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The Effect of Spirulina Supplementation Cultured in Different Nutrient Media on the Performance, Carcass Characteristics, Physiological Responses, and Economic Value of Broilers

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ABSTRACT

The high production cost of spirulina (*Arthrospira platensis)* presents a significant obstacle to its utilization as a feed supplement. This study aimed to evaluate the efficacy of alternative culture media specifically tempeh wastewater and chicken manure for spirulina production and to investigate the effects of its supplementation on the performance, carcass characteristics, physiological responses, and economic value in broiler chickens. A total of 200 unsexed Loughmann MB 202 broiler chicks were allocated into a Completely Randomized Design (CRD) with five treatment groups: T0 (Control), T1 (spirulina in NPK medium), T2 (spirulina in tempeh wastewater medium), T3 (spirulina in chicken manure medium), and T4 (Commercial spirulina). The results demonstrated that spirulina supplementation, particularly from the tempeh wastewater medium (T2) and commercial sources (T4), significantly ($P<0.05$) enhanced total body weight gain and improved the feed conversion ratio (FCR). Treatments T2 and T4 also yielded the highest carcass and breast meat percentages, while significantly reducing abdominal fat content, total cholesterol, LDL, HDL, and Malondialdehyde (MDA) levels in both blood and meat. Furthermore, nutrient digestibility and Income Over Feed Cost (IOFC) values were highest in the T2 and T4 groups, with no significant difference observed between them. It was concluded that spirulina cultured in tempeh wastewater possesses an efficacy equivalent to that of commercial spirulina in improving broiler performance, carcass characteristics, and physiological responses, thereby offering an economical and sustainable production alternative.

Key words: Broiler chickens, Spirulina, *Arthrospira platensis,* Tempeh wastewater, Oxidative stress, IOFC.

INTRODUCTION

Spirulina (*Arthrospira platensis*), a species of photosynthetic microalgae, has been recognized as a "superfood" (Podgórska-Kryszczuk 2024) owing to its exceptional nutritional profile, which includes 60-70% protein (Shao et al. 2019), a wide array of B vitamins, beta-carotene, essential minerals (Rashmi 2020; Sharma et al. 2024) and antioxidants such as phycocyanin and zeaxanthin (Citi et al. 2024). This rich nutritional composition establishes spirulina as an ideal candidate for a functional supplement in poultry feed (Kumar 2020; Tufan & Kutlu 2021). Its application in poultry has been demonstrated to enhance growth rates, productivity, and overall health (Deepika et al. 2021). The inherent antioxidants and bioactive compounds can bolster the immune system (Abotaleb et al. 2020), rendering poultry more resilient to diseases (Khafaga & El-Sayed 2018). Consequently, spirulina holds significant potential to replace synthetic antibiotic growth promoters (Rubel et al. 2019), aligning with the increasing consumer demand for safe, antibiotic-residue-free food products (Rafiq et al. 2022). Despite its numerous benefits, a primary constraint to the widespread adoption of spirulina in the livestock industry is its high production cost (Lane 2022), which encompasses cultivation, harvesting, and biomass

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processing (Kurpan et al. 2024). The use of commercial culture media, such as Bold's Basal Medium (BBM) or BG-11, is not only expensive but also poses potential environmental concerns (Ragaza et al. 2020; Lim et al. 2021). To overcome these challenges, innovation in more economical and sustainable cultivation systems is imperative. A promising approach involves the utilization of organic waste as an alternative nutrient source (Yuan et al. 2019; Doan et al. 2020).

Spirulina possesses a unique ability to thrive in diverse environments and utilize nutrients from wastewater (Doan et al. 2020). Food industry effluents, such as tempeh wastewater which is rich in organic matter (Puspawati & Soesilo 2018; Susi et al. 2022; Maro'ah et al. 2024). The tempeh industry wastewater is considered as the most effective medium for cultivating local microalgae. The success of this medium is attributed to its sufficient supply of essential nutrients, such as carbohydrates, protein, and fat, which serve as the primary energy source required for the microalgae's growth and cell division, ultimately leading to the highest population density among the tested mediums. While livestock waste like chicken manure, containing high levels of nitrogen, phosphorus, and potassium, represent potential, low-cost, and readily available media (Kumari 2024; Baishya et al. 2025). Previous research has indicated that other microalgae, such as *Chlorella*, can be optimally grown in food industry wastewater media (Widayat et al. 2018). In light of these considerations, this study aims to evaluate the effectiveness of using tempeh wastewater and chicken manure as alternative culture media for spirulina. Furthermore, this research investigates the influence of supplementation with spirulina produced from these various media on growth performance, carcass characteristics, and physiological responses, while also analyzing its economic value in broiler chickens.

