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EFFECTS OF EUBIOTIC LIGNOCELLULOSE SUPPLEMENTAION ON PRODUCTION, BLOOD BIOMARKERS, INTESTINAL HEALTH, AND LITTER QUALITY IN BROILER CHICKENS

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ABSTRACT

There is a great concern with maintaining the integrity of the digestive tract and limiting pathogenic load, which is critical for poultry performance. The dietary inclusion of new natural fiber sources can improve the poultry's gut health and the overall performance. The goal of the current research was to study the effect of a fiber supplement (eubiotic lignocellulose; insoluble and partially fermentable) on the production, carcass traits, blood metabolites, antibody titers, intestinal health state, and litter condition of broiler chickens. 450 day-old unsexed Cobb 500 chicks (3 repetitions) were divided at random into three dietary treatments as follows: control (T1) was fed the standard diet (no supplementation of lignocellulose), the second treatment (T2) was supplemented with 0.8% lignocellulose on top of the standard diet, and the third group (T3) received a reformulated standard diet that included 0.8% lignocellulose. Eubiotic lignocellulose dietary supplementation resulted in a notable improvement ($P \leq 0.05$) in production metrics, including body weight, body weight gain, and the European Production Efficiency Factor, compared to the control birds. Additionally, eubiotic lignocellulose supplemented birds had significantly higher levels of total proteins and lower levels of cholesterol and triglycerides ($P < 0.01$). Birds fed a diet supplemented with 0.8% eubiotic lignocellulose showed a considerable increase in the length of three small intestinal villi sections ($P < 0.001$). Diet formulation with 0.8% eubiotic lignocellulose showed relatively enhanced serum antibody titers and lowered caecal *Clostridia* counts in birds. Based on our findings, the incorporation of 0.8% eubiotic lignocellulose in broiler diets, whether on top of or integrated into the diet, led to improved body weight gain, gut health, blood biochemical profile, and litter quality parameters.

Keywords: eubiotic fiber, broiler chickens, performance metrics, biochemical profile, intestinal morphometry, caecal *clostridial* count

INTRODUCTION

Since poultry is one of the top markets worldwide, increasing its production is a must due to the marked increase in populations **(Mottet & Tempio 2017)**. Finding a new feed ingredient to minimize the diet cost while keeping the broiler chicken growth performance or even enhancing it will be of great value to the broiler chicken industry. Such newly developed feed ingredients include new fiber sources. Dietary fibers can improve digestive function, litter quality and general bird health **(Mateos *et al.*, 2012; Farran *et al.*, 2017; Kheravii *et al.*, 2017)**. Choosing the correct fiber for birds is essential, as soluble fiber increases the viscosity of the intestine and hinders feed from passing through the gut at the proper rate. In contrast, insoluble fibers help maintain healthy gut motility, which prevents chime from backing up into the small intestine and potentially causing an infection **(Sacranie *et al.*, 2012; Kroismayr, 2014)**. Fermentable fibers also shift the fermentation process to the large intestine, where beneficial bacteria proliferate **(Jha *et al.*, 2010)**.

In modern poultry nutrition, specialized fiber products known as lignocellulose are incorporated in poultry diets with low inclusion rates, because they improve the digestibility and general intestinal health, as noted by **Slavin (2013)** and **Kheravii *et al.* (2017)**. Lignocellulose is among the best sources of fiber used in poultry feed. It originates from special fresh wood and contains higher levels of insoluble cellulose and hemicelluloses (>55%) and lignin (25–30%) than typical fiber sources **(Kroismayr, 2014)**. A novel category of lignocellulose products has recently entered the market, known as eubiotic lignocellulose. Contrary to the first generation of lignocellulose products, which were consisted entirely of non-fermentable hemicellulose, cellulose, and lignin, eubiotic lignocellulose comprises a harmonious blend of both non-fermentable (insoluble) and fermentable fiber components. It has a fiber content of up to 85% **(Sun *et al.*, 2021)**.

Previous literatures have studied the lignocellulose effects on broiler and laying hen productive parameters, nutrient absorption, gut sound, and microbial flora in the intestine. **Kroismayr *et al.* (2009)** demonstrated that 1.0–1.5% eubiotic lignocellulose significantly improved growth outcomes and economic returns by 9%. Furthermore, an increase in egg production of 1.7 and a 40% decrease in mortality were seen in research at a commercial layer farm that used 1% eubiotic lignocellulose starting at week **50 (Kroismayr, 2014)**. Broilers' gut microbial environments were improved by the application of eubiotic lignocellulose products (0%, 1%, or 2%), which enhanced gut health and litter quality **(Kheravii *et al.*, 2017)**.

Many studies added dietary lignocellulose on top of feed; only a few studies integrated it into the diet **(Rohe *et al.*, 2020)**. Based on the above-mentioned studies, the lignocellulose inclusion rate and feed formulation of these experiments differed. It is challenging to make a conclusive determination of eubiotic lignocellulose as an innovative insoluble fiber source since the results are contradictory; feeding lignocellulose might have positive, neutral, or negative impacts. Therefore, this study evaluates and compares the impact of dietary lignocellulose on performance parameters, blood biomarkers, and intestinal soundness in broiler chickens that were fed a control diet, eubiotic lignocellulose supplement on top of the feed, and chickens that received eubiotic lignocellulose supplement based on feed formulation.

