 8th weeks	22.20^{a}± 0.45	23.40^{a}± 0.52	24.80^{b}± 0.60	22.40^{a}± 0.40	22.80^{a}± 0.55	27.80^{b}± 0.75	0.025	0.020	0.412
Mortality Rate	1st to 4th weeks	0.00^{a}± 0.00	0.00^{a}± 0.00	0.00^{a}± 0.00	3.33^{b}± 0.10	0.00^{a}± 0.00	0.00^{a}± 0.00	0.022	0.010	0.215
	5th to 8th weeks	0.00^{a}± 0.00	0.00^{a}± 0.00	0.00^{a}± 0.00	0.00^{a}± 0.00	0.00^{a}± 0.00	6.67^{b}± 0.22	0.040	0.080	0.350
Fertilization Rate	1st Batch	76.66^{a}± 0.80	80^{a}± 0.90	83.66^{a}± 0.92	73.33^{b}± 1.10	76.67^{a}± 0.90	90^{c}± 1.00	0.025	0.030	0.140
	2nd Batch	83.33^{a}± 0.67	83.33^{a}± 0.67	86.66^{a}± 0.72	80^{b}± 1.00	83.33^{a}± 0.67	93.33^{c}± 0.88	0.018	0.040	0.150
Hatching Rate	1st Batch	80^{a}± 1.30	76.67^{a}± 1.50	86.67^{a}± 1.15	83.33^{a}± 1.10	80^{a}± 1.30	90^{b}± 1.00	0.021	0.025	0.160
	2nd Batch	76.67^{a}± 1.50	83.33^{a}± 1.10	90^{a}± 1.00	80^{a}± 1.30	86.67^{a}± 1.20	93.33^{b}± 1.00	0.015	0.040	0.120

Note: CK, no additives control group; BV-L1, *B. velezensis* level one; BV-L2, *B. velezensis* level two; INU-L1, Inulin level one; INU-L2, Inulin level two; SYN, combination of BV-L1, and INU-L1. Values are indicating as mean± SEM, where in the same row, means with different letters differ significantly (*P* <0.05), whereas means sharing the same letter are not significantly different. ANOVA designates the overall treatment effect, and Linear and Quadratic *P*-values indicate the linear and quadratic responses to dietary inclusion levels.

Intestinal histomorphology and morphometry assessment: The histomorphology of the intestinal ileum was examined, and the analysis revealed no apparent pathological changes in any of the treatment groups (Fig.3). The morphometric measurements of the duodenum, jejunum and ileum of intestine have assessed and the following parts were measured e.g., villus height, crypt depth, mucosal thickness, intestine wall thickness, and villus width (Fig.4, A1-4). The results of the crypt depth measurements in the duodenum showed no significant differences ($P>0.05$), with all groups exhibiting similar values. However, in the jejunum and ileum, significant differences ($P<0.05$) are observed. In both the jejunum and ileum, the SYN group showed the highest crypt depth followed by BV-L2 group (Fig.4B). The intestinal wall thickness of duodenum part in CK exhibited the thinnest intestinal wall, with a significantly lower thickness compared to all treated groups ($P<0.05$). In the jejunum, the BV-L1 and SYN groups demonstrated the thickest walls. Similarly, in the ileum, the SYN group had the thickest intestinal walls, followed by BV-L1 (Fig.4C). In the duodenum, jejunum and ileum the SYN group exhibited the tallest villi ($P<0.05$), followed by BV-L2 group, (Fig.4D). Duodenum, BV-L1 group showed the higher mucosal thickness, whereas in jejunum and ileum, the SYN group demonstrated the thickest mucosal layer (Fig.4E). The BV-L1 treatment exhibited the higher villus width, similarly, in the jejunum, BV-L1 and BV-L2 treatments led to the highest villus width. In the ileum, the SYN group demonstrated the higher villus width (Fig.4F). The muscle thickness in the duodenum part had no significant differences among the treatments while in the jejunum, ileum the SYN group showed a significant increase ($P<0.05$) (Fig.4G).

