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Simple Summary

Poultry farming faces the challenge of producing eggs efficiently while ensuring the health and welfare of laying hens. Nutrition is one of the main factors influencing performance, egg quality, and intestinal health. In this study, we evaluated the combined effects of a stimbiotic feed additive and different levels of dietary fiber in laying hens. The results showed that the combination of stimbiotic and higher fiber levels improved bird performance, egg quality, and intestinal morphology. This research provides practical insights for poultry producers seeking more efficient and environmentally responsible strategies to support animal health and food production.

Abstract

Moderately fermentable dietary fiber, especially when combined with stimbiotic (STB) supplementation, can enhance intestinal health, nutrient utilization, and overall performance in laying hens, although effects depend on fiber type, level, and diet composition. To investigate this, 1,200 Bovans White laying hens (32 weeks old) were assigned to a 2 × 6 factorial experiment with two levels of supplementation (without or with 0.01% STB) and six dietary fiber treatments: Control (corn–soybean), Wheat–High CF, 75:25 wheat–corn, 50:50 wheat–corn, 25:75 wheat–corn, and Corn–soybean–Low CF. The study spanned five 28-day periods, evaluating productive performance, egg quality, and intestinal morphology. Dietary fiber levels significantly improved feed intake, egg production, egg mass, feed conversion, and intestinal structure, while STB alone had limited effects. Hens fed 75:25 and 50:50 wheat–corn diets consumed more feed, and the highest egg production and mass were observed in birds receiving Control, 75:25 wheat–corn, and Wheat–High CF diets. Egg quality benefited from the fiber–STB interaction, producing heavier eggs with higher yolk pigmentation, thicker shells, and greater Haugh unit and specific gravity. STB supplementation increased jejunal villus width and absorptive area, whereas fiber type affected ileal morphology. In conclusion, high dietary fiber improves performance, egg quality, and intestinal health in laying hens, and supplementation with 0.01% STB further enhances these effects.

Keywords: fermentable fiber; feed efficiency; gut health; xylanase; wheat bran

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1. Introduction

Historically, dietary fiber has been considered a low-value component in poultry nutrition, viewed as an energy diluent and associated with the presence of antinutritional factors [1]. However, recent studies show that its effect is highly dependent on solubility and fermentability, which can positively or negatively impact performance and gut health [2,3]. Moderately fermentable fiber can stimulate gastrointestinal tract development, enhance endogenous enzyme production, and modulate the microbiota, resulting in improved nutrient utilization and immune responses [4–6].

In laying hens, this issue is even more relevant due to their prolonged production cycle, increasing animal welfare demands, and the frequent use of fibrous ingredients in commercial diets [7]. Nevertheless, the presence of soluble non-starch polysaccharides (NSPs), especially arabinoxylans, can increase digesta viscosity, impair digestion and microbiota balance, and predispose birds to enteric disorders [8–10].

Commercial diets are most commonly based on corn, which contain low levels of arabinoxylans and fiber, limiting the effectiveness of xylanase in enhancing fiber fermentation. Conversely, the inclusion of wheat bran tends to increase the availability of substrates for enzyme activity. Wheat bran is an insoluble fiber source rich in arabinoxylans—estimated at around 23.2% [11]. Studies have shown that moderate inclusion of wheat bran can improve intestinal health in poultry, as it provides energy for enterocytes through the fermentation of arabinoxylo-oligosaccharides by the microbiota, producing short-chain fatty acids (SCFAs) [12,13]. According to Suriano et al. (2018), wheat bran also exerts anti-inflammatory effects and improves intestinal barrier function and microbial composition.

The use of exogenous enzymes, such as xylanase, has emerged as a strategy to hydrolyze NSPs and release fermentable oligosaccharides, thereby improving digestibility and reducing the negative effects of viscosity [14,15]. In this context, stimbiotic (STB) supplementation—a combination of β−1,4-endo-xylanase and xylo-oligosaccharides (XOS)—is noteworthy, as it has a dual mode of action: degrading fibrous fractions and providing prebiotic substrates for cecal fermentation, resulting in SCFA production with beneficial effects on gut health [16,17,25].

