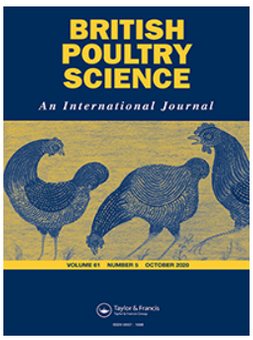

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Polyethylene glycol inactivates red grape pomace condensed tannins for broiler chickens

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ABSTRACT

1. This study was conducted to investigate the effect of inactivating GP condensed tannins using graded levels of polyethylene glycol (PEG) on feed intake, physiological, carcase, and meat quality traits of male Cobb 500 broilers.
2. Three hundred, two-week old, male Cobb 500 broilers (334.6 ± 21.43 g live weight) were allocated to 30 pens carrying 10 birds each. Five isonitrogenous and isoenergetic diets were formulated for grower (14–35 d) and finisher (36–42 d) phases by diluting a commercial broiler diet with untreated GP (PEG0) at 6.5% (w/w) or with the same amount of GP but pre-treated with PEG at 2.5% (PEG1), 5% (PEG2), 10% (PEG3) or 15% (w/w) (PEG4) and randomly allocated to pens in a four-week feeding period.
3. Feed intake, weight gain, feed conversion efficiency (FCE), and blood, carcase and meat quality parameters were determined. Weekly weight gain and FCE linearly ($P < 0.05$) increased in week 4 and linearly ($P < 0.05$) decreased in week 6 in response to PEG treatment levels.
4. Mean corpuscular volume linearly ($P < 0.05$) decreased in response to PEG levels, whereas blood urea nitrogen/creatinine ratio, urea, total protein, globulin and cholesterol showed quadratic trends in response to PEG levels. Spleen and ileum weights tended ($P < 0.1$) to linearly decrease with PEG levels. Heart weight and meat redness tended ($P < 0.1$) to quadratically respond to increasing levels of PEG.
5. It was concluded that PEG treatment partially inactivated GP condensed tannins without compromising the health status of broiler chickens. An optimum PEG inclusion level could not be determined for feed intake, weight gain and FCE. However, the presence of other antinutrients such as fibre and low molecular weight phenolics in GP may be responsible for the linear decreases observed in this study.

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Broiler; condensed tannin; growth; meat quality; polyethylene glycol; red grape pomace

Introduction

The demand for healthy, organically produced, high quality poultry products is increasing as meat consumers become more discerning. As such, the use of antibiotic growth promoters (AGPs) and synthetic feed additives in poultry production is increasingly being frowned upon (Mnisi et al. 2017). Plant bioactive compounds with nutraceutical properties such as those found in red grape pomace (GP) have potential to replace AGPs (Durmic and Blache 2012) and other synthetic feed additives designed to boost growth performance and product quality. Red grape pomace is particularly rich in a number of phenolics (Isaak et al. 2013; Aditya et al. 2018) that have antioxidant and antimicrobial properties (Makkar et al. 2007) as well as immune modulatory activity (Ben Saad et al. 2017). Indeed, Abu Hafsa and Ibrahim (2018), reported no adverse effects in the well-being of broiler chicks fed grape seeds and recommended their use as herbal supplements to improve performance and enhance antioxidant capacity and gut integrity of broilers. However, GP is currently been viewed as an environmental nuisance due to disposal in landfills or through incineration. Thus, the use of GP as a feed ingredient in broiler diets represents an ingenious strategy to valorise this waste product, reduce cost of broiler production and enhance the quality of broiler meat while simultaneously protecting the environment (Kumanda et al. 2019a). When included as ingredients in poultry diets, the bioactive compounds in GP have the potential to enhance bird health and productivity as well as the quality and sensory properties of products (Chamorro et al. 2019). Indeed, supplementing chicken diets with GP has been reported to increase weight gain, and efficiency of feed utilisation in chickens (Chamorro et al. 2013). In addition, supplementing broilers with GP improved antioxidant capacity (Goñi et al. 2007) and shelf life (Brannan 2008; Garrido et al. 2011; Jeronimo et al. 2012) of meat.

While there is ample evidence that bioactive compounds in GP have beneficial effects when included in broiler diets, the pomace also contains condensed tannins (CT), which have negative nutritional effects in broilers. Condensed tannins limit the amount of GP that can be included in broiler diets since they are known to reduce nutrient digestibility (Medugu et al. 2012). Indeed, Kumanda et al. (2019a) found that dietary inclusion of GP at more than 7.5% reduced feed intake in broilers. In order to facilitate the inclusion of higher levels of GP in chicken diets, it is important to ameliorate the negative nutritional effects of CT. A potentially effective strategy is to treat GP with polyethylene glycol (PEG), a known tannin-binding compound. Polyethylene glycol has a high affinity for CT and as such readily forms

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complexes with them thereby preventing them from binding with dietary nutrients (Mansoori et al. 2007; Mlambo et al. 2009), endogenous enzymatic proteins and other chemical components of the diet. Ameliorating CT will ensure that GP can be used as a source of beneficial phytochemicals without reducing the growth performance of broiler chickens (Kumanda et al. 2019b). This approach may promote higher intake of beneficial non-tannin phenolics and other bioactive compounds by the birds. However, the use of PEG to ameliorate anti-nutritional effects of red GP condensed tannins in broiler chickens has not been investigated until now. This represents the first ever attempt to determine the optimum quantity of PEG required for complete neutralisation of CT in order to valorise GP as a nutraceutical in Cobb 500 broiler chickens. We hypothesised that treating GP with PEG before including it in Cobb 500 broiler diets improves growth performance, haematology, serum biochemistry, carcase characteristics and meat quality traits.

