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Effect of Pre-Treating Dietary *Moringa oleifera* Leaf Powder with Fibrolytic Enzymes on Physiological and Meat Quality Parameters in Jumbo Quail

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Abstract: High fiber levels (165 g neutral detergent fibre (NDF)/kg DM) in *Moringa oleifera* leaf powder (MOLP) could limit its utilization as a nutraceutical source in Jumbo quail diets. Pre-treating MOLP with exogenous fibrolytic multi-enzymes could reduce the nutrient-encapsulating effect of non-starch polysaccharides and subsequently increase nutrient and bioactive compound utilization. Thus, this study investigated the effect of pre-treating dietary MOLP with an exogenous fibrolytic enzyme mixture on some physiological parameters and meat quality characteristics in Jumbo quail. A total of 396 Jumbo quail were randomly distributed to 6 experimental diets, with 6 replicate pens each and 11 birds per replicate. The experimental diets were: CON = a standard grower diet (156.5 g NDF /kg) without MOLP; ENZ0 = CON + 10% MOLP; and CON + MOLP pre-treated with 0.25% (ENZ25), 0.50% (ENZ50), 0.75% (ENZ75) and 1% (ENZ100) fibrolytic enzymes. There were no significant linear or quadratic effects on growth performance parameters and carcass characteristics in response to incremental levels of fibrolytic enzymes. However, neutrophils linearly increased, while breast meat lightness and 24 h hue angle linearly declined with enzyme levels. Quadratic effects were observed on gizzard weights and 1 h hue angle in response to enzyme levels. All the hemato-biochemical values fell within the normal ranges for healthy quail. It was concluded that the maximum fibrolytic multi-enzyme application rate of 1% may not have been adequate to enhance feed utilization and positively affect weight gain in Jumbo quail, thus higher levels may need to be investigated further.

Keywords: carcass characteristics; *Coturnix coturnix*; feed additives; growth performance; phytogenic plants

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1. Introduction

In the South African poultry sector, Jumbo quail (*Coturnix coturnix*) farming is a new venture [1] that can substantially contribute to food and nutrition security, as well as economic growth, if sustainably developed. Jumbo quail is the largest and fastest-growing meat-type breed of quail, which has been recently developed from the traditional Japanese quail line. The birds are noted for early attainment of sexual maturity (six weeks of age), high reproductive rates (±300 eggs per year), disease resistance and low feed requirements (35–45 g/day/bird) [2]. However, the growth of the poultry sector has generated additional pressure on feed ingredients and increased the competition between humans and birds for conventional nutrient sources, such as maize and soybean. Therefore, emerging quail farmers struggle to profitably meet the birds' nutrient requirements for maximum production [2]. *Moringa oleifera* leaves have been found to contain substantial amounts

of protein, amino acids, n-3 fatty acids, minerals, and vitamins [3], as well as beneficial bioactive compounds. The moringa foliage has been used as an organic additive in animal and human diets to supply biologically active substances, such as carotenoids, ascorbic acid, and phenolic compounds [4]. In addition, moringa leaves are a rich source of antioxidants [5], which can be used to enhance quail meat quality and control inflammation [6]. The extract of MOLP contains tannins, flavonoids, and glycosides that have medicinal properties [5]. In south Asia and some parts of Africa, moringa leaves are used to treat a variety of diseases [7], while their consumption has been reported to improve nutrient absorption, increase immunological response, strengthen immune functions, and promote health in broiler chickens due to high concentrations of micronutrients and polyphenols [8]. In addition, the leaves have been shown to improve quality and oxidative stability of broiler meat due to their high antioxidant activity [7,9]. However, the presence of antinutritional factors, such as tannins, saponins, phytate, lectins, and cyanogenic glucosides, in moringa [10] can compromise utilization of nutrient and bioactive compounds and, consequently, quail performance. Indeed, other scholars have reported that the inclusion of MOLP in quail diets should not exceed 25 g/kg due to the presence of antinutritional factors [11,12].

Moreover, high fiber (NDF) content (165 g/kg DM) in moringa leaves negatively affects nutrient availability [13,14] and limits their level of inclusion in simple non-ruminant diets [15]. High intake of dietary fiber has been reported to reduce nutrient digestibility and cause acute toxicosis and hepatocellular damage to birds [16], suggesting a need to ameliorate the negative effect of fiber to allow the birds to fully benefit from the bioactive components of MOLP. Indeed, birds, such as the quail, have no capacity to produce the enzymes, such as beta-glucanase, hemi-cellulase, and cellulase, needed to digest β-glycosidic linkages in non-starch polysaccharides present in moringa leaves [17]. Fiber reduces nutrient intake and growth rate and may compromise biochemical parameters when included at high levels in animal diets [18]. Indeed, dietary fiber is known to increase intestinal transit time and intestinal viscosity, which reduces diffusion and assimilation rates of nutrients and beneficial bioactive compounds in birds [19].