Although studies in broilers have consistently shown positive effects of STB, results in laying hens remain limited and inconsistent, varying according to fiber type and level, diet composition, and production conditions [2,3,6]. Thus, a knowledge gap remains regarding which dietary fiber levels, with or without STB supplementation, can simultaneously optimize performance, egg quality, and intestinal integrity in commercial layers.

Therefore, this study evaluated the effect of different dietary high-fiber levels, with or without STB supplementation, on productive performance, egg quality, and intestinal morphology of laying hens.

2. Materials and Methods

2.1. Local and Animals

This study was conducted at Campus II of the Federal University of Paraíba, located in the city of Areia, at a latitude of 6°57′48″ S, a longitude of 35°41′30″ W, and an altitude of 618 m in Paraíba, Brazil. All protocols and procedures followed animal welfare guidelines and were approved by the local Ethics Committee of the Federal University of Paraíba (Areia, Paraíba, Brazil).

A total of 1,200 Bovans White laying hens, 32 weeks of age, were used in the study. These birds were obtained at one day of age and managed according to the instructions described in the strain manual until the beginning of the experimental phase. The hens were housed in conventional laying facilities with clay-tiled roofs, equipped with trough feeders and nipple drinkers. They were kept in galvanized wire cages measuring 100 × 45 × 45 cm.

2.2. Experimental Diets and Design

The diets were formulated to meet the nutritional requirements of the Bovans White strain, considering an average intake of 120 g/bird/day, according to [19]. The experimental design was

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conducted in a 2 × 6 factorial arrangement, with two levels of supplementation (without or with 0.01% stimbiotic - STB) and six fiber levels, consisting of: 1. Control (corn–soybean); 2. Wheat–High CF; 3. 75:25 wheat–corn; 4. 50:50 wheat–corn; 5. 25:75 wheat–corn; 6. Corn–soybean–Low CF; 7. Control (corn–soybean) + STB; 8. Wheat–High CF + STB; 9. 75:25 wheat–corn + STB; 10. 50:50 wheat–corn + STB; 11. 25:75 wheat–corn + STB; 12. Corn–soybean–Low CF + STB (Table 1). The STB (Signis, β-1,4-endo-xylanase and xylo-oligosaccharides, AB Vista, Marlborough, UK) was supplemented at 100 mg/kg of feed, providing an activity of 16,000 BXU/kg. One BXU (xylanase unit) corresponds to the amount of enzyme required to release 1 nmol of reducing sugars from birchwood xylan per second at 50 °C and pH 5.3.

Table 1. Feedstuff and chemical composition (g/kg) of diets for Bovans White laying hens.

Items	Control (corn–soybean)	Corn–soybean – Low CF	75:25 wheat–corn	50:50 wheat–corn	25:75 wheat–corn	Wheat – High CF
Wheat	169.0	0.0	449.2	299.5	149.7	598.9
Wheat bran	0.0	0.0	128.4	85.6	42.8	171.2
Corn	535.3	565.1	145.3	285.2	425.1	5.4
Corn gluten meal	0.0	221.0	55.3	110.5	165.8	0.0
Soybean meal	195.1	111.3	111.5	111.4	111.4	111.5
Soybean oil	1.3	3.7	8.7	7.0	5.3	10.3
Coarse limestone	48.0	47.7	43.6	43.3	43.0	48.5
Fine limestone	32.0	31.9	37.0	37.0	37.0	32.3
Dicalcium phosphate	8.7	6.9	7.4	7.3	7.1	7.6
Salt	2.5	1.0	1.6	1.4	1.2	1.8
Sodium bicarbonate	1.0	1.0	1.6	1.4	1.2	1.7
L-Lysine HCl, 780 g/kg	1.0	2.6	2.4	2.5	2.6	2.3
DL-Methionine, 999 g/kg	1.8	1.8	2.0	1.9	1.8	2.0
L-Threonine, 985 g/kg	0.0	0.2	0.6	0.4	0.3	0.7
L-Tryptophan, 980 g/kg	0.0	0.6	0.2	0.3	0.5	0.0
L-Valine, 990 g/kg	0.0	0.0	0.4	0.2	0.1	0.5
L-Isoleucina	0.1	0.9	0.8	0.9	0.9	0.8
Choline chloride, 600 g/kg	2.3	2.3	2.3	2.3	2.3	2.3
Vitamin premix and trace mineral[1]	2.0	2.0	2.0	2.0	2.0	2.0
Stimbiotic (STB) [2]	0.0	0.0	0.0	0.0	0.0	0.0
Total	1000	1000	1000	1000	1000	1000
ME kcal/kg	2830	2670	2670	2670	2670	2670
Crude protein	163	164	164	164	164	165
Met + Cys dig	7.0	7.0	7.0	7.0	7.0	7.0
Lys dig	7.4	7.4	7.4	7.4	7.4	7.4
Tre dig	5.3	5.3	5.3	5.3	5.3	5.3
Tryp dig	1.8	1.8	1.8	1.8	1.8	1.8
Val dig	6.0	6.0	6.0	6.0	6.0	6.0
Ca	334	334	334	334	334	334
Available P	3.9	3.9	3.9	3.9	3.9	3.9
Na	1.7	1.7	1.7	1.7	1.7	1.7
CF	23.4	37.3	29.9	32.4	34.9	27.4
Neutral detergent fiber	101	161	132	142	151	122
Acid detergent fiber	43.2	58.2	58.4	58.3	58.3	58.4