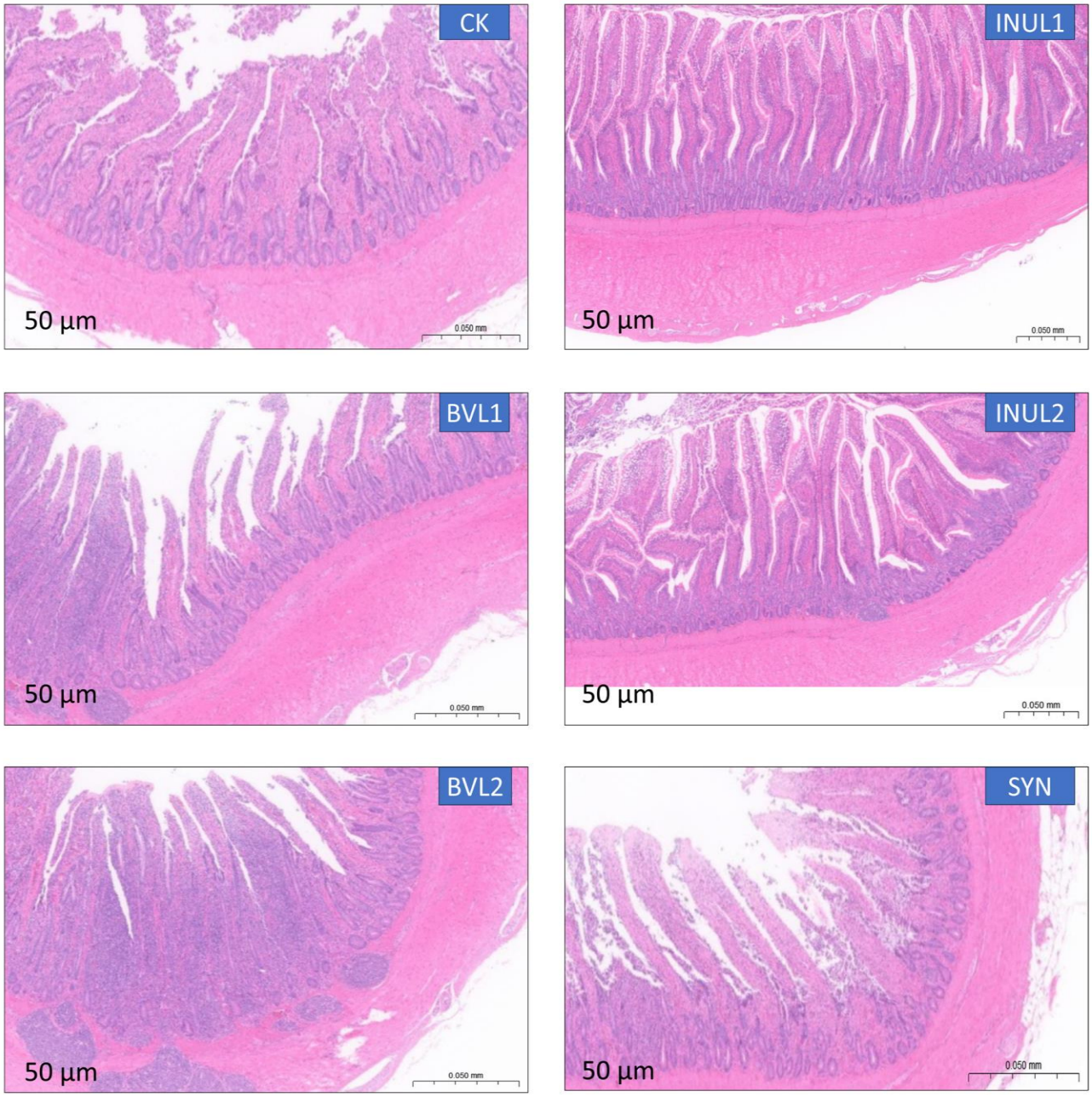


Fig. 3: Histomorphology of the intestinal ileum of geese fed, containing different levels of *B. velezensis*, inulin, and their combination (synbiotic). Scale bars = 50µM (×400).

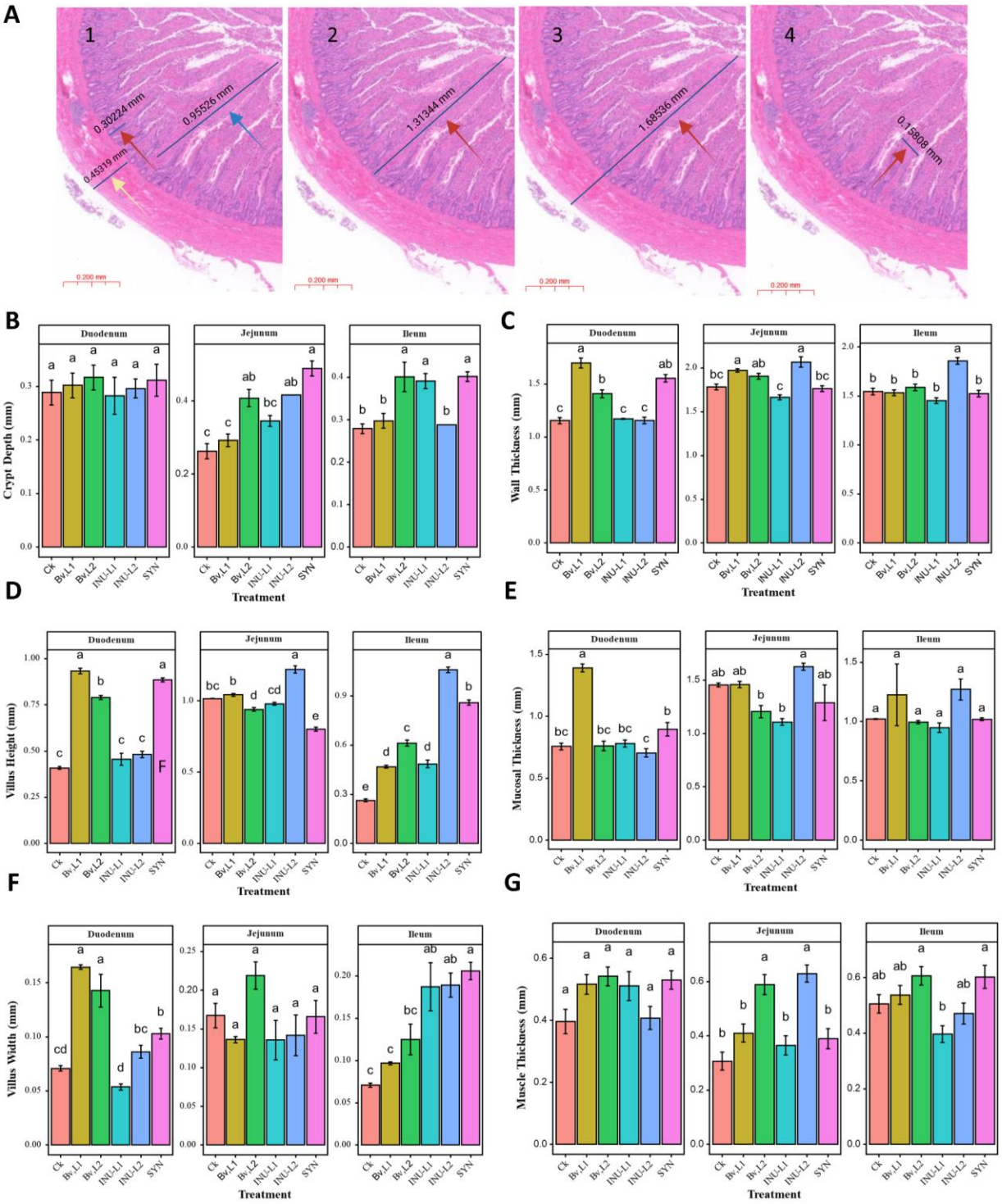


Fig. 4: Small intestine histomorphometry of geese, fed with varying levels of *B. velezensis* and Inulin inclusion. (A) visualization of intestinal crypt depth, muscularis thickness, villus height, mucosal thickness, wall thickness and the villus width. (B) crypt depth measurements of small intestinal parts. (C) wall thickness measurement of duodenum, jejunum and ileum. (D) measurement of villus height in different parts of small intestine. (E) morphometry of mucosal thickness. (F) measurement of villus width, (G) measurement of intestinal muscle thickness. Different superscripts letters above the bar graphs show significant difference among the treated groups within the same panel ($P < 0.05$), whereas groups sharing the same letter are not significantly different ($P \geq 0.05$).

Microbial SCFAs analysis: In total nine distinct SCFAs were detected where the concentration of 4-methylvaleric acid (4-MVA), acetic acid (AA), and butyric acid (BA) in the cecal content of geese are highlighted in (Fig.5A). Concentrations of 4-MVA and AA were highest in the SYN group, while BA levels were significantly ($P<0.05$) elevated in both the SYN and BV-L2 treatments. SYN inclusion substantially increased the concentrations of caproic acid (CA). Mean levels of CA, isobutyric acid (IBA), and 3,5,5-trimethylhexanoic acid (INA) were measured to quantify this effect. Similarly, BV-L2 and INU-L2 treatments markedly increased IBA levels, with the most pronounced effect observed for INU-L2 (Fig.5B). Isovaleric (IVA), propionic (PA), and valeric (VA) acids were also detected, where IVA reached the highest concentrations, particularly in the BV-L2 and

SYN groups (Fig.5C). The heatmap visualized significant upregulation (red) and downregulation (blue) of differential metabolites across the experimental groups (Fig.5D). The Coefficient of Variation (CV) for SCFA Quality Control (QC) samples was calculated, and its distribution is illustrated. The y-axis represents the percentage of peaks, and the x-axis indicates the Coefficient of Variation (CV) values, which range from 0 to 1. The red line represents the CV distribution for the QC samples, showing that approximately 80% of peaks have a CV value below 0.3. This indicated low variability and high measurement consistency (Fig.5E). The stacked bar plot compared three variables molecular weight (blue), the specific compound value Q1 (orange), and KEGG pathway (green) for various SCFA, and MCFAs metabolites, and reflecting their unique biochemical profiles (Fig.5F).