One potential solution to this problem is the use of exogenous fibrolytic enzymes to break down the cell wall components in moringa leaves prior to their inclusion in quail diets. Exogenous fibrolytic enzyme supplementation reduces the intestinal viscosity and the nutrient encapsulating effect of cell walls, and it subsequently increases protein and energy utilization [20]. The use of fibrolytic enzymes could enhance the utility of MOLP as a source of nutrients and bioactive compounds in quail diets by reducing the antinutritional activities of fiber in moringa and thus promote improved blood parameters. Furthermore, using fibrolytic enzymes could allow MOLP to be included at higher amounts in Jumbo quail diets without negatively affecting their productivity and meat quality. Therefore, this work evaluated the effect of pre-treating MOLP with graded levels of a fibrolytic multi-enzyme on feed utilization and physiological and meat quality responses in Jumbo quail. We tested the hypothesis that pre-treatment of MOLP with fibrolytic enzymes would improve growth performance, blood parameters, and meat quality attributes in Jumbo quail.

2. Materials and Methods

2.1. Study Resources

The experiment was carried out from March to April 2021 at Molelwane Farm (25°86′00″ S; 25°64′32″ E) of the North-West University (Mafikeng, South Africa), with ambient temperatures ranging from 27 °C to 35 °C. Fresh green *M. oleifera* leaf powder was bought from Origin Organics Investments (PTY) LTD in Centurion, South Africa. Viscozyme® L is a multi-enzyme complex containing a wide range of carbohydrases, including arabanase, cellulase, *β*-glucanase, hemicellulase, and xylanase. It has an enzyme activity of 100 fungal *β*-glucanase per gram and a density of 1.2 g/mL and was supplied by Sigma-Aldrich

(Modderfontein, Gauteng, South Africa). The other feed ingredients were bought from Nutroteq (PTY) LTD in Centurion, South Africa.

2.2. Enzyme Treatment of Moringa and Analyses

In our previous work, the inclusion of dietary MOLP beyond 2.5% compromised growth performance in Japanese quail [11]. Thus, in this study, a higher level of 10% MOLP was pre-treated with Viscozyme® L at the rate of 0, 0.25, 0.50, 0.75, and 1% before being incorporated into a standard quail diet. Exactly 6 kg of milled (2 mm; Polymix PX-MFC 90D, Kinematica AG, Switzerland) MOLP per treatment was sprayed and hand mixed with 6000 mL of distilled water in which 12.5, 25, 37.5, and 50 mL of Viscozyme® L (density: 1.2 g/mL) was dissolved [21]. The untreated MOLP (6 kg) was sprayed with 6000 mL of distilled water only. During this mixing process, efforts were made to avoid the leaching of MOLP chemical components by ensuring that no excess liquid ran off the samples in partially opened containers [22]. Treated and untreated MOLP were then stored at room temperature (average 30 °C) for 24 h to allow time for the enzyme to predigest fiber [22] in MOLP. Thereafter, the untreated and treated MOLP were sun dried until constant weight and then crushed (2 mm) before being used in diet formulation. The nutrient composition (Table 1) of untreated and enzyme-pre-treated MOLP was determined using the methods by the Association of Official Analytical Chemists [23] for dry matter (DM), ash, organic matter (OM), and crude protein (CP). The fiber detergent method by Van Soest et al. [24] was used to determine the neutral detergent fiber (NDF) and acid detergent fiber (ADF). Metabolizable energy (ME) was calculated using the following equation, $ME = 0.821 \times DE\ (MJ)$, by Khalil et al. [25].

Table 1. Nutrient composition (g/kg DM, unless stated otherwise) of untreated and fibrolytic enzyme pre-treated *Moringa oleifera* leaf powder.

	[1] Substrates				
Nutrients	**MENZ0**	**MENZ25**	**MENZ50**	**MENZ75**	**MENZ100**
Dry matter (g/kg)	918.4	921.4	925.8	913.9	910.1
Ash	9.51	9.55	9.43	8.68	8.08
Organic matter	827.3	825.8	823.3	820.5	892.2
Metabolizable energy (MJ/kg)	11.9	11.9	11.9	11.8	11.9
Crude protein	217.0	213.0	213.2	212.8	206.0
Neutral detergent fiber	165.4	161.3	160.4	155.5	159.6
Acid detergent fiber	147.2	148.2	146.6	145.7	141.9
Acid detergent lignin	134.7	132.5	131.9	130.1	133.2

[1] Substrates: MENZ0 = MOLP without enzyme pre-treatment; MENZ25 = MOLP pre-treated with 0.25% fibrolytic enzyme; MENZ50 = MOLP pre-treated with 0.50% fibrolytic enzyme; MENZ75 = MOLP pre-treated with 0.75% fibrolytic enzyme; MENZ100 = MOLP pre-treated with 1% fibrolytic enzyme.