Materials and methods

Ethical clearance was sought and obtained from the Animal Research Ethics Committee of the North-West University (Approval number. NWU-00414-18-A5).

Study site and ingredient sources

The feeding trial was conducted at the North-West University Molelwane Farm (25°86′00”S, 25°64′32”E) in the North West province of South Africa with ambient temperatures ranging between 3°C and 37°C. Sun-dried red grape (*Vitis vinifera* L. Var. Shiraz) pomace was acquired from Blaauwklippen Wine Estate (33.9741°S, 18.8423°E) (Stellenbosch, South Africa). The environmental conditions of the Estate are as described by Kumanda et al. (2019a). Polyethylene glycol (PEG, Mr 4000) was supplied by Merck (PTY) LTD (Gauteng, South Africa), whereas the other feed ingredients were bought from Optifeeds (PTY) LTD (Lichtenburg, South Africa).

Polyethylene glycol treatment

Four PEG solutions were made by dissolving 125, 250, 500 or 500 g PEG in 5000 mL of distilled water. Each PEG solution was subsequently sprayed onto 5 kg of GP thus producing PEG treatment rates of 2.5, 5, 10, and 15% prior to its inclusion in the experimental diets. The maximum level of PEG treatment was selected in order to completely neutralise GP condensed tannins by exceeding a 1:1 ratio of CT to PEG. Condensed tannin levels of GP were determined to constitute 10% of DM. The untreated sample of GP (5 kg) was sprayed with 5000 mL of distilled water only. The amount of distilled water used to dissolve the PEG was arrived at through an iterative process with the objective of avoiding run-off liquid that would leach the GP of its chemical components. Both the PEG-treated and water-treated (no PEG) GP were kept at room temperature for 24 hours to allow the PEG to react with the tannins in GP. After this incubation period both the PEG-treated and water-treated GP were oven-dried at 60°C until a constant weight was reached. After drying the GP was ground milled and included in commercial grower and finishing diets as described below. The untreated and PEG-treated GP substrates were chemically analysed and the results are presented in Table 1.

Diet formulation

Five isonitrogenous and isoenergetic diets were formulated for grower and finisher phases by Nutroteq (PTY) LTD (Gauteng, South Africa). For the two phases, the five diets were designed as follows: a commercial broiler diet with 6.5% untreated GP (PEG0) and the commercial broiler diet with the same amount of GP but treated with PEG at 2.5% (PEG1), 5% (PEG2), 10% (PEG3), or 15% (PEG4) (w/w) inclusion levels as shown in Table 2. The choice of PEG treatment levels were based on the desire to completely neutralise the condensed tannins by ensuring a 1:1 ratio of CT to PEG. Condensed tannin levels of red grape pomace used in this study were determined to constitute 10% of DM.

Experimental design

A total of 300, day-old male Cobb 500 broiler chicks were purchased from Mimosa chicks (Mafikeng, South Africa). The chicks were evenly allocated to 30 pens (experimental units) such that each pen (3.5 × 1.0 × 1.85 m) had 10 birds. A commercial starter diet was provided to the day-old broiler chicks for 10 days. From day 11 to 13, the birds were adapted to the experimental grower diets with measurements commencing from day 14 to day 35 (grower phase) and day 36 to day 42 (finisher phase). Sunflower husks were used as bedding in all the pens. The broiler house was fitted with semi-automatic curtains

Table 1. Chemical composition (g/kg DM, unless otherwise stated) of untreated and polyethylene glycol-treated red grape pomace.

	Substrates[1]					
Chemical components[2]	GPPEG0	GPPEG1	GPPEG2	GPPEG3	GPPEG4	SEM
Dry matter (g/kg)	966.1	964.0	964.9	966.6	955.7	0.996
Ash	67.35	67.49	60.65	66.98	59.41	1.889
Organic matter	898.8	896.5	913.2	899.6	896.3	3.455
Neutral detergent fibre	409.3	386.1	405.1	416.9	421.2	34.38
Acid detergent fibre	323.3	319.7	324.5	339.2	345.8	21.58
Acid detergent lignin	182.3	187.3	193.3	174.8	197.3	26.94
Ether extract	70.99	64.49	62.97	58.35	57.31	5.372
SCT ($AU_{550\ nm/200\ mg}$)	1.21	1.08	1.21	1.06	1.28	0.314
iCT ($AU_{550\ nm/200\ mg}$)	0.36	0.29	0.32	0.39	0.47	0.147
TSP (g TAE/kg)	16.43	19.75	19.33	16.45	13.79	6.745
TiPh (g TAE/kg)	6.50	6.27	6.40	6.00	5.35	1.391
Crude protein	113.7	114.2	115.8	112.8	108.7	1.94

[1]Substrates: GPPEG0 = untreated grape pomace; GPPEG1 = GP treated with PEG at 2.5%; GPPEG2 = GP treated with PEG at 5.0%; GPPEG3 = GP treated with PEG at 10%; GPPEG4 = GP treated with PEG at 15%.
[2]Chemical components: SCT = Soluble condensed tannins; iCT = Insoluble condensed tannins; TiPh = Total insoluble phenolics; TSP = Total soluble phenolics.