Expression of genes linked to gut tight junctions: The genes related to tight junctions, like *Occludin*, *Claudins1* and Tight Junction Protein 1 were optimized (Fig.6A). Dietary inclusion significantly influenced the duodenal tight junction genes *Claudin 1* (CLDN1), *Occludin* (OCLN), and *Tight Junction Protein 1* (TJP1) in geese. The treatments, especially BV-L2, significantly upregulate *CLDN1* and *TJP1* where the *OCLN* expression is also notably higher with BV-L2, indicating its positive influence on tight junction formation (Fig.6B). The tight junction gene *CLDN1*, and *TJP1* in the jejunum also expressed and SYN leads to the increase upregulated expression, (Fig.6C). The genes were also expressed in the ileum. The SYN produces the highest upregulated expression for *CLDN1* (5-fold) and *OCLN* (2.5-fold) (Fig.6D). These findings highlight the potential for *B. velezensis* and Inulin to improve tight junction gene expression in intestine of geese.

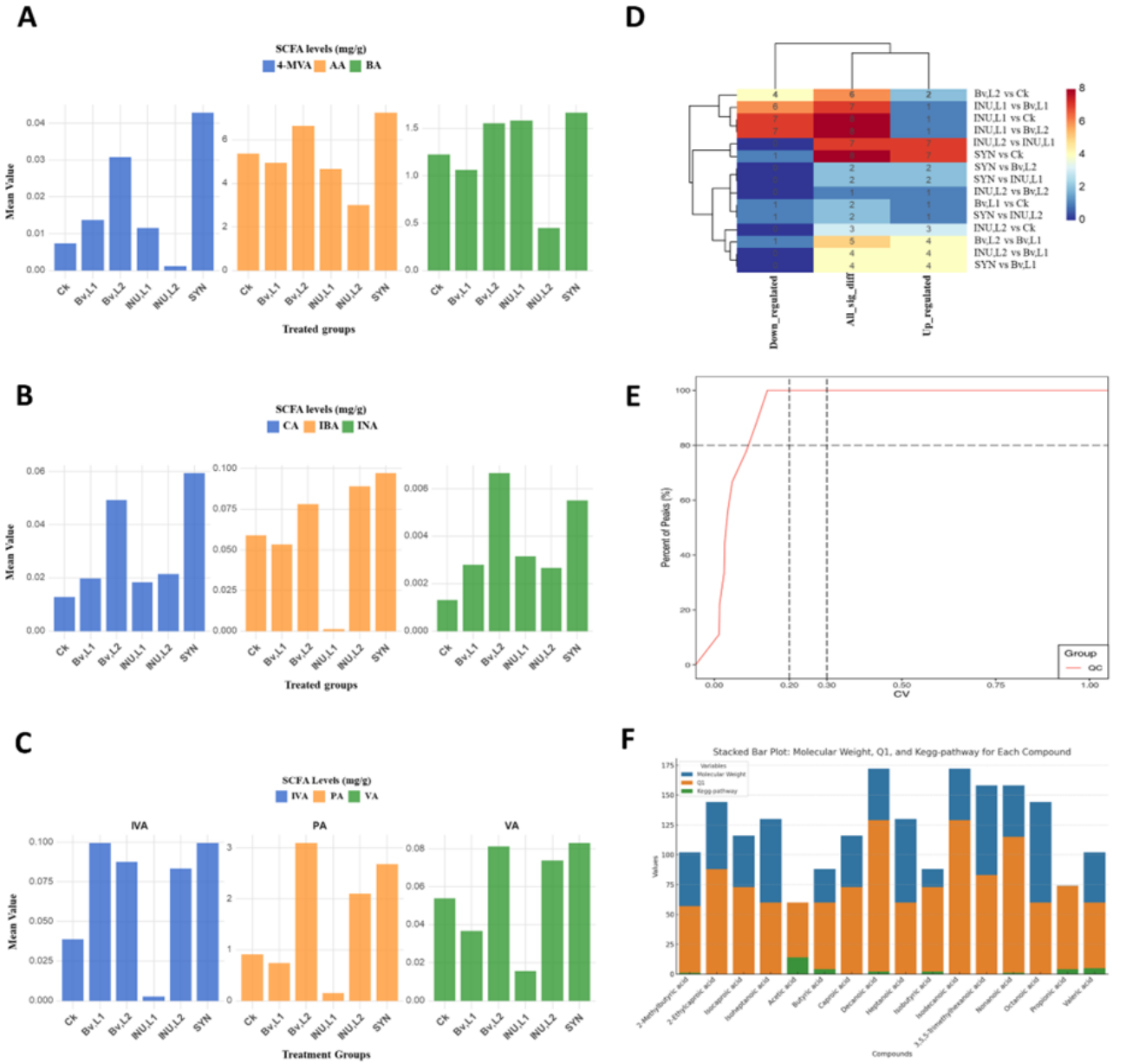


Fig. 5: SCFAs are produced in the cecum of diet treated geese. (A) concentrations of 4-methylvaleric acid (4-MVA), acetic acid (AA), and butyric acid (BA). (B) concentrations of SCFAs including caproic acid (CA), isobutyric acid (IBA), and 3,5,5-trimethylhexanoic acid (INA). (C) measured concentrations of isovaleric (IVA), propionic (PA), and valeric (VA) acids. (D) a heatmap of the significantly up and down-regulated metabolites across the different treatment groups. (E) coefficient of variation (CV) values for the quality control (QC) samples, confirming high data quality and strong analytical consistency. (F) specific biochemical characteristics of the short- and medium-chain fatty acid (SCFA and MCFA) metabolites.

