



Article

The Effect of Green Tea (Camellia sinensis) Leaf Powder on Growth Performance, Selected Hematological Indices, Carcass Characteristics and Meat Quality Parameters of Jumbo Quail

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Abstract: Green tea leaves contain a wide range of active bio-compounds that are essential for sustainable quail intensification; however, its feed value is not known for the Jumbo quail. Therefore, this study evaluated the effect of different levels of green tea leaf powder (GTLP) on physiological and meat quality parameters of the Jumbo quail. One-week-old chicks (n = 350; 56.1 ± 2.12 g live-weight) were evenly distributed to 35 replicate pens and reared on five experimental diets formulated as follows: a standard grower diet with zinc-bacitracin (PosCon), a standard grower diet without zinc-bacitracin (NegCon), and NegCon diet treated with 10 (GT10), 25 (GT25) and 50 g/kg (GT50) of GTLP. Weight gain linearly decreased in week 2 but increased in week 4, whereas feed conversion efficiency linearly declined in weeks 2 and 3 as GTLP levels increased. Overall feed intake, carcass yield, and caecum and colon weights showed a linear increase with GTLP levels. Hematological parameters fell within the normal ranges reported for healthy quail. The GT10 group showed larger liver weights than the PosCon and NegCon groups. It was concluded that dietary inclusion of GTLP enhances overall feed intake and carcass performance but not feed efficiency, hematological and meat quality parameters of Jumbo quail.

Keywords: *Coturnix coturnix*; feed additives; meat quality; physiological responses; phytogenic plants; sustainable intensification

1. Introduction

Sustainable intensification of quail (*Coturnix coturnix*) birds could be an alternative vehicle through which the poultry industry can continue to contribute immensely to global food and nutrition security. Quail birds possess great potential for increasing the supply of dietary protein for humans through the consumption of meat and eggs [1–3]. The quail sector has recently seen the evolution of the Jumbo quail, developed from the traditional Japanese quail (*Coturnix coturnix japonica*) for meat production [2]. This bird is the fastest-growing and largest of all quail birds, weighing between 250 g and 300 g at six weeks of age [3]. Generally, quail birds have inherent immunity and strong resistance against several poultry diseases [4], which allows limited use of in-feed antibiotic growth promoters (AGP) in their diets. However, the commercial game-bird diet recommended for optimum quail production is expensive and not easily accessible. As a consequence, farmers resort to the use of commercial chicken diets that contain AGP such as zinc-bacitracin, a mixture of high molecular weight polypeptides (bacitracin A, B and C and several minor components) [5].

Zinc-bacitracin is a synthetic AGP that is commonly used to improve performance, biological efficiency and general health of intensively reared animals [6]. However, the continued use of AGPs has negative effects on the well-being of consumers due to the



Citation: Mahlake, S.K.; Mnisi, C.M.; Lebopa, C.; Kumanda, C. The Effect of Green Tea (*Camellia sinensis*) Leaf Powder on Growth Performance, Selected Hematological Indices, Carcass Characteristics and Meat Quality Parameters of Jumbo Quail. Sustainability 2021, 13, 7080. https:// doi.org/10.3390/su13137080

Academic Editor: Alessandra Durazzo

Received: 30 April 2021 Accepted: 15 June 2021 Published: 24 June 2021

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development of pathogenic bacterial resistance and the presence of antibiotic residues in poultry meat [7,8]. Nonetheless, the use of AGPs is still rife in tropical countries due to high prevalence of infectious diseases in these regions, while research on possible replacements continues to lag behind [6]. It is, therefore, essential that safe and efficient phytotherapeutics with growth-stimulating, meat-boosting and antimicrobial activities be identified and evaluated for intensive quail production.

Phytogenic products such as green tea (Camellia sinensis) leaves have putative antibacterial, antiviral and antioxidative activities [9-11] that can improve quail performance and the quality of quail meat. Indeed, Khan [12] stated that green tea leaves (GTL) possess high polyphenolic antioxidants that have the ability to modify and improve meat quality attributes. Cao et al. [13] and Shomal et al. [14] reported improved performance and health status of broiler chickens when reared on diets containing green tea by-products. These beneficial effects could be due to the presence of bioactive compounds such as catechins, flavonoids and flavanols in GTL [12,15]. Moreover, these leaves contain protein, amino acids, vitamins, calcium, phosphorus, selenium and zinc that can be exploited for sustainable quail production [16–18]. Despite the nutraceutical properties of GTL, there are limited studies that have investigated its effect on growth performance, hematology, and meat quality traits in Jumbo quail diets, which could be because this bird was only recently developed. In addition, the presence of phenolic acids such as tannins in the GTLP could interfere with the utilization of protein and other nutrients, and potentially impair the birds' performance. Thus, it is crucially important that a maximum inclusion level of GTLP in Jumbo quail diets be determined so as not to compromise their performance. Therefore, this study evaluated the effect of graded levels of green tea leaf powder (GTLP) as an alternative to zinc-bacitracin on growth performance, blood parameters, internal organs, and carcass and meat quality traits of Jumbo quail. We hypothesized that dietary inclusion of GTLP would improve physiological responses and meat quality attributes of Jumbo quail.