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[1] Vitamin premix provided per kg of product: vitamin A, 9637 UI; vitamin D3, 2409 UI; vitamin E, 36.1 UI; vitamin K3, 1.93 mg; vitamin B1, 2.59 mg; vitamin B12, 0.016 mg; vitamin B6, 3.61 mg; vitamin B5, 12.95 mg; vitamin B3, 39.0 mg; vitamin B9, 0.90 mg; biotin, 0.09 mg; Trace mineral provided per kg of product: Mn, 64.20 mg; Zn, 59.63 mg; Fe, 45.85 mg; Cu, 9.14 mg; I, 0.927 mg; Se, 0.275 mg. [2] Signis, β−1,4-endo-xylanase and xylooligosaccharides, AB Vista, Marlborough, UK.

2.3. Experimental Variables

Performance

The experiment consisted of five periods of 28 days each. The variables evaluated included feed intake (FI, g/bird/day). To determine FI, the residual feed was weighed and subtracted from the amount of feed initially provided for the entire period. At the end of each 28-day period, the amount of feed consumed was divided by the number of hens in each treatment and by the number of days to calculate the average grams of feed consumed per hen per day.

Egg production (EP, %) was determined by recording the number of eggs produced per day, including broken, cracked, and abnormal eggs (e.g., soft-shelled eggs). This value represented the average EP of the hens during each period.

Egg mass (EM, g) was calculated by multiplying the average egg weight by the total number of eggs produced during the experimental period.

Feed conversion ratio per egg mass (FCR-EM, g/g) was calculated as the total FI divided by the total EM produced (kg/kg). Feed conversion ratio per dozen eggs (FCR-DZ, g/dozen) was calculated as the total FI (kg) divided by the number of dozens of eggs produced.

Body weight variation (BWV, g) was obtained by weighing the hens at the beginning and at the end of the experimental phase. The average BWV is expressed in grams per hen.

Egg Quality

Egg quality analyses were performed during the last three days of each 28-day period. Three eggs with average weight from each replicate were collected, individually identified, and weighed on an analytical balance. Subsequently, the eggs were broken onto a flat surface to measure albumen height (mm) using a depth micrometer (model S-8400, Ames®) [20].

Yolk and albumen weights were then recorded. The shells were dried in a forced-air oven at 45 °C for 48 h and subsequently weighed. Percentages of each component were calculated by dividing the weight of the component by the total egg weight and multiplying by 100.

Yolk color was assessed using the DSM Yolk Color Fan scale (DSM, São Paulo, Brazil).

The Haugh Unit was determined using the equation proposed by [21]: HU = 100 × log (H – 1.7 × W^0.37 + 7.57), where HU = Haugh Unit, H = albumen height (mm), and W = egg weight (g).

Eggshell thickness was assessed with a digital micrometer at three evenly spaced locations on the equatorial region of the shell, and the arithmetic mean was used as the representative value.

