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Performance, intestinal development and cecal microbial composition in laying hens fed different fiber sources

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ABSTRACT - Laying hens' gut health and microbiota are directly linked to their productivity, egg quality, and overall welfare. This study evaluated the impact of different fiber sources on the performance Dekalb laying hens (63 to 71 weeks of age) and egg quality at 67 and 71 weeks. At the end of the trial (71 weeks), organ development was assessed, and the cecal microbiota was characterized using 16S rRNA sequencing. Birds were divided into four groups: a diet with elephant grass (*Pennisetum purpureum* Schum), a diet with commercial insoluble fiber, a control diet without added fiber, and a diet with soybean hulls, with five replicates of 26 hens each. Fiber inclusion did not affect egg production, egg mass, feed conversion per dozen or per egg box, or eggshell quality (P>0.05). However, hens fed elephant grass had higher final body weight (P≤0.05), while soybean hulls improved feed conversion, reduced costs, and increased egg weight in the first evaluation (67 weeks; P≤0.05). Additionally, diets with soybean hulls led to a higher yolk percentage but a lower albumen percentage (P≤0.05). Beta diversity analysis revealed greater bacterial diversity in hens fed elephant grass compared with those in the control and soybean hull groups (P≤0.05). Soybean hulls increased the abundance of the *Selenomonadaceae* family, while elephant grass favored the genera *Bacteroides*, *Megamonas*, and the species *Faecalibacterium prausnitzii.* These findings indicate that elephant grass, soybean hulls, and commercial insoluble fiber are viable alternatives in layer hen diets to improve microbial diversity and gut health in poultry, with soybean hulls standing out as a cost-effective option for maintaining performance and egg quality.

Keywords: 16S rRNA, bacterial diversity, commercial insoluble fiber, elephant grass, gut health, soybean hulls

1. Introduction

Various types of fiber derived from agricultural by-products have been tested as feed ingredients in laying hen nutrition to adjust the dietary energy density, increase dietary neutral detergent fiber (NDF) content, and consequently reduce costs (Mateos et al., 2012; Rodríguez et al., 2012; Sousa et al., 2019; Andrade et al., 2022; Sousa et al., 2025). The wide variety of fiber options, along with differences in their physicochemical composition and solubility, are factors that alter the positive effects on the production of volatile fatty acids, gut health, and the microbiological diversity of the intestine, making it difficult to determine the best option for improving performance (Singh and Kim, 2021; Sousa et al., 2025).

These alternative fiber sources should be free of soluble fractions, as these can reduce nutrient absorption. In addition, they must avoid antinutritional factors that encapsulate nutrients within cell walls and should be sufficiently efficient to replace wheat bran, which is commonly used as a fiber source but often has higher production costs due to the challenges of cultivation in tropical climates (Giroto, 2008). The experimental fibers used in this study, which are commercially available in Brazil and have a low acquisition cost, are primarily composed of soluble non-starch polysaccharides (NSP). Due to their physicochemical properties, NSP reduce reduce gastrointestinal content retention time and limit nutrient absorption, consequently decreasing the utilization of dietary nutrients (Montagne et al., 2003; Choct et al., 2010).

Besides affecting digestive physiology, fibers have a high capacity to positively influence gut development and health by preventing the adhesion of pathogenic bacterial populations to the epithelial mucosa (Jha et al., 2019). Other mechanisms through which fibers positively influence the intestine include increasing the population of bacteria responsible for polysaccharides breakdown and short-chain fatty acid production, which provide energy to colonocytes and stimulate cell proliferation (Garcia et al., 2012; Glowacki and Martens, 2021; Sousa et al., 2025).

While laying hens are crucial for global food production due to their efficient conversion of feed into a low-cost protein source (Barbosa et al., 2025), a significant challenge remains: identifying alternative fiber sources to replace costly ingredients like corn or wheat (Shuaib et al., 2022). Although this issue affects poultry production globally, the present study specifically addresses regional constraints by evaluating locally sourced fiber alternatives available in Brazil.

Existing physiological and microbiota studies offer no clear consensus on which fiber options can reduce costs while maintaining or even improving performance (Jha et al., 2019; Sousa et al., 2019). Since a laying hens' gut health and microbiota are directly linked to their productivity, egg quality, and overall welfare, a deeper investigation was warranted (Guo et al., 2022). Therefore, the objective of this study was to evaluate the influence of including various fiber sources in the diets of commercial laying hens (63 to 71 weeks of age) on key parameters, including performance, egg quality, intestinal morphology, and cecal microbiota.

2. Material and methods

The current experimental protocol was approved by the Ethical Principles in Animal Experimentation Committee under protocol N° 048/2023. The laying hens were reared at the Experimental Center of the company Mantiqueira Brasil, located in the city of Primavera do Leste, Mato Grosso, Brazil (20° 04' 13" S, 44° 18' 06" W). The bacteria isolated and identified in this study were registered in the National System for the Management of Genetic Heritage and Associated Traditional Knowledge (SisGen) under protocol number A2B7C2E.

2.1. Experimental design, treatments and management

The experimental design was completely randomized and consisted of four treatments: elephant grass (EG), commercial insoluble fiber (IF) containing cellulose, control (CO), and soybean hull (SH). The study included five replicates of 26 birds per experimental unit. A total of 520 Dekalb White laying hens, aged 63 to 71 weeks, were used. The laying hens were housed in 20 identical cages (50 × 44 × 40 cm), each containing 13 birds, resulting in a stocking density of 370 cm^2/bird. Each cage was equipped with trough feeders and nipple drinkers. The laying hens received a lighting program of 16L:8D per day during the entire experiment. Feed and water were provided *ad libitum*. Prior to the experimental period (63 to 71 weeks), the hens underwent a 15-d adaptation period to the diet and the poultry house facilities.

All experimental diets were formulated based on corn and soybean meal, following the nutritional recommendations for light laying hens established by Rostagno et al. (2017). The treatments were defined according to the type of fiber included in the diet: a diet with elephant grass (*Pennisetum* *purpureum Schum*) cultivar BRS Capiaçu, a diet with a commercial insoluble fiber (containing cellulose), a corn–soybean meal control diet without added fiber, and a diet with soybean hulls (Table 1). The inclusion levels of each fiber source were determined based on a literature review focused on fiber use in poultry nutrition (Sousa et al., 2019; Hou et al., 2020; Andrade et al., 2022; Sousa et al., 2025).