MATERIAL AND METHODS

The current trial was conducted at the Poultry and Animal Research Center of the Faculty of Veterinary Medicine, Cairo University. The Institutional Animal Care and Use Committee of Cairo University authorized all procedures, including the handling and treatment of birds (Ethical reference No: VetCU03162023743).

Broiler Management, Experimental protocol, and Diets formulation

450 Cobb-500 broiler chicks were divided at random into three different feeding groups, each containing three replicates of 50 chickens. For up to 33 days, the broiler chicks were grown on a floor enclosure with bedding composed of wood-shaving litter. Managing the housing's temperature, light, and humidity, as well as the vaccination schedule, was done in accordance with the Cobb-500 strain guidelines. Diets were provided with free access along with freshwater and given in two forms: crumbled for the beginning stage and pelleted for the growing and

finishing stages. The Cobb-500 breed's manual standards **(Aviagen, 2022)** were followed in the formulation of the experimental diets, which were designed to be iso-caloric and iso-nitrogenous. Specifically, the standard diet with 0% lignocellulose was fed to the control group (T1), 0.8% lignocellulose was added on top of the standard diet (T2), and a reformulated standard diet with 0.8% lignocellulose was fed to the third group (T3). **Table 1** illustrates the feed composition and nutrient analysis of conventional diets.

The eubiotic lignocellulose was obtained from Agromed GMBH distributor in Egypt (OptiCell®, Agromed GMBH Company, Austria) and used in this study. OptiCell® is micronized fiber concentrate from insoluble, fermentable lignocellulose originated from the tree trunk, including both: cambium and bark.

Wood is a naturally occurring, sustainable raw material that contains lignocellulose. OptiCell® natural fibers come from freshly cut, untreated wood that is harvested from Germany's sustainably managed forests. The trunk's various components are blended, carefully ground, and produced into standardized, superior products that boost productivity. When designing the third experimental diet (T3), the nutrient content of lignocellulose supplement was considered. The supplement has the following composition: 0% energy, 8% moisture, 0.9% crude protein, 0.8% crude fat, crude ash 1.0%, 1.3% minerals and trace elements, lignin 25–30%, 88% total dietary fiber (TDF), crude fiber 59%, 1.3% soluble TDF, Neutral detergent fiber (NDF) 78%, Acid detergent fiber (DF) 64%.

Table 1 Feed ingredients and nutrient contents of broiler diets from 1-33 days of the experiment.

Ingredients %	Control (T1)*			T3		
	Starter (0-14 days)	Grower (15-28 days)	Finisher (29-33 days)	Starter (0-14 days)	Grower (15-28 days)	Finisher (29-33 days)
Yellow corn	57.43	61.23	65.93	56.70	61.40	66.18
Corn gluten 60%	3.00	2.00	2.00	1.00	2.00	2.00
Soybean meal (46% CP)	36.0	32.20	27.00	38.00	32.18	27.00
Opticell	0.00	0.00	0.00	0.8	0.8	0.8
Soy-oil	0.00	1.20	2.0	0.00	0.3	1.00
Di-calcium phosphate	0.90	0.80	0.50	0.90	0.80	0.50
Limestone	1.40	1.30	1.25	1.47	1.30	1.25
Common salt	0.35	0.35	0.35	0.35	0.35	0.35
Sod bicarbonate	0.15	0.15	0.15	0.15	0.15	0.15
DL-Methionine	0.20	0.20	0.20	0.18	0.20	0.20
Lysine	0.20	0.20	0.25	0.08	0.15	0.20
Unike**	0.05	0.05	0.05	0.05	0.05	0.05
Phytase	0.01	0.01	0.01	0.01	0.01	0.01
Xylanase	0.01	0.01	0.01	0.01	0.01	0.01
Broiler premix***	0.30	0.30	0.30	0.30	0.30	0.30
Total (%)	100	100	100	100	100	100
Chemical composition						
Metabolizable Energy (Kcal/kg)	3010.06	3105.84	3201.51	3016.30	3092.76	3199.25
Crude protein (%)	23.11	21.04	19.05	23.38	21.01	19.04
Crude fat (%)	2.57	3.83	4.73	2.53	2.95	3.75
Crude fiber (%)	2.36	2.30	2.22	2.84	2.77	2.70
Calcium (%)	1.02	0.95	0.86	1.05	0.95	0.86
Available phosphorus (%)	0.50	0.47	0.41	0.50	0.47	0.41

*T1: Control: Standard diet (no lignocellulose supplementation); T3: broilers fed a standard diet reformulated with 0.8% lignocellulose. T2 was the same for the control group with the addition of 0.8% lignocellulose on top of the feed. **Toxin binder (Addisseo). ***Vitamin-mineral premix per Kg of diet: 1200000 IU vit.A, 350000 IU vit.D 3,4000 mg vit.E,250mg vit.B1,800 mg vit.B2,600 mg vit.B6,3.2mg vit.B12, 450 mg vit.K3,4.5g nicotinic acid,1.5g Ca pantothenate,120 mg folic acid, 5mg biotin, 55 mg choline chloride,3g Fe,2 g Cu, 10 g Mn, 8 g Zn,120 mg I, 40 mg Co.

