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Selection-Specific Bioactive Metabolites from *Vernonia hymenolepis* Wash Water Dictate Broiler Growth and Metabolic Efficiency in a Yeast-Dominant Fermentation System

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Abstract

The combined application of fermentation, phytobiotics, and endogenous probiotics offers a promising, sustainable alternative to conventional feed strategies, particularly for small-scale poultry production. This study evaluated the effects of fermenting a corn/soy diet using wash water (VhWW) derived from four distinct chemotypes of *Vernonia hymenolepis* (Green Bitter [GBVh, T4], Green Sweet [GSVh, T5], Purple Bitter [PBVh, T6], and Purple Sweet [PSVh, T7]) on broiler performance, nutrient utilization, and metabolism. A mandatory daily aeration step in the 72-hour staggered fermentation system created a novel Yeast-dominant, Lactic Acid Bacteria (LAB)-suppressed ecology across all VhWW treatments (≥8.00 $\log_{10}$CFU/mL *Saccharomyces cerevisiae*). This ecology conferred antimicrobial selectivity, achieving the elimination of *E. coli* in T4, T5, and T6. The PBVh selection (T6) yielded the highest Body Weight Gain (BWG: 2191.00 g, $P < 0.001$) and best Feed Conversion Ratio (FCR: 1.65, $P = 0.053$). However, its superior growth was decoupled from bulk nutrient absorption, as its corrected metabolizable dry matter (cMDM) was only intermediate. Conversely, the GBVh selection (T4) achieved the highest nutrient utilization (cMDM: 188.37 mg/g, $P < 0.001$) but one of the lowest BWGs, indicative of metabolic antagonism. This antagonism was supported by an adverse lipid profile (highest LDL and lowest HDL, $P < 0.001$). The PSVh selection (T7) induced metabolic stress (highest Relative Liver Weight, $P < 0.001$), poorest utilization, yet showed a repartitioning effect with the highest Relative Breast Yield (26.03%, $P < 0.001$). We conclude that the efficacy of VhWW fermentation is strain-dependent, with the PBVh selection maximizing performance by optimizing post-absorptive metabolic efficiency through bioactive metabolites derived from its unique phyto-microbial interaction.

Keywords

Fermentation, *Vernonia hymenolepis*, Chemotype, Phytobiotics, Yeast-Dominant, Metabolic Antagonism, Nutrient Decoupling, Broiler

1. Introduction

Human efforts to improve livestock feeds and feeding strategies have continuously evolved, driven by the need to enhance animal health, growth, productivity, and overall food production efficiency [1] [2]. This evolution has led to sophisticated, science-based approaches combining techniques such as fermentation, nutritional supplementation, and the use of feed additives [3] [4].

Fermentation is a critical advancement, enhancing a feed's nutritional profile by increasing nutrient bioavailability and breaking down anti-nutritional factors (ANFs) [5]. Concurrently, feed additives (e.g., prebiotics, probiotics, enzymes, phytobiotics) are used to optimize gut health and improve performance [6]. Despite the known synergy of these techniques, their combined application in developing novel feed resources-particularly via simple, small-scale methods—is scarcely documented [7]. Most studies on fermented feeds utilize plain water or incorporate specific, pure microbial cultures (e.g., *Lactobacillus*), which can be technically challenging and costly for small-scale producers [8].

A novel, practical alternative is to exploit the endogenous probiotic and phytobiotic potential locked within certain plant-based resources. Building on the concept of auto-detoxification observed in *Jatropha curcas* seed cake [9], the wash water derived from edible Vernonia species—such as *Vernonia hymenolepis* Wash Water (VhWW) [10] or *Vernonia amygdalina* Wash Water (VaWW) [11] offers a unique, low-cost platform. This wash water is known to possess prophylactic properties and retains numerous nutrients and phytochemicals [11] [12] effectively acting as both a natural supplement and feed additive.

Fermenting the precise daily feed ration with VhWW provides a unique opportunity to simultaneously explore the benefits of fermentation, supplementation, endogenous probiotics, phytobiotics, and precision feeding. Afui *et al.* [13] previously identified four distinct selections of *V. hymenolepis*: green bitter (GBVh), green sweet (GSVh), purple bitter (PBVh), and purple sweet (PSVh). However, it remains unclear whether the wash water derived from each specific selection will differentially influence the microbial ecology of the fermentation process or the subsequent growth, carcass yield, and metabolic response of broiler chickens fed the resulting diets.

This study, therefore, seeks to evaluate the effects of fermenting a standard corn/soy-based diet with wash water from these four selections of *V. hymenolepis* on the growth performance, carcass characteristics, nutrient utilization, and serum lipid profile of broiler chickens. The central goal is to identify a strain-specific VhWW selection that maximizes feed utilization and growth, providing a practical, sustainable, and integrated feeding strategy for small-scale poultry farmers. Figure 1 represents a graphical abstract of the study.