MATERIALS AND METHODS

Ethical approval

All experimental procedures involving the use of animals were approved by the Center for Research and Community Service of the State Agricultural Polytechnic of Payakumbuh and were conducted in accordance with the Government of the Republic of Indonesia Regulation No. 95 of 2012 concerning Veterinary Public Health and Animal Welfare.

Location and time of study

The research was conducted at the Nutrition Laboratory and the Experimental Cage Unit (Farm) of the State Agricultural Polytechnic of Payakumbuh.

Animals, housing and management

A total of 200 unsexed Day-Old Chicks (DOC) of the Loughmann MB 202 broiler strain, with a uniform initial body weight (39-41g), were used in this study. The chickens were reared in an open-house raised-floor system with slatted floors. The housing was divided into 20 experimental pen units, each measuring 1mx1m and housing 10 chickens. Each pen was equipped with a manual feeder and drinker. A brooder (artificial heat source) was used during the brooding period (1-14 days of age).

A basal diet was formulated to meet the nutritional requirements of broiler chickens according to standard recommendations for each growth phase (pre-starter, starter, and finisher) and was provided *ad libitum*. The composition and proximate analysis of the basal diet are presented in Table 1. Drinking water was also provided *ad libitum* throughout the research period.

Spirulina culture and application

The preparation of culture media involved creating three types of nutrient stock solutions. First, a standard Nitrogen-Phosporus-Potassium (NPK) stock solution was made by dissolving 10g of Nitrogen fertilizer (Urea), 1g of Phosphorus fertilizer (TSP), and 5g of Potassium fertilizer (KCl) in 1L of distilled water. For the alternative media, a tempeh wastewater stock solution was prepared from the liquid residue of the soybean boiling process, while a chicken manure stock solution was made from an extract of finely ground and filtered dry chicken manure. Both alternative stock solutions derived from organic waste were then analyzed for their N, P and K content and subsequently diluted with distilled water until their concentrations were equivalent to the standard NPK stock solution. The nutrient composition of the three culture media is presented in Table 5.

Spirulina sp. was cultured in transparent containers under conditions of pH 6.5-7.5 and a temperature of 20-30°C. The culture medium was prepared by adding 1mL of the stock solution to each liter of water, resulting in a final nutrient concentration in the medium of 10mL/L N, 1mL/L P, and 5mL/L K. The culture was initiated with a spirulina inoculum to achieve an initial density of 1-2 x 10^5cells/mL. Cultivation was carried out for 10 days until the culture reached a stationary phase. The fresh spirulina culture (without biomass separation) was then mixed into the chickens' drinking water according to the treatment at a concentration of 10% (v/v).

Table 1: Composition and Analyzed Nutrient Content of the Basal Diet (g/kg)

Item	Pre- starter 1-15d	Starter 16-30d	Finisher 31-45d
Dry mater (g/kg)	879	876	879
Crude protein (g/kg)	205.16	190.43	181.13
Ether extract (g/kg)	23	31	36
Crude fiber (g/kg)	24	32	36
Lysine (g/kg)	12	12	11
Methionine (g/kg)	5	5	4
Ash (g/kg)	49	54	57
Calsium (g/kg)	9	8	8
Phosphorus (g/kg)	5	5	5
Apparent metabolizable energy (kcal/kg)	3.000	3.000	3.100