Sampling and measurements

Production metrics

For 33 days, the birds' body weights (BW), feed intake (FI) were recorded on weekly basis, and daily mortality data was noted. The starting and final body weights were subtracted to get the average body weight gain (BWG). The feed conversion ratio (FCR) and the European Production Efficiency Factor (EPEF) were then calculated using this data.

Carcass characteristics

After the experiment ended (lasting 33 days), five birds per treatment group were chosen from each replicate. These birds were euthanized to measure the carcass, breast, thigh, drum, and abdominal fat weights, which were then represented as a percentage of the live body weight **(Shirzadegan *et al*., 2014)**.

Blood biochemical profile

Samples of blood were obtained from each bird at 33 days of age at the slaughter. (3-4 animals were chosen at random from each replicate of all the groups, but only 8/10 animals from each group were used for biochemical analysis because of hemolysis and/or sample volume. Following coagulation, serum was obtained by centrifuging the blood samples for 10 minutes at 5,000 rpm. The serum was then stored at -20°C until analysis. Using standardized kits purchased from Spectrum in Germany, the following parameters have been measured: serum, blood urea nitrogen, creatinine, uric acid, total proteins, albumin, glucose, triglycerides, and cholesterol. All tests were conducted in duplicate. The difference between TP and albumin serum levels was used to compute serum globulin levels, and the albumin/globulin ratio was estimated.

Small intestine morphometric parameters

On day 33, small intestines were collected from 4 birds per group after slaughtering, flushed with a buffer solution, and sliced into the three parts of small intestine. Then samples were fixed, processed, and stained according to **(Bancroft & Gamble 2008)**. At 40x magnification, using a Leica Quin 500 analyzer computer system (Leica Microsystems, Switzerland) at Cairo University's Faculty of Veterinary Medicine, the stained sections were examined to measure the length of the villi and the depth of the intestinal glands (crypt) for each group.

Caecal *Clostridia* count

Fecal contents from four birds' caeca/group were aseptically collected on the day of slaughter (day 33). Fecal matter (1 g) was ten-fold serially diluted in tubes of 9 ml sterile saline solution till the 10^{-12} dilution, and 100 µl was picked from the last three dilutions and spread on Reinforced Clostridial Agar (Oxoid Ltd, Basingstoke, Hants, UK) plates **(Esmaeilipour *et al*., 2012)**. Inoculated plates were anaerobically incubated (Oxoid™ AnaeroGen™, Thermo Scientific Inc., USA) at 37° C for 24 – 48 h. *Clostridia* colonies were counted and reported as $\log_{10}$- colony-forming units (CFU)/g.

Vaccinal Newcastle Disease virus (NDV) immune status

Using the hemagglutination inhibition (HI) test, the NDV-antibody titers were evaluated on day 33 in eight serum samples per group **(WOAH, 2023)**. Two-fold serial dilutions were performed for 25 µl of each serum sample in 99-well microtiter plates. A commercial NDV-Lasota antigen with four hem agglutinanion units (4 HAU) was distributed in wells at a rate of 25 µl per well. The plates were left at room temperature for 20 minutes to allow for the antigen-antibody reaction. Then, 25 µl of packed chicken RBCs 1% suspension was applied to each well and left for 30 to 45 minutes, or until the RBCs precipitated in the control wells. The readings were recorded as log2 HI titers.

Litter quality examinations

From each replicate on day 33, litter was pooled for the upper 7 cm of three various spots in sterile plastic bags and thoroughly mixed. The moisture of the litter was obtained by subjecting 10 g of each litter sample to 100 ± 5 °C in a hot air oven for 24 - 48 h, then subtracting the dried weight from the initial weight **(Dumas**

et al., 2011; Lopes *et al.*, 2013). Total litter nitrogen was assessed through the total Kjeldahl nitrogen **(Jackson, 1973)**.

Statistical analysis

The results were presented as means ± standard error of means. To statistically compare the experimental groups, the One-way ANOVA and Tukey post-hoc tests were employed with a significance level set at $P \le 0.05$. Statistical analysis was performed using Version 18.0 of PASW Statistics Software (SPSS Inc., Chicago, IL, USA). The R Foundation for Statistical Computing software (R version 4.4.3; 2025-02-28 ucrt) was used for computing the effect sizes (Eta squared, η^2) by the *'effectsize'* package to assess the magnitude of treatment effects **(Ben-Shachar et al., 2020)**. According to Cohen's criteria **(Cohen, 1988)**, η^2 values of 0.01, 0.06, and ≥0.14 were considered small, medium, and large effect sizes, respectively. Additionally, boxplots were generated using with the 'ggplot2' and 'ggpubr' packages **(Wickham, 2016; Kassambara, 2020)**.

RESULTS

Broiler performance

Table 2 illustrates the influence of eubiotic lignocellulose on broiler performance. Significant variations ($P \le 0.01$) were observed in body weight, average weight gain, and EPEF between the control group and those receiving eubiotic lignocellulose either added on top (T2) or integrated into the diet (T3). Moreover, eubiotic lignocellulose supplemented groups showed a numerical enhancement in FCR. Among them, T3 exhibited the best performance when fed a reformulated conventional diet with 0.8% lignocellulose. However, feed intake did not change significantly ($P > 0.05$). Additionally, birds fed eubiotic lignocellulose had a lower mortality rate than those fed a standard diet (no lignocellulose addition). Moreover, the effect sizes for body weight, weight gain, feed intake, FCR, EPEF, and mortality rate were large, with η^2 values of 0.89, 0.89, 0.37, 0.52, 0.76, and 0.29, respectively. These values indicate that the treatment groups explained 89%, 89%, 37%, 52%, 76%, and 29% of the variance in each corresponding parameter.