2.3. Diet Formulation

Six experimental diets, in a mash form, were formulated by hand to meet the nutritional requirements for grower quails, as stated by the National Research Council [26], by incorporating treated and untreated MOLP into a standard grower diet as follows: a standard grower diet without MOLP (CON); a standard grower diet containing 10% MOLP without enzyme pre-treatment (ENZ0); and a standard grower diet containing 10% MOLP pre-treated with 0.25% (ENZ25), 0.50% (ENZ50), 0.75% (ENZ75), and 1% fibrolytic enzymes (ENZ100), as shown in Table 2. The nutrient composition of the dietary treatments was analyzed as described above for the untreated and enzyme-treated MOLP.

Table 2. Gross ingredient and nutrient composition of the dietary treatments.

	[1] Diets					
	CON	**ENZ0**	**ENZ25**	**ENZ50**	**ENZ75**	**ENZ100**
Ingredients (g/kg *as fed* basis)						
Viscozyme® L	0.0	0.0	2.5	5	7.5	10
Moringa oleifera leaf powder	0.0	100.0	100.0	100.0	100.0	100.0
Yellow maize fine	698.6	626.9	626.9	626.9	626.9	626.9
Prime gluten 60	18.0	18.0	18.0	18.0	18.0	18.0
Full-fat soya powder	50.7	148.6	148.6	148.6	148.6	148.6
Soybean powder	196.7	70.5	70.5	70.5	70.5	70.5
Limestone powder	14.5	14.5	14.5	14.5	14.5	14.5
Mono calcium phosphate	7.2	7.2	7.2	7.2	7.2	7.2
Salt fine	3.2	3.2	3.2	3.2	3.2	3.2
Sodium bicarbonate	1.7	1.7	1.7	1.7	1.7	1.7
Choline powder	0.8	0.8	0.8	0.8	0.8	0.8
Lysine	2.8	2.8	2.8	2.8	2.8	2.8
L-Threonine	0.4	0.4	0.4	0.4	0.4	0.4
Methionine	1.9	1.9	1.9	1.9	1.9	1.9
Grower phytase	1.7	1.7	1.7	1.7	1.7	1.7
Vitamin and mineral premix [2]	0.5	0.5	0.5	0.5	0.5	0.5
Olaquindox antibiotic	0.4	0.4	0.4	0.4	0.4	0.4
Nutrient composition (g/kg DM, unless stated otherwise)						
Dry matter (g/kg)	913.6	917.9	919.9	922.2	926.7	929.0
Ash	4.66	4.34	4.58	4.53	4.63	4.16
Organic matter	864.9	861.6	867.9	872.8	873.8	877.5
Metabolizable energy (MJ/kg)	11.9	11.8	11.8	11.8	11.8	11.8
Crude protein	187.2	187.7	188.6	188.4	187.7	188.8
Neutral detergent fiber	156.5	155.3	148.3	152.2	154.1	154.3
Acid detergent fiber	141.1	144.1	142.3	149.2	144.1	148.7
Acid detergent lignin	131.3	138.6	134.2	134.4	134.4	136.2

[1] Diets: CON = standard quail grower diet without *Moringa oleifera* leaf powder (MOLP); ENZ0 = control diet containing 10% MOLP without enzyme pre-treatment; ENZ25 = control diet containing 10% MOLP pre-treated with 0.25% fibrolytic enzymes; ENZ50 = control diet containing 10% MOLP pre-treated with 0.50% fibrolytic enzymes; ENZ75 = control diet containing 10% MOLP pre-treated with 0.75% fibrolytic enzymes; ENZ100 = control diet containing 10% MOLP pre-treated with 1% fibrolytic enzymes; [2] Premix: vitamin A (11000 IU), vitamin B1 (2.5 mg), vitamin E (25 IU), vitamin D3 (2500 IU), vitamin K3 (2.0 mg), vitamin B6 (5.1 mg), vitamin B2 (4.5 mg), niacin (30 mg), folic acid (0.7 mg), pantothenic acid (10 mg), biotin (0.12 g), magnesium sulphate (100 mg), copper sulphate (8.0 mg), zinc sulphate (79 mg), ferrous sulphate (80 mg), potassium iodide (0.34 mg), and sodium selenite (0.25 mg).