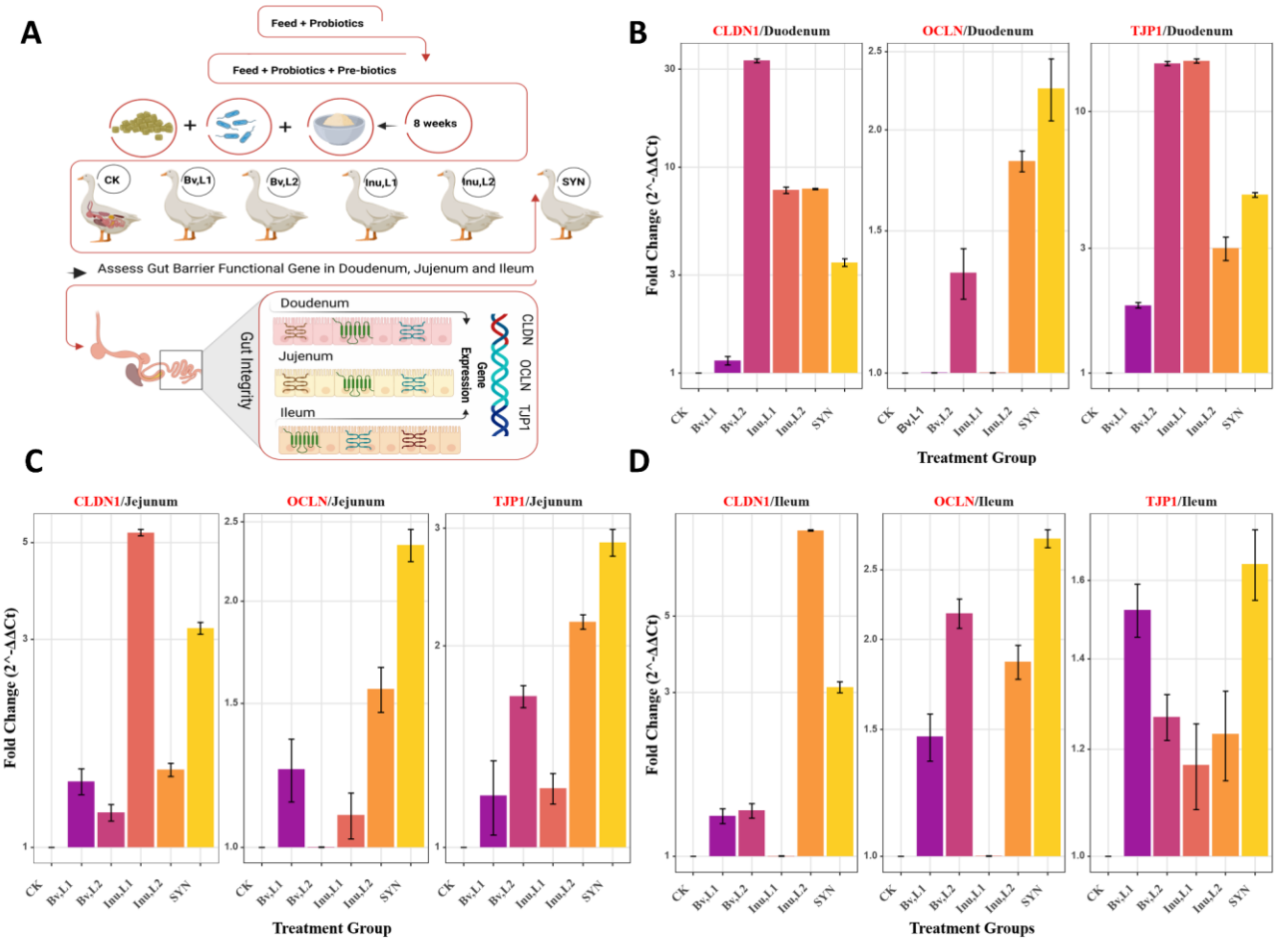


Fig. 6: Gut barrier functionality by the tight junction linked gene validation. (A) flow of feed treated with *B. velezensis* and inulin demonstrates their positive impact on gut integrity. (B) expression of key tight junction gene like CLDN1, OCLN, and TJP1 in the duodenum of geese. (C) expression of tight junction linked gene in the jejunum of geese. (D) gene expression in the ileum of geese fed different *B. velezensis* and inulin levels. Data are presented as mean ± SEM.

Microbial profile and relative abundance in geese cecal content: Hierarchical clustering revealed the unique abundance patterns of bacteria taxa among the experimental groups. At the family level, *Peptococcaceae* showed higher relative abundance in the CK group but decreased in the treatment groups. In contrast, *Bacteroidaceae* and *Prevotellaceae* showed higher relative abundance in the BV-L2 group. *Bacteroidaceae* and *Rikenellaceae* also exhibited treatment-dependent variations in relative abundance. The color gradient in (Fig. 7A) represents relative abundance, with red indicating higher and blue indicating lower abundance. At the genus level, the abundance of *Blautia* was greater in the CK and BV-L2, whereas the levels of *Peptococcus* decreased in most treatments. There was a lot of variances in abundance of *Erysipelotrichaceae*_UCG-003 between groups, with the highest in BV-L2 group. The abundance of *Faecalibacterium* was found to differ among treatments, but *Desulfovibrio* was found to increase in the BV-L1 and BV-L2 groups (Fig.7B). Circos plot visually illustrates changes in microbial abundance and interactions on the level of families. There were specific families that were more abundant in specific treatments and *Ruminococcaceae* (green) and *Bacteroidaceae* (red) were the most abundant in *Bacillus* and inulin treatments (Fig.7C). The venn flower represented the distribution of Operational Taxonomic Units (OTUs). The outer segments, labeled A, B, C, indicated biological replicates like control (CK1, CK2 and CK3) to R denoted SYN., correspond to individual treatments, and the numbers within each segment represent the OTUs unique to each treatment group (Fig.7D). The relative abundance of bacterial families is shown in the bar plot. The *Bacteroidaceae* (blue) and unclassified family-level taxon-*Incertae_Sedis* (orange) were the most prevalent microbes in the microbial community, in both replicas of the *B. velezensis* and SYN treatments (Fig.7E). The BV-L1 and BV-L2 addition enhanced the bacterial genus, where the *Peptococcaceae* and *Rikenellaceae*_RC9_gut_group and *Bacteroides* were prominent. The SYN treatment exhibited patterns similar to the *Bacillus* treatments, particularly in its effect on *Bacteroides* abundance (Fig.7F).

Relative alpha and beta diversity in geese cecal content: Alpha diversity measured by indices such the ACE (Abundance-based Coverage Estimator) index, Chao1 and Simpson. Together, these indices, provide a comprehensive view of microbial diversity by considering both the richness (number of species) and the evenness (distribution of species) within a microbial community.

The box plot depicting the ACE index shows significant differences ($P<0.05$) in microbial richness in the treatment groups. In this case, the ACE index is relatively high in BV-L1, BV-L2 and SYN, indicating that *B. velezensis* and their combine with inulin significantly enhances microbial richness (Fig.8A). The Chao1 index also measured high species richness in BV-L1 and BV-L2 treated group, whereas, the SYN group, showed higher Chao1 index (Fig.8B). The BV-L2 and SYN groups showed the highest Simpson index values, then CK and others groups (Fig.8C). To assess differences in cecal microbiota composition, *β*-diversity analysis was conducted. The NMDS plot (Jaccard test) illustrated the *β*-diversity of the cecal microbiota across treatment groups. The CK is tightly clustered, showing less variability, while BV-L1, BV-L2, INU-L1, INU-L2, form a separate cluster, with clear differentiation. The SYN forms a distinct cluster as well, separate from other groups (Fig.8D). The NMDS (Curtis's test) demonstrated the distinct separation between the groups, whereas the R value of 1 and the *P*-value of 0.001 confirmed that the differences observed between the groups are statistically significant (Fig.8E). The Unweighted-Unifrac Test indicated that each treatment results in distinct shifts in microbial composition (Fig.8F), while the Weighted –Unifrac Test followed the same significant trend (Fig.8G). The principal component analysis consistently revealed that the SYN diet induced the substantial alterations in both the microbial community and physiological metrics among all tested conditions. The Cluster-tree, Jaccard Test image depicts the taxonomic *β*-diversity of the cecal microbiota in geese. The color-coded bars represent the relative *β*-diversity such as *Bacteroidaceae* (light blue), *Incertae_Sedis* (blue), *Peptococcaceae* (green), and *Faecalibacterium* (pink). *B. velezensis,* inulin and SYN replicates are characterized by an elevated abundance of *Bacteroidaceae* and *Peptococcaceae*. The CK showed a stable microbiota composition (Fig.8H). Cluster-tree, weighted–Unifrac Test followed by the same trend of beta diversity which showed that these treatments also contributed to alterations in the microbiota composition (Fig.8I).