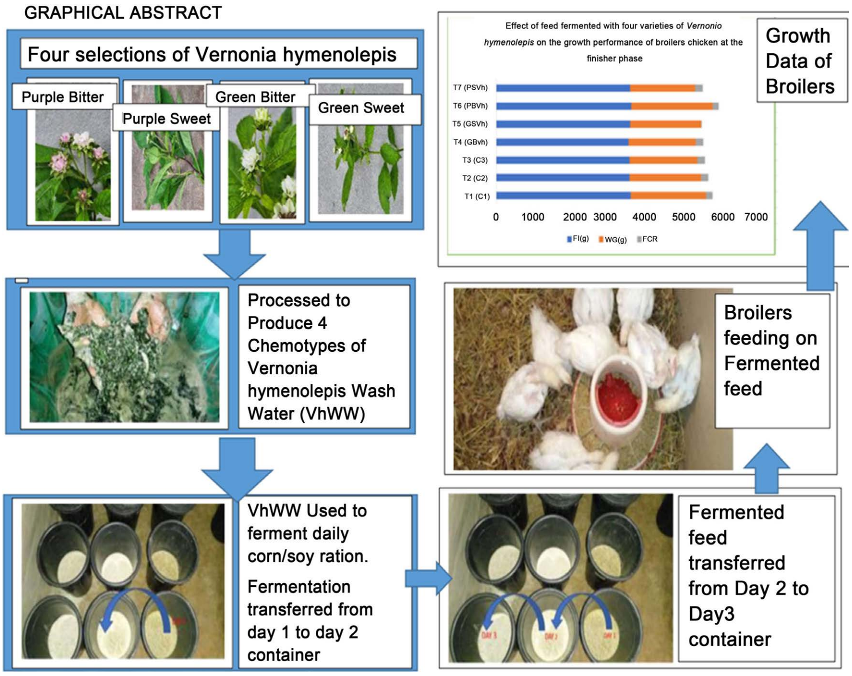

Figure 1. Selective efficacy of *Vernonia hymenolepis* wash water fermented corn/soy diets for broilers.

2. Methodology

2.1. Study Location and Duration

The 42-day feeding trial, including a 7 day acclimatization period, was conducted at the Faculty of Agriculture and Veterinary Medicine Teaching and Research Farm (FAVM-TRF), University of Buea, Cameroon (Latitude 4˚4' N-4˚12'N; Longitude 9˚9' E-9˚19'E). The site is characterized by a humid equatorial climate (altitude 870 - 1000 m a.s.l., annual rainfall 3000 - 5000 mm, temperature 20˚C - 30˚C).

2.2. Source and Preparation of *Vernonia hymenolepis* Wash Water (VhWW)

2.2.1. Source of *Vernonia hymenolepis* Selections

Four distinct selections were sourced from the established Vernonia unit at the FAVM-TRF [10]: Green Bitter (GBVh), Green Sweet (GSVh), Purple Bitter (PBVh), and Purple Sweet (PSVh).

2.2.2. Preparation of Wash Water

Leaves were harvested daily and prepared using a modified method [14] [15]. For 1000 g of fresh leaves:

1) Leaves were manually abraded (squeezed and rubbed) without water until foaming was observed.

2) Tap water was added sequentially in descending volumes (3000 mL, 2000 mL, 1000 mL, and 500 mL) to rinse out the foam.

3) For sweet varieties (GSVh, PSVh), rinsing ceased immediately upon foam clearance.

4) For bitter varieties (GBVh, PBVh), the process continued until the bitterness was barely perceptible to the human palate.

5) The total VhWW collected from the initial 1000 g of fresh leaves averaged 6000 ± 1500 mL across all selections and was used immediately for fermentation

2.3. Experimental Diets and Treatments

2.3.1. Preparation of Broiler Basal Diet

Starter (Days 1 - 21) and Finisher (Days 22 - 42) mash diets were formulated to meet NRC (1994) requirements (Table 1). Titanium dioxide (0.25%) was included in the finisher diet as an indigestible marker.

Table 1. Composition of the starter and finisher feed used in the experiment.

Ingredient	Starter (%)	Finisher (%)
Corn	55.20	61.00
Soybean meal	40.1	34.94
Dicalcium phosphate	2.80	2.00
DL-Methionine	0.15	0.06
Premix (vitamin/mineral)	1.50	1.50
Salt	0.25	0.25
Titanium dioxide	0.00	0.25
Total	**100**	**100**
Calculated Chemical composition		
ME* (Kcal/kg DM)	2962.00	3200.00
Crude protein (%DM)	23.00	20.00
Ether extract (%DM)	7.17	6.18
Crude fiber (%DM)	2.42	2.55
Methionine (%DM)	0.52	0.38
Ca (%DM)	1.00	0.90
Available P (%DM)	0.45	0.35

ME = Metobolisable Energy, CP = Crude Protein, CF = Crude Fiber. Premix* Premix Composition (per kg):Vit A 3000,000 UI, VIT D3 600,000 UI, Vit E 4000 mg, Vit K3 500 mg, Vit B1 320 mg, Vit B2 1000 mg, Vit B3 2400 mg, Vit B6 400 mg, Vit B12 7 mg, Vit PP/Niacin 4800 mg, Biotin 10 mg, Choline chloride 100,000 mg, Folic acid 160 mg, Copper (II) sulfate 200 mg, Zinc oxide 10,000 mg, Manganese oxide 1400 mg, Calcium iodate 200 mg, Methionine 200,000 mg, Iron sulfate 8000 mg, Sulfate 2000 mg.

2.3.2. Dietary Treatment Groups

A total of seven dietary treatments were tested (Table 2). All groups received the same basal diet, but differed in the fermentation medium and prophylactic coverage:

- T1 (Conventional Control): Fermented with tap water; received full conventional prophylactic protocol (detailed in Table 3).
- T2 (Positive Control): Fermented with tap water; received Oxy-tetracycline 80 (0.5 g/L water) + Vaccines.
- T3 (Negative Control): Fermented with tap water; received only tap water + Vaccines.
- T4-T7 (VhWW Test Diets): Fermented with respective VhWW selections (GBVh, GSVh, PBVh, PSVh); received only tap water + Vaccines.

Table 2. Description of treatment groups.

Treatment Code	Fermentation Medium	Prophylaxis via drinking water
T1: (Conventional Control)	Fermented with tap Water only	Conventional Prophylaxis Protocol (Table 3)
T2: Positive Control	Fermented with tap Water only	Oxyteracycline 80 at 0.5 g/L water + Vaccines
T3: Negative Control	Fermented with tap Water only	Tap water + Vaccines
T4: (GBVh)	Fermented with Green Bitter VhWW	Tap water+ Vaccines
T5: (GSVh)	Fermented with Green Sweet VhWW	Tap water+ Vaccines
T6: (PBVh)	Fermented with Purple Bitter VhWW	Tap water+ Vaccines
T7: (PSVh)	Fermented with Purple Sweet VhWW	Tap water+ Vaccines

Table 3. Conventional prophylactic protocol.