Experimental design and treatments

This study was designed using a Completely Randomized Design (CRD) consisting of five treatment groups with four replicates, where each replicate unit housed 10 chickens. The treatment period lasted from day 16 to day 45. The treatment was initiated on day 16 because the pre-starter phase (0–14 days) involves ongoing digestive and immune system development, making responses less stable. Beginning supplementation at day 16 ensures birds have entered the starter-finisher phase, where nutrient utilization is more consistent. The treatment groups were as follows: T0 as the negative control, receiving the basal diet and drinking water without supplements; T1, T2, and T3, which each received the basal diet plus drinking water containing 10% (v/v) spirulina culture from NPK medium, tempeh wastewater medium, and chicken manure medium, respectively; and T4 as the positive control, receiving the basal diet plus drinking water containing 0.1% (w/v) commercial spirulina powder.

Sample collection and analytical procedures

Performance parameters

Broiler performance data were collected during the treatment period from day 16 to day 45. The parameters observed included feed intake, body weight gain (BWG), and feed conversion ratio (FCR). Feed intake was calculated daily by subtracting the amount of remaining feed from the total feed provided, with the results being accumulated. Body weight gain was calculated as the difference between the final body weight on day 45 and the initial body weight on day 16, with weighing performed after a 12-hour fasting period. Subsequently, the feed conversion ratio was calculated by dividing the total feed intake by the total body weight gain during the treatment period using the formula:

$$FCR = Total\ Feed\ Intake\ /\ Total\ BWG$$

Carcass characteristics

Carcass analysis was conducted at the end of the study (day 45) by randomly selecting two chickens from each pen unit (a total of 8 chickens per treatment) whose body weights were close to the group average. The selected chickens were slaughtered, bled, de-feathered, and eviscerated. The parameters measured included carcass weight, which is the weight of the chicken after the removal of blood, feathers, head, feet, and internal organs (except for the lungs and kidneys). Subsequently, the carcass percentage was calculated using the formula:

$$Carcass\ Percentage = (Carcass\ Weight\ /\ Live\ Weight) \times 100\%$$

Additionally, the percentage of carcass parts was determined, where the weights of the breast, thigh, and visceral organs (liver, heart, spleen) were recorded and their percentages relative to the carcass weight were calculated.

Physiological responses

Physiological response analysis was conducted through several measurements. For blood analysis, blood samples (±5mL) were collected from one chicken per replicate on day 45 via the jugular vein using a sterile syringe and placed in tubes containing EDTA. Blood plasma was obtained by centrifugation at 3000 rpm for 15 minutes. From the plasma, the blood lipid profile was measured using an enzymatic colorimetric method with commercial diagnostic kits to determine the levels of triglycerides, total cholesterol, and HDL, while LDL levels were calculated using the Friedewald formula:

$$LDL = Total\ Cholesterol\ -\ HDL\ -\ (Triglycerides\ /\ 5)$$

Antioxidant activity was evaluated by measuring Malondialdehyde (MDA) levels as an indicator of lipid peroxidation in blood plasma and breast muscle tissue, using a reaction with TBA and TCA solutions, with absorbance read at a wavelength of 532nm. Furthermore, a nutrient digestibility trial was conducted from day 38 to 45 using one chicken per replicate placed in a metabolic cage. After a three-day adaptation period, feces were collected quantitatively for five consecutive days, and then feed and fecal samples were analyzed for their content of dry matter, crude protein, crude fat, and crude fiber according to AOAC (2005) methods.

Economic analysis

An economic analysis was performed by calculating the Income Over Feed Cost (IOFC) per chicken during the treatment period, using the formula:

$$IOFC\ \left(\frac{IDR}{Broiler}\right) = \left(Total\ BWG\ (kg) \times Live\ chicken\ price\ \left(\frac{IDR}{kg}\right)\right) - (Total\ feed\ intake\ (kg)\ \times\ Feed\ price\ (IDR/kg)).$$

Statistical analysis

All quantitative data obtained were analyzed using Analysis of Variance (ANOVA) for a Completely Randomized Design (CRD) with the aid of statistical software. If the ANOVA results indicated a significant difference among treatments (P<0.05), a Duncan's Multiple Range Test was performed to compare the mean values between treatments. Pearson correlation analysis was used to measure the relationship between variables.