2.4. Ethics Statement and Feeding Trial

The care and use of the birds were approved (NWU-01884-19-S5) by the Animal Production Ethics Committee of the North-West University, and the feeding trial was conducted in a manner that avoided unnecessary discomfort to the birds. Mixed-gender Jumbo quail chicks (n = 396; one-week old) were purchased from Golden Quail Farm (Randfontein, South Africa). In a completely randomized design, the chicks were distributed to 36 replicate pens (experimental unit), such that the experimental diets were represented 6 times per pen. The pens, each holding 11 chicks, were built using wire mesh (30 cm H × 60 cm W × 100 cm L), and polythene plastics were used as bedding. The birds were accustomed to the experimental treatments in their replicate pens until they were two weeks old, and stress packs (consisting of soluble vitamins and electrolytes) were given for the first three days. The quail house had an average temperature of 30 °C and humidity of 60%, which was monitored daily. During the four-week experiment, the birds had unlimited access to the experimental diets and fresh, clean water, and rearing was performed under natural lighting (from 06 h00 am to 18 h00 pm). Initial live weights (170.9 ± 4.40 g) were recorded at two weeks of age and then measured weekly until six weeks of age by weighing all the birds in a pen to establish average weekly body weight gain (ABWG). From week 2 to week 6, the average weekly feed intake (AWFI) was calculated by subtracting the

weight of feed refusals from the weight of feed supplied. Weight gain was divided by feed consumed to calculate feed conversion efficiency (FCE). No mortalities were recorded in this study, giving a 100% survival rate.

2.5. Slaughtering Procedure and Blood Analyses

At day 42 of age, all the birds in each experimental unit were weighed to determine final body weight (FBW) and then transported to a local poultry abattoir (Rooigrond, North West, South Africa), where they were stunned and then slaughtered by cutting the jugular vein. About 2 mL of blood samples were randomly collected from two birds per experimental unit and immediately transferred into whole blood tubes (with EDTA) and serum tubes for analyses of hematological and serum biochemical parameters using the Automated Hematology Analyzer and the Automated Vet Test Chemistry Analyzer from IDEXX Laboratories (Pty) Ltd. (Gauteng, South Africa), respectively. The blood was handled, processed, and stored as described by Washington and van Hoosier [27]. For hematological analyses, the blood samples were stored in a cooler box containing ice packs and analyzed within 48 h of collection, whereas for serum analyses, the samples were centrifuged after clotting and then analyzed [28].

2.6. Carcass Characteristics and Internal Organs

All the carcasses were initially weighed to determine hot carcass weight (HCW) and then re-weighed after chilling in a cold-room for 24 h to obtain cold carcass weight (CCW). Carcass yield was calculated as the proportion of HCW on FBW. Weights of all carcass parts (breast, wing, drumstick, and thigh) and internal organs (liver, gizzard, proventriculus, and small intestine) were measured using a digital weighing scale (Explorer® EX224, OHAUS Corporation, NJ, USA) and expressed as proportion of the HCW.

2.7. Meat Quality Parameters

All the carcasses in this study were used to measure meat quality parameters. Color coordinates of breast meat (L^* = lightness, a^* = redness, and b^* = yellowness) were taken 1 h and 24 h post-mortem using a color spectrophotometer (CM 2500c, Konika Minolta, Osaka, Japan) according to the Commission Internationale de l'Eclairage [29]. Hue angle and chroma values were calculated using a^* and b^* [30]. Meat pH was measured on the pectoralis major muscle 1 h and 24 h post-mortem using a Corning Model 4 pH-temperature meter (Corning Glass Works, Medfield, MA, USA), which was calibrated using standard pH solutions after each experimental unit.

Breast meat samples were pre-weighed and then cooked to a core temperature of 75 °C to determine cooking loss Honikel [31]. Raw breast meat samples were sheared using a Meullenet-Owens Razor Shear Blade (A/MORS) installed in a Texture Analyzer to estimate shear force (TA.XT plus, Stable Micro Systems, Surrey, UK). Grau and Hamm [32] filter-paper press method was used to assess the water-holding capacity (WHC) of breast meat slices under pressure of 60 kg.

2.8. Data Analysis

Coefficients for linear and quadratic effects of enzyme levels were determined using response surface regression procedure of SAS (Proc RSREG; SAS, [33]). CON data were not used in the regression analysis. Weekly feed intake, weight gain, and feed conversion efficiency were analyzed using the repeated measures analysis option in the general linear models of SAS [33] to determine the interaction effects between diet and time (in weeks). Data for overall feed intake, physiological, and meat quality parameters were analyzed using one-way ANOVA (PROC GLM; SAS, [33]), with diet as the only factor. Significance was declared at $p < 0.05$, and treatment means were separated using the probability of difference option in SAS.