Specific gravity was determined using the saline flotation method. Eggs were immersed in sodium chloride (NaCl) solutions with densities ranging from 1.0700 to 1.0975 g/cm^3, with a gradient of 0.0025 between successive solutions. The density of the solutions was regularly verified using an oil densimeter.

Intestinal Morphology

At the end of the experiment, one bird per replicate was euthanized for the subsequent collection of biological material. A 1 cm fragment was collected from the middle portion of the duodenum, jejunum, and ileum of each bird, with each treatment comprising; these fragments were fixed by immersion in 10% formaldehyde. The tissue fragments were embedded in paraffin according to standard histological procedures. Next, 5 µm sections were cut from each paraffin block in a microtome, and the histological slides were stained with "periodic acid–Schiff" (PAS) and scanned with a Motic camera (Motic Instruments Inc., Xiamen, China) coupled to an Olympus BX-53

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microscope (Olympus Corporation, Tokyo, Japan) with Motic Image Plus 2.0 image analyzer software (Motic Instruments Inc., Xiamen, China).

For each photomicrograph, three measurements of the intestinal villus and crypt depth were taken, totaling 90 measurements (10 animals × 3 photomicrographs × 3 measurements) for each variable mentioned above per treatment. The villus width and height (μm) was measured from the region of the intestinal mucosa that coincided with the upper portion of the crypts until its apex. The crypt depth (μm) was the distance between the villus base to the crypt–villus transition region. The villus–crypt ratio was determined by the ratio of the villus height to the crypt depth. The absorptive surface area (μm) was estimated by considering a villus as a cylindrical structure. Villus absorptive surface area was calculated using the formula: Villus absorptive surface area = 2π × (average villus width/2) × villus height [23].

2.4. Statistical Analysis

Data were analyzed as a 2 × 6 factorial using the PROC GLM procedure of SAS (version 9.4, SAS Institute Inc., Cary, NC, USA). The factors included stimbiotic supplementation (0 or 100g/ton of feed) and levels of dietary fiber (Control (corn–soybean); 2. Wheat–High CF; 3. 75:25 wheat–corn; 4. 50:50 wheat–corn; 5. 25:75 wheat–corn; 6. Corn–soybean–Low CF). Significance was set at $p \leq 0.05$ and tendency was declared at $0.05 < p \leq 0.1$. Significantly different means were separated using Tukey's HSD.

3. Results

3.1. Performance

There was no significant interaction between dietary fiber levels and STB supplementation ($p > 0.05$). Independently, STB supplementation did not affect feed intake (FI), egg production (EP), egg mass (EM), feed conversion per egg mass (FCR-EM) or per dozen eggs (FCR-DZ), or body weight variation (BWV) ($p > 0.05$). However, dietary fiber levels significantly influenced all variables evaluated ($p < 0.05$; Table 2).

Hens fed the 50:50 wheat–corn and 75:25 wheat–corn diets had higher FI compared with the other treatments ($p = 0.0029$). The highest EP and EM were obtained from hens fed the Control (corn–soybean), 75:25 wheat–corn, and Wheat–High CF diets ($p < 0.0001$). Consequently, these diets also promoted better FCR-EM ($p < 0.0001$) and FCR-DZ ($p < 0.0001$). Regarding BWV, hens fed the Corn–soybean–Low CF, 25:75 wheat–corn, and 75:25 wheat–corn diets exhibited less body weight loss at the end of the experimental period ($p = 0.0152$).

Table 2. Influence of dietary fiber levels and STB supplementation on the performance of laying hens.