RESULTS

Spirulina culture density

The different culture media significantly (P<0.05) supported the growth of Spirulina. As shown in Table 2, the cell densities in all culture media (T1, T2, T3) were significantly higher than the control (T0 and T4). Although there was no statistically significant difference among the three culture media, the tempeh wastewater medium (T2) yielded the highest numerical cell density.

Broiler performance

The effects of Spirulina supplementation on broiler performance are detailed in Table 2. There was no significant difference (P>0.05) in water consumption or feed intake across all groups. However, treatments had a highly significant effect (P<0.001) on body weight gain (BWG) and feed conversion ratio (FCR). The T4 and T2 groups showed the highest overall BWG and the lowest (most efficient) overall FCR, with no significant difference between them.

Table 2: Spirulina's Cell Density and Broiler Performance Parameters

Parameter	T0 (Control)	T1	T2	T3	T4	SEM	Pr>F
Spirulina's Cell Density (sel/mL)	0.00^{a}	13.73^{b}	15.64^{b}	14.51^{b}	0.00^{a}	3.782	*
Broiler Weight Gain (BWG)							
BWG Starter (g)	535.13^{a}	585.95^{b}	619.58^{b}	588.95^{b}	631.35^{b}	9.95	***
BWG Finisher (g)	1721.98^{a}	1733.08^{a}	1808.80^{b}	1746.78^{a}	1833.13^{b}	9.17	***
BWG Overall (g)	2502.05^{a}	2563.38^{b}	2672.38^{c}	2581.40^{b}	2709.68^{c}	16.87	***
Feed Intake							
Feed Intake Initial (g)	426.34	404.71	400.31	411.55	401.95	6.22	NS
Feed Intake Starter (g)	739.77	799.99	799.27	783.12	793.89	16.03	NS
Feed Intake Finisher (g)	3194.35	3193.06	3233.29	3200.94	3285.85	23.87	NS
Feed Intake Overall (g)	4360.44	4397.73	4432.84	4395.58	4481.41	26.37	NS
Feed Conversion Rate							
FCR Initial	1.74	1.65	1.64	1.67	1.64	0.027	NS
FCR Starter	1.38^{d}	1.37^{cd}	1.29^{ab}	1.33^{bc}	1.26^{a}	0.016	***
FCR Finisher	1.86^{b}	1.84^{b}	1.80^{ab}	1.83^{b}	1.79^{a}	0.016	*
FCR Overall	1.74^{c}	1.71^{b}	1.65^{a}	1.70^{b}	1.65^{a}	0.008	***
Water Consumption	7285.05	7299.3	7190.5	7205.03	7219.2	34.23	NS

Treatments consisted of T0=Control (without spirulina); T1=spirulina cultured in NPK medium; T2=spirulina cultured in tempeh wastewater; T3=spirulina cultured in chicken manure; T4=Pure spirulina powder. Means with different superscripts (a,b,c,d,e) in the same row are significantly different (P<0.05) by Duncan's Multiple Range Test. SEM=Standard Error of the Mean (Pooled). Pr>F=Significance level of the treatment effect. NS=Not Significant (P>0.05); *=P<0.05; **=P<0.01; ***=P<0.001.

Table 3: Broiler Carcass Characteristics (%)

Parameter	T0 (Control)	T1	T2	T3	T4	SEM	Pr>F
Whole Carcass	65.99^{a}	68.05^{b}	69.81^{c}	68.02^{b}	70.69^{c}	0.3	***
Breast Muscle	34.20^{a}	35.02^{b}	36.28^{c}	35.25^{b}	36.28^{c}	0.249	***
Tigh and Drumstick	22.80^{a}	23.54^{b}	25.36^{c}	23.89^{b}	26.50^{d}	0.319	***
Wing	11.42	11.29	11.95	11.62	12.06	0.366	NS
Proventriculus	0.51	0.52	0.51	0.51	0.51	0.008	NS
Gizzard	2.01	2	2.02	2	2.05	0.035	NS
Liver	2.28^{b}	2.25^{b}	2.08^{a}	2.08^{a}	2.09^{a}	0.022	***
Intestine	4	3.98	4.1	4.08	3.88	0.144	NS
Abdominal Fat	2.67^{c}	2.32^{b}	1.49^{a}	2.04^{b}	1.22^{a}	0.119	***
Spleen	4.80^{b}	3.50^{a}	4.53^{b}	3.28^{a}	3.20^{a}	0.257	***