3. Results

Repeated measures analysis did not show significant week × diet interaction effects on average weekly body weight gain (p = 0.184) and average weekly feed conversion efficiency (p = 0.417) but on average weekly feed intake (p = 0.011). Table 3 shows that there were neither linear nor quadratic responses ($p > 0.05$) for average weekly feed intake, overall weigh gain, and overall FCE to incremental levels of fibrolytic enzymes. Similarly, no enzymatic effects ($p > 0.05$) were observed on overall weight gain, overall FCE, and feed intake in weeks 3, 4, 5, and 6.

Table 3. Effect of pre-treating dietary *Moringa oleifera* leaf powder (MOLP) with incremental levels of fibrolytic enzymes on growth performance (g/bird) in Jumbo quail (n = 396).

	[1] Diets							*p* Value	
[2] Parameters	CON	ENZ0	ENZ25	ENZ50	ENZ75	ENZ100	[3] SEM	Linear	Quadratic
Overall BWG	148.8	137.6	146.3	145.9	138.9	144.5	4.625	0.651	0.452
Overall FCE	0.203	0.187	0.203	0.197	0.189	0.200	0.005	0.525	0.587
Average weekly feed intake									
Week 3	139.5	139.2	143.1	140.5	143.8	140.0	3.564	0.082	0.443
Week 4	150.7	157.6	151.3	151.5	154.2	160.2	3.750	0.492	0.054
Week 5	232.2	229.1	223.5	231.8	230.0	223.4	3.781	0.712	0.447
Week 6	209.2	205.8	202.6	214.0	206.7	195.5	5.043	0.337	0.093

[1] Diets: CON = standard grower diet without MOLP; ENZ0 = control diet containing 10% MOLP without enzyme pre-treatment; ENZ25 = control diet containing 10% MOLP pre-treated with 0.25% fibrolytic enzymes; ENZ50 = control diet containing 10% MOLP pre-treated with 0.50% fibrolytic enzymes; ENZ75 = control diet containing 10% MOLP pre-treated with 0.75% fibrolytic enzymes; ENZ100 = control diet containing 10% MOLP pre-treated with 1% fibrolytic enzymes; [2] Parameters: BWG = body weight gain; FCE: feed conversion efficiency; [3] SEM = standard error of the mean.

Table 4 indicates that there were neither linear nor quadratic effects ($p > 0.05$) for all the hemato-biochemical parameters, with the exception neutrophils [y = 1.18 (±0.875) + 0.037 (±0.039) x; R^2 = 0.206, p = 0.017], which linearly increased with enzyme levels. Similarly, significant dietary effects were observed only on neutrophils, where birds on diet ENZ100 (4.47×10^9/L) had higher ($p < 0.05$) neutrophil levels than those on diets ENZ0 and ENZ75. Nonetheless, the CON diet promoted similar ($p > 0.05$) neutrophils levels as all the other dietary treatments.

Table 5 shows that there were neither significant linear nor quadratic effects for carcass characteristics and internal organ weights of Jumbo quails as enzyme treatment level increased. However, a quadratic effect was observed for gizzard weight [y = 2.02 (±0.075) + 0.009 (±0.003) x − 0.00008 (±0.00003) x^2; R^2 = 0.181, p = 0.023] in response to incremental levels of the enzymes. Similarly, no dietary effects ($p > 0.05$) were observed on carcass characteristics and internal organ weights of the birds.

Table 6 shows that there was a significant quadratic response for breast meat hue angle measured 1 h post-mortem [y = 1.17 (±0.025) − 0.002 (±0.001) x + 0.00002 (±0.00001) x^2; R^2 = 0.166, p = 0.027] in response to enzyme treatment levels. Significant linear decreases for breast meat lightness (L^*) [y = 50.6 (±0.873) − 0.125 (±0.041) x; R^2 = 0.340, p = 0.0001] and for breast meat hue angle measured 24 h post-mortem [y = 1.06 (±0.033) − 0.001 (±0.001) x; R^2 = 0.187, p = 0.019] were observed as fibrolytic enzyme levels increased. Diet CON promoted a lower 1-hour hue angle value (1.02) than all the other diets whose hue angle value did differ ($p > 0.05$). Birds on diet CON had a higher 24-hour hue angle than birds on diets ENZ0, ENZ50, ENZ75 and ENZ75, which had statistically similar hue $angle_{24}$ values.

Table 4. Effect of pre-treating dietary *Moringa oleifera* leaf powder (MOLP) with incremental levels of fibrolytic enzymes on blood parameters in Jumbo quail (n = 72).