Day/Age	Type of Medication	Mode of Administration	Dosage	Function
1	Avinew (A), Bioral (B) and Galivac (G)	Beak dipping or Intra ocular	1000 D in 10 L	Prevention of NCD, IB and Gumboro
1 - 5	Anti-stress and vitamin	Drinking water	5 g in 5 L	Against stress
6 - 8	Antibiotic (oxy)	Drinking water	5 g in 2.5 L	Disease prevention
8	Vaccine; A, B, G	Drinking water	1000 D in 10 L	Booster against viral infection
8 - 10	Vitamin (Amin total)	Drinking water	5 g in 10 L	Growth promoter
11 - 13	Anti-coccidiosis	Drinking water	5 g in 10 L	Prevention of coccidiosis
14 - 16	Vitamin (Amin total)	Drinking water	5 g in 10 L	Growth promoter
17 - 19	Antibiotic (oxy)	Drinking water	5 g in 10 L	Anti-infectious
20 - 22	Vitamin	Drinking water	5 g in 10 L	Growth promoter
21	Vaccine; A, B, G	Drinking water	1000 D in 10 L	Booster against viral infection
23 - 25	Anti-coccidiosis	Drinking water	5 g in 10 L	Prevention of coccidiosis
26 - 29	Vitamin	Drinking water	5 g in 10 L	Growth promoter
30	Dewormer (anthelmintic)	Drinking water	5 g in 2.5 L	Against worms
35 - 37	Liver protector	Drinking water	1 ml in 1 L	Diuretic
38 - 42	Vitamin	Drinking water	5 g in 10 L	Growth promoter

Vaccine: A: Avinew, B: Bioral, G: Galivac, NCD; New castle disease, IB; Infectious bronchitis.

2.3.3. Fermentation of Experimental Diets

Fermentation followed a 72-hour continuous, staggered cycle adapted from Heres *et al.* [16], using a feed-to-liquid ratio of 1:3.4. Fermentation was conducted daily with the precise quantity of feed required for the next day's feeding, using three sets of containers (Day 1, Day 2, Day 3).

The daily manual transfer of the mash from one container to the next served as a mandatory aeration step, ensuring homogenization and maximal oxygen exposure throughout the 72-hour period. The decanted liquid from the Day 3 container was used to initiate the Day 1 cycle for the next batch, establishing a continuous fermentation culture.

2.3.4. Identification of Microbial Diversity in the Fermentation Media

Decanted fermentation fluid from the Day 3 containers (72 hours) was collected and serially diluted using sterile physiological saline. 100 µL of the appropriate dilutions was spread-plated on selective and non-selective media. Plates were incubated at 37˚C for bacteria (24 - 48 hours) and 25˚C for yeast (48 - 72 hours).

- Media Used: 5% Blood Agar, MacConkey Agar, Eosin Methylene Blue (EMB) Agar, Sabouraud Dextrose Agar (SDA), and Cysteine Lactose Electrolyte Deficiency (CLED) Agar.
- Identification: Bacterial colonies were characterized using morphological characteristics, Gram staining, and a battery of biochemical tests (e.g., Catalase test, Coagulase test, Indole, Citrate, Triple Sugar Iron) to identify genus and species. Yeast colonies were confirmed via Gram staining and characteristic morphology on SDA.
- Quantification: The number of viable colonies was counted on plates with 30 - 300 CFU and results were reported as $\log_{10}$CFU/mL using the formula:

$$\text{CFU/mL} = \frac{\text{Number of colonies} \times \text{Dilution factor}}{\text{Volume of inoculum } (\text{mL})}$$

The limit of detection (LOD) was set based on the lowest dilution (10^1) and volume plated (0.1 mL), resulting in:

- LOD for LAB (on CLED): 2.00 $\log_{10}$CFU/mL.
- LOD for E. coli (on EMB/MacConkey): 2.30 $\log_{10}$CFU/mL. Counts below this LOD were considered undetectable.

2.4. Experimental Design and Management

A total of 250 Ross 308 broiler day-old chicks were procured. Following acclimatization for 7 days on dry mash (non-fermented feed), 231 chicks were randomly assigned to seven dietary treatments in a Completely Randomized Design (CRD). Each treatment had three replicates of 11 birds each (7 treatments × 3 replicates × 11 birds = 231 birds). Fermented feed and the respective treatment water were offered *ad libitum*.

2.5. Nutrient Digestibility

To maintain maximal statistical power for the primary outcome (growth performance), the experimental unit (pen) was kept intact until the end of the main study (Day 42). The digestibility trial was subsequently conducted on a sub-sample of birds (n = 12 per treatment group) from Day 42 to Day 48 using the total excreta collection method. An additional "unfed" (blank) group was included to estimate basal endogenous nutrient losses. To prevent starvation-induced catabolism and ensure metabolic data was not skewed by muscle breakdown, birds in the "unfed" group were provided with a 5% glucose solution in their drinking water *ad libitum* as a non-protein energy source.

Protocol and Calculations

Birds were individually housed in metabolic cages for a 3-day acclimatization (Day 42 - 44), followed by a 4-day quantitative collection period (Day 45 - 48). Total excreta and feed consumption were measured. Nutrient utilization was calculated as:

- Apparent Digestibility Coefficient (ADC):

$$\text{ADC}(\%) = \frac{\text{Nutrient Input }(\text{g}) - \text{Nutrient Excreted }(\text{g})}{\text{Nutrient Input }(\text{g})} \times 100$$

- Nutrient Metabolizability Corrected for Endogenous Losses (MDc)

$$\text{MDc}(\text{mg/g}) \; = \frac{\text{Nutrient Input} - (\text{Nutrient Output FED} - \text{Nutrient Output UNFED})}{\text{Nutrient input }(\text{g})}$$

where Nutrient Output FED − Nutrient Output UNFED represents the estimated total endogenous nutrient losses from the unfed group [17].