Table 1 - Ingredients and analyzed nutrient composition of laying hen diets

Ingredients (g/kg)	Elephant grass	Insoluble fiber	Control	Soybean hulls
Corn grain 7.5% CP	640.0	642.0	642.0	637.0
Soybean meal 48% CP	201.0	203.0	203.0	203.0
Soybean hull	0.0	0.0	0.0	20.0
Elephant grass	20.0	0.0	0.0	0.0
Commercial insoluble fiber	0.0	15.0	0.0	0.0
Inert (washed sand)	0.0	0.0	16.0	0.0
Limestone	98.0	99.2	98.2	99.3
Meat and bone meal	32.0	32.0	32.0	32.0
Common salt	3.5	3.5	3.5	3.5
Mineral-vitamin premix[1]	2.0	2.0	2.0	2.0
DL-Methionine 980 g/kg	1.9	1.9	1.9	1.9
Adsorbent	1.0	1.0	1.0	1.0
Choline chloride	0.3	0.3	0.3	0.3
L-Lysine 980 g/kg	0.2	0.1	0.1	0.0
Cost (US$/kg)	0.330	0.353	0.332	0.333
Calculated nutritional levels				
Calcium (%)*	4.19 (3.88)	4.23 (4.82)	4.20 (3.61)	4.24 (3.22)
Metabolizable energy (MJ/kg)	11.27	11.29	11.29	11.20
Neutral detergent fiber (%)	9.28	8.11	8.11	9.22
Available phosphorus (%)	0.29	0.29	0.29	0.29
Dig. lysine (%)	0.75	0.75	0.75	0.76
Dig. methionine+cysteine (%)	0.64	0.64	0.64	0.65
Dig. methionine (%)	0.41	0.41	0.41	0.41
Crude protein (%)*	16.50 (18.33)	16.47 (17.49)	16.47 (18.37)	16.62 (20.89)
Sodium (%)	0.17	0.17	0.17	0.17
Digestible threonine (%)	0.53	0.53	0.53	0.54

[1] Choline (min): 67.2 g/kg; vitamin A (min): 4,050,000 IU/kg; vitamin D3 (min): 1,500,000 IU/kg; vitamin E (min): 3,500 IU/kg; vitamin K3 (min): 1,000 mg/kg; vitamin B1 (min): 500 mg/kg; vitamin B2 (min): 1,750 mg/kg; vitamin B6 (min): 500 mg/kg; vitamin B12 (min): 5,000 mcg/kg; niacin (min): 10.5 g/kg; calcium pantothenate (min): 3,300 mg/kg; folic acid (min): 200 mg/kg; biotin (min): 7.7 mg/kg; iron (min): 25 g/kg; copper (min): 5,000 mg/kg; manganese (min): 50 g/kg; zinc (min): 40 g/kg; iodine (min): 600 mg/kg; selenium (min): 140 mg/kg; and zinc bacitracin, 14 g/kg.
* In parenthesis analyzed levels.

The elephant grass was manually cut when it reached 120 d of regrowth, with a total of approximately 500 kg harvested. The grass was then shredded and moved to a drying area, where it was left for 15 d, turned daily. After drying, the grass was ground to an approximate size of 3 mm and incorporated into the other feed ingredients.

To update the feed cost from the first quarter of 2023, when the cost data were originally collected from suppliers in Minas Gerais, Brazil, to 2025, the year of publication, the following two-step procedure was applied. First, the original BRL costs were converted to U.S. Dollars (USD 2023) using the average 2023 exchange rate (BRL 4.96/USD 1). Second, the converted USD 2023 values were adjusted to account for general inflationary effects over the period by applying the accumulated variation of the Consumer Price Index (IPCA), providing a reliable estimate of the adjusted USD 2025 cost. This methodology ensures cost comparability across different time periods and allows for broader international comparisons.

$$\text{Value 2025 (USD)} = [(\text{Value 2023 (BRL)} / \text{Exchange rate 2023}) * (1 + i\ 2024) * (1 + i\ 2025)]$$

2.2. Performance and egg quality

Egg production was recorded daily to calculate the average laying rate (%) during the experimental period (63 to 71 weeks). The laying rate was determined by dividing the number of eggs collected daily by the total number of hens in the respective replicate/group, multiplied by 100. The amount of feed offered and the leftovers for each replicate were weighed weekly to determine feed intake and feed conversion ratio. Mortality was recorded daily to obtain the viability percentage and the number of eggs produced per hen. Egg weights were recorded weekly, with all eggs from each replicate being weighed. This allowed for the calculation of both egg weight and feed conversion per egg mass produced. At the end of the experimental period, feed conversion per egg carton (360 eggs) and feed cost were determined.

At 67 and 71 weeks of age, 30 eggs from each treatment were analyzed to determine the percentage of yolk, shell, and albumen, shell thickness, Haugh unit, shell strength, and yolk coloration. Shell thickness was measured using a Digimess digital micrometer (Digimess, São Paulo, Brazil), with 0.001 mm precision. Measurements were taken at three distinct points on the eggshell (apical, equatorial, and basal regions). The result was the average of the three measurements, expressed in millimeters.

The Haugh unit was calculated using the egg weight and albumen height and according to the formula: $HU = 100 \log_{10} (h - 1.7 W^{0.37} + 7.56)$, where h = albumen height and W = egg weight (Brant et al., 1951). To assess shell strength, compression fracture testing was performed using the Bröring fast-egg-shell-tester (Bröring Technology GmbH®, Oldenburg, Germany). The whole egg was placed longitudinally on a metal ring (5 cm in diameter) inside a porcelain crucible. The shell was pressed until fracture occurred. Yolk coloration was determined using yolks from the eggs used to measure the proportions of egg components, Haugh unit, and shell thickness.

The DSM Yolk Color Fan (2005 – HMB 51548, DSM-Firmenich, Maastricht, The Netherlands) color chart was used, and the yolk color was immediately compared to the closest color on the palette, which ranges from 1 to 15. These evaluations were made by the same person, in the same location, to avoid variation, especially since this is a subjective analysis. At the beginning and at the end of the experimental period, all birds were weighed to assess weight gain.