2. Materials and Methods

2.1. Study Area and Diet Formulation

The feeding trial was conducted between October and November 2020 at Molelwane Research Farm (25°40.459′ S, 26°10.563′ E) of North-West University (North-West, South Africa). Dry green tea leaves were purchased from Mountain Herb Estate (25°43'27.6" S, 27°57′54.8" E) in Kameeldrift-West (Pretoria, Gauteng, South Africa). The leaves were milled (2 mm; Polymix PX-MFC 90 D, Kinematica AG, Malters, Switzerland) to produce the powder (GTLP) prior to blending with the other ingredients. The GTLP was analyzed according to the Association of Official Analytical Chemists methods [19] in triplicates, and contained 972.6 g/kg dry matter, 924.8 g/kg organic matter, 295.8 g/kg DM crude protein, 200.5 g/kg DM neutral detergent fiber, 145.7 g/kg DM acid detergent fiber, and 52.53 g/kg DM acid detergent lignin. The GTLP was further analyzed using the methods described by Makkar [20] and Porter et al. [21] and contained 328.3 g TAE/kg DM total soluble phenolics and 1.963 AU soluble condensed tannins, respectively. Five isoenergetic and isoproteic experimental diets (Table 1) were formulated to meet the nutritional requirements for broiler quail birds [22]. The diets were formulated using a nutritional software as follows: (1) a standard grower diet with zinc-bacitracin (PosCon), (2) a standard grower diet without zincbacitracin (NegCon), (3) NegCon diet treated with 10 g/kg of green tea leaf powder (GT10), (4) NegCon diet treated with 25 g/kg of green tea leaf powder (GT25), and (5) NegCon diet treated with 50 g/kg of green tea leaf powder (GT50).

2.2. Nutritional Composition of the Diets

The experimental diets were analyzed (Table 2) for dry matter (DM), ash, ether extracts and crude fiber using the Association of Official Analytical Chemists methods [19]. Mineral contents (calcium, phosphorus, sodium and chloride) were determined following the guidelines by the Agri-Laboratory Association of Southern Africa [23]. Total soluble phenolics (TSPh) were determined following the Folin–Ciocalteau method [20]. Absorbance

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was recorded at 725 nm wavelength using a spectrophotometer (T60 UV-Visible spectrophotometer, PG Instruments Limited, Lutterworth, UK) and expressed as tannic acid equivalent (g TAE/kg DM). Soluble condensed tannins (SCT) were determined using the Butanol-HCl method [21], and absorbance was measured at 550 nm wavelength using the spectrophotometer described above. Crude protein and apparent metabolizable energy corrected for nitrogen (AMEn) were predicted using models from the near infrared reflectance spectroscopy (SpectraStar XL, Unity Scientific, Milford, MA, USA).

Table 1. Gross	s ingredient o	composition (g/kg.as	fed basis)	of expe	erimental diets.
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Ingredients	PosCon	NegCon	GT10	GT25	GT50
Green tea leaf powder	0.0	0.0	10.0	25.0	50.0
Soya oil cake 47%	213.9	213.9	210.8	206.2	219.9
Extra soya oil cake 40%	40.0	40.0	40.0	40.0	40.0
Sunflower 38%	30.0	30.0	30.0	30.0	30.0
Canola oil cake 34%	100.0	100.0	100.0	100.0	70.0
Yellow maize	550.6	550.6	543.8	533.5	523.8
Soya oil	33.1	33.1	33.0	33.0	31.5
Salt (fine)	6.0	6.0	6.0	6.0	6.0
Monocalcium phosphate	9.0	9.0	9.0	9.0	9.0
Limestone	7.0	7.0	7.0	7.0	7.0
Valine	0.10	0.10	0.10	0.10	2.50
Lysine HCL	3.30	3.30	3.30	3.30	3.30
Methionine-DL	2.60	2.60	2.60	2.50	2.60
Threonine	0.40	0.40	0.40	0.40	0.40
² Vitamin premix	3.0	3.0	3.0	3.0	3.0
Quantum blue phytase	0.50	0.50	0.50	0.50	0.50
Zinc-Bacitracin	0.50	-	-	-	-

 $^{^1}$ Experimental diets: PosCon = a standard grower diet with zinc-bacitracin; NegCon = a standard grower diet without zinc-bacitracin; GT10 = NegCon diet treated with 1 0 g/kg of green tea leaf powder; GT25 = NegCon diet treated with 2 5 g/kg of green tea leaf powder; GT50 = NegCon diet treated with 2 5 g/kg of green tea leaf powder; GT50 = NegCon diet treated with 2 5 g/kg of green tea leaf powder. 2 7 Vitamin premix: biotin (0.12 g), folic acid (0.7 mg), niacin (30 mg), pantothenic acid (10 mg), vitamin A (11,000 IU), vitamin B1 (2.5 mg), vitamin B2 (4.5 mg), vitamin B6 (5.1 mg), vitamin D3 (2500 IU), vitamin E (25 IU), vitamin K3 (2.0 mg).

Table 2. Nutritional compositional of experimental diets on an as fed basis (g/kg, unless stated otherwise).