2.6. Data Collection (Carcass, Organs, and Serum)

On Day 49, following a 6 to 8-hour fasting period, birds were euthanized. Data collected included:

- Carcass Characteristics: Dressed Weight (DW) and weight of primal cuts (Head + Neck, Thigh + Drumstick, Breast, Wing). Cuts were expressed as a percentage of Final Live Weight (LW).
- Organ Weights: Absolute and relative weights of gizzard, heart, lungs, liver, and intestine.
- Serum Lipid Profile: Blood samples were collected, and serum was analyzed for Total Triglycerides (TG), Total Cholesterol (TC), High-Density Lipoprotein (HDL), and Low-Density Lipoprotein (LDL).

2.7. Statistical Data Analysis

Data were analyzed using IBM SPSS Statistics, version 22. Assumptions of normality (Shapiro-Wilk) and homogeneity of variances (Levene's test) were verified. Treatment differences were assessed via One-Way Analysis of Variance (ANOVA). When Levene's test indicated heterogeneity ($P < 0.05$), Welch's ANOVA was applied. Post-hoc separation was performed using Duncan's Multiple Range Test (DMRT). Results are reported as Means, with significance set at $P < 0.05$. To enhance practical significance of the findings, Effect Sizes (Partial η^2) and 95% Confidence Intervals (95% CI) are reported for all parameters.

3. Results

3.1. Microbial Diversity in Fermentation Media

The comparative microbial load in the 72-hour fermented corn/soy diets is presented in **Table 4**. The observed microbial profile of the 72-hour fermented corn/soy media was characterized by a Yeast-dominant, Lactic Acid Bacteria (LAB)-suppressed ecology in the VhWW groups (T4 - T7).

Populations of *Lactobacillus* were below the limit of detection

(<2.00 $\log_{10}$CFU/mL) across all treatment groups (T1 - T7), indicating that the daily, mandatory aeration strategy failed to support the detectable growth and establishment of LAB.

Saccharomyces cerevisiae showed significant proliferation, particularly in VhWW treatments. T4, T5, and T6 (GBVh, GSVh, and PBVh) resulted in a high load of ≥8.00 log10CFU/mL, significantly exceeding the Tap Water Controls (T1 - T3: 4.00 $\log_{10}$CFU/mL). The maximum count reliably quantifiable was 8.00 $\log_{10}$CFU/mL.

The VhWW treatments demonstrated a substantial inhibitory effect on *E. coli*. Tap Water Controls (T1 - T3) showed high counts (6.00 $\log_{10}$CFU/mL), but T4, T5, and T6 achieved the greatest suppression, dropping *E. coli* below the detection limit (<2.30 $\log_{10}$CFU/mL). The T7 (PSVh) treatment was less effective, recording 2.00 $\log_{10}$CFU/mL.

Table 4. Comparative microbial load in the 72-hour fermented corn/soy diets by tap water and VhWW.

Treatment (Vh Selection)	*Lactobacillus* ($\log_{10}$CFU/mL)	*Escherichia coli* ($\log_{10}$CFU/mL)	*Saccharomyces cerevisiae* ($\log_{10}$CFU/mL)
T1: Conventional Control	<2.00	6.00	4.00
T2: Positive Control (Antibiotic)	<2.00	6.00	4.00
T3: Negative Control	<2.00	6.00	4.00
T4: Green Bitter Vh (GBVh)	<2.00	<2.30	≥8.00
T5: Green Sweet Vh (GSVh)	<2.00	<2.30	≥8.00
T6: Purple Bitter Vh (PBVh)	<2.00	<2.30	≥8.00
T7: Purple Sweet Vh (PSVh)	<2.00	2.00	6.00

Note: CFU = Colony-Forming Units. ($\log_{10}$CFU/mL) is the concentration of viable microorganisms in the media. <2.00 and <2.30 denote below the limit of detection. ≥8.00 denotes at or above the reliable quantification limit. The threshold for robust, beneficial microbial cultures in feed is generally considered > 6.00 $\log_{10}$CFU/mL.

3.2. Growth Performance

The comparative effect of corn/soy diets fermented with tap water and VhWW selections on broiler growth performance is presented in **Table 5**. Dietary treatments had a highly significant effect on Body Weight Gain (BWG) ($P < 0.001$), with a large effect size (Partial $\eta^2 = 0.589$).

The T6 group (PBVh) achieved the highest BWG (2191.00 g), which was statistically superior ($P < 0.05$) to T4 (GBVh) and T7 (PSVh), and numerically superior to all control groups. The T7 group (PSVh) recorded the lowest BWG (1742.33 g).

A significant association ($P = 0.053$) was observed between the treatment diet and Feed Conversion Ratio (FCR), with the best FCR (highest efficiency) observed in T6 (1.65), which was substantially better than the poorest FCR in T7 (2.02).

Feed Intake (FI) and Initial Weight showed no significant differences ($P > 0.8$), confirming successful randomization and comparable diet consumption.

Table 5. Comparative effect of corn/soy diets fermented with tap water and VhWW selections on broiler growth performance.