2.3. Digestive organs development

At the end of the experimental period (71 weeks of age), one laying hen per replicate (selected from within a ±10% range of the replicate's average weight) was euthanized by cervical dislocation for intestinal morphology and cecal microbiota analyses. For the intestinal morphology data, the birds' gastrointestinal tracts were removed and evaluated by weighing the gizzard (after cleaning and removing all residual feed), measuring the intestinal length, and then dissecting the intestine. The duodenum, jejunum, and ileum were separated to analyze villus height and crypt depth. A total of 20 samples were collected from each segment per treatment, resulting in 60 samples per treatment. These intestinal fragments, each approximately three centimeters in length, were fixed in a 10% formalin solution and sent to the laboratory for analysis. Cross sections (5 mm thick) of each intestinal segment were processed in low-melt paraffin and stained with hematoxylin and eosin (Adibmoradi et al., 2006). The morphological indices evaluated were villus height from the tip of the villus to the crypt, crypt depth from the base of the villi to the submucosa (Zang et al., 2009).

2.4. Cecal microbiota analyses

The cecal contents from these same laying hens used for digestive organs development were collected for cecal microbiota analyses, resulting in a total of 20 samples. The samples were collected aseptically and stored in tubes containing a RNA*later*. DNA was extracted using the commercial kit ZR Fecal DNA MiniPrep (Zymo Research, Irvine, California, USA) following the manufacturer's protocol and quantified by spectrophotometry at 260 nm. To assess DNA integrity, all samples were run on a 1% agarose gel.

For microbiota analyses, a segment of approximately 460 bp from the V3-V4 hypervariable region of the 16S rRNA gene was amplified using universal primers under the following PCR conditions: 95 °C for 3 min; 25 cycles of 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s; with a final extension at 72 °C for 5 min. From these amplicons, a metagenomic library was constructed using the Nextera DNA Library Preparation Kit (Illumina®, San Diego, CA, USA). The amplicons were pooled and subsequently sequenced on the Illumina® MiSeq platform (Degnan and Ochman, 2012).

The reads obtained were analyzed using only the forward strand on the QIIME2 platform (Quantitative Insights into Microbial Ecology) (Caporaso et al., 2011). The analysis workflow included the removal of low-quality sequences, filtering, chimera removal, and taxonomic classification. Sequences were classified into bacterial genera by identifying Amplicon Sequence Variants (ASVs), which are based on sequence homology by comparison against a reference database. For sequence comparison, the 2021 update (GTDB 202) of the Genome Taxonomy Database (Chaumeil et al., 2022) was used. To standardize the data and ensure comparability among samples, 20,239 reads per sample were used for ASV-based classification, resulting in the analysis of 20 samples.

2.5. Statistical analyses

The experimental design was completely randomized, comprising four treatments with five replicates of 26 birds each per experimental unit for performance evaluation. For morphological analysis, one bird per replicate was used, totaling 20 birds per treatment. For egg quality analysis, 30 eggs per treatment were used, with each egg considered as a replicate.

All data were analyzed using the following fixed effect model:

$$Y_{ij} = \mu + T_i + e_{ij},$$

in which Y is the response variable, μ is the overall mean, T_i is the fixed effect of treatment (i = elephant grass, commercial insoluble fiber, control and soybean hulls), and e is the residual error.

Means were subjected to ANOVA, and when significant, means were compared using Tukey's test ($P \leq 0.05$). The exception was the beta diversity analysis and yolk color at 67 and 71 weeks, these data did not meet the normality assumption and were therefore analyzed using the Kruskal-Wallis test and Dunn's post hoc test ($P \leq 0.05$). All analysis were evaluated using R software.

For the microbiota analysis, the statistical comparison of alpha diversity for each group was performed using the non-parametric Kruskal-Wallis test followed by Dunn's post hoc test, considering results with $P \leq 0.05$. Beta diversity analyses were conducted using PERMANOVA within the QIIME2 pipeline, employing 10,000 permutations. All figures and statistical analyses were generated using R. Alpha diversity metrics were calculated using the "phyloseq" (McMurdie and Holmes, 2013), "vegan" (Oksanen et al., 2015), and "Microbiome" (Lahti and Shetty, 2018) libraries. Differences in the relative abundances of taxa among groups were assessed using the Kruskal-Wallis test and Dunn's post hoc test ($P \leq 0.05$).

3. Results

3.1. Performance and egg quality

The initial body weights of the layers were similar across all treatments ($P>0.05$), confirming the uniformity of the birds at the start of the experimental period (Table 2). At the end of the experiment, final body weight showed a significant difference. Hens fed elephant grass had a higher final weight than those fed insoluble fiber ($P \leq 0.05$). The final weights of the control and soybean hulls groups were similar to those of all other treatments ($P>0.05$). For weight gain between 63 and 71 weeks, no significant differences were observed among any the treatments ($P>0.05$; Table 2).

Treatments did not affect egg production, feed intake, egg weight, or egg mass from 61 to 73 weeks (P>0.05; Table 3). Laying hens fed soybean hulls exhibited a lower feed conversion per kilogram compared to those in the other treatments (P≤0.05). Similarly, laying hens fed soybean hulls achieved a lower feed conversion ratio compared to birds fed the control diet or commercial insoluble fiber (P≤0.05), while those fed elephant grass showed similar results to the other treatments (P>0.05). Moreover, the fiber source impacted feed cost, with the soybean hull diet resulting in a lower feed cost compared to the insoluble fiber and control treatments (P≤0.05); diets with elephant grass and the control treatment showed no significant differences between them (P>0.05). There was no effect of the fiber source on laying hens' viability (P>0.05; Table 3).

In the initial egg quality evaluation at 67 weeks, laying hens fed soybean hulls produced significantly heavier eggs than those fed elephant grass or insoluble fiber (P≤0.05). No differences were observed among treatments for yolk, albumen, and shell percentages, yolk color, Haugh units, shell strength, or shell thickness (P>0.05; Table 4).