Table 2. Gross composition (g/kg. *as is* basis) of experimental diets offered to Cobb 500 broilers in the grower and finisher phases.

	Grower diets					Finisher diets				
Ingredients	PEG0	PEG1	PEG2	PEG3	PEG4	PEG0	PEG1	PEG2	PEG3	PEG4
Polyethylene glycol	0	1.63	3.25	6.5	9.75	0	1.63	3.25	6.5	9.75
Red grape pomace	65	65	65	65	65	65	65	65	65	65
Soya olicake (46.5%)	99	99	99	99	99	17	17	17	17	17
Fullfat soya	118	118	118	118	118	238	238	238	238	238
Lysine (Sint 78%)	2.76	2.76	2.76	2.76	2.76	1.71	1.71	1.71	1.71	1.71
Methionine (dL 98%)	0.99	0.99	0.99	0.99	0.99	1.17	1.17	1.17	1.17	1.17
Threonine (98%)	0.05	0.05	0.05	0.05	0.05	0.01	0.01	0.01	0.01	0.01
Maize yellow	651	651	651	651	651	655	655	655	655	655
Feed lime	13.6	13.6	13.6	13.6	13.6	11.8	11.8	11.8	11.8	11.8
MDCP (WS >70%)	7.7	7.7	7.7	7.7	7.7	2.3	2.3	2.3	2.3	2.3
Salt (fine)	3.25	3.25	3.25	3.25	3.25	2.99	2.99	2.99	2.99	2.99
Sodium bicarbonate	1.6	1.6	1.6	1.6	1.6	1.51	1.51	1.51	1.51	1.51
Phytase (100 g/t sk)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Olaquindox (10%)	0.4	0.4	0.4	0.4	0.4	0.2	0.2	0.2	0.2	0.2
[1]Premix no spec	0.5	0.5	0.5	0.5	0.5	-	-	-	-	-
Choline CL (60%)	0.8	0.8	0.8	0.8	0.8	-	-	-	-	-
Salinomycin (12%)	0.5	0.5	0.5	0.5	0.5	-	-	-	-	-
Gluten 60	34	34	34	34	34	-	-	-	-	-
Premix no spec[1] + Choline Cl	-	-	-	-	-	2.5	2.5	2.5	2.5	2.5
Zinc bacitracin (15%)	-	-	-	-	-	0.5	0.5	0.5	0.5	0.5

[1]Premix no spec: A non-disclosure agreement was signed with the feed manufacturer.

that were rolled down in the morning (07:00 AM) to allow natural lighting and closed in the evening (17:00 PM). Throughout the study, temperatures in the house were constantly monitored using a thermometer and lighting was provided via fluorescent bulbs and the birds had free access to clean fresh water.

Chemical analyses of untreated and PEG-treated grape pomace and experimental diets

The untreated and PEG-treated GP substrates and experimental diets were sampled and dried in an oven at 60°C until constant weight and then milled (Polymix PX-MFC 90 D) to pass through a 1 mm sieve in preparation for chemical analyses (Tables 1 and 3). Untreated GP, treated GP and diets (PEG0, PEG1, PEG2, PEG3 and PEG4) were analysed using AOAC international methods (AOAC 2005) for dry matter, organic matter, ash, crude protein, neutral detergent fibre, acid detergent fibre, acid detergent lignin, crude fibre and crude fat. For experimental diets only, minerals (Ca, P, Na, Cl and K) were analysed following Agri Laboratory Association of Southern Africa guidelines (AGRILASA 1998). Metabolisable energy of experimental diets was predicted using models from NIRs SpectraStar XL (Unity Scientific, Australia). For untreated and treated GP, the Folin-Ciocalteau method (Makkar 2003) was utilised to determine the concentration of total soluble phenolics (TSP), which were expressed as tannic acid equivalents (g TAE/kg). Total insoluble phenolics (TiPh) fraction was determined by repeating the same procedure using the residues of phenolic extraction. Soluble condensed tannins (SCT) were analysed using a mixture of 95% butanol and 5% HCl (Porter et al. 1986) and reported as absorbance units (AU) per 200 mg sample. The insoluble condensed tannins (iCT) fraction was also determined by reacting butanol-HCl with the sample residue from TSP extraction (Makkar 2003).

Table 3. Chemical composition (g/kg. unless otherwise stated) of experimental diets for grower and finisher phases.