Table 2 Cumulative performance changes in response to dietary eubiotic lignocellulose in broiler chickens (days 1-33).

Groups	Final Body weight (g)	Weight gain (g)	Feed intake (g)	FCR[1] (g/g)	EPEF[2]	Mortality (%)
T1	1905^{b}	1859^{b}	2892	1.56	313^{b}	13.33
T2	2171^{a}	2125^{a}	3054	1.44	401^{a}	10.67
T3	2090^{a}	2044^{a}	2848	1.39	413^{a}	7.33
SEM[3]	41.70	41.70	51.42	0.03	17.96	1.63
Eta^2 (η^2)	0.89	0.89	0.37	0.52	0.76	0.29
***P*- value**	0.001	0.001	0.247	0.110	0.013	0.365

[a,b] Mean values with different superscripts in the same column indicate a significant difference (Tukey's test; $P \le 0.05$). T1: Control: Standard diet (no lignocellulose supplementation); T2: broilers supplemented with 0.8 % lignocellulose on top of feed; T3: broilers supplemented with 0.8 % reformulated lignocellulose.
[1] FCR: Feed Conversion Ratio (g of feed/g of weight gain),[2] EPEF: European Production Efficiency Factor= (livability × live weight (kg)/ (age in days × FCR) × 100, [3] SEM: Standard error of the mean. Eta^2: According to Cohen's criteria, η^2 values of 0.01, 0.06, and ≥0.14 were considered small, medium, and large effect sizes, respectively.

Carcass characteristics

The illustrated data in **Table 3** revealed that lignocellulose didn't significantly impact dressing percentage, breast, thigh, or drum yield. The effect sizes (η^2) indicated small effects for thigh yield (0.02), breast yield (0.03), and abdominal fat (0.03), and medium effects for drumstick (0.08) and dressing percentage (0.10).

Table 3 Impact of dietary eubiotic lignocellulose on carcass traits of broiler chickens.

Groups	Dressing (%)	Breast (%)	Thigh (%)	Drumstick (%)	Abdominal fat (%)
T1	71.36	32.20	25.52	12.85	1.76
T2	74.16	32.50	25.70	13.30	1.58
T3	73.09	33.43	25.43	12.66	1.61
SEM[1]	0.73	0.55	0.26	0.18	0.11
Eta^2 (η^2)	0.10	0.03	0.02	0.08	0.03
***P*- value**	0.299	0.720	0.790	0.390	0.786

Mean values with different superscripts in the same column indicate a significant difference (Tukey's test; $P \le 0.05$). T1: Control: Standard diet (no lignocellulose supplementation); T2: broilers supplemented with 0.8% lignocellulose on top of feed; T3: broilers supplemented with 0.8% reformulated lignocellulose.
[1] SEM: Standard error of the mean. Eta^2: According to Cohen's criteria, η^2 values of 0.01, 0.06, and ≥0.14 were considered small, medium, and large effect sizes, respectively.

Blood biochemical profile

As demonstrated by the findings in **Table 4**, the groups fed a diet enriched with 0.8% lignocellulose-on top (T2) and 0.8% lignocellulose reformulated (T3) exhibited a substantial increase in total protein levels compared to the control group ($P = 0.004$). Additionally, birds that received 0.8% lignocellulose-on top (T2) had significantly higher total protein levels compared to the group that received a reformulated conventional diet containing 0.8% lignocellulose (T3). Furthermore, the current study found that serum triglycerides and cholesterol ($P<0.01$) were significantly reduced in the groups that received either 0.8% lignocellulose on top (T2) or reformulated (T3) within the basal diet, comparable to the control group. Moreover, the birds supplemented with 0.8% eubiotic lignocellulose on top (T2) showed significantly lower cholesterol levels and triglycerides compared to the group that was given the reformulated basal diet with 0.8% lignocellulose (T3). However, the other measured parameters, including albumin, globulin, A/G ratio, urea, uric acid, creatinine, and glucose, did not show any statistically significant variations between the groups.

The effect sizes (η^2) for serum biochemical parameters varied from negligible to large, indicating differential treatment influence. A large effect was observed for cholesterol ($\eta^2 = 0.65$) and triglycerides ($\eta^2 = 0.61$), with both showing highly significant differences among groups ($P < 0.0001$). A moderate effect was found for total protein ($\eta^2 = 0.35$, $p = 0.004$), indicating that 35% of the variance in serum protein levels was explained by the treatment. Other parameters showed small or negligible effect sizes, including albumin ($\eta^2 = 0.09$), globulin ($\eta^2 = 0.15$), A/G ratio ($\eta^2 = 0.05$), BUN ($\eta^2 = 0.02$), creatinine ($\eta^2 = 0.12$), uric acid ($\eta^2 = 0.06$), and glucose ($\eta^2 = 0.03$), none of which were statistically significant ($P > 0.05$).

Table 4 Effect of dietary eubiotic Lignocellulose on different biochemical and metabolic parameters.