Parameter	T1: Conventional Control	T2: Positive Control	T3: Negative Control	T4: GBVh	T5: GSVh	T6: PBVh	T7: PSVh	95% CI Range (All Treatments)	ANOVA p-value	Effect Size (Partial η^2)
Initial Weight (g)	172.67	173.67	173.00	172.00	173.00	173.00	173.00	172.26 - 173.48	0.654	0.048
Body Weight Gain (g)	2033.33^{ab}	1913.66^{ab}	1817.00^{ab}	1811.00^{a}	1897.66^{ab}	2191.00^{b}	1742.33^{a}	1850.7 - 1963.7	<0.001*	0.589
Feed Intake (g)	3614.16	3594.66	3594.66	3557.50	3607.83	3626.16	3609.83	3581.1 - 3617.3	0.885	0.032
Feed Conversion Ratio	1.77	1.87	1.97	1.96	1.90	1.65	2.07	1.83 - 1.93	0.053	0.174

Note: Means sharing the same superscript within a row are not significantly different ($p > 0.05$). 95% CI = 95% confidence intervals range for all treatments; *Welch's ANOVA was used when Levene's test indicated a violation of the assumption of homogeneity of variances ($p < 0.05$).

3.3. Nutrient Utilization

The Effect of Corn/Soy Diets Fermented with Tap water and VhWW Selections on Corrected Metabolizable Nutrient Content are indicated in **Table 6**. Dietary treatments resulted in highly significant differences ($P < 0.001$) for all corrected metabolizable nutrient parameters (cMDM, cMOM, cMCP, and cMCC). The Partial η^2 values were exceptionally high (0.952 to 0.974), indicating that the *V. hymenolepis* selection overwhelmingly dictated nutrient utilization.

The T4 (GBVh) group demonstrated the highest metabolizable content for cMDM (188.37 mg/g), cMOM (186.47%), and cMCP (48.30±%). This group showed superior breakdown and absorption for all major nutrients. The T7 (PSVh) group recorded the lowest utilization for cMDM, cMOM, and cMCP, aligning with its poor growth performance.

The T6 (PBVh) diet, which delivered the best growth (Section 3.2), showed only intermediate cMDM and cMCP values, similar to the control groups. Furthermore, T6 and T7 recorded the lowest cMCC values (0.53 % and 0.35 %, respectively), suggesting these specific fermentations impaired the metabolization of cellulose.

Table 6. Effect of Corn/Soy diets fermented with tap water and VhWW selections on corrected metabolizable nutrient content.

Parameter	T1: Conventional Control	T2: Positive Control	T3: Negative Control	T4: GBVh	T5: GSVh	T6: PBVh	T7: PSVh	95% CI Range (All Treatments)	ANOVA p-value	Effect Size (Partial η^2)
Corrected Metabolisable Dry Matter (cMDM (mg/g))	154.76^b	151.41^b	151.41^b	188.37^c	137.05^a	151.90^b	133.40^a	148.27 - 156.33	<0.001*	0.952
Corrected Metabolisable Organic Matter (cMOM (%))	55.96^c	52.34^c	52.58^c	86.47^d	37.28^b	51.70^c	32.03^a	48.25 - 56.46	<0.001*	0.961
Corrected Metabolisable Crude Protein (cMCP (%))	43.57^d	42.13^{cd}	42.18^{cd}	48.30^e	39.53^c	40.87^c	34.87^a	40.28 - 42.64	<0.001*	0.974
Corrected Metabolisable Cellulose (cMCC (%))	2.13^c	2.00^{bc}	2.08^{bc}	2.23^c	1.72^b	0.53^a	0.35^a	1.29 - 1.76	<0.001*	0.956

Note: Means sharing the same superscript within a row are not significantly different ($p > 0.05$). 95% CI = 95% confidence intervals range for all treatments; *Welch's ANOVA was used when Levene's test indicated a violation of the assumption of homogeneity of variances ($p < 0.05$).

3.4. Carcass Characteristics and Yield

The Effect of Corn/Soy Diets Fermented with Tap water and VhWW Selections on carcass characteristics and yield of broiler chickens are shown in Table 7. The dietary treatments significantly influenced Live Weight ($P < 0.001$). T6 (PBVh) recorded the highest Live Weight (2364.00 g), consistent with its superior BWG. The T6 consistently recorded the highest absolute weights for all major primal cuts (Head + Neck, Thigh + Drumstick, Breast, and Wing), reflecting its overall larger size.

Despite having one of the lowest Live Weights, the T7 (PSVh) group achieved the significantly highest Relative Breast Yield (26.03%, $P < 0.001$, Partial $\eta^2 = 0.574$), which was statistically greater than all other groups. This suggests a repartitioning of muscle mass favoring the breast.

The T6 had the lowest Relative Head + Neck Yield (5.14% ± 0.01%), indicating a more commercially desirable proportion.

3.5. Organ Weights

The Effect of Corn/Soy Diets Fermented with Tap water and VhWW Selections on Organ Weights are indicated in Table 8. Highly significant differences were observed for absolute gizzard weight, absolute liver weight, relative liver weight, absolute lung weight, absolute intestine length, and relative intestine length ($P < 0.001$ for all).

The T6 (PBVh) recorded the highest absolute gizzard weight (68.66 g) and the highest absolute liver weight (46.00 g), significantly greater than most controls. This indicates enhanced organ mass, likely proportional to its superior body size and metabolic rate, or a physiological response to feed factors.

The T7 (PSVh) recorded the highest Relative Liver Weight (2.26%), suggesting possible liver hypertrophy (hepatomegaly) and metabolic stress, despite having the lowest Live Weight. This group also recorded the highest relative intestine length (117.26 mm/kg).

3.6. Serum Lipid Profile

The Effect of Corn/Soy Diets Fermented with Tap water and VhWW Selections on Broiler Serum Lipid Profile are indicated in Table 9. The dietary treatments significantly affected serum Triglycerides (TG) ($P = 0.002$), HDL ($P < 0.001$), and LDL ($P < 0.001$).