Chemical components	Grower diets	Finisher diets
Dry matter	901.0	899.8
Organic matter	852.5	858.8
Metabolisable energy (MJ/kg)	11.9	12.2
Crude protein	170.0	160.0
Crude fat	53.28	72.70
Crude fibre	56.09	65.20
Ash	48.47	41.00
Calcium	8.20	6.60
Phosphorus	4.81	3.30
Sodium	1.80	1.60
Chloride	3.00	2.50
Potassium	7.08	7.10

Feed intake and growth performance

Feed offered was weighed before feeding and refusals were weighed in the morning prior to the next feeding. Average weekly feed intake (AWFI) was determined in weeks 2 to 6. The birds were weighed weekly to determine weekly live weight and weight gain (AWG). Feed conversion efficiency (FCE) was calculated as a proportion of weight gain to feed intake. All weights were taken using a digital weighing scale (Explorer EX224, 0.01 g readability (2 decimal places) OHAUS Corp, Parsippany, NJ, USA).

Blood parameters

At 41 days of age, blood samples were collected from two chickens randomly selected from each replicate. The brachial vein was punctured using a five mL scalp vein needle set. Blood was collected into two sets of sterilised bottles, one containing ethylene diamine tetra acetic acid as the anti-coagulant. Haematological parameters (erythrocytes, haemoglobin, haematocrits, red blood cell distribution width (RDW), mean corpuscular volume (MCV), and mean corpuscular haemoglobin (MCH)) were determined using an automated IDEXX LaserCyte Haematology Analyser (IDEXX Laboratories, Inc., Maine, US). Mean corpuscular haemoglobin concentration (MCHC) was calculated as MCH divided by MCV. Clotted blood (collected in red top tubes) was centrifuged to generate serum for biochemical analysis. Glucose, creatinine, calcium, albumin, urea, total protein, globulin, cholesterol, amylase, total bilirubin, phosphorus, alanine

transaminase (ALT), blood urea nitrogen/creatinine ratio (BUN/CREA ratio), and albumin/globulin ratio (ALB/GLOB ratio) were analysed using an automated IDEXX Vet Test Chemistry Analyser (IDEXX Laboratories, Inc., Maine, US).

Slaughter procedure

At 42 days of age, all broilers were deprived feed for a period of 13 hours to empty the crop and then taken to an abattoir for slaughter. At the abattoir, chickens were stunned and then slaughtered by cutting the jugular vein with a sharp knife and left hanging upside down until bleeding ended. Thereafter, the chickens were de-feathered and packaged according to experimental unit. Carcases were taken to the Animal Science laboratory of the North-West University for measurements.

Carcase characteristics and internal organs

Hot carcase weights (HCW) were recorded immediately after slaughter. After chilling for 24 hours at 4°C, the carcases were reweighed to determine cold carcase weight (CCW). The dressing percentage was determined as the proportion of HCW on slaughter weight (SW). Weights of the wings, thighs, drumsticks, livers, gizzards and gizzard fats, hearts, spleens, proventriculi, small (duodenum, jejunum and ileum) and large (caeca) intestines were determined and expressed as the proportion of HCW.

Meat pH and colour

The central area of the breast muscle was used to measure meat pH 24 hours after slaughter using a Corning Model 4 pH-temperature metre (Corning Glass Works, Medfield, MA) equipped with an Ingold spear-type electrode (Ingold Messtechnik AG, Udorf, Switzerland). The pH metre was calibrated after every 10 samples as described by Kumanda et al. (2019a). Colour of the meat (L* = lightness, a* = redness and b* = yellowness) was determined using a Minolta colour-guide (Spectrophotometer CM 2500 c, Konika Minolta, Osaka, Japan) as described by Kumanda et al. (2019a). Hue angle and chroma values were calculated as described by Priolo et al. (2002).

Cooking loss and Warner-Bratzler shear force

Raw pectoralis major muscle samples were weighed individually (w1) and then oven broiled at 140°C for 20 minutes. The broiled samples were cooled for 20 minutes and reweighed to obtain the cooked weight (w2). Cooking loss was calculated according to the following equation:

$$Cooking\ loss(\%) = \frac{w1 - w2}{w1} \times 100$$

Cooked muscle samples were sheared perpendicular to the fibre direction using a Warner-Bratzler shear device mounted on a Texture Analyser (TA XT plus, Stable Micro Systems, Surrey, UK) to determine the average shear force of each sample, expressed in Newtons.