Treatments consisted of T0=Control (without spirulina); T1=spirulina cultured in NPK medium; T2=spirulina cultured in tempeh wastewater; T3=spirulina cultured in chicken manure; T4=Pure spirulina powder. Means with different superscripts (a,b,c,d,e) in the same row are significantly different (P<0.05) by Duncan's Multiple Range Test. SEM=Standard Error of the Mean (Pooled). Pr>F=Significance level of the treatment effect. NS=Not Significant (P>0.05); *=P<0.05; **=P<0.01; ***=P<0.001.

Carcass characteristics

Spirulina supplementation significantly impacted most carcass characteristics (Table 3). Whole carcass, breast muscle and thigh percentages were significantly highest in the T4 and T2 groups. Conversely, abdominal fat percentage was significantly lowest in the T4 and T2 groups. The relative weight of the liver was also significantly lower in the T2, T3 and T4 groups compared to the control.

Physiological responses

All measured physiological parameters showed a highly significant response to the treatments (Table 4). Digestibility of crude protein, crude fat, and crude fiber was highest in the T4 and T2 groups. For the blood profile, T4 and T2 groups exhibited the most favorable outcomes, with the lowest levels of triglycerides, total cholesterol, LDL, and glucose, and the highest levels of HDL and total protein. Similarly, antioxidant activity was highest in the T4 and T2 groups, as indicated by the lowest MDA levels in both serum and meat.

Economic analysis

The economic analysis, measured by Income Over Feed Cost (IOFC), is presented in Table 4. The treatments had a highly significant effect (P<0.001) on profitability. The T4 and T2 groups generated the highest IOFC, with no significant difference between them.

DISCUSSION

Culture media effectiveness

The initial evaluation of culture media established that tempeh wastewater (T2) is a superior substrate for Spirulina proliferation, achieving the highest cell density (15.64 x 10^5 cells/mL) compared to inorganic NPK (T1) and chicken manure (T3). Although all media supported growth (P<0.05), the numerical superiority of T2 is attributed to its complex nutritional profile. Unlike T1, which provides only defined inorganic macronutrients, T2 contains dissolved organic carbon, essential amino acids, peptides, vitamins, and bioavailable trace minerals (Pakpahan et al. 2021; Pramaningsih et al. 2022). Despite equalized N-P-K concentrations across treatments, these organic components facilitate mixotrophic growth (Chaerun 2009; Pakpahan et al. 2021; Pramaningsih et al. 2022; Susi et al. 2022), enabling spirulina to simultaneously utilize photosynthesis and organic nutrient assimilation for energy (Chainapong et al. 2012). Furthermore, natural growth factors in the wastewater act as metabolic catalysts. In contrast, while T3 is nutrient-rich, its potential for compositional inconsistency and inhibitory compounds may limit maximal density. Thus, tempeh wastewater is validated not just as an alternative, but as a highly effective medium for biomass production.