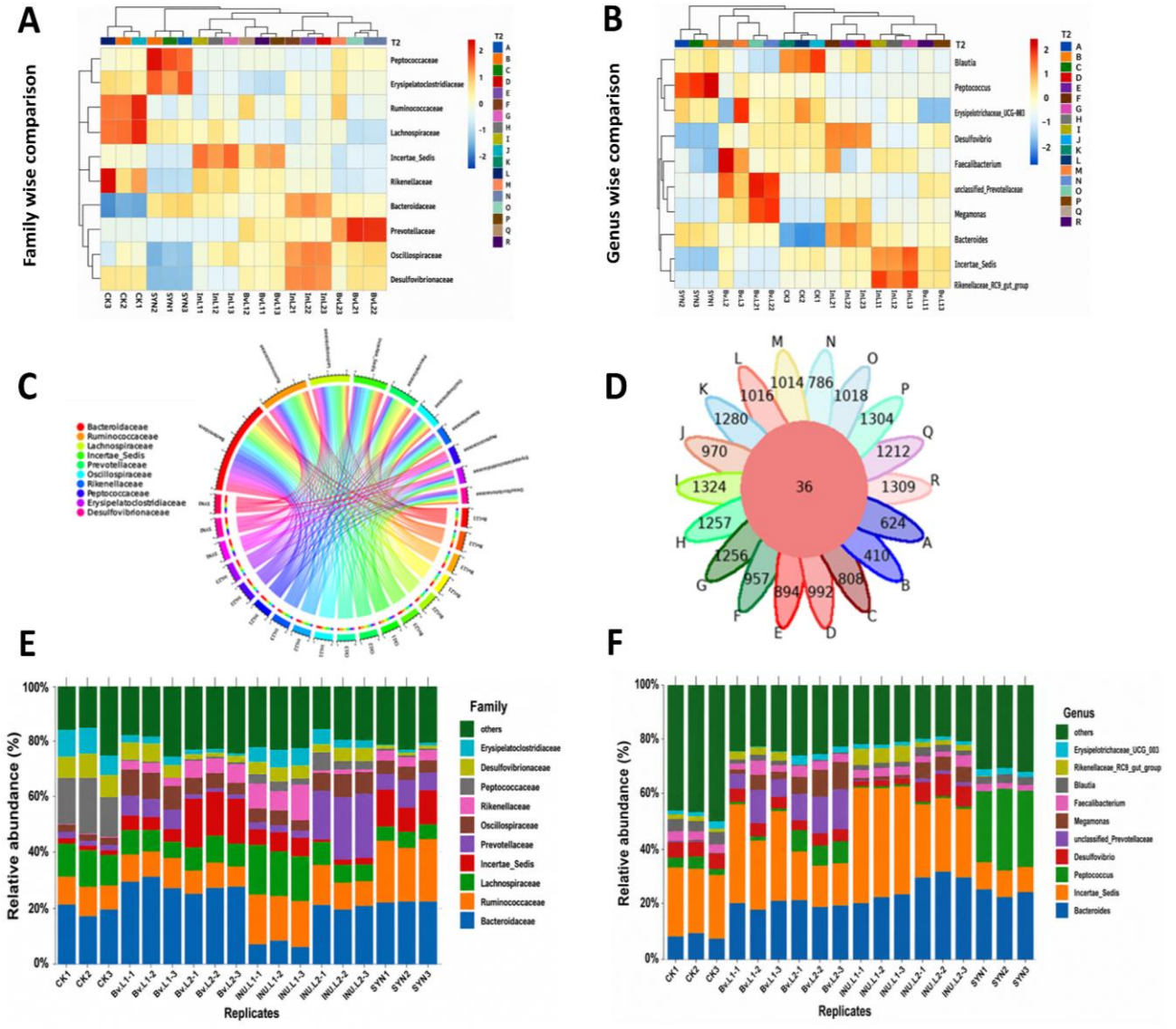


Fig. 7: Cecal microbiota diversity in terms of families and genera across the different dietary inclusion groups. (A) bacterial family distribution via heatmap visualization. (B) genus distribution via heatmap. (C) circos plot provides a visual swift of the bacterial family. (D) distribution of Operational Taxonomic Units (OTUs). (E) relative bacterial family distribution via bar plot. (F) relative bacterial genus via bar plot.