Water holding capacity and drip loss

The water holding capacity (WHC) of the meat was measured on the surface of a freshly cut slice of the pectoralis major muscle and determined as the amount of water expressed from fresh meat held under pressure (60 kg pressure) using the filter-paper press method developed by Grau and Hamm (1957). The WHC was calculated as follows:

$$WHC(\%) = \frac{Initial\ weight - Weight\ after\ pressing}{Initial\ weight} \times 100$$

Drip loss was determined using a method adapted from Zhang et al. (2010), where pieces of muscle from the pectoralis major muscle weighing approximately 2 grams (wet weight, w1) were hooked and suspended using wire steel in a plastic bottle and sealed properly without touching the sides of the bottle. The samples were reweighed to obtain weight after drip (w2). The difference in weight of each sample before and after drip was conveyed as percentage drip loss and calculated as follows:

$$Drip\ loss(\%) = \frac{w1 - w2}{w1} \times 100$$

Statistical analysis

Polynomial contrast was used to evaluate data for linear and quadratic effects of PEG treatment. Response surface regression analysis (Proc RSREG; SAS 2010) was applied to determine the optimum PEG treatment level applied to GP according to the following quadratic model: $y = ax^2 + bx + c$, where y = response variable; a and b are the coefficients of the quadratic equation; c is intercept; x is PEG levels (%) and $-b/2a$ is the x value for optimal response. Average weekly FCE, AWFI and AWG data were analysed using the repeated measures analysis procedure of SAS (2010). The following linear statistical model was employed:

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

Where, Y_{ijk} = dependant variable, μ = population mean constant common to the observation, D_i = effect of diet, W_j = effect of time, $(D \times W)_{ij}$ = interaction effect of diet and time and E_{ijk} = random error term associated with observation ijk, assumed to be normally and independently distributed.

Growth performance, blood parameters, internal organs, carcase characteristics and meat quality data was analysed using the GLM procedure of SAS (2010). The linear statistical model was as follows:

$$Y_{ij} = \mu + D_i + E_{ij}$$

Where, Y_{ij} = dependant variable, μ = population mean, D_i = effect of diets, and E_{ij} = random error associated with observation ij, assumed to be normally and independently distributed. For all statistical tests, significance and tendency were declared at $P \leq 0.05$ and $0.05 < P \leq 0.1$, respectively.

Results

Feed intake and growth performance

Table 3 shows the chemical composition of experimental diets. Repeated measures analysis showed a significant week

Table 4. The effect of pre-treatment of dietary red grape pomace with polyethylene glycol on overall feed intake (g/bird), average weekly weight gain (g/bird) and average weekly feed conversion efficiency (g:g) of Cobb 500 broiler chickens.

	Dietary treatments[1]						Significance	
	PEG0	PEG1	PEG2	PEG3	PEG4	SEM	Linear	Quadratic
Overall FI[2]	3325.9	3289.7	3368.1	3267.9	3312.2	50.33	0.896	0.610
Average weekly weight gain (g/bird)								
Week 3	314.3	321.3	321.5	289.3	305.3	10.41	0.327	0.211
Week 4	426.4	426.1	453.4	454.6	463.6	9.41	0.011	0.368
Week 5	483.1	493.4	491.4	487.4	506.9	8.18	0.162	0.538
Week 6	743.8	740.4	691.9	665.4	666.1	28.9	0.047	0.490
Average weekly FCE (g:g)								
Week 3	0.603	0.620	0.609	0.574	0.599	0.019	0.508	0.285
Week 4	0.573	0.566	0.588	0.617	0.614	0.009	0.009	0.144
Week 5	0.534	0.539	0.529	0.534	0.548	0.005	0.248	0.257
Week 6	0.643	0.671	0.606	0.598	0.592	0.017	0.009	0.696

[1]Dietary treatments: PEG0 = a commercial diet with untreated grape pomace; PEG1 = a commercial diet with GP pre-treated with PEG at 2.5%; PEG2 = a commercial diet with GP pre-treated with PEG at 5%; PEG3 = a commercial diet with GP pre-treated with PEG at 10%; PEG4 = a commercial diet with GP pre-treated with PEG at 15%.
[2]Overall FI = feed intake (g/bird) from 14–42 d of age.

× diet interaction for AWG and FCE, except for AWFI. There were neither linear nor quadratic responses ($P > 0.05$) of overall feed intake to incremental levels of PEG. Table 4 shows that in week 4 and 6, there were significant linear PEG effects for AWG and FCE. Average weekly weight gain and FCE linearly increased in week 4 [$y = 0.54\ (\pm\ 0.31)x + 422\ (\pm\ 8.73)$; $R^2 = 0.346$, $P = 0.011$; $y = 0.0008\ (\pm\ 0.0003)x + 0.57\ (\pm\ 0.0095)$; $R^2 = 0.339$, $P = 0.009$, respectively] and linearly decreased in week 6 [$y = 736\ (\pm\ 25.74) - 1.2\ (\pm\ 0.92)x$; $R^2 = 0.232$, $P = 0.047$; $y = 0.65\ (\pm\ 0.143) - 0.0006\ (\pm\ 0.0005)x$; $R^2 = 0.371$, $P = 0.009$, respectively] with PEG levels.