	[1] Diets							*p* Value	
Parameters	**CON**	**ENZ0**	**ENZ25**	**ENZ50**	**ENZ75**	**ENZ100**	**[2] SEM**	**Linear**	**Quadratic**
Hemoglobin (g/dL)	6.25	9.20	8.66	12.0	7.60	9.05	1.17	0.211	0.779
Neutrophils ($\times 10^9$/L)	3.36 ^{ab}	0.980 ^{b}	2.69 ^{ab}	3.26 ^{ab}	1.39 ^{b}	4.47 ^{a}	1.153	0.017	0.827
Monocytes ($\times 10^9$/L)	0.558	0.243	1.23	1.15	0.301	0.825	0.505	0.675	0.340
Eosinophils ($\times 10^9$/L)	0.826	1.002	0.773	0.282	0.407	0.669	0.340	0.237	0.862
Basophils ($\times 10^9$/L)	0.040	0.147	0.110	0.063	0.043	0.101	0.068	0.554	0.259
Glucose (mmol/L)	6.74	7.87	11.6	12.1	6.70	6.56	2.775	0.285	0.105
Phosphorus (mmol/L)	4.74	5.00	4.93	4.20	4.71	4.55	0.306	0.158	0.246
Total protein (g/L)	54.5	57.5	74.1	65.0	66.6	54.5	10.56	0.530	0.074
Albumin (g/L)	29.0	18.4	22.7	19.4	19.0	17.3	3.272	0.289	0.172
Globulin (g/L)	35.0	38.9	51.4	47.9	47.5	40.6	8.747	0.984	0.076
Alkaline phosphatase (U/L)	123.5	232.9	197.7	141.6	149.5	201.3	44.46	0.329	0.084
Amylase (U/L)	224.7	282.0	336.7	263.8	292.6	460.3	86.20	0.167	0.220
Lipase (U/L)	332.3	238.7	244.1	220.8	201.0	198.7	31.13	0.068	0.878

[a,b] Means in the same row with different superscripts are significantly different ($p < 0.05$); [1] Diets: CON = standard grower diet without MOLP; ENZ0 = control diet containing 10% MOLP without enzyme pre-treatment; ENZ25 = control diet containing 10% MOLP pre-treated with 0.25% fibrolytic enzymes; ENZ50 = control diet containing 10% MOLP pre-treated with 0.50% fibrolytic enzymes; ENZ75 = control diet containing 10% MOLP pre-treated with 0.75% fibrolytic enzymes; ENZ100 = control diet containing 10% MOLP pre-treated with 1% fibrolytic enzymes; [2] SEM = standard error of the mean.

Table 5. Effect of pre-treating dietary *Moringa oleifera* leaf powder (MOLP) with incremental levels of fibrolytic enzymes on internal organs and carcass characteristics (g/100 g HCW, unless stated otherwise) of Jumbo quail (n = 396).

	[1] Diets							*p* Value	
Parameters	**CON**	**ENZ0**	**ENZ25**	**ENZ50**	**ENZ75**	**ENZ100**	**[2] SEM**	**Linear**	**Quadratic**
Final body weight (g)	239.5	229.8	232.5	230.5	226.6	230.2	5.163	0.725	0.995
Hot carcass weight (g)	150.1	143.8	146.5	138.6	146.9	139.2	2.968	0.359	0.682
Cold carcass weight (g)	146.3	139.5	141.3	137.1	142.1	134.4	2.970	0.305	0.365
Carcass yield (%)	62.8	62.6	63.0	60.1	64.9	60.5	1.493	0.648	0.751
Breast	17.0	16.4	17.0	17.6	15.0	16.4	0.701	0.400	0.577
Wing	4.37	4.70	4.91	4.79	4.73	4.80	0.137	0.988	0.676
Thigh	6.19	6.22	7.60	6.57	6.30	6.23	0.484	0.455	0.298
Drumstick	4.28	4.76	4.63	4.48	4.20	4.50	0.157	0.086	0.259
Gizzard	2.20	1.99	2.22	2.24	2.18	2.13	0.104	0.598	0.023
Liver	2.89	2.69	2.84	2.90	2.85	2.64	0.385	0.501	0.532
Proventriculus	0.614	0.610	0.598	0.609	0.696	0.534	0.064	0.501	0.533
Small intestine	4.28	3.45	4.16	3.52	3.47	4.60	0.564	0.684	0.379

[1] Diets: CON = standard grower diet without MOLP (control diet); ENZ0 = control diet containing 10% MOLP without enzyme pre-treatment; ENZ25 = control diet containing 10% MOLP pre-treated with 0.25% fibrolytic enzymes; ENZ50 = control diet containing 10% MOLP pre-treated with 0.50% fibrolytic enzymes; ENZ75 = control diet containing 10% MOLP pre-treated with 0.75% fibrolytic enzymes; ENZ100 = control diet containing 10% MOLP pre-treated with 1% fibrolytic enzymes; [2] SEM = standard error of the mean.