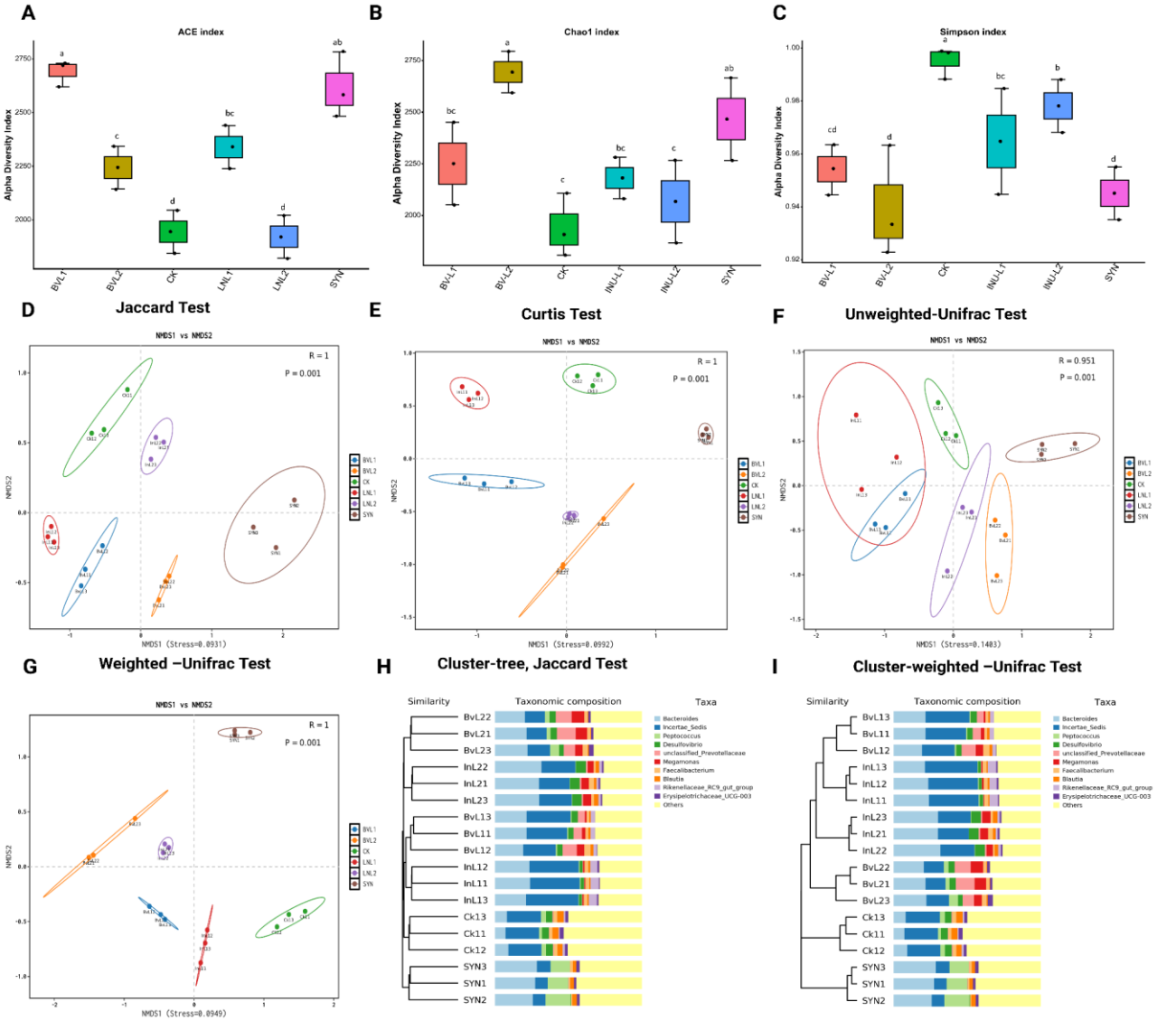


Fig. 8: Relative abundance analysis of α and β-diversity. (A) ACE index analysis of alpha diversity. (B) Chao1 index analysis of alpha diversity. (C) Simpson analysis. (D) Jaccard test analysis of β-diversity. (E) Curtis test analysis. (F) analysis of Unweighted-Unifrac test. (G) analysis of Weighted-Unifrac test. (H) Cluster-tree analysis of Jaccard test. (I) Cluster-weighted-UniFrac test analysis. Data in Panels A–C were analyzed using ANOVA followed by Tukey's post hoc test for multiple comparisons. Significance levels are indicated by different letters (a, b, c, d) above the bars (P<0.05).

DISCUSSION

In the present study, we assessed the effects of *B. velezensis*, including BV-L1 and BV-L2, and inulin, including INU-L1 and INU-L2, while their combination (SYN) on the productive, reproductive, and gut health performance of breeding geese. The effects of the different dietary inclusion were observed on performance parameters, as well as on their gut health status, with notable changes in microbiota composition, and diversity. Probiotics have become a major focus due to their potential to enhance gut health, improve nutrient absorption, and modulate immune responses, ultimately optimizing reproductive performance in breeder broilers (Adli *et al.*, 2025). Early probiotic administration helps establish stable gut microflora in chicks, and their use in breeder broilers may benefit progeny by reducing pathogenic colonization and enhancing immune function (Forder *et al.*, 2024). Additionally, probiotics have been associated with improvements in egg production (Darsi and Zhaghari, 2021).

Egg morphometric and nutritional parameters were assessed and the significant change were found in the treated groups during the 5th to 8th week of feed trial. *B. velezensis* modulates the gut microbiota, thereby influencing digestion, nutrient absorption, and immune response. These integrated physiological effects often require several weeks to translate into measurable performance improvements (Cao *et al.*, 2022; Li *et al.*, 2022). Similarly, prebiotics like inulin, which promote the growth of beneficial gut bacteria, may require time to alter the microbial community sufficiently to impact egg production and shape (Li *et al.*, 2025b). The geese treated with BV-L2 and their combination of (e.g., BV-L1 and INU-L1) with inulin showed noticeably higher values compared to the control CK. Research from (Ye *et al.*, 2020), exhibited similar finding where the *B. velezensis* inclusion improved the egg production and shape indices in laying hen.

Reproductive traits, including fertilization and hatchability rates, were significantly higher in the SYN group, suggesting that the SYN inclusion positively

impacts reproductive success. A similar study by Khan *et al.* (2025), found that diet supplementation with synbiotics (probiotics with MOS) and MOS alone improved fertility characteristics in quails. Another report on the use of probiotics, *Lactobacillus spp*. or *Lactobacillus spp*. combined with mushroom extract as a synbiotic formulation in farmed fish showed significantly higher gonadosomatic index, absolute fecundity, and relative fecundity compared to the control (Zakariaee *et al.*, 2021; Khan *et al*., 2024). Consistent with these findings, a combination of prebiotics and probiotics, including mannan-oligosaccharides, *Lactobacillus rhamnosus*, and *Bifidobacterium longum*, improved FSH and LH levels in quails (Wang *et al.*, 2021; Zakariaee *et al.*, 2021).

The histological and morphometric analyses of the small intestine showed significant improvement, indicating a healthier and more functional intestinal lining. Where (Sun *et al.*, 2022) reported that probiotic supplementation improves intestinal tissue morphology, as evidenced by increased villus height and a greater villus height-to-crypt depth (VH:CD) ratio. These morphological changes are indicative of an expanded absorptive surface area and enhanced digestive and absorptive function. In poultry, the comparatively short length of this organ means that its total absorptive capacity is largely determined by the surface area provided by the intestinal villi (Bogusławska-Tryk *et al.*, 2021). The integrity of the gut barrier is essential for overall health, growth, and disease resistance (Yaseen *et al.*, 2025). To assess gut functionality in the present study, genes associated with tight junctions were examined. *TJP1* is crucial for maintaining the stability and integrity of intercellular junctions (Gilani *et al.*, 2018) while *Claudin-1* and *Occludin* are key proteins that form tight junctions between epithelial cells, regulating permeability and the tightness of cell-cell interactions (Song *et al.*, 2018). The gut lumen is lined with a mucus layer that surrounds a mixture of nutrients, metabolites, and a diverse microbial community, which work together to maintain gastrointestinal health and homeostasis (Borda-Molina *et al.*, 2018).