Haematological and serum biochemistry parameters

For haematological parameters, there were no significant linear and quadratic trends for erythrocytes, haematocrits, haemoglobin, MCV, MCH, MCHC and RDW in response to incremental levels of PEG (Table 5). Table 5 also shows that there were no significant linear and quadratic trends for all serum biochemical parameters except for urea, BUN/CREA ratio, total protein, globulin and cholesterol. Blood urea nitrogen/creatinine ratio [$y = 9.77\ (\pm\ 1.029) + 0.007\ (\pm\ 0.038)x - 0.0002\ (\pm\ 0.00024)$; $R^2 = 0.21$, $P = 0.039$], urea [$y = 0.00013\ (\pm\ 0.0000044)x^2 - 0.00157\ (\pm\ 0.00069)x + 0.723\ (\pm\ 0.0204)$; $R^2 = 0.311$, $P = 0.010$], total protein [$y = 61.29\ (\pm\ 3.208) + 0.353\ (\pm\ 0.1087)x - 0.0023\ (\pm\ 0.00069)x^2$; $R^2 = 0.439$, $P = 0.004$], globulin [$y = 40.3\ (\pm\ 3.1) + 0.31\ (\pm\ 0.109)x - 0.00205\ (\pm\ 0.00067)x^2$; $R^2 = 0.386$, $P = 0.008$] and cholesterol [$y = 6.67\ (\pm\ 0.306) + 0.019\ (\pm\ 0.0104)x - 0.00014\ (\pm\ 0.000066)x^2$; $R^2 = 0.230$, $P = 0.046$] showed quadratic ($P < 0.05$) trends in response to PEG levels.

Internal organs and carcase and meat quality traits

Table 6 shows that there were no significant linear and quadratic trends for size of internal organs of Cobb 500 broilers in response to PEG levels. However, there was

Table 5. The effect of pre-treatment of dietary red grape pomace with polyethylene glycol on blood parameters of Cobb 500 broiler chickens.

	Dietary treatments[1]						Significance	
Parameters[2]	PEG0	PEG1	PEG2	PEG3	PEG4	SEM	Linear	Quadratic
Haematological parameters								
Erythrocytes ($\times 10^{12}$/L)	1.86	1.34	2.45	1.99	1.42	0.552	0.551	0.829
Haematocrit (L/L)	18.0	18.0	19.4	18.3	23.4	2.14	0.147	0.188
Haemoglobin (g/dL)	9.21	8.79	9.56	9.13	8.63	0.437	0.937	0.779
MCV (fL)	74.6	73.3	73.5	76.4	87.02	5.67	0.048	0.096
MCH (pg)	38.2	37.5	36.2	40.7	35.9	2.38	0.530	0.366
MCHC (g/dL)	1.51	0.52	0.49	0.51	0.41	0.811	0.211	0.470
RDW (%)	25.11	27.4	26.6	24.4	21.3	2.90	0.189	0.366
Serum biochemical parameters								
Glucose (mmol/L)	18.5	17.6	20.1	19.5	19.0	1.12	0.746	0.240
Creatinine (μmol/L)	25.8	20.5	25.0	26.5	35.0	5.71	0.462	0.143
Calcium (mmol/L)	2.35	2.06	2.51	2.21	2.13	0.141	0.256	0.917
Albumin (g/L)	22.2	20.0	23.3	25.10	22.2	1.25	0.316	0.123
ALT (IU/L)	87.8	50.8	83.1	65.5	55.6	10.22	0.194	0.587
Urea (mmol/L)	0.73	0.70	0.69	0.71	0.78	0.024	0.053	0.010
BUN/CREA ratio	9.83	10.50	8.50	9.00	5.20	1.580	0.022	0.038
Total protein (g/L)	65.0	68.8	73.1	74.6	60.1	3.02	0.839	0.004
Globulin (g/L)	43.8	47.0	49.8	53.0	39.8	2.74	0.983	0.008
ALB/GLOB ratio	1.12	0.45	0.47	0.40	0.91	0.362	0.564	0.136
Cholesterol (mmol/L)	6.65	7.01	7.49	7.02	6.54	0.301	0.351	0.046
Amylase (IU/L)	525.5	398.2	515.0	420.0	518.8	57.43	0.988	0.232
Total bilirubin (μmol/L)	17.8	16.2	21.3	22.9	20.1	1.73	0.566	0.544
Phosphorus (mmol/L)	3.97	3.46	4.10	3.73	3.60	0.187	0.175	0.774

[1]Dietary treatments: PEG0 = a commercial diet with untreated grape pomace; PEG1 = a commercial diet with GP pre-treated with PEG at 2.5%; PEG2 = a commercial diet with GP pre-treated with PEG at 5%; PEG3 = a commercial diet with GP pre-treated with PEG at 10%; PEG4 = a commercial diet with GP pre-treated with PEG at 15%.
[2]Parameters: MCV = mean corpuscular volume; MCH = mean corpuscular haemoglobin; RDW = red blood cell distribution width; ALT = alanine aminotransferase; BUN/CREA ratio = blood urea nitrogen/creatinine ratio; ALB/GLOB ratio = albumin/globulin ratio.

Table 6. The effect of pre-treatment of dietary red grape pomace with polyethylene glycol on internal organs (g/100 g HCW) of Cobb 500 broiler chickens.