	¹ Experimental Diets					
Composition	PosCon	NegCon	GT10	GT25	GT50	
Dry matter	894.6	894.6	894.9	895.4	896.6	
Crude protein	226.0	226.0	226.0	226.0	226.0	
Ether extract	66.80	66.80	67.32	68.29	65.60	
Crude fiber	35.80	35.80	40.20	42.73	44.80	
Ash	19.70	19.70	20.71	22.20	22.70	
² AMEn (MJ/kg)	12.56	12.56	12.56	12.56	12.56	
Calcium	5.70	5.70	6.10	6.80	7.80	
Phosphorus	6.90	6.90	7.10	7.20	7.40	
Sodium	2.40	2.40	2.35	2.40	2.40	
Chloride	3.50	3.50	3.50	3.51	3.50	
Total soluble phenolics (g TAE/kg)	18.53	16.31	24.37	32.28	53.43	
Soluble condensed tannins (AU)	0.056	0.079	0.163	0.229	0.351	

¹ Experimental diets: PosCon = a standard grower diet with zinc-bacitracin; NegCon = a standard grower diet without zinc-bacitracin; GT10 = NegCon diet treated with 10 g/kg of green tea leaf powder; GT25 = NegCon diet treated with 25 g/kg of green tea leaf powder; GT50 = NegCon diet treated with 50 g/kg of green tea leaf powder. ² AMEn = nitrogen-corrected apparent metabolizable energy.

2.3. Experimental Design and Bird Management

Three hundred and fifty one-week-old unsexed quail chicks were purchased from Golden Quail Farm (Randfontein, Gauteng, South Africa). The chicks were randomly allocated to 35 replicate pens (experimental units), with each pen (100 cm L \times 60 cm W \times 30 cm H)

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holding 10 birds, which were replicated seven times per dietary treatment. The pens, standing 20 cm above ground floor, were made of wire-mesh, and polythene plastics were used as bedding. The dietary treatments were offered *ad libitum* using 10-hole hinged chick feeders. The birds had free access to fresh, clean water provided using Poltek poultry water fountain drinkers. The quail house was cleaned once a week, and the birds were regularly observed for any abnormalities. No mortalities were recorded during the experimental period, giving a 100% survival rate. The experiment was conducted under natural lighting (12 h of daylight) with house temperatures ranging between 25 $^{\circ}$ C and 30 $^{\circ}$ C and average indoor humidity of 60%.

2.4. Measurements of Feed Intake and Growth Performance

All the birds in each pen (experimental unit) were put in a box and weighed (LBK 6 kg Adam scale, 1 g readability, Adam Equipment S.A. (Pty) Ltd, Milton Keynes, UK) to determine average initial body weight (56.1 ± 2.12 g live-weight). Average live-weights were measured weekly by weighing all the birds in each pen until the age of six weeks, to determine average weekly body weight gain (ABWG) per bird. Average weekly feed intake (AWFI) per bird was measured by subtracting the weight of feed refusals from that of the feed offered (which were measured daily from beginning of week 1 to the end of week 6), and dividing the difference by the total number of birds in the pen. Average weekly feed conversion efficiency (FCE) was calculated as body weight gain over feed consumed.

2.5. Slaughter Procedures and Blood Analyses

At the age of 42 days, all the birds in a pen were weighed to determine final body weight and thereafter transported to a commercial poultry abattoir (Rooigrond, North West, South Africa) for slaughter. At the abattoir, the birds were electrically stunned and then slaughtered by cutting the jugular vein with a sharp knife. During bleeding, about 2 mL of blood samples were collected from two birds randomly selected from each experimental unit using 5 mL disposable syringes fitted with 23 g needles. The blood samples were immediately transferred into hematological tubes containing ethylenediaminetetraacetic acid (EDTA), an anti-coagulant, for hematological analyses. The tubes containing blood were stored in a cooler bag and the blood samples were analyzed within 48 h after collection [24]. Hematological indices (erythrocytes, hemoglobin, hematocrit, neutrophils, red blood cells distribution width (RDW), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), white blood cells, lymphocytes, monocytes, eosinophils, reticulocytes, platelets, platelets distribution width (PDW), mean platelet volume (MPV)) were determined using an automated IDEXX LaserCyte Hematology Analyzer (IDEXX Laboratories SA, Midrand, Gauteng, South Africa).

2.6. Carcass Characteristics and Internal Organs

After slaughter, the carcasses were plucked by hand and manually eviscerated to determine internal organ sizes, carcass characteristics, and meat quality attributes. Hot carcass weight (HCW) was measured 1 h after slaughter, whereas cold carcass weight (CCW) was determined after chilling the carcasses at 16 °C for 24 h. Carcass cuts (wing, drumstick, breast and thigh) and internal organs (liver, gizzard, proventriculus, spleen, small intestine, caecum and colon) were weighed using a digital weighing scale (Explorer®EX224, OHAUS Corporation, Parsippany, NJ, USA) and expressed as a proportion of HCW (%HCW). Carcass yield was calculated as the proportion of HCW on final body weight using the following formula:

$$\textit{Carcass yield (\%)} = \frac{\textit{Hot carcass weight}}{\textit{final body weight}} \times 100$$

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2.7. Meat Quality Measurements