Table 2 - Effects of dietary fiber source on laying hens' final weight and gain weight at 63 and 71 weeks of age

Treatment	Initial weight (kg)	Final weight (kg)	Weight gain (kg)
Elephant grass	1.56	1.68a	0.12
Insoluble fiber	1.55	1.61b	0.06
Control	1.58	1.67ab	0.08
Soybean hulls	1.59	1.65ab	0.08
SEM	2.14	2.04	26.28
P-value	0.36	0.02	0.09

SEM - standard error of mean.
a-b - Means followed by different letters within the column were considered different by the Tukey test (P≤0.05).

Table 3 - Effects of dietary fiber source on production performance in laying hens from 61 to 73 weeks of life

Treatment	Egg production (%)	Feed intake (g/day)	Egg weight (g)	Egg mass (g/bird/day)
Elephant grass	89.30	115.82	60.32	53.90
Insoluble fiber	89.94	118.78	59.90	53.88
Control	88.70	116.70	59.98	53.24
Soybean hull	93.32	115.74	61.70	57.66
SEM	7.21	5.55	0.96	4.95
P-value	0.15	0.73	0.09	0.07
	FCR (kg egg:kg feed)	FCR per box (FCR/Box)	Cost/FCR (US$)	Viability (%)
Elephant grass	2.18b	47.48ab	0.721ab	96.94
Insoluble fiber	2.23b	48.30b	0.789c	98.48
Control	2.22b	48.13b	0.738b	99.24
Soybean hull	2.03a	45.38a	0.678a	96.16
SEM	0.04	2.01	3.53	5.27
P-value	0.03	0.01	<0.01	0.98

FCR - feed conversion ratio; SEM - standard error of mean.
Feed cost was calculated based on feed conversion and viability.
a-c - Means followed by different letters within the column were considered different by the Tukey test (P≤0.05).

At 71 weeks, laying hens that received soybean hull or control diets produced heavier eggs than those on the commercial insoluble fiber diet (P≤0.05), while the elephant grass group was similar to the other groups (P>0.05; Table 5).

Additionally, hens fed soybean hulls exhibited a higher yolk percentage and a lower albumen percentage compared to the other treatments (P≤0.05). All other treatments were similar to each other for both yolk and albumen percentages (P>0.05). Yolk color, Haugh units, shell strength, and shell thickness remained unaffected by any of the treatments (P>0.05; Table 5).

Table 4 - Effects of dietary fiber source on internal and external egg quality on 67 weeks of life

Treatment	Egg weight (g)	Yolk (%)	Albumen (%)	Yolk color*
Elephant grass	59.80b	27.18	63.52	4.90
Insoluble fiber	59.86b	26.41	63.40	4.90
Control	61.20ab	26.50	64.04	4.96
Soybean hull	63.60a	27.13	64.27	4.66
SEM	0.37	1.21	1.28	0.55
P-value	<0.01	0.34	0.38	0.32
	Haugh unit	Shell (%)	Resistance (kgf)	Thickness (µm)
Elephant grass	85.97	9.28	4,032.38	40.51
Insoluble fiber	89.02	9.46	4,084.99	42.38
Control	86.77	9.44	3,658.55	41.01
Soybean hull	86.16	9.30	3,888.32	41.49
SEM	3.98	0.67	455.03	2.01
P-value	0.16	0.68	0.08	0.27

SEM - standard error of mean.
* Parameter submitted to Kruskal Wallis's test (P≤0.05).
a-b - Means followed by different letters within the column were considered different by the Tukey test (P≤0.05).

Table 5 - Effects of dietary fiber source on internal and external egg quality on 71 weeks of life

Treatment	Egg weight (g)	Yolk (%)	Albumen (%)	Yolk color*
Elephant grass	62.16ab	22.26b	68.37a	4.90
Insoluble fiber	60.23b	22.34b	68.28a	4.90
Control	63.80a	22.60b	68.31a	4.96
Soybean hull	63.53a	26.48a	64.29b	4.66
SEM	7.55	13.32	4.92	13.65
P-value	0.01	<0.01	<0.01	0.32
	Haugh unit	Shell (%)	Resistance (kgf)	Thickness (µm)
Elephant grass	85.97	9.37	4,163.04	40.51
Insoluble fiber	89.02	9.35	3,995.09	42.38
Soybean hull	86.16	9.22	3,895.19	41.49
Control	86.16	9.07	3,763.99	41.01
SEM	6.71	7.25	16.86	9.24
P-value	0.16	0.27	0.13	0.27

SEM - standard error of mean.
* Parameter submitted to Kruskal Wallis's test (P≤0.05).
a-b - Means followed by different letters within the column were considered different by the Tukey test (P≤0.05).

3.2. Organ development and intestinal morphology

Laying hens fed commercial insoluble fiber exhibited a longer ileum compared to those on the control diet and soybean hull diet (P≤0.05), while laying hens fed elephant grass showed ileum lengths similar to those of the other treatments (P>0.05). No significant differences were observed among the treatments for total intestinal length, duodenum and jejunum lengths, or gizzard relative weight (P>0.05; Table 6).

Laying hens fed commercial insoluble fiber had the greatest duodenal villus height, while those fed elephant grass had the lowest (P≤0.05), the control and soybean hull groups showed similar results to each other and to the other treatments (P>0.05). No significant effects were observed for duodenal villus width (P>0.05; Table 7).

Table 6 - Effects of dietary fiber source on laying hens' intestinal length and percentage of gizzard at 71 weeks of age

Treatment	Intestine (cm)	Duodenum (cm)	Jejunum (cm)	Ileum (cm)	Gizzard (%)
Elephant grass	160.43	22.81	72.81	53.49ab	13.41
Insoluble fiber	163.83	22.78	73.62	59.89a	15.31
Control	165.95	20.48	78.54	46.61b	13.90
Soybean hull	168.21	17.80	78.94	44.61b	12.58
SEM	23.0	4.51	5.72	3.95	6.21
P-value	0.75	0.60	0.76	0.19	0.43

SEM - standard error of mean.
a-b - Means followed by different letters within the column were considered different by the Tukey test (P≤0.05).