Groups /parameters	T1	T2	T3	SEM[1]	Eta^2 (η^2)	*P*-value
Total proteins(g/dl)	4.00 ^{a}	4.12 ^{a}	4.47 ^{b}	0.07	0.35	0.004
Albumin (g/dl)	1.87	1.91	1.99	0.03	0.09	0.309
Globulin (g/dl)	2.13	2.21	2.47	0.08	0.15	0.125
A/G Ratio	0.89	0.92	0.82	0.04	0.05	0.550
BUN (mg/dl)	2.57	2.42	2.51	0.74	0.02	0.750
Creatinine (mg/dl)	1.18	1.16	1.05	0.35	0.12	0.214
Uric acid (mg/dl)	4.47	4.22	4.58	0.12	0.06	0.480
Glucose (mg/dl)	228.01	224.25	222.30	3.03	0.03	0.660
Triglycerides (mg/dl)	57.10 ^{a}	37.25 ^{b}	48.100 ^{c}	1.94	0.61	<0.0001
Cholesterol (mg/dl)	146.20 ^{a}	133.11^{b}	123.07 ^{c}	2.34	0.65	<0.0001

[a,b,c] Mean values with different superscripts in the same row indicate a significant difference (Tukey's test; $P \le 0.05$). T1: Control: Standard diet (no lignocellulose supplementation); T2: broilers supplemented with 0.8% lignocellulose on top of feed; T3: broilers supplemented with 0.8% reformulated lignocellulose.
[1] SEM: Standard error of the mean. Eta^2: According to Cohen's criteria, η^2 values of 0.01, 0.06, and ≥0.14 were considered small, medium, and large effect sizes, respectively.

Small intestine morphometric parameters

Table 5 shows that the 0.8% Lignocellulose-on top group (T2) had significantly longer villi in the ileum and duodenum than the control group did ($P<0.01$). When comparing the jejunum of the group (T3) given a reformulated 0.8% lignocellulose to the control, a substantial rise was seen ($P<0.01$). The depth of the intestinal gland in the ileum was significantly higher in the 0.8% lignocellulose-on-top group (T2) than in the control. The effect sizes (η^2) for intestinal morphology parameters ranged from medium to very large, reflecting varying levels of treatment influence across gut segments. In the duodenum, large effects were noted for villus length ($\eta^2 = 0.73$) and crypt depth ($\eta^2 = 0.43$), with a moderate effect on the villus/crypt ratio ($\eta^2 = 0.24$). In the jejunum, the treatment effect was very large for villus length ($\eta^2 = 0.78$), moderate for the villus/crypt ratio ($\eta^2 = 0.41$), and small for crypt depth ($\eta^2 = 0.15$). In the ileum, villus length ($\eta^2 = 0.99$) and crypt depth ($\eta^2 = 0.84$) showed very large effects, while the villus/crypt ratio exhibited a large effect ($\eta^2 = 0.53$).

Table 5 Effect of dietary supplementation with dietary eubiotic lignocellulose on histomorphometry of small intestine of broiler chickens (mean ± SEM; µm).

Groups	***Duodenum***			***Jejunum***			***Ileum***		
	Villi length	**Crypt depth**	**Villus crypt ratio**	**Villi length**	**Crypt depth**	**Villus crypt ratio**	**Villi length**	**Crypt depth**	**Villus crypt ratio**
T1	1596.97^b	114.91^{ab}	14.00^a	1062.84^b	88.24^a	12.30^{ab}	574.80^b	76.51^b	7.53^a
T2	1720.31^a	124.80^a	13.86^a	882.62^c	80.14^a	11.02^b	838.92^a	105.32^a	8.01^a
T3	1563.17^b	102.18^b	15.38^a	1188.27^a	89.86^a	13.28^a	531.07^c	79.80^b	6.68^b
SEM[1]	29.26	7.53	0.83	47.24	7.05	0.78	9.86	3.97	0.36
Eta^2 (η^2)	0.73	0.43	0.24	0.78	0.15	0.41	0.99	0.84	0.53
***P*-value**	<0.001	0.034	0.194	<0.001	0.367	0.043	<0.0001	<0.0001	0.011

[a,b,c] Means in the same column showing different superscripts point to significant differences ($P \leq 0.05$). T1: Control - Standard diet (no lignocellulose supplementation); T2: broilers supplemented with 0.8% lignocellulose on top of feed; T3: broilers supplemented with 0.8% reformulated lignocellulose. [1] SEM: Pooled standard error of the mean. Eta^2: According to Cohen's criteria, η^2 values of 0.01, 0.06, and ≥0.14 were considered small, medium, and large effect sizes, respectively.

Caecal *Clostridia* count

Clostridia count for caecal fecal matter was presented in **Fig.1**. The lowest count was found in group T3 (8.70 ± 0.70 $\log_{10}$/g), then control T1 (9.67 ± 1.20 $\log_{10}$/g), and T2 (10.15 ± 0.66 $\log_{10}$/g). The differences, however, did not vary significantly ($P = 0.684$). The effect size for caecal *Clostridia* was small to medium ($\eta^2 = 0.17$), indicating that 17% of the variance in *Clostridia* levels could be attributed to the treatment groups.