B. velezensis and inulin significantly influenced the cecum microbiota and their metabolites production in geese, where these additives promote the beneficial microbiota and inhibiting harmful ones, which can lead to better gut health, improved digestion, and overall performance in geese. A research conducted by (Markowiak-Kopeć and Śliżewska, 2020) whereas the 42-day supplementation with synbiotics significantly elevated the concentrations of lactic acid and total SCFAs including (acetic, propionic, butyric, valeric, and formic acids) in chicken excreta compared to a control group. The authors note that these SCFAs, produced by promoting beneficial saprophytic flora, can inhibit pathogens like *Salmonella*, *E. coli*, and *Campylobacter* through competitive exclusion for colonization sites (Markowiak-Kopeć and Śliżewska, 2020). Higher concentrations of SCFAs are widely recognized as beneficial for animals, supporting intestinal mucosal health, regulating pH, and modulating energy metabolism by promoting glucose utilization and fatty acid oxidation (Li *et al.*, 2023b). The cecum, due to its longer transit time, is known to harbor a complex microbiota that facilitates extensive fermentation (Qu *et al.*, 2008). The obtained findings provide a comprehensive characterization of the cecal microbiota in geese fed *B. velezensis* and inulin. The observed increase in both α- and *β*-diversity in the treated group suggests that these dietary inclusions can effectively enhance the richness, evenness, and overall diversity of the cecal microbiota. Similar improvements in microbiota diversity were observed in ducks supplemented with dietary ferulic acid products (Liu *et al.*, 2021). In our study some bacterial families were dominant among the other cecal microbial diversity like, *Bacteroidaceae*, *Prevotellaceae*, *Incertae Sedis*, and *Peptococcaceae*. The *Bacteroidaceae* family ferment polysaccharides that the host cannot digest, producing SCFAs like butyrate, propionate, and acetate, which support intestinal health, regulate pH, and aid in energy metabolism. The *Prevotellaceae* family also ferments carbohydrates, especially plant-based fibers, contributing to SCFA production, particularly acetate and propionate, while helping modulate the immune system and maintain gut microbiota balance (Ma and Lee, 2025). These results further indicate that the effect of probiotics bacteria, prebiotics and their combination (synbiotics) on geese health and performance is regulated by multiple factors, such as the obvious changes in beneficial microbiota of gut, production of SCFAs, different dietary level of *B. velezensis* and inulin, duration of the feeding trails.

Conclusions: In conclusion, this study demonstrates that dietary inclusion of *B. velezensis* level two, and the synbiotic group, significantly improved the productive and reproductive performance of Wanxi White breeding geese while modulating intestinal microbiota biodiversity. The synbiotic effects of *B. velezensis* and inulin, showed promising potential and can be consider as potential alternative. Future research should explore different levels of synbiotics and extend the feeding trials to evaluate gut dynamics, histological changes, and to better understand the mechanisms behind this dietary inclusion, ultimately optimizing dosage formulations for commercial use.

Ethical approval: This experimental design was carried out in compliance with the regulations and guidelines established by the Animal Care Committee of the School of Life Sciences at Anhui Agricultural University, with every effort taken to reduce animal suffering. The study methods were authorized by Anhui Agricultural University's Animal Experiment Committee (KJLLXM2025114).

CRediT authorship contribution statement: IM. Khan and N. Nassar; Writing–original draft, editing draft, Software, Methodology, Formal analysis. D. She and Q. Zhang; Methodology, Formal analysis, Validation. S. Khan; Visualization, Formal analysis. A. Khalid, N. Du, and X. Zhu; Formal analysis, Validation, methodology. Z. Wang; Writing–review & editing, Supervision, Re-sources, Project administration, Funding acquisition, Conceptualization. All authors read and approved the final manuscript. Visualization

Data and model availability statement: None of the data were deposited in an official repository. All data are available from the authors upon request.

Declaration of interest: None

Financial support statement: This work was supported by the Natural Science Foundation of Anhui Province (2308085MC99), the National Key Research and Development Program Project (2024YFD1300705), the Post Doctor's Project of Anhui Agricultural University (03087003), the Major Science and Technology Program of Anhui Province (201903a06020020), and the Anhui Industry and Technology System of Poultry Science (AHCYJSTX—06).

REFERENCES

Abd El-Ghany WA, 2020. Paraprobiotics and postbiotics: Contemporary and promising natural antibiotics alternatives and their applications in the poultry field. Open Veterinary Journal 10: 323–330.

Abdullah RM, Ali S, Aslam B and Arshad MI, 2024. Molecular characterization and drug resistance pattern of *Pseudomonas aeruginosa* isolated from poultry meat and meat products. Pakistan Veterinary Journal 44(3):812-818.

Adli DN, Sholikin MM, Sitaresmi PI, *et al.*, 2025. Dose-Dependent Effects of Probiotics on the Reproductive Performance, Egg Characteristics, and Seminal Traits of Broiler Breeders: A Model-Based Meta-Analysis. Journal of Animal Physiology and Animal Nutrition 109: 1101-1119.

Akhtar MF, Shafiq M and Ali I, 2021. Improving gander reproductive efficacy in the context of globally sustainable goose production. Animals 12, 44.

Akram FSM, Ashraf M, Ali S, *et al*., 2017. Isolation of gram-positive bacteria from different sources and evaluation of their probiotic properties. Journal of Medical Microbiology and Infectious Diseases 5(1):12-16.

Alqahtani FS, Bahshwan S, AL-Qurashi MM, *et al.*, 2024. Impact of dietary *Bacillus toyonensis* M44 as an antibiotic alternative on growth, blood biochemical properties, immunity, gut microbiota, and meat quality of IR broilers. Pakistan Veterinary Journal 44(3):637-646.

Bianchi F, Dall'Asta M, Del Rio D, *et al*., 2011. Development of a headspace solid-phase microextraction gas chromatography–mass spectrometric method for the determination of short-chain fatty acids from intestinal fermentation. Food Chemistry 129: 200-205.

Bogusławska-Tryk M, Ziółkowska E, Sławińska A, *et al.*, 2021. Modulation of intestinal histology by probiotics, prebiotics and synbiotics delivered in ovo in distinct chicken genotypes. Animals 11: 3293.

Borda-Molina D, Seifert J and Camarinha-Silva A, 2018. Current perspectives of the chicken gastrointestinal tract and its microbiome. Computational and Structural Biotechnology Journal 16: 131-139.

Cao Y, Xun M, Ren S, *et al*., 2022. Effects of dietary organic acids and probiotics on laying performance, egg quality, serum antioxidants and expressions of reproductive genes of laying ducks in the late phase of production. Poultry Science 101: 102189.

Chen X, Liu X, Du Y, *et al.*, 2018. Green forage and fattening duration differentially modulate cecal microbiome of Wanxi white geese. PLoS One 13: e0204210.

Darsi E and Zhaghari M, 2021. Effects of Bacillus subtilis PB6 supplementation on productive performance, egg quality and hatchability in broiler breeder hens under commercial farm condition. Journal of Applied Animal Research 49: 109-117.

Forder RE, Willson NL, Angove JA, *et al*., 2024. Dietary inclusion of a Saccharomyces cerevisiae metabolite improved reproductive performance but did not affect intestinal permeability in two chicken meat breeder lines. Poultry Science 103: 103595.

Gilani S, Howarth G, Nattrass G, *et al.*, 2018. Gene expression and morphological changes in the intestinal mucosa associated with increased permeability induced by short-term fasting in chickens. Journal of Animal Physiology and Animal Nutrition 102: e653-e661.