A digital pH meter (HI98163, Hanna Instruments, Woonsocket, RI, USA) fitted with a spear-type electrode was used to measure breast meat pH at 1 h and 24 h post-slaughter on the central area of the breast muscles. The pH meter was cleaned and calibrated using standard solutions (pH 4 and 7) provided by the manufacturer for this purpose. The guidelines from the Commission Internationale de l'Eclairage [25] were used to determine color coordinates (L^* = lightness, b^* = yellowness, and a^* = redness) on the surface of the breast muscle using a Minolta color spectrophotometer (BYK-Gardener GmbH, Geretsried, Germany), with a 20 mm diameter measurement area and illuminant D65-day light, 10° observation angle. The spectrophotometer was calibrated as prescribed by the manufacturer before the measurements, and after measuring each experimental unit. Hue angle and chroma values were determined as described by Priolo et al. [26]. The filter-paper press method by Grau and Hamm [27] was used to determine the water holding capacity (WHC), in which pre-weighed breast meat samples are placed in-between two filter papers on a flat surface and pressed with ~60 kg weight for 5 min. The method described by Honikel [28] was used to measure cooking loss, in which pre-weighed breast meat samples are cooked to reach a core temperature of 74 °C. Breast meat samples were used to determine shear force (N), a measure of meat tenderness, by shearing (crosshead speed 200 mm/min, one shear in the centre of each core) each sample using a Meullenet-Owens Razor Shear Blade (A/MORS) mounted on a Texture Analyzer (TA XT plus, Stable Micro Systems, Surrey, UK) as described by Lee et al. [29].

2.8. Statistical Analysis

Average weekly feed intake, body weight gain and FCE data were analyzed using the repeated measures analysis option in the general linear model (GLM) procedure of Statistical Analysis System (SAS) version 9.4 [30] using the following statistical model:

$$Y_{ijk} = \mu + D_i + T_j + (D \times T)_{ij} + E_{ijk}$$

where Y_{ijk} = dependent variable, μ = population mean, D_i = effect of diet, W_j = effect of time (in weeks), $(D \times T)_{ij}$ = interaction effect between diet and time, and E_{ijk} = random error associated with observation ijk, assumed to be normally and independently distributed.

Growth performance, hematology, and meat quality traits data (except for PosCon data) were tested for linear and quadratic effects using polynomial contrasts (PROC RSREG [30]). Data for overall feed intake, physiological parameters, and meat quality traits were analyzed using one-way analysis of variance (PROC GLM [30]) with diet as the only factor. All tested parameters were declared statistically significant at p < 0.05 and the least squares means were compared using the probability of difference option in SAS.

3. Results

3.1. Feed Intake and Physiological Responses

Repeated measures analyses showed significant week \times diet interaction effects on ABWG (p = 0.002) and FCE (p < 0.0001), but not on AWFI (p = 0.408). Overall feed intake linearly increased (y = 0.295 (± 0.936) x + 792.8 (± 8.62); $R^2 = 0.324$; p = 0.004) with increasing dietary GTLP levels. There were significant dietary effects on overall feed intake, where quail birds reared on diet GT50 (839.5 g/bird) had the highest overall feed intake, compared to those reared on diets NegCon and GT10, which were statistically similar.

Table 3 shows that weight gain linearly decreased in week 2 ($y = 50.78 \pm 0.978$) – 0.287 (± 0.106) x; $R^2 = 0.358$; p = 0.006), but increased in week 4 ($y = 0.418 \pm 0.201$) $x + 42.14 \pm 0.47$; $R^2 = 0.288$; p = 0.012) as GTLP levels increased. For the 5-week study period, no significant quadratic trends were observed for weight gain in response to dietary GTLP levels.

No significant dietary effects were observed on weight gain in weeks 3, 5 and 6. However, in week 2, diet PosCon showed the highest weight gain compared to the GTLP-

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containing diets, but the PosCon group had similar weight gain as the NegCon group. Four-week old birds reared on diet GT25 (51.15 g/bird) had the highest weight compared to those reared on NegCon and GT10 diets. However, the PosCon group had statistically similar weight gain compared to all the other treatment groups.

	Table 3. Average weekly	oody weight gain (g/bird) of Jumbo quail r	reared on diets containing green tea leaf po	owder.
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		¹ E:	xperimental D		pΝ	/alue		
	PosCon	NegCon	GT10	GT25	GT50	² SEM	Linear	Quadratic
Week 2	52.36 ^c	51.05 b,c	47.73 ^{a,b}	46.39 ^a	45.20 ^a	0.989	0.006	0.070
Week 3	58.75	59.14	58.07	56.38	56.32	1.136	0.107	0.440
Week 4	44.74 ^{a,b}	43.48 a	43.00 a	51.15 ^b	49.19 ^{a,b}	1.750	0.012	0.191
Week 5	26.36	24.92	28.32	27.91	26.37	2.283	0.828	0.220
Week 6	22.84	17.17	27.99	23.59	26.44	2.936	0.120	0.410

 a,b,c In a row, means with different superscripts significantly differ (p < 0.05). ¹ Experimental diets: PosCon = a standard grower diet with zinc-bacitracin; NegCon = a standard grower diet without zinc-bacitracin; GT10 = NegCon diet treated with 10 g/kg of green tea leaf powder; GT25 = NegCon diet treated with 25 g/kg of green tea leaf powder; GT50 = NegCon diet treated with 50 g/kg of green tea leaf powder. ² SEM = standard error of the mean.

Feed conversion efficiency linearly declined in week 2 ($y = 0.518 \pm 0.009 - 0.003 \pm 0.001$) x; $R^2 = 0.543$; p < 0.0001) and week 3 ($y = 0.417 \pm 0.006$) -0.000 ± 0.001) x; $R^2 = 0.437$; p = 0.0004) as GTLP levels increased (Table 4). No significant quadratic effects were observed for FCE with dietary GTLP levels for the 5-week study period.