Table 7 - Effects of dietary fiber source on laying hens' intestinal histomorphometry at 71 weeks of age

Treatment	Villus height	Villus width	Crypt diameter	Wall thickness
		Duodenum (µm)		
Elephant grass	1,174.45b	171.23	130.10a	117.95b
Insoluble fiber	1,635.56a	193.45	70.99b	212.90a
Control	1,610.01ab	154.91	95.31ab	221.45a
Soybean hulls	1,591.01ab	251.33	103.09ab	205.11ab
SEM	0.18	0.09	0.02	0.88
P-value	0.03	0.09	0.01	<0.01
		Jejunum (µm)		
Elephant grass	925.25	963.23	75.99	125.33
Insoluble fiber	971.10	869.38	67.34	139.78
Control	991.04	961.67	71.11	143.44
Soybean hulls	948.61	991.43	75.51	130.38
SEM	0.05	0.09	0.10	0.22
P-value	0.81	0.76	0.24	0.45
		Ileum (µm)		
Elephant grass	671.35	135.23ab	77.35	101.34
Insoluble fiber	768.25	125.39ab	58.34	115.48
Control	689.23	89.90b	61.67	104.70
Soybean hulls	685.91	181.33a	79.77	105.44
SEM	0.05	0.08	0.05	0.08
P-value	0.78	<0.01	0.23	0.45

SEM - standard error of mean.
a-b - Means followed by different letters in the column were considered different by the Tukey test (P≤0.05).

For the duodenal crypt diameter, laying hens fed elephant grass exhibited greater values compared with those fed commercial insoluble fiber (P≤0.05), while the control and soybean hull groups showed similar results to the other groups (P>0.05). Additionally, laying hens on the control and insoluble fiber diets had thicker duodenal walls than those fed elephant grass (P≤0.05; Table 7).

No significant differences were observed in the jejunal villus height, villus width, crypt diameter, or wall thickness (P>0.05). For the ileum villi, laying hens fed soybean hulls exhibited greater villus width than the control group (P≤0.05), while the elephant grass and insoluble fiber groups displayed similar values to the other treatments (P>0.05). No significant effects were observed for ileal villus height, ileal crypt diameter, and in the ileal wall thickness (P>0.05; Table 7).

3.3. Cecal microbiota analysis

No significant differences were observed among treatments across multiple alpha diversity indices (Shannon, Pielou's Evenness, Simpson, Fisher, observed OTUs, and Chao1) (P>0.05; Figure 1). Beta diversity was evaluated using Bray-Curtis, Jaccard, UniFrac, and weighted UniFrac metrics. The elephant grass treatment was significantly higher than both the control and soybean hull groups based on Bray-Curtis distances (P = 0.03), and from the soybean hull group when assessed by weighted UniFrac (Figure 2). In contrast, no significant differences were detected with the Jaccard and unweighted UniFrac metrics (P>0.05; Figure 2).

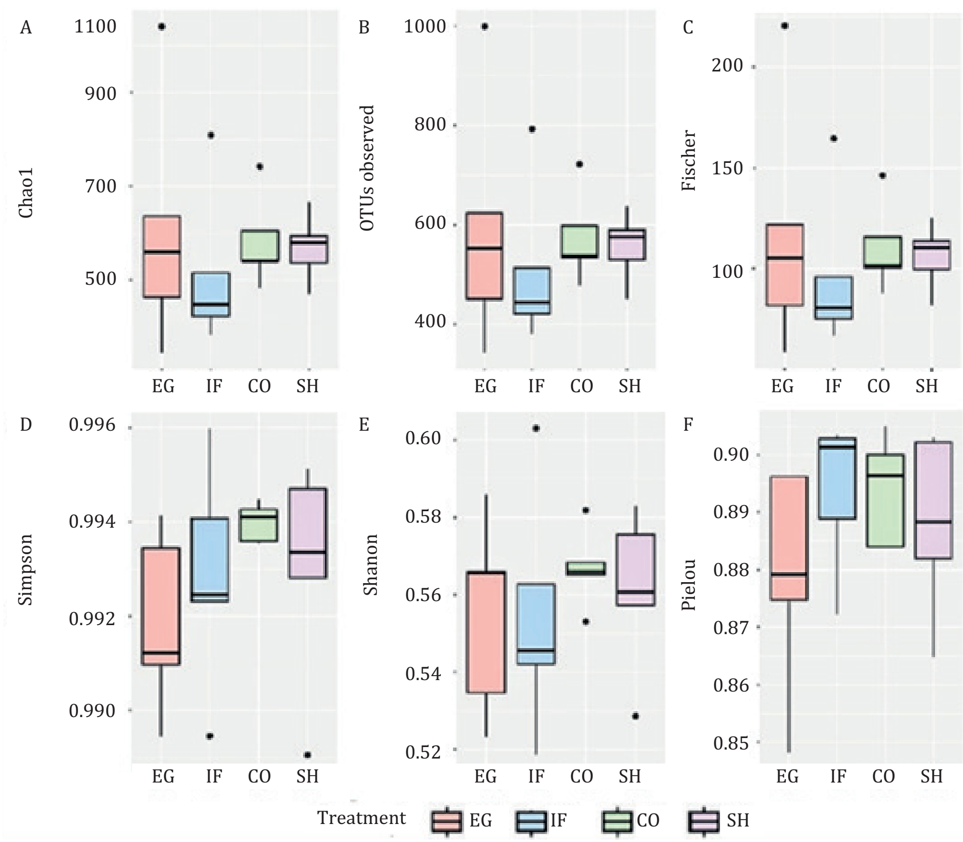

Figure 1 - Alpha-diversity estimated by the parameters Chao1 (A), Observed OTUs (B), Fisher Index (C), Simpson Index (D), Shannon (E), and Evenness (F). The treatments include elephant grass (EG), commercial insoluble fiber (IF), control (CO), and soybean hulls (SH).