	Dietary treatments[1]						Significance	
	PEG0	PEG1	PEG2	PEG3	PEG4	SEM	Linear	Quadratic
Duodenum	0.88	0.92	0.83	0.87	0.92	0.031	0.652	0.338
Jejunum	1.54	1.42	1.36	1.50	1.47	0.074	0.655	0.388
Ileum	0.92	0.92	0.88	0.95	0.94	0.039	0.072	0.116
Caeca	1.01	0.99	1.00	1.08	1.03	0.040	0.350	0.610
Gizzard	1.84	1.71	1.76	1.87	1.84	0.051	0.805	0.794
Gizzard fat	0.70	0.84	0.98	0.84	0.94	0.050	0.165	0.281
Heart	0.62	0.65	0.61	0.62	0.60	0.015	0.189	0.061
Large intestine	1.35	1.34	1.36	1.37	1.30	0.051	0.531	0.313
Liver	2.23	2.18	2.14	2.25	2.21	0.040	0.549	0.809
Proventriculus	0.40	0.39	0.39	0.41	0.39	0.012	0.664	0.664
Spleen	0.13	0.13	0.13	0.14	0.16	0.022	0.077	0.386

[1]Dietary treatments: PEG0 = a commercial diet with untreated grape pomace; PEG1 = a commercial diet with GP pre-treated with PEG at 2.5%; PEG2 = a commercial diet with GP pre-treated with PEG at 5%; PEG3 = a commercial diet with GP pre-treated with PEG at 10%; PEG4 = a commercial diet with GP pre-treated with PEG at 15%.

a tendency for spleen [y = 0.130 (± 0.009) – 0.0001 (± 0.00033)x; R^2 = 0.227, P = 0.077] and ileum [y = 0.930 (± 0.0304) – 0.001 (± 0.0011); R^2 = 0.303, P = 0.072] weights to linearly decrease with PEG levels. Heart weight tended (P = 0.061) to quadratically respond to increasing levels of PEG. Similarly, there were neither linear nor quadratic trends ($P > 0.05$) for carcase characteristics, meat pH, colour, cooking loss, shear force, WHC and drip loss in response to PEG levels (Tables 7 and 8). Meat redness showed a tendency (P = 0.072) to quadratically respond to PEG inclusion levels.

Discussion

Feed utilisation and growth performance

Red grape pomace has been used as a nutraceutical in broiler diets to boost growth performance, enhance antioxidant capacity and meat quality (Chamorro et al. 2013; Abu Hafsa and Ibrahim 2018). However, the CT present in GP have detrimental post-ingestive effects if consumed in large quantities because they are known to decrease the digestion and absorption of nutrients as well as other dietary compounds (Kumanda et al. 2019b). The presence of these anti-nutritional compounds limits the amount of GP that can be incorporated into poultry diets as a source of the much-needed bioactive compounds. Indeed, Kumanda et al. (2019a) reported a reduction in feed intake when GP was included in broiler diets at levels above 7.5%. Polyethylene glycol (PEG), a tannin-binding compound, is known to ameliorate the negative effects of CT by breaking pre-formed tannin-nutrient complexes (Besharati and Taghizadeh, 2009; Mlambo et al. 2009) thus improving nutrient and phytochemical bioavailability. This study represents the first attempt to determine the optimum PEG treatment

Table 7. The effect of pre-treatment of dietary red grape pomace with polyethylene glycol on carcase characteristics of Cobb 500 broiler chickens.

	Dietary treatments[1]						Significance	
Parameters[2]	PEG0	PEG1	PEG2	PEG3	PEG4	SEM	Linear	Quadratic
SW (g)	2304.0	2321.2	2302.5	2237.7	2276.1	39.08	0.542	0.553
CCW (g)	1676.4	1688.4	1712.9	1665.7	1697.9	31.29	0.578	0.908
HCW (g)	1709.5	1688.8	1730.6	1656.3	1700.5	28.43	0.996	0.504
Dressing %	75.56	73.34	75.57	74.92	75.30	0.853	0.710	0.541
Wing (g/100 g HCW)	5.0	4.90	4.90	4.94	4.91	0.073	0.742	0.545
Thigh (g/100 g HCW)	6.18	5.91	5.90	6.53	6.33	0.121	0.198	0.497
Drumstick (g/100 g HCW)	5.57	5.40	5.55	5.70	5.60	0.101	0.704	0.939

[1]Dietary treatments: PEG0 = a commercial diet with untreated grape pomace; PEG1 = a commercial diet with GP pre-treated with PEG at 2.5%; PEG2 = a commercial diet with GP pre-treated with PEG at 5%; PEG3 = a commercial diet with GP pre-treated with PEG at 10%; PEG4 = a commercial diet with GP pre-treated with PEG at 15%.
[2]Parameters: SW = slaughter weight; CCW = cold carcase weight; HCW = hot carcase weight.

Table 8. The effect of pre-treatment of dietary red grape pomace with polyethylene glycol on meat quality parameters of Cobb 500 broiler chickens.