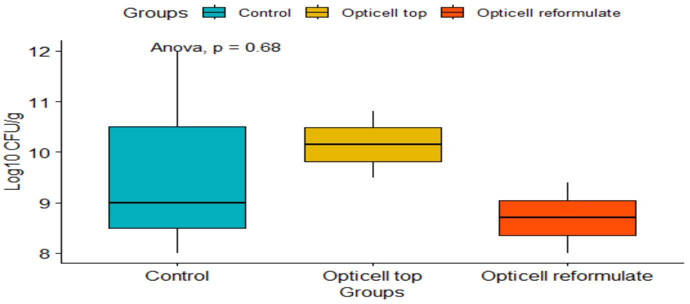

Figure 1 Effect of dietary supplementation with dietary eubiotic lignocellulose on caecal *clostridial* count of broiler chickens (day 33)

Vaccinal Newcastle Disease virus (NDV) HI titers

Antibody HI-titers against NDV on day 33 revealed slight differences between groups. Group T3 reported the highest value (5.29 ± 0.68 $\log_2$), followed by T2 (4.67 ± 0.49 $\log_2$), then the control T1 (4.50 ± 0.67). The difference between HI-titers of the three groups did not show significance ($P = 0.645$). The effect size for NDV HI titers was small ($\eta^2 = 0.05$), indicating that only 5% of the variance in HI titers was explained by the treatment groups.

Litter moisture and nitrogen percentages

There was no significant variation between the groups on day 33; litter moisture% (**Fig. 2**) recorded means of 41.03 ± 5.17 % for control (T1), 53.37% ± 0.78 for T2, and 42.10% ± 1.33 for T3 ($P = 0.056$). The effect size for litter moisture between groups was large ($\eta^2 = 0.62$), indicating that 62% of the variance in moisture content was attributable to the treatment effect. Nitrogen content revealed levels of 2.01% for control (T1), 2.60% for T2, and 2.24% for T3.

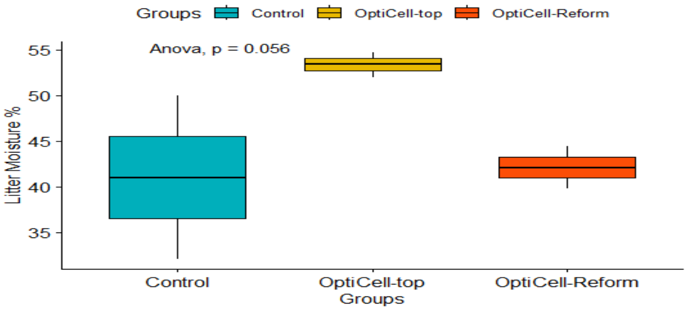

Figure 2 Effect of dietary supplementation with dietary eubiotic lignocellulose on litter moisture percentage of broiler chickens (day 33)

DISCUSSION

Nutrition plays a key role in maintaining gut health in birds, particularly by influencing the villus height to crypt depth ratio, a crucial measure of nutrient absorption in the small intestine **(Mateos *et al.*, 2012)**. Overall health depends on a healthy gut and a proper balance between beneficial and harmful bacteria, which improves broiler performance and boosts feed conversion. To support gut health, broiler diets are often supplemented with alternatives to antibiotics, such as phytobiotics, including the insoluble crude fibers that are applied to improve broiler intestinal health. However, research on the effects of cellulose and lignin, either in their pure forms or from various sources, on poultry growth and performance has produced inconsistent results **(Roberts *et al.*, 2015)**. These conflicting results point to the need for more research to fully comprehend the potential use of lignocellulose as a growth stimulant in chicken production. According to **Slavin (2013)**, consuming more dietary fiber improves gut health by fortifying the intestinal barrier, enhancing immunological response, and reducing pathogenic bacteria like *Clostridium*. Part of the challenge is that existing studies vary widely in terms of lignocellulose inclusion levels and the overall nutrient composition of the diets being tested.