Table 4: Broiler Physiological Response Parameters and Income Over Feed Cost

Parameter	T0 (Control)	T1	T2	T3	T4	SEM	Pr>F
Broiler Nutrient Digestibility (%)							
Crude Protein Digestibility	71.59^{a}	76.45^{b}	77.03^{cd}	76.87^{bc}	77.48^{d}	0.162	***
Crude Fat Digestibility	69.34^{a}	69.70^{a}	71.32^{b}	70.49^{a}	72.21^{c}	0.164	***
Crude Fiber Digestibility	29.17^{a}	31.77^{b}	34.48^{d}	33.09^{c}	34.69^{d}	0.331	***
Broiler Blood Profile							
Triglycerides (mg/dL)	66.95^{d}	57.75^{c}	54.35^{b}	57.64^{c}	52.74^{a}	0.331	***
Total Cholesterol (mg/dL)	152.52^{e}	139.97^{d}	128.61^{b}	136.48^{c}	126.02^{a}	0.769	***
LDL (mg/dL)	83.72^{e}	75.34^{d}	65.58^{b}	72.31^{c}	59.82^{a}	0.589	***
HDL (mg/dL)	41.96^{a}	43.07^{a}	48.00^{c}	45.06^{b}	50.77^{d}	0.393	***
Glucose (mg/dL)	142.14^{d}	132.78^{c}	125.33^{b}	131.62^{c}	122.74^{a}	0.605	***
Total Protein (g/dL)	3.00^{a}	3.34	3.63	3.16	3.47	0.079	NS
Broiler Antioxidant Activity							
Serum MDA (nmol/mL)	2.51^{c}	1.92^{b}	1.65^{a}	1.82^{b}	1.59^{a}	0.036	***
Meat MDA (nmol/mL)	1.34^{d}	1.15^{c}	1.04^{b}	1.14^{c}	0.95^{a}	0.021	***
IOFC (Rp)	36.29^{a}	37.71^{b}	40.48^{c}	38.23^{b}	41.14^{c}	0.35	***

Treatments consisted of T0=Control (without spirulina); T1=spirulina cultured in NPK medium; T2=spirulina cultured in tempeh wastewater; T3=spirulina cultured in chicken manure; T4=Pure spirulina powder. Means with different superscripts (a, b, c, d, & e) in the same row are significantly different (P<0.05) by Duncan's Multiple Range Test. SEM=Standard Error of the Mean (Pooled). Pr>F=Significance level of the treatment effect. NS=Not Significant (P>0.05); *=P<0.05; **=P<0.01; ***=P<0.001.

Table 5: Nutrient Composition (N,P,K) of NPK, Tempeh Wastewater, and Chicken Manure Media After Standardization

Culture Medium	N (mg L^{-1})	P (mg L^{-1})	K (mg L^{-1})	Notes
NPK Stock Solution	4.665	200.8	2.623	10g urea, 1g SP-36, 5g KCl per L
Tempeh Wastewater (raw)	2.927	125.4	1.253	Before concentration
Tempeh Wastewater (5:1 concentrated)	4.278	176.3	2.405	Within ±20% of NPK
Chicken Manure Extract (1:10 w/v)	3.524	164.2	2.050	Before concentration
Chicken Manure Extract (2:1 concentrated)	4.309	196.9	2.438	Within ±20% of NPK

Broiler growth performance

Spirulina supplementation via drinking water maintained consistent water consumption across all groups (P>0.05), indicating excellent palatability. In terms of growth metrics, the commercial powder (T4) and tempeh wastewater culture (T2) groups consistently delivered the highest Body Weight Gain (BWG) and the most efficient Feed Conversion Ratio (FCR) (P<0.01), significantly outperforming T1, T3, and the control (T0). The biological equivalence of T2 and T4 is driven by spirulina's high protein content (50-70%) (Gumbo & Nesamvuni 2017; Jiang et al. 2023), lipid reserves and rich antioxidant profile including vitamins B12 and pro-vitamin A (Sharoba 2014; Gumbo & Nesamvuni 2017; Farg et al. 2021). The superior FCR (1.65 for both T2 and T4) suggests a metabolic shift where improved gut health and enzymatic activity partition nutrients towards muscle synthesis rather than maintenance (Park et al. 2018; Ramukhithi et al. 2023; Irshad et al. 2024). Additionally, the higher feed intake observed in T4 reflects a robust physiological state where reduced oxidative stress facilitates optimal appetite signaling (Iqbal et al. 2004; Iqbal et al. 2005; Cimpean et al. 2025).

Carcass characteristics

The improvements in growth were mirrored in carcass quality. Supplementation significantly increased carcass yield and the composition of high-value cuts (P<0.01), with T4 and T2 groups exhibiting the highest percentages of breast muscle and thigh. This supports the concept of nutrient partitioning, where bioactive compounds modulate hormonal pathways to favor protein synthesis over adiposity (Lopez & Leeson 2008; Rafiq et al. 2022). Concurrently, abdominal fat was drastically reduced in T4 (1.22%) and T2 (1.49%) compared to the control (2.67%), aligning with spirulina's known hypolipidemic effects (Abed et al. 2023). Furthermore, the lower relative liver weight in treated groups indicates a reduced metabolic workload for detoxification. By lowering systemic oxidative stress, spirulina relieves the liver's burden (Allameh et al. 2023), suggesting the observed anatomical changes are driven by metabolic optimization.