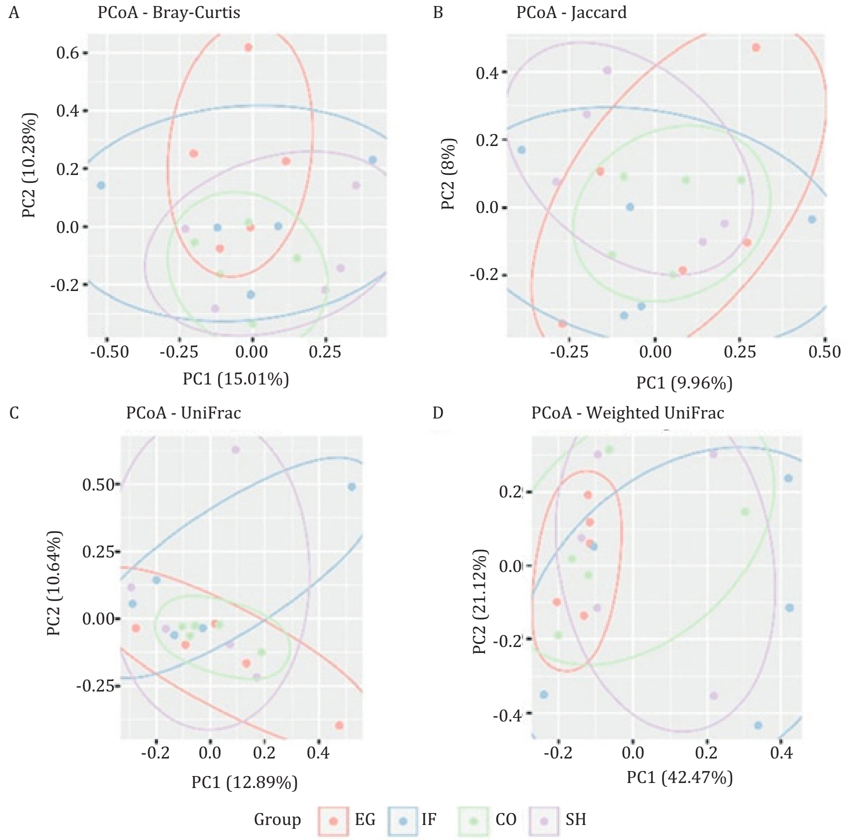

Figure 2 - Beta diversity estimated by the parameters Bray-Curtis (A), Jaccard (B), UniFrac (C), and Weighted UniFrac (D). Colored ellipses have been added automatically via the ggforce library in R. Treatments include elephant grass (EG), commercial insoluble fiber (IF), control (CO), and soybean hulls (SH).

The genera *Phocaeicola*, *Faecalibacterium*, *Bacteroides*, *Olsenella*, and *Lawsonibacter* were dominant (Figure 3), while at the species level, *Phocaeicola sp900066445, Faecalibacterium sp002160895, Bacteroides sp002160055, Olsenella sp002159625*, and *Phocaeicola salanitronis* were most abundant (Figure 4).

Statistical analyses (Kruskal-Wallis with Dunn's post hoc test, P≤0.05) revealed specific differences in the relative abundance of certain taxa. At the genus level, *Bacteroides* and *Megamonas* showed higher abundance in the elephant grass treatment compared to the soybean hull and commercial insoluble fiber treatments (P≤0.05), but not compared to the control (P>0.05; Figure 5). At the species level, *Faecalibacterium prausnitzii* showed higher abundance in the elephant grass group compared with the soybean hull treatment (P≤0.05), and *Megamonas funiformis* showed higher abundance in the elephant grass treatment compared with both the soybean hull and commercial insoluble fiber treatments (P≤0.05), but not compared with the control (P>0.05; Figure 6).

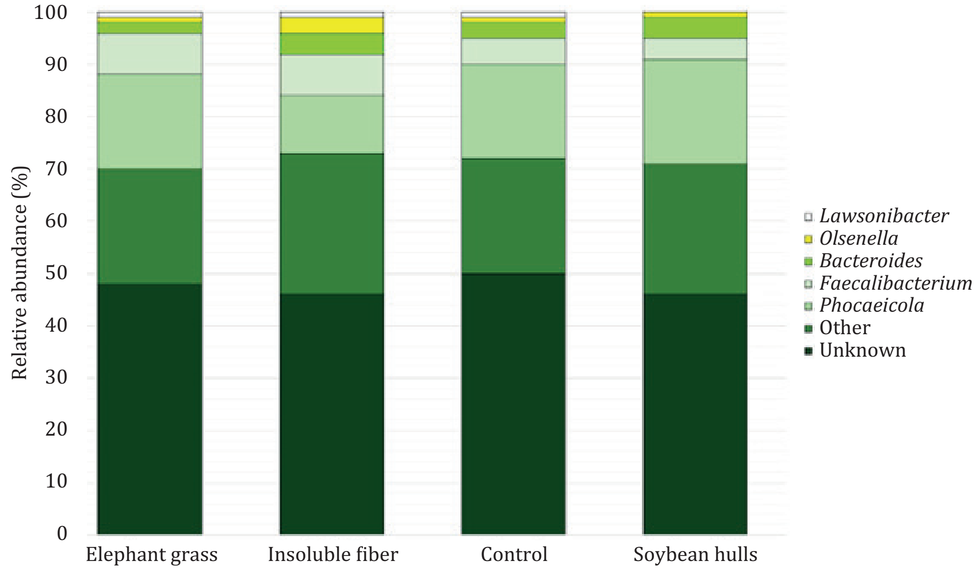

Figure 3 - Effects of dietary fiber source on relative abundance of genera detected in cecum contend of laying hens.

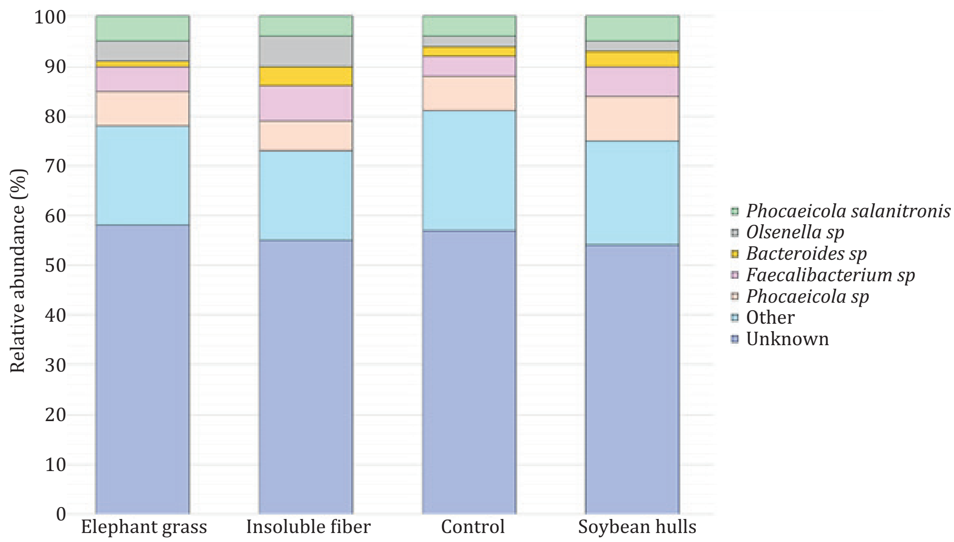

Figure 4 - Effects of dietary fiber source on relative abundance of species detected in cecum contend of laying hens.