Table 4. Average weekly feed conversion efficiency of Jumbo quail reared on diets containing green tea leaf powder.

		¹ E:		p Value				
	PosCon	NegCon	GT10	GT25	GT50	² SEM	Linear	Quadratic
Week 2	0.525 ^c	0.514 ^c	0.503 b,c	0.463 ^{a,b}	0.436 ^a	0.010	0.0001	0.292
Week 3	0.413 ^b	0.416 ^b	0.415 ^b	0.404 ^b	0.383 ^a	0.005	0.0001	0.413
Week 4	0.269 a,b	0.263 ^{a,b}	0.261 ^a	0.299 ^b	0.277 ^{a,b}	0.009	0.070	0.118
Week 5	0.144	0.137	0.154	0.150	0.139	0.012	0.666	0.254
Week 6	0.106	0.083	0.130	0.110	0.119	0.012	0.165	0.382

 a,b,c In a row, means with different superscripts significantly differ (p < 0.05). ¹ Experimental diets: PosCon = a standard grower diet with zinc-bacitracin; NegCon = a standard grower diet without zinc-bacitracin; GT10 = NegCon diet treated with 10 g/kg of green tea leaf powder; GT25 = NegCon diet treated with 25 g/kg of green tea leaf powder; GT50 = NegCon diet treated with 50 g/kg of green tea leaf powder. ² SEM = Standard error of the mean.

In week 2, the control groups (PosCon and NegCon) had higher FCE compared to the GT25 and GT50 groups. Three-week old birds reared on diet GT50 had the lowest FCE (0.383) compared to those reared on the other diets. Four-week old birds fed diet GT25 had higher FCE (0.299) than those fed diet GT10 (0.261). Nonetheless, the control diets (PosCon and NegCon) promoted similar FCE to the GTLP-containing diets. No dietary effects (p > 0.05) were observed on FCE in weeks 5 and 6.

Table 5 shows that there were significant quadratic trends for hematocrits (y = 0.025 (± 0.008) $x^2 - 1.272$ (± 0.415) x + 36.86 (± 3.644); $R^2 = 0.428$; p = 0.008) and MCV (y = 0.024 (± 0.010) $x^2 - 1.305$ (± 0.508) x + 70.72 (± 4.454); $R^2 = 0.337$; p = 0.028), as GTLP levels increased. No significant dietary effects were observed on all hematological parameters of Jumbo quail.

3.2. Carcass Characteristics and Internal Organs

Table 6 shows that there were significant linear increases for carcass yield (y = 0.088 (± 0.090) x + 63.82 (± 0.833); $R^2 = 0.315$; p = 0.004), HCW (y = 0.699 (± 0.456) x + 160.9 (± 4.195); $R^2 = 0.229$; p = 0.021) and CCW (y = 0.759 (± 0.437) x + 154.1 (± 4.02); $R^2 = 0.220$; p = 0.032) with increasing GTLP levels. Similarly, linear increases were observed for caecum (y = 0.921 (± 0.075) x + 0.011 (± 0.009); $R^2 = 0.635$; p < 0.0001) and colon (y = 0.001 (± 0.008) x + 0.351 (± 0.077); $R^2 = 0.187$; p = 0.035) weights as GTLP levels increased. No quadratic

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trends (p > 0.05) were observed for all the carcass characteristics and internal organs of the Jumbo quail.

Table 5. Hematological parameters of Jumbo quail reared on diets containing gr

		¹ Ex	perimental		p Value			
² Parameters	PosCon	NegCon	GT10	GT25	GT50	³ SEM	Linear	Quadratic
Erythrocytes ($\times 10^{12}/L$)	4.26	4.75	4.71	4.49	4.59	0.433	0.886	0.057
Hematocrits (%)	25.89	30.43	30.16	24.43	29.56	4.685	0.751	0.008
Hemoglobin (g/dL)	9.143	11.04	9.94	9.94	11.10	0.880	0.942	0.051
MCV (fL)	60.57	63.35	62.75	55.89	63.29	4.823	0.487	0.028
MCH (fL)	38.90	24.31	21.75	24.47	24.81	10.47	0.924	0.345
MCHC (g/dL)	31.95	31.11	28.55	34.85	30.30	2.487	0.790	0.406
RDW (%)	24.02	25.14	25.09	23.54	24.37	2.467	0.557	0.283
Reticulocytes (%)	168.5	132.2	148.4	72.78	89.09	36.56	0.802	0.288
White blood cells ($\times 10^9/L$)	122.9	131.0	126.3	83.82	108.4	29.38	0.338	0.569
Neutrophils (%)	11.42	8.39	12.57	6.98	6.57	2.132	0.143	0.548
Lymphocytes ($\times 10^9/L$)	107.0	121.5	109.0	75.96	100.9	28.02	0.380	0.522
Monocytes ($\times 10^9/L$)	1.68	1.66	1.64	1.32	1.38	0.348	0.573	0.565
Eosinophils ($\times 10^9/L$)	1.37	1.21	1.33	0.841	0.673	0.318	0.060	0.975
Platelets (K/μL)	1455	1387	1251	1161	1350	291.3	0.600	0.673
PDW (%)	22.24	21.15	20.99	16.34	21.03	2.028	0.890	0.282
MPV (fL)	3.91	3.71	3.77	6.07	3.99	1.007	0.531	0.162

¹ Experimental diets: PosCon = a standard grower diet with zinc-bacitracin; NegCon = a standard grower diet without zinc-bacitracin; GT10 = NegCon diet treated with 10 g/kg of green tea leaf powder; GT25 = NegCon diet treated with 25 g/kg of green tea leaf powder; GT50 = NegCon diet treated with 50 g/kg of green tea leaf powder. ² Parameters: RDW = red blood cell distribution width, MCV = mean corpuscular volume, MCH = mean corpuscular hemoglobin, MPV = mean platelet volume, PDW = platelets distribution width, MCHC = mean corpuscular hemoglobin concentration. ³ SEM = standard error of the mean.