Diets		FI (g/bird/day)	EP (%)	EM (g)	FCR- EM (kg/kg)	FCR-DZ (kg/dozen)	BWV (g)
Means for main effect of STB							
STB	+	117.55a	94.46a	56.41a	2.10a	1.50a	6.81a
	-	117.27a	95.05a	56.87a	2.08a	1.48a	8.08a
Means for main effect of fiber levels							
Control (corn– soybean)		115.65b	96.10a	57.92a	2.01d	1.45c	11.07a
Corn–soybean – Low CF		117.13ab	91.72c	54.24c	2.17a	1.54a	6.00b
50:50 wheat–corn		118.46a	94.98ab	56.68ab	2.10bc	1.50ab	7.44ab
25:75 wheat–corn		117.6ab	93.62bc	55.52bc	2.12ab	1.51ab	6.16b
75:25 wheat–corn		118.30a	95.97a	57.66a	2.07bcd	1.48bc	6.13b
Wheat – High CF		117.49ab	96.15ª	57.83ª	2.05cd	1.47bc	7.88ab
Pooled SEM		0.216	0.243	0.174	0.007	0.005	0.478
p-Value	STB	0.5042	0.1637	0.0908	0.0743	0.0697	0.16
	Fiber levels	0.0029	<.0001	<.0001	<.0001	<.0001	0.0152
	STB* Fiber levels	0.4499	0.4077	0.8681	0.4592	0.2274	0.2084

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STB, stimbiotic; CF, crude fiber; FI, Feed intake; EP, Egg production; EM, Egg mass; FCR-EM, Feed Conversion Ratio per egg mass; FCR-DZ, feed conversion dozen eggs; BWV, Body weight variation; [a,b,c,d] Means in a column, within a group, with different superscripts are significantly different ($p < 0.05$).

3.2. Egg Quality

Interactions between STB supplementation and dietary fiber levels were observed for yolk color, shell thickness, and eggshell specific gravity ($p < 0.0001$) (Tables 3 and 4). The main effect of STB supplementation significantly affected Haugh unit ($p = 0.0222$) and specific gravity ($p < 0.0001$).

Laying hens fed the Control (corn–soybean), 25:75 wheat–corn, and 75:25 wheat–corn diets produced heavier eggs ($p < 0.0001$). More intensely pigmented yolks were obtained from hens fed the Control (corn–soybean) and Wheat–High CF diets ($p < 0.0001$). The highest Haugh unit and specific gravity values were observed in eggs from hens fed the Wheat–High CF diet ($p < 0.0001$), whereas thicker eggshells were produced by hens fed the 75:25 wheat–corn diet ($p = 0.0198$). Dietary fiber levels did not influence the percentage of yolk, albumen, or eggshell.

Table 4 details the interactions between dietary fiber levels and STB supplementation. Eggs from hens fed the 25:75 wheat–corn diet supplemented with STB exhibited darker yolk pigmentation, similar to those from hens receiving the 50:50 wheat–corn diet without STB. The Control (corn–soybean) diet supplemented with STB resulted in thicker eggshells ($p < 0.0001$). Moreover, STB supplementation in the Corn–soybean–Low CF, 50:50 wheat–corn, or 25:75 wheat–corn diets significantly increased specific gravity ($p < 0.0001$).

Table 3. Influence of dietary fiber levels and STB supplementation on the egg quality in laying hens.

Diets		Egg weight (g)	Yolk (%)	Albume (%)	Eggshell (%)	Yolk color	Haugh unit	Shell thickness (mm)	Specific gravity (g/cm3)
Means for main effect of STB									
STB	+	59.63	26.01	63.84*	10.15	4.08*	94.10a	0.411	1.18a*
	-	59.90	25.88	63.94*	10.17	4.17*	93.70b	0.411	1.16b*
Means for main effect of fiber levels									
Control (corn–soybean)		60.35a	26.05a	63.81a	10.17a	4.85a	93.14d	0.412ab	1.159c
Corn–soybean – Low CF		58.82c	26.39a	63.41a	10.18a	5.03a	94.59a	0.409b	1.185a
50:50 wheat–corn		59.66ab	25.44a	64.43a	10.11a	4.22b	94.50ab	0.410ab	1.173ab
25:75 wheat–corn		59.31bc	25.76a	63.97a	10.27a	4.76ab	94.10abcd	0.411ab	1.166bc
75:25 wheat–corn		60.08a	26.05a	63.81a	10.10a	3.05c	93.46cd	0.410ab	1.170ab
Wheat – High CF		60.29a	26.96a	63.92a	10.10a	2.84c	93.77bcd	0.413a	1.166bc
Pooled SEM		0.088	0.106	0.197	0.021	0.104	0.093	0.001	0.003
p-Value	STB	0.1097	0.5632	0.6779	0.5797	0.4853	0.0222	0.8505	<.0001
	Fiber levels	<.0001	0.2586	0.319	0.1419	<.0001	<.0001	0.0198	<.0001
	STB* Fiber levels	0.6820	0.3484	0.4067	0.1310	<.0001	0.0674	<.0001	<.0001

STB, stimbiotic; CF, crude fiber; *, interaction effect; [a,b,c,d] Means in a column, within a group, with different superscripts are significantly different ($p < 0.05$).