Table 6. Effect of pre-treating dietary *Moringa oleifera* leaf powder (MOLP) with incremental levels of fibrolytic enzymes on breast meat quality parameters in Jumbo quail (n = 396).

	[1] Diets							*p* Value	
[2] Parameters	CON	ENZ0	ENZ25	ENZ50	ENZ75	ENZ100	[3] SEM	Linear	Quadratic
pH_1	5.83	5.83	5.87	5.81	5.97	5.94	0.056	0.055	0.648
L^*_1	46.4	51.3	48.4	48.1	47.3	48.9	1.270	0.164	0.084
a^*_1	5.41	5.37	5.64	5.98	5.83	5.27	0.346	0.989	0.092
b^*_1	8.89	12.5	12.0	11.8	11.0	12.4	0.507	0.484	0.127
$Chroma_1$	10.4	13.6	13.3	13.3	12.4	13.5	0.500	0.466	0.313
Hue $angle_1$	1.02 ^{b}	1.16 ^{a}	1.12 ^{a}	1.10 ^{a}	1.08 ^{a}	1.16 ^{a}	0.027	0.702	0.027
pH_{24}	5.69	5.84	5.86	5.85	5.83	5.84	0.026	0.534	0.738
L^*_{24}	54.0	50.7	47.7	46.5	45.7	45.9	5.125	0.000	0.057
a^*_{24}	8.10	8.21	7.48	9.07	9.19	10.19	0.868	0.057	0.561
b^*_{24}	10.1	14.8	13.0	13.7	14.2	13.5	0.495	0.437	0.264
$Chroma_{24}$	13.0	16.9	15.0	16.4	17.0	17.2	0.783	0.338	0.255
Hue $angle_{24}$	0.895 ^{b}	1.06 ^{a}	1.04 ^{ab}	0.988 ^{a}	0.998 ^{a}	0.950 ^{a}	0.034	0.019	0.947
Cooking loss (%)	19.7	17.8	12.9	16.3	20.2	18.3	2.410	0.211	0.403
Shear force (N)	3.26	2.85	2.51	2.69	3.10	3.53	0.468	0.197	0.319
WHC (%)	87.3	88.2	87.9	87.1	83.5	85.8	1.631	0.102	0.734

^{a,b} Means in the same row with different superscripts are significantly different ($p < 0.05$); [1] Diets: CON = quail grower diet without MOLP (control diet); ENZ0 = control diet containing 10% MOLP without enzyme pre-treatment; ENZ25 = control diet containing 10% MOLP pre-treated with 0.25% fibrolytic enzymes; ENZ50 = control diet containing 10% MOLP pre-treated with 0.50% fibrolytic enzymes; ENZ75 = control diet containing 10% MOLP pre-treated with 0.75% fibrolytic enzymes; ENZ100 = control diet containing 10% MOLP pre-treated with 1% fibrolytic enzymes; [2] Parameters: L^* = lightness; a^* = redness; b^* = yellowness; WHC = water-holding capacity; [3] SEM = standard error of the mean.

4. Discussion

Moringa foliage has the potential to be used as a source of bioactive compounds and nutrients in quail diets. However, the amount of the leaf meal that could be added to quail diets is limited by its high fiber content, among other factors [11]. High levels of dietary fiber have detrimental effects on digestion, absorption, and utilization of nutrients and bioactive compounds in birds [19,34]. Thus, this study used a fibrolytic multi-enzyme pretreatment to alleviate the negative effects of fiber in dietary moringa leaf meal. Repeated measures analysis revealed a significant week × diet interaction effect only on the average weekly feed intake, indicating that the dietary effect on feed intake was not consistent as the birds grew older. It was expected that birds fed the standard diet containing MOLP pre-treated with fibrolytic enzymes would have higher overall feed intake. This is due to the ability of fibrolytic enzymes to improve the digestibility of fiber-rich diets, allowing the birds to ingest more feed [35]. The results, however, did not support this hypothesis, as there were no variations in feed intake across the experimental diets. Similarly, enzyme pre-treatment of MOLP had no effect on weight gain and feed conversion efficiency. These findings are in line with those of Huseein et al. [36] who studied growth, carcass characteristics, and meat quality of broilers and recorded no effect on weight gain and feed conversion ratio in birds fed a low-energy diet supplemented with a multi-enzyme. Contrary to these results, Hana et al. [37] and Hajati et al. [38] reported that adding multi-enzymes to standard poultry diets significantly improved body weight gain and feed conversion ratio and ileal digestibility of crude protein in broilers.