The T4 (GBVh) achieved the lowest TG concentration (122.03 mg/dL-a beneficial effect) but was simultaneously associated with the lowest HDL (21.03 mg/dL) and the highest LDL (73.36 mg/dL). The T3 (Negative Control) group recorded the highest TG concentration (193.93 ± 10.99 mg/dL), suggesting a baseline susceptibility to high lipid accumulation in the absence of intervention.

Table 7. Effect of Corn/Soy Diets Fermented with Tap water and VhWW Selections on carcass characteristics and yield of broiler chickens.

Parameter	T1: Conventional Control	T2: Positive Control	T3: Negative Control	T4: GBVh	T5: GSVh	T6: PBVh	T7: PSVh	95% CI Range (All Treatments)	ANOVA p-value	Effect Size (Partial η^2)
Live Weight (g)	2206.00^{ab}	2087.33^{ab}	1990.00^{ab}	1983.00^{a}	2070.66^{ab}	2364.00^{b}	1915.33^{a}	1841.9 - 1983.1	<0.001*	0.352
Slaughter Weight (g)	2025.33	1941.00	1.906	1801.66	1886.00	2069.66	1806.33	1698.4 - 1801.9	0.054	0.179
Dressed Weight (g)	1930.33	1870.66	1836.33	1725.33	1773.00	1929.66	1719.33	1608.2 - 1703.3	0.078	0.159
Head + Neck (g)	113.33^{b}	106.66^{ab}	110.00^{ab}	110.00^{ab}	113.33^{b}	112.66^{ab}	93.33^{a}	106.3 - 111.0	<0.001*	0.367
Thigh + Drumstick (g)	180.00^{ab}	170.00^{ab}	153.00^{ab}	161.66^{ab}	136.66^{a}	190.00^{b}	144.33^{a}	153.4 - 171.7	<0.001*	0.521
Breast (g)	443.33^{ab}	410.00^{a}	433.33^{ab}	400.00^{a}	423.33^{ab}	470.00^{b}	453.33^{ab}	416.3 - 438.4	<0.001*	0.574
Wing (g)	79.66^{ab}	75.66^{ab}	59.33^{ab}	68.33^{ab}	64.00^{ab}	85.66^{b}	54.00^{a}	64.5 - 73.8	<0.001*	0.532
Rel. Slaughter Weight (%)	91.14	92.41	90.63	89.96	90.29	86.56	93.75	89.5 - 91.6	0.054	0.179
Rel. Dressed Weight (%)	86.48	88.72	86.96	85.76	84.33	80.18	88.76	84.1 - 86.6	0.078	0.159
Rel. Head + Neck (%)	5.57	5.57	5.75	6.07	5.97	5.14	5.36	5.42 - 5.98	0.005*	0.367
Rel. Thigh + Drumstick (%)	8.85	8.88	8.00	8.93	7.20	8.67	8.29	7.90 - 8.80	0.003*	0.228
Rel. Breast (%)	20.10^{a}	19.65^{a}	21.78^{ab}	20.18^{a}	20.44^{a}	19.88^{a}	23.67^{b}	20.24 - 21.45	<0.001*	0.492
Rel. Wing (%)	3.92	3.95	3.10	3.77	3.37	3.91	3.10	3.32 - 3.95	0.013*	0.22

Note: Means sharing the same superscript within a row are not significantly different ($p > 0.05$). 95% CI = 95% confidence intervals range for all treatments; *Welch's ANOVA was used when Levene's test indicated a violation of the assumption of homogeneity of variances ($p < 0.05$).

Table 8. Effect of corn/soy diets fermented with tap water and VhWW selections on the internal organs.

Parameter	T1: Conventional Control	T2: Positive Control	T3: Negative Control	T4: GBVh	T5: GSVh	T6: PBVh	T7: PSVh	95% CI Range (All Treatments)	ANOVA p-value	Effect Size (Partial η^2)
Gizzard (g)	61.66^{ab}	58.00^{a}	58.00^{a}	58.66^{a}	62.00^{ab}	68.66^{b}	59.33^{a}	59.6 - 62.3	<0.001*	0.447
Rel. Gizzard (%)	3.03	3.03	3.03	3.24	3.27	3.13	3.41	3.07 - 3.31	0.154	0.121
Heart (g)	10.00	9.00	8.33	7.66	8.33	9.33	7.66	8.1 - 8.9	0.179	0.105
Rel. Heart (%)	0.49	0.47	0.44	0.42	0.44	0.43	0.44	0.42 - 0.47	0.179	0.105
Liver (g)	38.00^{a}	38.16^{a}	38.00^{a}	38.66^{a}	37.66^{a}	46.00^{b}	39.33^{a}	38.2 - 40.3	<0.001*	0.624
Rel. Liver (%)	1.87^{a}	1.99^{ab}	1.99^{ab}	2.13^{ab}	1.98^{ab}	2.10^{ab}	2.26^{b}	1.99 - 2.11	<0.001*	0.305
Lungs (g)	8.66^{ab}	8.66^{ab}	7.66^{a}	8.00^{ab}	8.00^{ab}	9.00^{b}	7.66^{a}	7.8 - 8.4	<0.001*	0.271
Rel. Lungs (%)	0.43	0.45	0.40	0.44	0.42	0.41	0.44	0.41 - 0.45	0.545	0.079
Intestine Length (mm)	201.33^{bcd}	207.66^{d}	192.66^{a}	195.00^{ab}	197.33^{abc}	207.00^{d}	204.33^{cd}	198.2 - 203.6	<0.001*	0.712
Rel. Intestine (mm/kg)	99.03^{bcd}	108.53^{d}	100.68^{a}	107.66^{ab}	104.00^{abc}	94.43^{d}	117.26^{cd}	102.1 - 107.3	<0.001*	0.712

Note: Means sharing the same superscript within a row are not significantly different ($p > 0.05$). 95% CI = 95% confidence intervals range for all treatments; *Welch's ANOVA was used when Levene's test indicated a violation of the assumption of homogeneity of variances ($p < 0.05$).