Table 4. Interactions between dietary fiber levels and STB supplementation on the egg quality in laying hens.

Fiber levels	Yolk color		Shell thickness (mm)		Specific gravity (g/cm3)	
	STB					
	+	-	+	-	+	-
Control (corn– soybean)	4.770Aa	4.936Aab	0.419Aa	0.405Bb	1.154Ac	1.164Aa
Corn–soybean – Low CF	4.731Aa	5.337Aa	0.404Bc	0.414Aa	1.212Aa	1.159Ba
50:50 wheat–corn	3.704Bc	4.729Aab	0.407Ab	0.413Aa	1.185Ab	1.162Ba
25:75 wheat–corn	5.476Aa	4.038Bbc	0.411Ab	0.411Aab	1.179Ab	1.155Ba
75:25 wheat–corn	3.270Abc	2.822Ac	0.406Ab	0.410Aab	1.179Ab	1.162Aa
Wheat – High CF	2.542Ac	3.131Ac	0.417Aab	0.411Aab	1.165Aac	1.168Aa
Pooled SEM	0.104		0.001		0.003	
p-Value						

STB*Fiber levels	<.0001	<.0001	<.0001

[AB], uppercase letters compare the inclusion or not of STB within columns; [abc], lowercase letters compare dietary fiber levels within rows; significantly different ($p < 0.05$) according to Tukey's test.

3.3. Intestinal Morphology

Interactions between STB supplementation and dietary fiber levels were observed for duodenum and ileum morphology in laying hens ($p < 0.0001$) (Tables 5 and 6). The main effect of STB supplementation promoted wider villi ($p < 0.0001$) and a greater absorptive area in the jejunum ($p = 0.0063$). In the ileum, hens not receiving STB supplementation exhibited taller villi ($p = 0.0092$) and deeper crypts (p = 0.0054). The Control (corn–soybean), Corn–soybean–Low CF, and Wheat–High CF diets also influenced ileal morphology, promoting wider villi ($p = 0.0025$) and a greater absorptive area.

Table 6 shows the specific interactions between dietary fiber levels and STB supplementation. In the duodenum, hens fed the Corn–soybean–Low CF diet with STB exhibited wider villi ($p = 0.0106$). Conversely, the 25:75 wheat–corn diet with STB reduced villus width, while the 50:50 wheat–corn diet without STB resulted in shallower crypts ($p = 0.0011$). Additionally, the Corn–soybean–Low CF diet with STB decreased the villus-to-crypt ratio ($p = 0.0058$), whereas the Wheat–High CF diet with STB increased the absorptive area ($p = 0.0086$). In the ileum, hens receiving the 50:50 wheat–corn diet with STB presented narrower villi ($p = 0.0011$), a lower villus-to-crypt ratio ($p = 0.0058$), and a reduced absorptive area ($p = 0.0086$).

Table 6. Influence of dietary fiber levels and STB supplementation on the intestinal morphology in laying hens.