Blood parameters are crucial in determining the pathophysiological status and the quality and safety of feed ingredients for farm animals [1,39]. Due to the presence of antinutritional components, such as condensed tannins and fiber, it was hypothesized that MOLP would have deleterious impact on hematological and biochemical parameters. However, no diet-induced changes were observed for all hematological parameters, except neutrophils, which linearly increased in response to enzyme pre-treatment levels. Nonetheless, the CON diet promoted similar neutrophils levels as all the other dietary treatments, indicating that the increase in neutrophil levels was not induced by moringa.

Indeed, the concentration of blood parameters found in this study were within normal ranges for healthy quail birds [1,11]. Similarly, no variations in serum total protein or liver enzyme alkaline phosphatase were observed, with all reported values falling within the normal ranges for adult Japanese quail [39,40], indicating that the diets had no negative effect on bird health.

In this study, the size of the gizzards showed a quadratic response by first increasing and then decreasing with enzyme levels. Theoretically, the consumption of high fibrous diets induces changes in the size of intestines and gizzards in birds as an adaptation mechanism [21]. According to Musa et al. [41], consuming high-fiber feed causes gizzard enlargement, which leads to improved muscular grinding of feed particles and higher nutrient digestibility. These findings also reveal that fibrolytic enzymes have no influence on carcass yields and any of the carcass parts, which is consistent with the results by Saleh et al. [42], who found a similar lack of fibrolytic enzyme effect on broiler carcass yields. Carcass yield is affected by genetics, feed, slaughtering conditions, body weight, and gender of birds [43,44]. The effects of fibrolytic enzymes on breast, wing, thigh, and drumstick weights are consistent with those reported by Hajati et al. [38] in broilers given untreated and multi-enzyme-treated diets. The growth of the liver, breast, and proventriculi was not affected by the enzyme-treated MOLP diets. When injured, the liver produces enzymes, such as alanine aminotransferase (ALT) and alkaline phosphatase (ALKP), which are released into the circulation [45]. Elevated levels of these serum enzymes are associated with altered hepatic membrane permeability, which can arise as a result of circulatory hypoxia, exposure to toxins and toxemia, inflammation, metabolic abnormalities, or hepatocyte growth [45]. As a result, normal levels of these enzymes in quail fed MOLM-containing diets reflect normal quail liver and intestinal processes, implying that the moringa is a safe feed ingredient.

Enzyme pre-treatment of dietary MOLP resulted in a linear decrease in breast meat lightness and hue angle measured 24 h post- slaughter and a quadratic response for breast meat hue angle measured 1 h post-slaughter. The increased oxidative stability of breast meat seen after pre-treatment with dietary MOLP could be attributed to MOLP's antioxidant capabilities [10]. *Moringa oleifera* leaf powder is high in metals, including selenium, manganese, copper, and zinc, all of which are important in the action of antioxidant enzymes. In addition, tocopherol, ascorbic acid, carotenoids, polysaccharide, flavonoids, saponins, phenolics, tannins, and proanthocyanidins are among the natural antioxidant compounds found in MOLP [10,46]. In contrast, Kumanda et al. [21] found no differences in meat lightness between broilers given red grape pomace pre-treated with a fibrolytic enzyme mixture and those fed an untreated control diet. One of the most critical factors influencing meat quality indicators, such as cooking loss, tenderness, drip loss, and WHC, is the pH value of the meat [47]. The pH drop after slaughter is measured by the ultimate pH. The range is dictated by the amount of glycogen in breast muscle prior to slaughter and the rate at which the remaining glycogen is converted to lactic acid after slaughter [48]. Diets had no effect on meat pH, shear fore, cooking loss, drip loss, or water-holding capacity, measured 24 h post-mortem in this study. The lack of dietary effects suggests that enzyme-treated MOLP has the potential to promote normal oxidative stability for meat quality characteristics during storage. The shear force is an objective measure of meat toughness, with a lower shear force value suggesting tenderer meat [49]. However, there was no effect of food on shear force values in this investigation, even though shear force values tended to increase with rising enzyme-treated MOLP levels, implying that increased dietary enzyme-treated MOLP inclusion may have made the flesh tougher. The ability of meat to retain water is referred to as its water-holding capacity. It is a critical quality parameter that influences the amount of water lost during transit, storage, processing, and cooking [50]. Juices are expelled during cooking as a result of protein denaturation and muscle shrinkage [51]. In this investigation, there was no difference in WHC, cooking loss, or drip loss, indicating that substituting untreated or pre-treated MOLP did not impact meat quality.

5. Conclusions

The results showed that pre-treating MOLP with fibrolytic enzymes influenced neutrophils, gizzard weights, and meat color indicators but not growth performance and carcass parameters in Jumbo quail. The fibrolytic multi-enzyme application rate of 1% may not have been adequate to enhance feed utilization and positively affect weight gain in Jumbo quail; thus, higher levels may need to be investigated further.

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