	Dietary treatments[1]						Significance	
	PEG0	PEG1	PEG2	PEG3	PEG4	SEM	Linear	Quadratic
pH	7.08	7.11	6.92	6.90	6.72	0.123	0.260	0.809
Lightness (L^*)	51.3	52.1	51.0	50.6	52.3	1.04	0.686	0.529
Redness (a^*)	1.57	1.79	2.00	2.00	1.78	0.211	0.080	0.072
Yellowness (b^*)	11.6	12.4	11.7	12.2	11.9	0.424	0.161	0.223
Chroma	1.44	1.43	1.40	1.41	1.42	0.015	0.131	0.189
Hue angle	11.66	12.53	11.89	12.45	12.03	0.439	0.271	0.263
Cooking loss (%)	14.73	10.54	12.70	14.77	11.69	1.036	0.931	0.901
Shear force (N)	7.86	8.62	8.03	7.76	7.49	0.911	0.948	0.879
WHC[2] (%)	6.59	7.93	7.15	8.23	7.69	0.872	0.681	0.408
Drip loss (%)	34.04	27.80	29.22	29.01	33.97	3.584	0.805	0.414

[1]Dietary treatments: PEG0 = a commercial diet with untreated grape pomace; PEG1 = a commercial diet with GP pre-treated with PEG at 2.5%; PEG2 = a commercial diet with GP pre-treated with PEG at 5%; PEG3 = a commercial diet with GP pre-treated with PEG at 10%; PEG4 = a commercial diet with GP pre-treated with PEG at 15%.
[2]WHC = water holding capacity.

level required to completely ameliorate the anti-nutritional effects of CT and thus valorise GP as a nutraceutical in Cobb 500 broiler chickens. Repeated measures analysis revealed a significant week × diet interaction for AWG and FCE, which demonstrates that the efficiency of the birds in converting the dietary treatments into body mass was influenced by the age of the birds. Polyethylene glycol treatment of GP had positive linear effects on weight gain and FCE in week 4 indicating the potential of this compound to improve the performance of broiler chickens consuming tannin-rich GP. However, in week 6, both AWG and FCE linearly decreased with PEG levels, which was surprising because the finisher phase is meant to increase these parameters. Since this aberration was universal for all experimental diets, we speculated that this could have been due to an unfavourable rearing environment. Because of the linear effects, an optimal PEG treatment level could not be determined for both parameters thus there is a need to further increase the maximum level of PEG treatment for GP in future studies.

Blood metabolites, size of internal organs and meat quality

Blood parameters are used as indicators of pathological and nutritional status of animals, because they provide a clearer diagnosis of toxicosis and clinical monitoring of diseases (Karesh et al. 1997). No diet-induced changes were observed for erythrocytes, haematocrit, haemoglobin, MCV, MCH, MCHC and RDW, suggesting that dietary PEG inclusion had no negative effect on the physiological status of broilers. All the haematological parameters fell within the normal ranges reported for broiler chickens (Kumanda et al. 2019a). The effect of PEG treatment on GP was more pronounced on serum biochemical parameters such as urea, BUN/CREA ratio, total protein, globulin and cholesterol, demonstrating the ability of PEG to neutralise CT and increase the bioavailability of proteins (Makkar et al. 1995) and other nutrients. It was not surprising to see quadratic trends on blood urea, BUN/CREA ratio and globulin, since these indices have a strong positive correlation with serum total protein (Omidi and Ansari Nik 2012) and are associated with better nutritional status. In their study, Yang et al. (2017) reported that GP condensed tannins supplemented at a rate of 30 mg/kg had adverse effects on blood biochemical indices, indicated by an increase in liver enzymes viz., alkaline phosphatase (ALP) and alanine aminotransferase (ALT). In this study, however, no dietary effect was observed on the concentration of ALT, illustrating that PEG treatment of GP ameliorated the negative effects of CT in GP in broiler chickens. This finding was in line with those of Abu Hasfa and Ibrahim (2018), who found no significant changes in ALT levels of broilers supplemented with polyphenol-rich grape seed. However, more studies are required to validate this finding since ALT cannot be a reliable diagnostic value in birds due to its existence in other tissues (Harr 2002). Serum glucose, phosphorus and amylase were expected to increase with PEG treatment, however, they showed neither linear nor quadratic trends, revealing that PEG is not effective in improving bioavailability and hence concentrations of these metabolites in blood. Adding GP at 6.5% to a commercial broiler diet had no effect on size of internal organs, carcase and meat quality traits, which was in line with the findings of Aditya et al. (2018). Similarly, inactivating CT using PEG did not result in any changes in meat quality attributes, indicating the inertness of both PEG as a dietary ingredient and CT as far as broiler meat quality attributes are concerned. These findings were in agreement with those of Kumanda et al. (2019b) and Chikwanha et al. (2019), who reported a lack of dietary effect on meat lightness (L^*), redness (a^*) and yellowness (b^*). Nonetheless, the redness of the meat tended to decline with increasing PEG levels indicating that the highest PEG treatment of GP might have interfered with the concentration and/or activities of anthocyanins.

Conclusion

We concluded that the use of GP as a nutraceutical in broiler diets presents an opportunity to sustainably produce high quality meat products. The positive linear effects on weight gain and feed conversion efficiency in week 4 shows that PEG treatment successfully ameliorated the negative effects of GP condensed tannins when this by-product was included in broiler diets at a high level of 6.5%. An optimum PEG inclusion level could not be determined for growth performance traits due to the linear nature of their relationship.

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