Diets		Duodenum					Jejunum					Ileum				
		Villus width (µm)	**Villus height (µm)**	**Crypt depth (µm)**	**Villus-to-crypt ratio**	**Absorptive area**	**Villus width (µm)**	**Villus height (µm)**	**Crypt depth (µm)**	**Villus-to-crypt ratio**	**Absorptive area**	**Villus width (µm)**	**Villus height (µm)**	**Crypt depth (µm)**	**Villus-to-crypt ratio**	**Absorptive area**
Means for main effect of STB																
STB	+	227.99	1475.87	130.99	11.40*	335961.40	174.40a	1241.06*	123.31	10.26*	219140.03a*	120.28	689.01b	82.14b	8.48*	85329.04
	-	222.07	1472.02	128.90	11.53*	327658.78	147.96b*	1170.26*	118.79	9.91*	175594.70b*	122.95	755.23a	91.03a	8.47*	94820.98
Means for main effect of Fiber levels																
Fiber levels	Control (corn–soybean)	236.88	1450.11	124.57	11.72	343478.44	156.36	1113.23	116.55	9.58	172678.47	125.84a	759.63	90.45	8.56	96595.74a
	Corn–soybean – Low CF	236.31	1440.79	129.25	11.20	335886.31	163.44	1251.48	125.37	10.04	211014.41	130.93a	749.15	89.15	8.56	100142.94a
	50:50 wheat–corn	224.90	1409.99	130.40	10.93	319300.48	157.94	1176.85	125.20	9.56	186341.53	119.16ab	714.31	88.38	8.22	91060.62ab
	25:75 wheat–corn	220.41	1546.29	134.65	11.62	342153.02	168.73	1211.42	120.14	10.14	209486.93	107.2b	688.81	82.48	8.40	75438.83b
	75:25 wheat–corn	213.25	1444.77	129.15	11.31	308635.09	156.31	1230.10	119.06	10.47	194397.30	118.54ab	746.35	87.05	8.57	84733.48ab
	Wheat – High CF	223.16	1447.56	130.88	11.21	324053.74	146.12	1114.93	115.88	9.67	163737.46	126.10a	679.90	86.67	7.95	92480.37ab
Pooled SEM		0.052	0.053	0.037	0.035	0.073	0.086	0.080	0.053	0.042	0.133	0.066	0.070	0.061	0.038	0.114
p-Value	STB	0.2749	0.5182	0.9377	0.4139	0.6161	0.0001	0.6331	0.7308	0.4119	0.0063	0.5508	0.0092	0.0054	0.464	0.0641
	Fiber levels	0.0924	0.2949	0.1923	0.2771	0.2774	0.5587	0.3727	0.2715	0.0592	0.2404	0.0025	0.1829	0.5037	0.2029	0.0156
	STB* Fiber levels	0.0106	0.2262	0.0011	0.0058	0.0086	0.0742	0.848	0.1193	0.6487	0.1753	0.0011	0.2348	0.0058	0.1507	0.0086

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STB, stimbiotic; CF, crude fiber; *, interaction effect; [a,b] Means in a column, within a group, with different superscripts are significantly different ($p < 0.05$).

Table 7. Interactions between dietary fiber levels and STB supplementation on the intestinal morphology in laying hens.

	Diets	Duodenum								Ileum					
		Villus width (µm)		Crypt depth (µm)		Villus-to-crypt ratio		Absorptive area		Villus width (µm)		Crypt depth (µm)		Absorptive area	
	STB	+	-	+	-	+	-	+	-	+	-	+	-	+	-
Fiber levels	Control (corn–soybean)	235.64Aab	238.11Aa	120.22Aa	128.92Aab	11.70Aa	11.73Aa	331621.91Aab	355334.96Aa	133.52Aa	118.16Aa	92.57Aa	88.33Aa	101858.81Aa	91332.68Aa
	Corn–soybean – Low CF	258.01Aa	214.62Aa	133.83Ab	124.66Ab	10.53Bb	11.88Aa	355183.26Aa	316589.35Aab	124.20Aa	137.66Aa	84.05Aab	94.25Aa	91952.60Aa	108333.28Aa
	50:50 wheat–corn	223.50Aab	222.81Aa	137.66Ab	124.09Bb	11.22Aab	11.21Aab	339579.57Aab	308527.91Ab	94.00Bb	120.52Aa	73.64Bb	99.71Aa	57953.41Bb	92924.27Aa
	25:75 wheat–corn	208.04Ab	218.46Aa	128.11Aab	130.19Aab	11.20Aab	11.41Aab	296733.41Ab	320536.78Aab	121.55Aa	116.78Aa	84.69Aab	89.41Aa	93264.89Aa	88856.35Aa
	75:25 wheat–corn	211.68Aab	229.15Aa	131.06Ab	138.25Aa	11.60Aa	11.64Aa	320082.39Aab	364223.65Aa	116.62Aa	120.47Aa	79.79Aab	85.18Aa	80661.37Aab	88805.61Aa
	Wheat – High CF	239.72Aab	210.08Aa	128.36Aab	132.44Aab	11.32Aab	10.54Ab	346183.93Aab	292417.03Bb	134.53Aa	117.68Aa	88.21Aab	88.56Aa	95276.01Aa	89684.73Aa
Pooled SEM		0.052		0.066		0.061		0.114		0.066		0.061		0.114	
p-Value	STB*Fiber levels	0.0106		0.0011		0.0058		0.0086		0.0011		0.0058		0.0086	