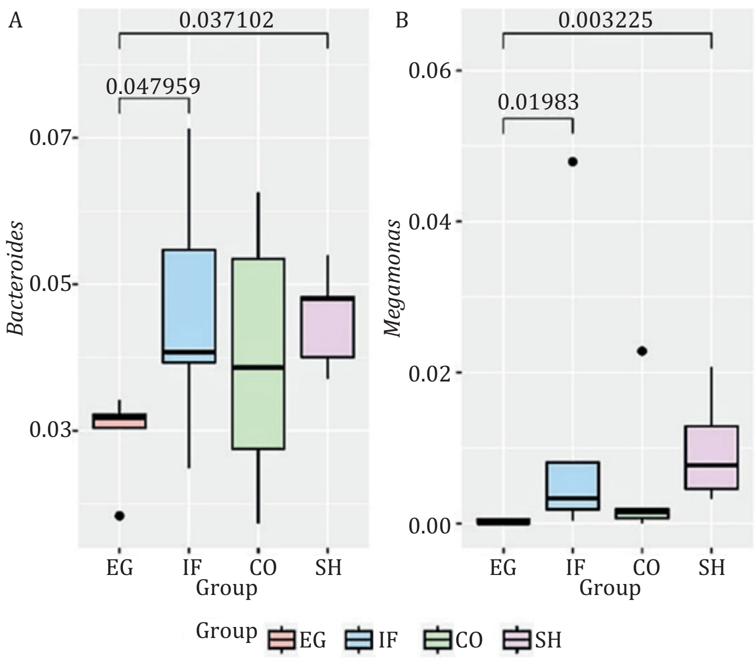

Figure 5 - Differential abundance of the genera *Bacteroides* (A) and *Megamonas* (B). Treatments include elephant grass (EG), commercial insoluble fiber (IF), control (CO), and soybean hulls (SH).

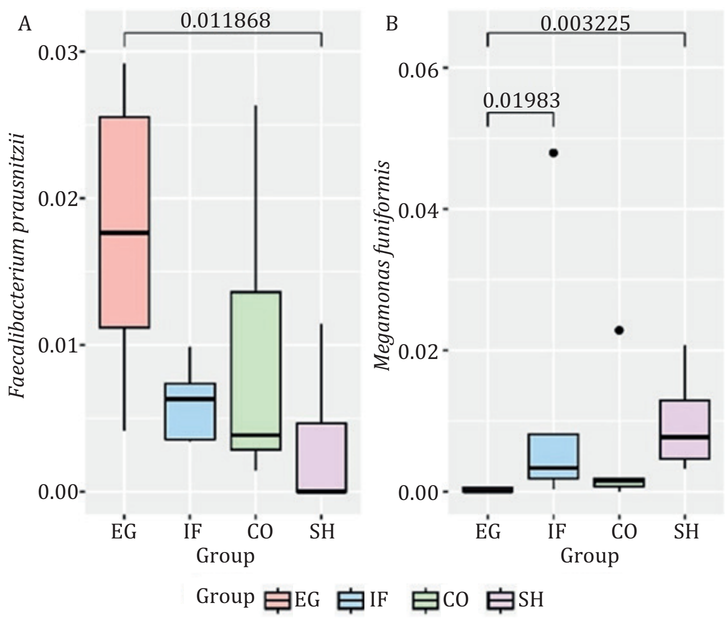

Figure 6 - Differential abundance for the species *Faecalibacterium prausnitzii* (A) and *Megamonas funiformis* (B). The treatments include elephant grass (EG), commercial insoluble fiber (IF), control (CO), and soybean hulls (SH).

4. Discussion

The present study demonstrated that including up to 2% of fiber sources: elephant grass, soybean hulls, and commercial insoluble fiber, in laying hen diets did not adversely affect performance parameters, such as egg production, eggs per housed hen, egg weight, egg mass, viability, or most egg quality indices. In fact, soybean hulls improved feed conversion efficiency, which may be associated with their relatively higher protein content. This fiber source, along with elephant grass, enhanced cecal microbiota diversity, promoting beneficial bacteria such as *Bacteroides* and *Faecalibacterium prausnitzii*. These microbiota shifts may contribute to improved nutrient utilization, intestinal health, and immune modulation, providing further support for incorporating diverse fiber sources in layer nutrition (Oakley et al., 2014).

Moreover, laying hens fed diets containing elephant grass achieved a higher final body weight compared to those receiving commercial fiber sources. The improved performance of the elephant grass group is likely due to its predominantly insoluble fiber, which may have stimulated endogenous enzyme production, thereby improving nutrient digestion and absorption. This finding aligns with other studies on dietary fiber in poultry, for instance, Panaite et al. (2016) found that supplementing broilers with 8% pelleted alfalfa increased their live weights. Similarly, Yokhana et al. (2016) and Sozcu et al. (2020) also reported improvements in body weight and feed efficiency with the inclusion of lignocellulose at moderate levels (1-2%).

However, weight gain in laying hens can be detrimental, as a positive energy balance may disrupt the finely coordinated processes of egg formation, potentially leading to reproductive dysfunctions such as expanded ovarian hierarchies and hyperovulation (Walzem and Chen, 2014). Taken together, these findings suggest that moderate fiber inclusion is ideal for supporting performance while avoiding profound negative effects on reproductive physiology.