Physiological responses

The observed phenotypic improvements are underpinned by significant enhancements in physiological markers. In Digestibility and Gut Health, Treatments T4 and T2 significantly increased the digestibility of crude protein, fat, and fiber (P<0.01). Spirulina likely functions as a prebiotic, stimulating beneficial gut microflora such as Lactobacillus (Park et al. 2018; Mamashli et al. 2025). These bacteria aid in fiber fermentation (So et al. 2018; Xu et al. 2022) and produce short-chain fatty acids that maintain intestinal epithelial health (Xiong et al. 2022), thereby expanding the surface area for nutrient absorption.

Blood Profile and Antioxidant Status, systemically, spirulina improved lipid profiles by lowering triglycerides and LDL while increasing HDL, and enhanced insulin sensitivity blood (Ji et al. 2020; Du et al. 2023), potentially by inhibiting cholesterol synthesis enzymes (Park et al. 2018). Crucially, Malondialdehyde (MDA) levels were significantly lower in T4 and T2 (P<0.01). This confirms potent antioxidant activity mediated by phycocyanin and superoxide dismutase (SOD), which neutralize free radicals and prevent lipid peroxidation (Park et al. 2018; Burhan et al. 2021).

The physiological data explains the performance outcomes. A strong negative correlation between meat MDA and BWG (r=-0.868) confirms that suppressing oxidative stress liberates metabolic resources (ATP, amino acids) for growth rather than repair (Tan et al. 2019; Rezar et al. 2023). Similarly, the correlation between MDA and feed intake (r=-0.685) suggests that reducing inflammation prevents cytokine-induced "sickness behavior," preserving normal appetite (He et al. 2018; Nawaz & Zhang 2021).

Economics

The biological benefits translated directly into economic advantages. Income Over Feed Cost (IOFC) analysis showed T4 and T2 groups generated the highest profitability (P<0.01), effectively offsetting the cost of supplementation. Importantly, T2 (tempeh wastewater culture) achieved economic parity with the expensive commercial product (T4). This demonstrates that utilizing waste-based media is not only a biologically effective strategy for sustainable broiler production but also an economically viable one.

Conclusion

Spirulina cultured in concentrated tempeh wastewater and chicken manure extract achieved nutrient levels comparable to the NPK medium and supported optimal microalgal growth. Supplementation of Spirulina, particularly from tempeh wastewater (T2) and commercial Spirulina (T4), significantly improved broiler body weight gain, feed efficiency, and carcass yield. These treatments also enhanced physiological responses by reducing lipid profile parameters and oxidative stress while increasing nutrient digestibility. Economic analysis showed that T2 and T4 generated the highest income over feed cost with no significant difference between them. Overall, Spirulina produced using tempeh wastewater provides an effective, economical, and sustainable alternative to commercial Spirulina for broiler production.

DECLARATIONS

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Conflict of Interest: The Authors declare that there is no conflict of interest.

Data Availability: All data supporting this study are stored at the Agricultural Polytechnic of Payakumbuh and can be accessed upon reasonable request.

Ethics Statement: All experimental procedures were reviewed and approved by the institutional ethics committee of the Agricultural Polytechnic of Payakumbuh and complied with national guidelines on animal welfare.

Author's Contribution: Salvia, Yeti Marlida, and Ilyanie H. were responsible for the conceptualization and study design. Salvia, Ramaiyulis, Muthia Dewi, Nadia Rahma, Dwi Ananta, and Andi Rifki Rosandy conducted the experiments and collected the data. Salvia, Yeti Marlida, and Kurnia Nastira Ningsih performed data analysis and interpretation. Salvia prepared the original draft of the manuscript, while all authors reviewed, revised, and approved the final version.

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