[AB], uppercase letters compare the inclusion or not of STB within columns; [abc], lowercase letters compare dietary fiber levels within rows; significantly different ($p < 0.05$) according to Tukey's test.

4. Discussion

This study evaluated the effect of different dietary high-fiber levels, with or without STB supplementation, on productive performance, egg quality, and intestinal morphology of laying hens. It was demonstrated that dietary fiber levels, rather than STB supplementation alone, were the main drivers of productive performance in laying hens. Hens fed diets with moderate wheat inclusion 75:25 wheat–corn or wheat–high CF presented higher EP and EM, which translated into improved FCR. These findings align with reports that moderately fermentable fiber stimulates gastrointestinal tract development and supports nutrient utilization through the production of short-chain fatty acids [5,18]. In contrast, excessive inclusion of wheat (50:50 wheat–corn) increased FI without improving egg output, suggesting that higher fiber levels may have diluted dietary energy, leading to compensatory FI but lower efficiency. Similar outcomes were observed by [2], who noted that high-fiber diets can impair nutrient digestibility in layers.

With respect to BWV, hens fed the 25:75 wheat–corn and 75:25 wheat–corn diets exhibited reduced weight loss. This effect may reflect a balance between adequate fermentable substrates and the metabolic benefits of short-chain fatty acid production, which provide energy to enterocytes and support gut health [12,24].

Regarding egg quality, both dietary fiber levels and their interaction with STB supplementation influenced yolk pigmentation, Haugh unit, shell thickness, and specific gravity. Heavier eggs and darker yolks were associated with corn–soybean and wheat–high CF diets, in agreement with [7], who reported that yolk pigmentation is directly linked to dietary pigment sources and gut absorptive capacity. The beneficial effect of STB on eggshell traits, particularly the increase in specific gravity when combined with low- or moderate-fiber diets, may result from enhanced mineral absorption following partial hydrolysis of arabinoxylans [14,16]. However, the inconsistent effects observed across fiber levels suggest that the efficacy of STB in laying hens is highly diet-dependent, confirming the variability reported in previous studies [3,6].

Intestinal morphology was also markedly influenced by fiber level and its interaction with STB supplementation. Wider villi and larger absorptive areas were observed in hens receiving the corn–soybean or wheat–high CF diets, indicating that these formulations supported epithelial development and nutrient uptake. The main effect of STB supplementation increased villus width and jejunal absorptive area, corroborating its proposed prebiotic role through stimulation of beneficial microbial fermentation and SCFA production [17]. Conversely, the narrower villi and reduced villus-to-crypt ratio observed in hens fed the 50:50 wheat–corn diet with STB suggest a potential imbalance in fiber fermentability, where excessive NSP hydrolysis may have led to intestinal irritation or altered microbiota composition. These findings reinforce that the benefits of STB depend on both the type and level of dietary fiber [3,6].

Taken together, these results demonstrate that dietary fiber exerts a stronger and more consistent influence on performance, egg quality, and gut morphology in laying hens compared to STB supplementation. Nevertheless, STB may provide targeted improvements in nutrient absorption and eggshell quality when combined with specific fiber levels. Further research is warranted to clarify the optimal combinations of fiber sources and STB supplementation capable of simultaneously enhancing productivity, intestinal health, and egg quality in commercial laying hens.

5. Conclusions

High dietary fiber levels improved laying hens' performance, egg quality, and intestinal morphology, and supplementation with 0.01% stimbiotic further enhanced these effects, highlighting it as an effective strategy to optimize production and gut health in commercial laying hens.

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