In the current study, adding eubiotic lignocellulose supplementation considerably enhanced the production parameters, including final body weight, average body weight growth, and EPEF, in addition to numerical improvements in FCR, as compared to the control group. These performance improvements are linked to enhanced gut morphology and function through several key mechanisms. Specifically, in our feeding trial, eubiotic lignocellulose significantly increased the length of the birds' three small intestinal villi sections. This increase in villus length directly expands the absorptive surface area of the intestine, thereby enhancing nutrient utilization and explaining the significant improvement in growth performance. Numerous investigations have demonstrated that sources of insoluble fiber may enhance gut health, which in turn improves the overall growth performance of chickens because of their unique physical structure **(Hetland *et al.*, 2005; Kheravii *et al.*, 2018)**. According to **Sacranie *et al.* (2012)**, insoluble fibers regulate the passage rate and maximize the surface area contact between the digesta and the digestive enzymes, facilitating more efficient enzymatic breakdown and subsequent nutrient absorption. Moreover, fermentable fractions within the eubiotic lignocellulose supplement may undergo microbial fermentation in the hind gut, promoting the synthesis of short-chain fatty acids (SCFAs), particularly butyric acid (Jha et al., 2010), which serves as a primary energy source for colonocytes and strengthens the intestinal barrier **(Jha *et al.*, 2010)**. Additionally, the results demonstrated that supplementing the birds with eubiotic lignocellulose increased their blood total protein levels significantly. This elevation likely reflects enhanced protein digestibility and absorption, further supporting the link between improved gut health (villus lengthening, enzymatic activity) and nutrient availability. The positive findings of our investigations are supported by **Sarikhan *et al.* (2010)** and **Rahmatnejad & Saki (2016)**, who observed that adding 0.25% to 2% lignocellulose to the broiler diets increased weight gain and enhanced the FCR. Furthermore, our results were consistent with those of **Makivic *et al.* (2019)**, who found that 0.6% dietary lignocellulose enhanced growth performance by increasing nutrient digestibility, a core function of gut health. It was believed that the positive effects observed at lower inclusion levels of lignocellulose were directly due to increased nutrient digestibility and enhanced gut function (**Rahmatnejad and Saki 2016; Makivic *et al.*, 2019)**. It is unclear whether lignocellulose addition led to an increase or decrease in feed intake **(Liebl *et al.*, 2022)**. The response of chickens to feed intake and FCR is likely influenced by the chicken age together with the amount and kind of fiber supplement in the diet **(González-Alvarado *et al.*, 2007)**. The best FCR (T3) was obtained when feeding a basal diet that had been reformulated with 0.8% lignocellulose. This could be because broilers' body weight and feed intake are more affected by diet formulation than by lignocellulose **(Liebl *et al.*, 2022)**. These findings are supported by the studies of **Makivic *et al.* (2019)** and **Zeitz *et al.* (2019)**, which also highlight the importance of diet formulation when lignocellulose is added to diets. In an experiment conducted by **Makivic *et al.* (2019)**, 0.6% lignocellulose was integrated into the diet twice, substituting 0.3% corn and 0.3% soybean meal in one instance, and 0.6% soybean meal in another. The results showed substantial increases in body weight. Therefore, even slight modifications in feed formulation can impact the results and diminish their comparability with other feeding studies **(Makivic *et al.*, 2019; Liebl *et al.*, 2022)**. To completely understand if it promotes additional advantages in avian performance and gut health, further research is still needed on the inclusion of various quantities, kinds, mechanism of action and applications of lignocellulose in poultry diets**.**

The dietary supplementation with eubiotic lignocellulose across all experimental groups showed no significant variation compared to the control group concerning carcass traits like carcass dressing, breast yield, thigh yield, drum yield, and abdominal fat percentage. However, lignocellulose supplemented groups showed a reduction in the percentage of fat in the abdomen. This specific reduction is linked to gut-level lipid metabolism as dietary insoluble fiber like cellulose affects bile acid recycling, lipid absorption, and micelle formation within the intestinal lumen, impairing fat absorption and consequently reducing the amount of fat stored in the abdomen (**Jimenez-Moreno *et al.*, 2010; Hussien *et al.*, 2022)**. The current findings concur with those of **Zeitz *et al.* (2019)** and **Liebl *et al.* (2022)**, who revealed that supplementing broiler diets with lignocellulose didn't affect carcass parameters such as slaughter weight or carcass evisceration. **Zeitz *et al.* (2019)** suggested a potential mechanism involving reduced jejunal pro-inflammatory cytokine gene expression due to lignocellulose fermentation, which could contribute to overall gut health maintenance without altering carcass yield. Moreover, the quantity and type of dietary fiber ingested may also have an impact on carcass evaluation **(Montagne *et al.*, 2003)**. On the other hand, **Sarikhan *et al.* (2010)** showed that broiler diet supplementation with lignocellulose at concentrations of 0.50 and 0.75% significantly increased the dressing and breast yield as compared to the control group. The observed disparity may be due to differences in the extent of fermentation and solubility of the lignocellulose provided in this research, which could lead to variations in energy harvest and nutrient partitioning within the gut, thereby influencing carcass characteristics and abdominal fat deposition **(Liebl *et al.*, 2022)**.

Previous literature supports the interplay between dietary fiber and the gastrointestinal system health in poultry, including, gut structure, development, enzyme production, and enzymatic activity as well as the process of nutrient digestion and absorption **(Montagne *et al.*, 2003; Hetland *et al.*, 2005; Bogusławska-Tryk *et al.*, 2016; Röhe and Zentek 2021).** Our findings on blood biochemistry provide direct evidence of these gut-mediated effects. In contrast to the control group, the blood lipid profile of all experimental birds supplemented with eubiotic lignocellulose in this investigation revealed a substantial decrease in serum total cholesterol and triglycerides. These results verify the beneficial hypolipidemic effect, mechanistically driven by the non-fermentable fiber's capacity to bind bile acids in the intestinal lumen **(Bogusławska-Tryk *et al.*, 2016; Safaa *et al.*, 2014)**. This binding action may augment cholesterol elimination through fecal excretion and consequently reduce the levels of lipids in the bloodstream. Moreover, the notable increase in serum total proteins among both groups of broilers that received eubiotic lignocellulose, in comparison to the control group, is likely attributable to enhanced protein and amino acid digestibility and absorption in the gut **(Farran *et al.*, 2017)**. We speculate that lignocellulose may stimulate enzymatic activities critical for protein breakdown in the gut, including increased intestinal amino-peptidase and pepsin, as well as boosted pancreatic trypsin and chymotrypsin proteolytic activity **(Yokhana *et al.*, 2016; Farran *et al.*, 2017)**, leading to better amino acid uptake and systemic protein availability.