Table 6. Carcass characteristics and internal organ weights (%HCW, unless stated otherwise) of Jumbo quail reared on diets containing green tea leaf powder.

		¹ Ex	perimental D		<i>p</i> Value			
² Parameters	PosCon	NegCon	GT10	GT25	GT50	³ SEM	Linear	Quadratic
Carcass yield (%)	65.64	64.28	63.74	66.61	67.38	0.959	0.004	0.974
Final weight (g)	262.3	251.1	260.1	261.9	260.1	4.884	0.288	0.281
HCW (g)	172.0	161.4	165.9	174.5	175.2	4.103	0.021	0.403
CCW (g)	165.7	154.7	159.5	168.0	167.1	3.973	0.032	0.272
Drumstick	4.40	4.48	4.49	4.38	4.30	0.095	0.090	0.951
Thigh	6.04	6.17	6.31	6.31	6.40	0.131	0.626	0.587
Wing	4.20	4.32	4.37	4.32	4.35	0.079	0.983	0.967
Breast	22.89	21.41	26.79	24.82	25.50	1.170	0.690	0.613
Liver	2.85 ^a	2.89 a	3.45 ^b	3.01 ^{a,b}	3.20 a,b	0.126	0.704	0.471
Gizzard	2.18	2.28	2.31	2.21	2.39	0.055	0.948	0.297
Proventriculus	0.558	0.524	0.593	0.571	0.634	0.040	0.147	0.770
Spleen	0.130	0.126	0.142	0.119	0.113	0.011	0.133	0.591
Small intestine	4.00	3.94	4.47	4.28	5.02	0.266	0.240	0.286
Caecum	0.938 a,b	0.901 a	1.117 ^{a,b}	1.264 b,c	1.552 ^c	0.085	0.0001	0.779
Colon	0.249 a	0.401 ^{a,b}	0.270 a	0.503 ^{a,b}	0.689 ^b	0.074	0.035	0.617

 $^{^{}a,b,c}$ In a row, means with different superscripts significantly differ (p < 0.05). ¹ Experimental diets: PosCon = a standard grower diet with zinc-bacitracin; NegCon = a standard grower diet without zinc-bacitracin; GT10 = NegCon diet treated with 10 g/kg of green tea leaf powder; GT25 = NegCon diet treated with 25 g/kg of green tea leaf powder; GT50 = NegCon diet treated with 50 g/kg of green tea leaf powder. ² Parameters: HCW = hot carcass weight, CCW = cold carcass weight. ³ SEM = standard error of the mean.

There were no significant dietary effects on all carcass traits and internal organs, with the exception of liver, caecum and colon weights. Quail birds reared on diet GT10 (3.45%HCW) had larger liver weights than those reared on diets PosCon (2.85%HCW) and NegCon (2.89%HCW). However, the control groups (PosCon and NegCon) had similar liver weights to the GT25 and GT50 groups. Birds reared on diet GT50 (1.552%HCW) had

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> the largest caecum weights compared to those reared on PosCon, NegCon and GT10, which were statistically similar. The largest colon weights were recorded from birds reared on diet GT50 (0.689%HCW) whereas the lightest colon weights were from birds reared on PosCon (0.249%HCW) and GT10 (0.270%HCW) diets.

3.3. Meat Quality Parameters

Breast meat lightness (L^*_1) measured 1 h post-slaughter quadratically responded $(y = 39.0 (\pm 2.21) + 0.525 (\pm 0.239) x - 0.010 (\pm 0.005) x^2$; $R^2 = 0.173$; p = 0.048) to increasing GTLP levels (Table 7). Hue angle₁ linearly increased ($y = 0.01 \pm 0.003$) $x + 1.134 \pm 0.026$); $R^2 = 0.290$; p = 0.012) as GTLP levels increased. Linear and quadratic trends (p < 0.05) were observed for cooking loss $(y = 0.01 (\pm 0.002) x^2 - 0.179 (\pm 0.095) x + 40.03 (\pm 0.871);$ $R^2 = 0.507$; p = 0.005) in response to increasing GTLP levels.

¹ Experimental Diets n Valua

Table 7. Meat quality parameters of Jumbo quail reared on diets containing green tea leaf powder.