Huang DW, Sherman BT and Lempicki RA, 2009. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nature protocols 4: 44-57.

Khalid F, Khalid A, Fu Y, *et al.*, 2021. Potential of *Bacillus velezensis* as a probiotic in animal feed: a review. Journal of Microbiology 59: 627-633.

Khan A, Kango N and Srivastava R, 2025. Impact of dietary probiotics on the immune and reproductive physiology of pubertal male Japanese quail (Coturnix coturnix japonica) administered at the onset of pre-puberty. Probiotics and Antimicrobial Proteins 17: 1399-1417.

Khan IM, Nassar N, Chang H, *et al.,* 2024. The microbiota: a key regulator of health, productivity, and reproductive success in mammals. Frontiers in Microbiology 15:1480811.

Khan IM, Liu H, Zhuang J, *et al.*, 2021. Circular RNA expression and regulation profiling in testicular tissues of immature and mature Wandong cattle (Bos taurus). Frontiers in Genetics 12: 685541.

Khosravi A, Boldaji F, Dastar B, *et al.*, 2025. A synbiotic improves performance and gut health in broiler chickens. Scientific Reports 15: 19164.

Kim YB, Park J, Lee HG, *et al.*, 2024. Dietary probiotic Lacticaseibacillus paracasei NSMJ56 modulates gut immunity and microbiota in laying hens. Poultry Science 103: 103505.

Li C, Li S, Dang G, *et al.*, 2023a. Screening and characterization of Bacillus velezensis LB-Y-1 toward selection as a potential probiotic for poultry with multi-enzyme production property. Frontiers in Microbiology 14: 1143265.

Li G, Wang X, Liu Y, *et al.*, 2023b. Bile acids supplementation modulates lipid metabolism, intestinal function, and cecal microbiota in geese. Frontiers in Microbiology 14: 1185218.

Li L, Lv X, Han X, *et al*., 2022. Effect of dietary bacillus licheniformis supplementation on growth performance and microbiota diversity of pekin ducks. Frontiers in Veterinary Science 9: 832141.

Li Y, Ko K, Liu X, *et al.*, 2025a. Effects of inulin on growth performance and intestinal health of broilers by modulating intestinal microbiota and metabolome. Animal Nutrition 23: 257-270.

Li Z, Kim E, Ko K, *et al.*, 2025b. Integrating microbiome and metabolome analyses to unravel the role of inulin in enhancing the meat quality and bone health of ducks. Scientific Reports 15: 15194.

Lin R, Chen H, Rouvier R, *et al*., 2016. Genetic parameters of body weight, egg production, and shell quality traits in the Shan Ma laying duck (Anas platyrhynchos). Poultry Science 95: 2514-2519.

Liu Y, Lin Q, Huang X, *et al.*, 2021. Effects of dietary ferulic acid on the intestinal microbiota and the associated changes on the growth performance, serum cytokine profile, and intestinal morphology in ducks. Frontiers in Microbiology 12: 698213.

Liu Y, Xiong M, Hu X, *et al*., 2024. Dietary *Bacillus velezensis* KNF-209 supplementation improves growth performance, enhances immunity, and promotes gut health in broilers. Poultry Science 103: 103946.

Ma ZF and Lee YY, 2025. The role of the gut microbiota in health, diet, and disease with a focus on obesity. Foods 14: 492.

Magnoli AP, Parada J, Luna MJ, *et al.*, 2024. Impact of probiotic Saccharomyces cerevisiae var. boulardii RC009 alone and in combination with a phytase in broiler chickens fed with antibiotic-free diets. Agrobiological Records 16: 1-10.

Markowiak-Kopeć P and Śliżewska K, 2020. The effect of probiotics on the production of short-chain fatty acids by human intestinal microbiome. Nutrients 12: 1107.

Qu A, Brulc JM, Wilson MK, *et al.*, 2008. Comparative metagenomics reveals host specific metavirulomes and horizontal gene transfer elements in the chicken cecum microbiome. PLoS One 3: e2945.

Sana SS, Atuahene D, Nagy V, *et al.*, 2025. The Rising Threat of Antibiotic Resistance in Poultry: Veterinary and One Health Perspectives. Veterinary Sciences 12: 1059.

Sharma MK, McDaniel CD, Kiess AS, *et al.*, 2022. Effect of housing environment and hen strain on egg production and egg quality as well as cloacal and eggshell microbiology in laying hens. Poultry Science 101: 101595.

Song J, Li Q, Li P, *et al.*, 2018. The effects of inulin on the mucosal morphology and immune status of specific pathogen-free chickens. Poultry Science 97: 3938-3946.

Sun H, Gu T, Li G, *et al*., 2022. Effects of compound probiotics on growth performance, serum biochemical and immune indices, antioxidant capacity, and intestinal tissue morphology of Shaoxing duck. Animals 12: 3219. Tavaniello S, De Marzo D, Bednarczyk M, *et al.*, 2023. Influence of a commercial synbiotic administered in ovo and in-water on broiler chicken performance and meat quality. Foods 12: 2470.

Wang J, Wan C, Shuju Z, *et al.*, 2021. Differential analysis of gut microbiota and the effect of dietary Enterococcus faecium supplementation in broiler breeders with high or low laying performance. Poultry Science 100: 1109-1119.

Wang Q, Wang Q, Wang C, *et al.*, 2024. Genetic improvement of duration of fertility in chickens and its commercial application for

extending insemination intervals. Poultry Science 103: 103438.

Wang S, Peng Y, Liu X, *et al*., 2025. Combining chicory root pulp with yeast selenium: a novel strategy for improving egg quality, enhancing immune function, and promoting gut health in laying hens. Poultry Science: 105975.

Yang L, Jia C, Li Y, *et al*., 2024. The hypothalamic transcriptome reveals the importance of visual perception on the egg production of Wanxi white geese. Frontiers in Veterinary Science 11: 1449032.

Yaseen K, Ali S, Rahman SU, *et al*., 2025. Comparative molecular virulence typing and antibiotic resistance of Campylobacter species at the human–animal–environment interface. Foodborne Pathogens and Disease 22(2):109-117.

Ye M, Wei C, Khalid A, *et al*., 2020. Effect of *Bacillus velezensis* to substitute in-feed antibiotics on the production, blood biochemistry and egg quality indices of laying hens. BMC veterinary research 16: 400.

Zakariaee H, Sudagar M, Hosseini SS, *et al*., 2021. In vitro selection of synbiotics and in vivo investigation of growth indices, reproduction performance, survival, and ovarian Cyp19α gene expression in Zebrafish Danio rerio. Frontiers in Microbiology 12: 758758.

Zhao G, Nyman M and Åke Jönsson J, 2006. Rapid determination of short-chain fatty acids in colonic contents and faeces of humans and rats by acidified water-extraction and direct-injection gas chromatography. Biomedical Chromatography 20: 674-682.