Our studies on the impact of eubiotic lignocellulose-on-top mirrored those conducted by **Sarikhan *et al.* (2010)**, who discovered that poultry fed diet containing lignocellulose at 0.25%, 0.5%, and 0.75% levels increased villus length and villus crypt ratio in the ileum. This morphological adaptation is a direct gut health response to insoluble fiber, likely mediated by its physical stimulation of the mucosa and regulation of digesta passage. Moreover, the increase in jejunal villi length observed in our reformulated group was consistent with **Sozcu (2019)**; using 2% lignocellulose further demonstrates the capacity of lignocellulose to enhance the intestinal absorptive surface area. The development of intestinal microarchitecture is significantly influenced by the amount and concentration of enteral nutrients **(Bedford 2000; Yamauchi 2007)**. **Bedford (2000)** and **Röhe (2020)** proposed that hens fed high-fiber diets may strive to increase the surface area of their mucosa to promote nutrient absorption. Therefore, the longer villi induced by lignocellulose supplementation are mechanistically linked to better intestinal nutrient absorption capacity and, consequently, increased nutrient utilization and growth performance **(Iji *et al.*, 2001; Röhe *et al.*, 2017)**. Similarly, **Jimenez-Moreno *et al.* (2010)** found that lower levels of insoluble fiber (pea hulls) improved performance and villus height: crypt depth ratio, while high levels (7.5%) decreased it, underscoring the importance of optimal inclusion levels for positive gut morphological effects.

In the current study, caecal *Clostridia* reported a relatively low count in T3 received diet reformulated with eubiotic lignocellulose. Nevertheless, no discernible variations were found between the bird groups. This variability highlights the complex interaction between fiber type, inclusion level, nutrient composition, and gut microbial fermentation dynamics. According to previous research **(Sun *et al.*, 2021)**, dietary eubiotic lignocellulose could relatively raise the quantity of the fiber-degrading bacteria, and 4% levels of lignocellulose boosted the concentrations of those butyric acid-producing bacteria, which are beneficial for intestinal cell integrity. According to **Zeitz *et al.* (2019)**, dietary fermentable lignocellulose enhanced the competitiveness of *Clostridiaceae* and raised its abundance 38-fold, potentially indicating enhanced fibrolytic activity but also raising questions about pathogenic risks within this family. **Kheravii *et al.* (2017)** stated that feeding 2% lignocellulose achieved significantly lower caecal *Clostridia* counts than 1% lignocellulose and was non-significantly lower than the control group. These contrasting results suggest that the effect on *Clostridia* is highly dependent on the specific lignocellulose source, inclusion level, and diet composition, influencing whether fermentation favors beneficial butyrate producers or other *Clostridia* members.

Results of NDV antibody were numerically higher in T3 supplemented with a diet reformulated with eubiotic lignocellulose, but the difference was not statistically significant. These findings agree with **Sozcu (2019)**, who observed no significant differences in IgM levels among groups that received lignocellulose at rates of 0.5, 1, or 2 kg/ton of feed and the control. This indicates that although lignocellulose improves gut barrier function and may influence local immunity, its effect on systemic humoral immunity, as measured by NDV antibodies, may be limited or variable.

Litter moisture and nitrogen levels did not show significant differences among different groups. Previous research stated that dietary fiber has the capacity to hold water, and that capacity depends on the type of fiber. While not significant in this study, **Kheravii *et al.* (2017)** and **Röhe *et al.* (2020)** observed that enhanced intestinal water absorption due to fiber could elevate the dry matter content of excreta, representing another gut-mediated mechanism influencing litter quality.

CONCLUSION

Eubiotic lignocellulose may play a beneficial role in the poultry industry. This type of lignocellulose acts as a dietary fiber source that can be fermented in the gut, potentially improving overall gut health and nutrient absorption. Adding 0.8% eubiotic lignocellulose to broiler diets has been shown to improve broiler growth, gut health, and metabolic efficiency. The current study found that including 0.8% eubiotic lignocellulose significantly enhanced broiler growth performance (body weight, weight gain, EPEF) and numerically improved FCR, whether added on top of the standard diet or incorporated through reformulation. However, reformulating diets to include lignocellulose (instead of top-dressing) is recommended for optimal FCR without increasing feed intake, thereby boosting profitability. Use lignocellulose as a natural alternative to antibiotics to enhance intestinal morphology (villi length), barrier function, and nutrient absorption, which can reduce reliance on antimicrobials. Eubiotic lignocellulose can also improve lipid profiles (lower serum cholesterol and triglycerides, reduce abdominal fat) and increase total protein levels to produce leaner meat aligned with consumer preferences. The trend toward drier litter (though not statistically significant here) could support better barn conditions and lower ammonia emissions. Although not statistically significant, reformulated lignocellulose (T3) numerically decreased caecal *Clostridia* counts and increased NDV antibody titers. Reformulation (T3) performed better than top-dressing (T2) in optimizing FCR and reducing *Clostridia*, though both methods were effective. Producers can choose either top-dressing (for simplicity) or reformulation (for precision) based on milling capabilities, ensuring broad applicability across operations. However, further research is needed to evaluate the effects of eubiotic lignocellulose in different formulations on broiler physiology, as well as biochemical and metabolic profiles. Incorporating eubiotic supplements into livestock diets can lead to improved health, better growth performance, and reduced reliance on antibiotics.

This results in healthier birds and potentially more sustainable livestock production systems.

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