		EX	perimentai	Diets			p	arue
Parameters	PosCon	NegCon	GT10	GT25	GT50	³ SEM	Linear	Quadratic
<u>р</u> Н ₁	6.0	6.0	5.97	5.94	5.94	0.035	0.259	0.471
pH_{24}	5.87	5.89	5.86	5.87	5.80	0.055	0.390	0.851
Lightness (L^*_1)	43.55	39.86	41.53	47.28	43.55	2.234	0.507	0.048
Lightness (L^*_{24})	45.22	47.45	47.39	50.71	49.30	2.156	0.751	0.324
Redness (a_1^*)	2.85	2.80	3.16	2.42	2.85	0.348	0.435	0.817
Redness (a^*_{24})	5.21	5.51	5.32	4.62	5.15	0.421	0.701	0.184
Yellowness (b^*_1)	7.64	6.47	7.24	7.42	7.64	0.799	0.570	0.557
Yellowness (b^*_{24})	5.91	5.31	6.10	6.20	6.27	0.777	0.179	0.810
Hue angle ₁	1.21 ^{a,b}	1.15 ^a	1.16 a	1.25 ^b	1.24 ^{a,b}	0.027	0.012	0.175
Hue angle ₂₄	0.807	0.754	0.831	0.914	0.875	0.062	0.073	0.216
Chroma ₁	8.17	7.06	7.91	7.82	7.60	0.848	0.700	0.628
Chroma ₂₄	8.03	7.70	8.22	7.78	8.17	0.744	0.409	0.725
² WHC (%)	90.72	89.88	90.38	91.47	91.37	0.760	0.197	0.407
Cooking loss (%)	37.80 a	39.92 a	39.04 a	38.93 a	45.03 ^b	0.943	0.001	0.005
Shear force (N)	2.26	2.27	2.30	2.25	2.24	0.021	0.221	0.922

 $^{^{}a,b}$ In a row, means with different superscripts significantly differ (p < 0.05). Experimental diets: PosCon = a standard grower diet with zinc-bacitracin; NegCon = a standard grower diet without zinc-bacitracin; GT10 = NegCon diet treated with 10 g/kg of green tea leaf powder; GT25 = NegCon diet treated with 25 g/kg of green tea leaf powder; GT50 = NegCon diet treated with 50 g/kg of green tea leaf powder. ² WHC = water holding capacity. ³ SEM = standard error of the mean.

No significant dietary effects were observed on meat quality traits, except on hue angle₁ and cooking loss. The GT25 group had the highest hue angle₁ value (1.25) compared to the NegCon (1.15) and GT10 (1.16) groups. Nonetheless, the PosCon diet led to a similar hue angle₁ to all the other treatment groups. Cooking losses were highest on the GT50 group (45.0%) compared to the other treatment groups, which were statistically similar.

4. Discussion

4.1. Feed Intake and Physiological Responses

In response to the public call for organic farming, intensification of Jumbo quail without the use of in-feed growth stimulating antibiotics could be restricted by suboptimal performance, disease outbreaks and high mortality rates [4,31]. Therefore, it is imperative that phytogenic plants with growth-promoting and meat-enhancing effects be identified and evaluated in Jumbo quail diets. Green tea leaf powder (GTLP) is a rich source of bioactive compounds that have the ability to enhance growth performance and product quality [32]. In this study, repeated measures analysis revealed significant week and diet interaction effects on weight gain and FCE, which indicates that the ability of the birds to utilize and convert the dietary treatments into body mass depended on their age. The inclusion of GTLP in the diets increased overall feed intake with treatment level 50 g/kg recording the highest overall feed intake. This increase in feed consumption could be due to the increase in dietary fiber as GTLP levels increased. Indeed, the GTLP-containing diets Sustainability **2021**, 13, 7080 9 of 13

tended to have higher crude fiber levels compared to the positive and negative control diets. High dietary fiber levels gradually decrease the relative amount of digestible dry matter in the diets, which causes compensatory feed intake [3]. At two weeks of age, weight gain showed a linear decrease, suggesting that the young birds had a challenge in utilizing the diets as GTLP levels increased. This could be because young birds have lower absolute and relative gastrointestinal tract volume compared to older birds, hence the chicks were more affected by high dietary fiber [33]. These results agreed with the findings of Kaneko et al. [34], who reported a reduction in body weight gain in broiler chicks when GTLP was included at a rate of up to 50 g/kg in their diets. Similarly, Khalaji et al. [35] reported reduced body weight in broilers fed 500 mg/kg of green tea extract-containing feed. This could be the reason why linear decreases were observed for FCE values at two and three weeks of age, which also corroborates the findings of Cao et al. [13], who reported that the supplementation of green tea by-products does not improve feed efficiency in broiler chickens. Nonetheless, at four weeks of age, weight gain was improved by the inclusion GTLP, confirming that the ability of the birds to utilize the diets depended on their age. In similar studies, Biswas and Wakita [36], as well as Kaneko et al. [34], reported an improvement in weight gain in broiler chickens reared on Japanese green tea powder. At five and six weeks of age, similar body weight gains and FCE values were observed between the control diets and the GLTP-containing diets. It was expected that the negative control diet without zinc-bacitracin would perform sub-optimally given that this antibiotic is known to have growth-stimulating effects [6].

The assessment of hematological parameters in quail birds is essential in order to evaluate the safety and effectiveness of diets in optimizing bird performance without compromising their health [37]. Khan and Zafar [38] reported that hematological parameters are clinical indices used to indicate the pathophysiological status of an animal. In the present study, quadratic trends were observed for hematocrits, a measure of total erythrocytes per total volume of blood [39], and MCV, a measure of the average size of erythrocytes [40]. This confirms that, indeed, nutrition affects the blood profile of the birds. Overall, the inclusion of dietary GTLP had no negative influence on the hematological indices of the quail, with all the values falling within the normal range reported for healthy quail [41,42].