Table 9. Effect of corn/soy diets fermented with tap water and VhWW selections on broiler serum lipid profile.

Parameter	T1: Conventional Control	T2: Positive Control	T3: Negative Control	T4: GBVh	T5: GSVh	T6: PBVh	T7: PSVh	95% CI Range (All Treatments)	ANOVA p-value	Effect Size (Partial η^2)
Cholesterol (mg/dL)	150.93	154.50	146.10	172.80	137.06	154.46	134.73	142.77 - 157.49	0.060	0.150
Triglyceride (mg/dL)	155.06^{a}	182.93^{b}	193.93^{b}	122.03^{a}	153.00^{ab}	152.93^{ab}	152.93^{ab}	148.18 - 169.76	0.002*	0.267
HDL (mg/dL)	44.40^{b}	32.40^{ab}	21.46^{a}	21.03^{a}	21.50^{a}	29.70^{ab}	39.66^{ab}	26.97 - 33.07	<0.001*	0.330
LDL (mg/dL)	50.91^{ab}	55.23^{ab}	39.87^{a}	73.361^{c}	47.70^{a}	60.22^{bc}	54.04^{a}	48.70 - 57.26	<0.001*	0.365

Note: Means sharing the same superscript within a row are not significantly different ($p > 0.05$). 95% CI = 95% confidence intervals range for all treatments; *Welch's ANOVA was used when Levene's test indicated a violation of the assumption of homogeneity of variances ($p < 0.05$).

4. Discussion

The key objective of this study was to evaluate the efficacy of corn/soy fermentation using *Vernonia hymenolepis* wash water (VhWW) from four distinct *V. hymenolepis* selections as a novel, integrated feeding strategy. Our findings reveal a complex interplay between the specific VhWW selection, the resulting microbial profile, and the broiler's physiological response, demonstrating that the success of this strategy hinges not merely on bulk nutrient digestibility but on the resulting bioactive metabolites [18].

The observed suppression of Lactobacillus species was a consequence of the aeration strategy. However, many LAB strains are aerotolerant; thus, the total inhibition observed suggests that competitive exclusion by the hyper-proliferating yeast biomass and the specific antimicrobial components of the VhWW (such as sesquiterpene lactones) played a significant role in preventing LAB establishment,

(<2.00 $\log_{10}$CFU/mL) [19]. The minimum threshold for a culture to be considered as providing a robust, beneficial effect in fermented feed is often cited at >6.00 $\log_{10}$CFU/mL [20]; thus, the LAB population was negligible. Conversely, *Saccharomyces cerevisiae,* a Crabtree-positive yeast, continued the fermentation robustly in the presence of oxygen [21], facilitated by the "Pasteur Effect" [22], which describes an organism's capacity to switch its metabolism when conditions change from anaerobic to aerobic. The more efficient aerobic respiration pathway resulted in the massive proliferation (≥ 8.00 $\log_{10}$CFU/mL) in T4, T5, and T6. This established a novel Yeast-dominant, LAB-suppressed aerobic system.

Crucially, the VhWW system demonstrated a synergistic antimicrobial selectivity. Unlike conventional fermented liquid feed (FLF) where pathogen control relies almost entirely on LAB-generated organic acids, the VhWW system combines: 1) the inherent antimicrobial properties of *V. hymenolepis* phytochemicals (e.g., sesquiterpene lactones, saponins) effective against enteropathogens [12] [23], and 2) the yeast's competitive exclusion and potential production of fungicidal/bactericidal peptides [24]. The complete elimination of *E. coli* in T4, T5, and T6 validates this sophisticated, multi-pronged approach. Furthermore, The T7 selection failed to support robust yeast growth, recording only 6.00 $\log_{10}$CFU/mL compared to ≥ 8.00 in other VhWW groups. This failure is likely due to the "sweet" variety possessing a lower concentration of the fermentable precursors required to hit the "Pasteur Effect" threshold, linking fermentation efficacy directly to the specific botanical selection. The resulting weak fermentation failed to degrade anti-nutritional factors (ANFs), leading to the recorded hepatomegaly (highest Relative Liver Weight) as the liver underwent hypertrophy to process undegraded phytochemical toxicity

This superior microbiological control, achieved through a single, low-cost, non-technical intervention (daily manual aeration), is highly practical. It removes the necessity for expensive, commercially pure starter cultures required for many LAB-based FLF systems, making the PBVh fermentation a uniquely sustainable and integrated feeding technology suitable for small-scale poultry producers.

Another significant finding is the decoupling of nutrient utilization efficiency and actual growth performance based on the VhWW selection:

The T4 (GBVh) group achieved the highest metabolizable energy (cMDM, cMOM) and protein utilization (cMCP), indicating superior breakdown and absorption. Yet, its BWG was one of the lowest. This paradox suggests that while nutrients were efficiently absorbed, the specific fermentation products in T4 might contain factors—likely bitter compounds or secondary metabolites—that are metabolically antagonistic or growth-inhibitory at the cellular level, nullifying the realized growth potential [25]. The metabolic antagonism observed in the T4 (GBVh) group, characterized by high nutrient digestibility but poor growth, is likely driven by high concentrations of selection-specific phytochemicals, such as sesquiterpene lactones [12] [15]. While these compounds may enhance initial nutrient breakdown in the gut, literature suggests they can significantly interfere with hepatic lipid metabolism. Specifically, sesquiterpene lactones have been shown to modulate the activity of peroxisome proliferator-activated receptor alpha (PPARα) or interfere with the assembly of Very-Low-Density Lipoproteins (VLDL) in the liver [26] [27] and [28]. This provides a plausible biochemical mechanism for the observed dyslipidemia (highest LDL and lowest HDL), where absorbed carbon skeletons are diverted toward abnormal lipid cycling and hepatic stress rather than being channeled into protein synthesis for skeletal muscle deposition.