Concerning production parameters, feed intake and egg quality traits (e.g., egg weight, egg mass), were largely unaffected by the fiber sources. This is consistent with Sousa et al. (2019), who observed that reductions in dietary energy via fiber inclusion did not compromise laying performance. However, soybean hulls yielded better feed conversion per kilogram and per egg carton, as well as the most favorable feed cost. Although all diets were formulated to be isonutritive, the soybean hull diet exhibited higher crude protein levels upon bromatological analysis, which may partly explain its enhanced conversion efficiency. In contrast, the other diets performed the poorest in this regard.

Regarding viability, it was not significantly influenced by the dietary treatments. Nevertheless, fiber inclusion has been linked in other studies to improved welfare and reduced cannibalism, likely due to prolonged feeding time and decreased pecking behavior (Hartini et al., 2002), although Sousa et al. (2019) have noted adverse effects on viability with certain fiber sources, specifically a 2.78% reduction in viability with the use of soybean hulls at a 5% inclusion level.

In the first assessment, hens fed soybean hulls produced heavier eggs, while in the second evaluation, both the soybean hull and control diets yielded superior egg weights. Additionally, at 71 weeks of age, the soybean hull treatment was associated with a higher yolk percentage but a lower albumen percentage. This change in albumen percentage may be influenced by various factors, such as pH, storage time, and the integrity of ovomucin bonds (Silversides and Budgell, 2004). However, as these specific parameters were not evaluated in the present study, it is not possible to definitively attribute the observed differences to any single factor. Other egg quality parameters, including shell percentage, shell thickness, shell strength, Haugh unit, yolk color, and specific gravity, remained unaffected.

It is noteworthy that the second egg weight recording, which showed no significant treatment differences, provides a more reliable indicator of overall performance than isolated assessments. While this long-term trend remained unaffected, point-in-time assessments revealed specific, temporal effects. For instance, in the first assessment, hens fed soybean hulls produced heavier eggs, a finding that may be associated with the diet's high protein levels (Leeson and Summers, 2005).

These observations demonstrate that certain fiber sources can induce minor and temporary shifts in performance at specific times during the production cycle, even if the overall long-term trend remains unchanged.

In line with this, Andrade et al. (2022) who used up to 2.5% wheat bran in diet formulation, also found no negative changes in the internal and external quality of eggs. These results reinforce that this inclusion level may represent an upper limit, and that fiber appears to have little influence on these parameters at moderate inclusion rates. It is noteworthy that weekly egg weight recordings, which showed no significant treatment differences, may provide a more reliable indicator of overall performance than isolated quality assessments.

Regarding intestinal morphology, the low inclusion level of fiber (2%) generally did not produce marked changes in the overall gastrointestinal structure. Although hens fed commercial fiber exhibited a longer ileum than those on the control or soybean hull diets, and subtle differences were noted in duodenal villus height and crypt diameter among treatments, these effects were limited in magnitude. The minimal morphological alterations suggest that at low inclusion rates, fiber does not detrimentally affect gut structure, a finding that aligns with previous work by Sittiya et al. (2019), who observed significant morphological changes only at higher fiber inclusion levels.

The cecal microbiota analysis revealed that overall alpha diversity (assessed via Shannon, Pielou's Evenness, Simpson, Fisher, observed OTUs, and Chao1 indices) remained stable across treatments, indicating that fiber inclusion did not affect microbial richness or evenness. Furthermore, weighted UniFrac metrics revealed significant differences between the elephant grass and soybean hull treatments, suggesting that specific fiber sources modulate the cecal microbiota structure. This distinction highlights the potential of the soybean hull treatment to increase the abundance of butyrate-producing bacteria, which have been associated with improved growth performance and intestinal health (Leung et al., 2018; Liu et al., 2021).

Taxonomic profiling showed an increase in beneficial bacteria in some diets, particularly in hens fed elephant grass, which exhibited a higher relative abundance of the genera *Bacteroides* and *Megamonas*, as well as an increased proportion of *Faecalibacterium prausnitzii* a butyrate-producing bacterium known to enhance intestinal blood flow, mucosal production, and epithelial cell proliferation (Hou et al., 2020; Sousa et al., 2025). These microbiota shifts, while subtle, may have functional implications for nutrient digestion and immune modulation. In line with Hou et al. (2020), our results suggest that dietary fiber can enhance the diversity and abundance of beneficial bacterial taxa in the ceca, which could contribute to improved gut health and nutrient utilization.

The superior cost-benefit ratio of the soybean hull-based diet, as observed, provides a compelling economic argument for its use. However, a key practical implication of this work is the potential for price volatility of feed ingredients, which can fluctuate significantly based on global market conditions, regional availability, and seasonality. Therefore, while the favorable market prices at the time of this study likely contributed to the exceptional economic outcome, the ultimate decision on diet formulation should be based on a comprehensive evaluation that also considers animal performance and intestinal health.

5. Conclusions

The inclusion of up to 2% of fiber sources in laying hen diets did not negatively impact essential performance parameters. Moreover, it was associated with notable changes in gut health as fiber sources positively influenced the cecal microbiota, with elephant grass being associated with an increased relative abundance of beneficial bacteria such as *Bacteroides* and *Faecalibacterium prausnitzii*, which are commonly associated with improved nutrient digestion and intestinal function.

Furthermore, soybean hulls stood out as a cost-effective option for maintaining performance and egg quality. These results highlight the potential of these fiber sources to improve microbial diversity and gut health in poultry, without compromising production and at a reasonable cost.

Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Author contributions

Conceptualization: Lara, L. J. C. **Data curation:** Meireles, I. M. and Barbosa, H. J. S. **Formal analysis:** Meireles, I. M.; Lobato, H. C. and Araújo, I. C. S. **Funding acquisition:** Lara, L. J. C. **Investigation:** Meireles, I. M.; Costa, B. T. A.; Barbosa, H. J. S. and Lobato, H. C. **Methodology:** Meireles, I. M. and Costa, B. T. A. **Project administration:** Lara, L. J. C. **Resources:** Lara, L. J. C. **Supervision:** Lara, L. J. C. **Visualization:** Meireles, I. M.; Costa, B. T. A.; Barbosa, H. J. S.; Araújo, I. C. S. and Lara, L. J. C. **Writing – original draft:** Meireles, I. M. and Lara, L. J. C. **Writing – review & editing:** Lobato, H. C.; Araújo, I. C. S. and Lara, L. J. C.

Conflict of interest

The authors declare no conflict of interest.

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