4.2. Carcass Characteristics and Internal Organs

Carcass yield is an economically important trait in the production, processing and marketing of animal products because it reflects how much of the live weight ends up as a saleable yield [43,44]. In this study, the inclusion of GTLP increased the hot and cold carcass weights, resulting in larger carcass yields compared to those of the control diets. Erener et al. [45] also reported increased carcass weights of broiler chickens fed diets containing green tea extract. This could be attributed to the presence of bioactive agents such as epigallocatechins and flavanols in GTL [46] which are known to have growth-stimulating and antioxidative effects [47]. Antioxidants from herbs improve carcass quality and performance by inhibiting reactive oxygen species or cell damage by free radicals that cause oxidative stress [48–50]. However, the carcass cuts were not improved by the inclusion of dietary GTLP. Likewise, Jelveh et al. [51] reported similar breast and drumstick weights in broiler chickens supplemented with up to 40 g/kg green tea powder.

Little is known about the effect of green tea products on visceral organ weights in Jumbo quail birds. Treatment with GTLP had no effect on the weights of the internal organs except on caecum, colon and liver weights of the birds. Mateos et al. [50] stated that birds fed with fibrous diets have enlarged gastrointestinal organs. Likewise, quail birds reared on the GTLP group had heavier caecum and colon weights, which indicates an anatomical adaptation response mechanism by the birds to efficiently utilize high dietary fiber levels [45,50]. This corroborated the finding of Yang et al. [52] who reported increased large intestine weights in broiler chicks supplemented with 5 and 10 g/kg of green tea by-products. The larger liver weights in quail birds fed the GTLP-containing

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diets could have been triggered by the high concentration of secondary plant compounds such as phenolics and condensed tannins in GTLP, which require detoxification by the liver. Similarly, Yang et al. [52] reported heavier liver weights in broiler chickens fed diets containing green tea by-products, compared to those in the control group. Polyphenols in plant by-products overwhelm the detoxification mechanism by the liver, which ultimately results in increased liver size [53].

4.3. Meat Quality Parameters

Quail meat from the GTLP group had similar attributes to meat from the control groups except for meat lightness and hue angle. A quadratic effect was observed for breast meat lightness (L^*_1) , measured 1 h post-mortem, which could be a pigmentation effect by chlorophyll and antioxidants in GTL [54]. According to Qiao et al. [55], meat lightness can be classified as pale ($L^* > 53$), normal ($48 < L^* < 51$) and dark ($L^* < 46$). Thus, the inclusion of GTLP promoted darker meat color at 1 h post-mortem, but tended to exhibit a normal color at 24 h post-mortem. Fletcher [56] stated that meat color and meat pH are correlated such that higher meat pH often gives darker meat color and visa-versa. Thus, color change from 1 h to 24 h post slaughter may have been influenced by the slight drop in meat pH across all treatments. Hue angle describes the color change from red to yellow [57], and higher hue angle values represent yellowish meat [58]. The inclusion of GTLP increased breast meat hue angle values, indicating its potential to alter meat coloration [45]. Water holding capacity (WHC) is a trait of economic importance because it affects storage and processing qualities [59]. In this study, breast meat WHC was not affected by the dietary treatments, indicating that the use of GTLP does not compromise the storage and processing qualities of quail meat. Results showed that the inclusion of GTLP at 50 g/kg increases breast meat cooking losses, which indicates a reduction of meat juiciness [60]. Shear force, a measure of meat tenderness, was not affected by the dietary treatments, suggesting that supplementing the diets with GTLP does not affect meat texture. Overall, the use of GTLP promoted similar meat pH, color, texture and water-binding capacity as the zinc-bacitracin antibiotic-containing control diet, which suggests that GTLP does not compromise the organoleptic quality of quail meat [61].

5. Conclusions

The inclusion of green tea leaf powder as an alternative to zinc-bacitracin improved overall feed intake and carcass performance, but did not enhance feed efficiency, hematology, and meat quality parameters of the Jumbo quail. An optimum inclusion level of the green tea leaf powder could not be determined using the physiological responses obtained in this study, suggesting a need for further evaluation of green tea by-products at higher inclusion levels to generate non-linear responses. However, higher inclusion levels of green tea leaf powder compromised the performance of young quail chicks, therefore, we recommend that higher levels be tested in adult Jumbo quail diets. Green tea powder can be used as a safe and efficient nutraceutical in diets of finisher quail. The inclusion of GTLP as a substitute for AGP in Jumbo quail diets is desirable for sustainable intensification and better product quality.

Author Contributions: Conceptualization, S.K.M., C.M.M., C.L. and C.K.; methodology, S.K.M. and C.M.M.; software, C.M.M.; validation, C.M.M.; formal analysis, S.K.M. and C.M.M.; investigation, S.K.M. and C.M.M.; resources, C.M.M.; data curation, S.K.M. and C.M.M.; writing—original draft preparation, S.K.M. and C.M.M.; writing—review and editing, S.K.M., C.M.M., C.L. and C.K. visualization, C.M.M., C.L. and C.K.; supervision, C.M.M., C.L. and C.K.; project administration, C.M.M.; funding acquisition, S.K.M. and C.M.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the National Research Foundation (grant number: 122328), and the North-West University Masters bursary. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Animal Production Research Ethics Committee of the North-West University (approval no. NWU-02004-20-A5 and date of approval: 17 September 2020).

Informed Consent Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

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