The T6 (PBVh) group, which delivered the best growth performance and FCR, showed only intermediate metabolizable energy and protein utilization. This demonstrates that the superior growth achieved by T6 is not solely due to increased bulk nutrient availability but rather to a specific, highly beneficial mechanism. This mechanism is likely mediated by the robust Yeast biomass, which supplies highly bioavailable amino acids, β-glucans, and B-vitamins, promoting growth and gut health, thus optimizing the use of absorbed nutrients (post-absorptive metabolic efficiency) rather than merely increasing total absorption [29]

The varying metabolic endpoints underscore the importance of strain-specific bioactive components: The T6 group's superior growth is corroborated by its proportional increase in organ size (absolute gizzard, liver, and lung weights). The significant increase in gizzard size often indicates higher physiological activity related to improved mechanical digestion, contributing to better feed efficiency [30] [31]. The combination of excellent growth, efficient FCR, and a highly hygienic Yeast-dominant fermentation makes the PBVh selection the most promising candidate.

The hypothesis of metabolic antagonism in the T4 (GBVh) group is strongly supported by its adverse Serum Lipid Profile. This group recorded the highest LDL ("bad cholesterol") and lowest HDL ("good cholesterol"). This suggests that while the feed was efficiently digested, the residual GBVh metabolites interfered with hepatic lipid homeostasis, diverting absorbed resources away from muscle synthesis toward abnormal or less productive lipid cycling pathways [32] [33]. In this context, the specific profile observed in T4 (GBVh) is an example of how chemotypes (chemical profiles of a plant genotype) and processing (fermentation) can create acquired dyslipidemia that is highly specific to a single metabolic pathway.

The T7 (PSVh) selection is deemed unsuitable due to its consistently poorest performance metrics. This poor outcome is further explained by the highest Relative Liver Weight (hepatomegaly), suggesting significant hepatic hypertrophy and metabolic stress [34]. This likely resulted from the liver working harder to detoxify potent anti-nutritional factors (ANFs) that were neither degraded by the selection's weak fermentation nor tolerated by the bird. Interestingly, despite the poor overall growth, the T7 group achieved the significantly highest Relative Breast Yield, suggesting a repartitioning effect where muscle deposition was disproportionately allocated toward the commercially valuable breast muscle relative to the total body mass [35].

5. Conclusions

The study demonstrates that corn/soy fermentation using *V. hymenolepis* wash water is a highly selective and strain-dependent method for modifying feed quality and broiler performance. The success of this strategy is contingent upon the resulting microbial ecology and the specific bioactive metabolites produced, which dictate whether absorbed nutrients are efficiently channeled toward muscle synthesis or diverted toward detoxification and maintenance.

The T6 (Purple Bitter Vh) selection is the most effective growth promoter, delivering the highest BWG and FCR, attributed to a robust, hygienic, Yeast-dominant fermentation system that promotes post-absorptive metabolic efficiency.

The T4 (Green Bitter Vh) selection, despite maximizing nutrient digestibility, failed to promote growth due to suspected metabolic inhibitors that adversely affected the LDL profile.

The T7 (Purple Sweet Vh) selection is unsuitable, failing to initiate effective fermentation, resulting in the poorest nutrient utilization, and inducing metabolic distress (high relative liver weight).

We conclude that the PBVh selection offers a viable, novel, and practical strategy for feed processing that maximizes broiler performance by enhancing nutrient utilization at the level of post-absorptive metabolism.

6. Recommendations

Focus on Purple bitter (PBVh) Elucidation: The PBVh selection should be advanced for further research, specifically focusing on its fermentation end-products (e.g., Short-Chain Fatty Acids, β-glucans, specific Yeast metabolites) to elucidate the precise mechanisms driving its superior growth.

Phytochemical Analysis: A detailed chemical fingerprinting study (e.g., HPLC-MS/MS) is required to compare the PBVh and GBVh wash waters post fermentation to identify the specific bitter compounds in GBVh responsible for the observed metabolic antagonism (high LDL) despite high digestibility.

Gut Morphology and Integrity: Future studies should investigate the effects of T6 on intestinal villus height, crypt depth, and tight junction protein expression to confirm the proposed improvement in gut health and integrity derived from the Yeast biomass.

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Data Availability

Data are available from the corresponding author, upon reasonable request.

Author Contributions

ED: Conceptualization; formal analysis; investigation; methodology; project administration; resources, supervision, validation, writing original draft, review and editing.

NLM: Conceptualization; formal analysis, investigation; project administration; resources supervision, validation writing original draft.

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NBA: Conceptualization Data curation, formal analysis, investigation, methodology, project administration, resources, validation, writing original draft.

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Consent to Publish

All Co-authors have given their full consent to publish.

Ethical Considerations

Unsexed Cobb500 broiler chickens were used as experimental animals for this study. The chickens were raised in standard pens and temperature and humidity were closely monitored using a thermo-hydrometer (model 288-ATH, SL Technologies). The experiments were carried out following the National Ethical Committee Guidelines (No. FWA-IRB00001954) and International (European Committee Council Directive of November 24, 1986 (86/69/EEC); Guide for the Care and Use of Laboratory Animals (U.S. National Research Council, 1996) for the care and use of laboratory animals. All efforts were made to minimize the suffering and stress of chickens used at each stage of the study. Ethical approval was given by the University of Buea Institutional Animal Care and Use Committee (UB-IACUC) via permit No.UB-IACUC No.49/2023.

Conflicts of Interest

The authors declare no competing interests in the research and the publication.

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