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Stress-alleviating properties of dietary red grape pomace in Ross 308 broilers reared at a high stocking density

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Abstract

Nutraceutical plant products such as red grape pomace (*Vitis vinifera L. var. Shiraz*) contain potent antioxidants that could mitigate stress caused by high stocking density. An experimental feeding trial was conducted using a total of 720 densely-stocked (30 birds/pen) Ross 308 broiler chickens (300.6 ± 9.30 g live weight) to evaluate their productive, physiological, and meat quality responses, as well as welfare indicators when supplemented with incremental levels of red grape pomace. The birds were randomly distributed to 24 pens, each with a floor space of 1.32 m^2 (1 m length × 1.2 m width × 1.55 m height). The birds were raised on standard chicken diets supplemented with 0, 15, 30, and 50 g/kg of red grape pomace. Overall feed intake, overall body weight gain, and overall gain-to-feed ratio were not influenced by experimental diets. No quadratic or linear trends were observed for blood parameters, except for neutrophils and mean platelet volume. Linear decreases were observed in the weights of duodenum, ileum, cecum, and colon as red grape pomace levels increased. With regards to meat quality, water-holding capacity increased linearly, whereas cooking loss decreased linearly as dietary red grape pomace levels increased. Increasing red grape pomace levels did not affect temperature, yellowness, lightness, chroma, and shear force, but affected initial pH and 24-hour redness and hue angle of the breast meat. The use of red grape pomace did not improve productive performance, physiology, meat quality and welfare parameters, neither did it alleviate high stocking density-induced stress.

Keywords: antioxidants, chickens, nutraceuticals, oxidative stress, welfare

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Introduction

Stocking density is a critical component of socially-sustainable and economically-viable broiler production (Beloor *et al*., 2010). The stocking densities in use vary based on breed, climate, and production system (Azzam & El-Gogary, 2015). Broilers need to be raised under ideal stocking densities if they are to reach their genetic potential in terms of growth performance. The use of high stocking density, although attractive, can lead to increased stress and reduced bird performance (Feddes *et al*., 2002; Bolacali et al., 2018). Most studies show that low stocking density enhances bird performance in terms of final body weight, weight gain, and feed utilization efficiency (Villagra *et al*., 2009; Abudabos *et al*., 2013; Nasr *et al*., 2021). Furthermore, litter quality and welfare indicators are generally improved when broilers are reared at lower stocking densities (Thomas *et al*., 2004; Buijs *et al*., 2009). However, higher stocking densities remain attractive to broiler producers because better profit margins can be obtained when more chickens are produced in a given space (Adeyemo *et al*., 2016).

Unfortunately, overcrowding of birds leads to high levels of oxidative stress that is detrimental to bird health, production (Simitzis *et al*., 2012; Li *et al*., 2019; Hasan et al., 2022), and, possibly, meat quality. According to Sugiharto *et al*. (2019), oxidative stress in broilers is due to increased production

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of free radicals or reactive oxygen species. Synthetic antioxidants have been successfully used as dietary additives to reduce the negative impact of oxidative stress in broiler production (Salami *et al*., 2015). However, overuse of synthetic antioxidants such as butylated hydroxyanisole and butylated hydroxytoluene can compromise the health of poultry consumers (Zhou *et al*., 2019) and increase the cost of broiler production and the carbon footprint of the enterprise. Therefore, it is important to identify and evaluate less expensive, natural antioxidant sources that can be used as alternatives to synthetic antioxidants to ameliorate oxidative stress in broiler chickens.

Extracts from some agro-wastes, such as red grape pomace (GP), are rich in phenolics with putative antioxidant properties (Reddy *et al*., 2018). Grape pomace is a by-product of winemaking that constitutes the fruit's skin, seeds, and stems. The pomace can further be used as a nutraceutical source in animal feeds (Viveros *et al*., 2011). It is an excellent source of a variety of biomolecules, such as flavonoids (catechins and procyanidins) and phenolic acids (Falowo *et al*., 2014). These biomolecules have antioxidant activities that can mitigate oxidative stress induced by high stocking density, particularly in broilers reared in tropical regions (Brenes *et al*., 2016). The GP also contains anthocyanins, which are touted for their ability to prevent oxidative damage to cells (Pandey & Rizvi, 2009). Moreover, the presence of anthocyanins and resveratrol in GP adds value to the by-product since these polyphenols have cardio-protective properties (Costabile *et al*., 2019).

Grape pomace also has immune-enhancing (Ebrahimzadeh *et al*., 2018), growth-stimulating (Viveros *et al*., 2011), and antilipidemic (Hosseini-Vashan *et al*., 2020) effects on broiler chickens. A useful waste management strategy for the food industry is the recycling of the GP as beneficial supplements in chicken diets to reduce adverse environmental consequences of this by-product (Kasapidou *et al*., 2015). Winery waste has higher antioxidant activity compared to synthetic food antioxidants, solvent extracts, butylated hydroxytoluene (BHT), vitamin E, and ascorbyl palmitate (Lafka *et al*., 2007). The waste can therefore be used as a source of dietary antioxidants to mitigate stocking density-induced stress in broiler chickens. Previously, Thema *et al*. (2022) reported that high stocking densities of more than 27.3 kg/m^2 decreased overall feed intake, which negatively affected final body weight in Ross 308 broilers. As a result, the current study examines the effect of incremental levels of dietary red GP on productive, physiological, and meat quality parameters, as well as welfare indicators in Ross 308 broilers raised under a stocking density higher than the recommended stocking density. It was hypothesized that supplementing broilers reared under a high stocking density with GP would positively affect productive performance, quality of meat, and bird welfare.

Materials and Methods

The red GP was purchased from the Blaauwklippen Wine Estate, Stellenbosch, South Africa (33,9692° S; 18,8444° E). The GP was chemically analysed as described by Kumanda *et al*. (2019). In a mash form, four iso-nitrogenous and iso-energetic experimental diets were formulated by including GP in standard broiler grower and finisher diets at 0 (GP0), 15 (GP15), 30 (GP30), and 50 g/kg GP (GP50) (Table 1).

Samples of the experimental diets were oven-dried (60 °C) and milled (1 mm, Retsch SM 100 cutting mill, Germany) in preparation for chemical analysis (Table 2). The organic matter, dry matter, crude fibre, crude protein, and crude fat were analysed according to Association of Official Analytical Chemists' methods (AOAC, 2005). The metabolizable energy values were predicted using models from near infrared spectroscopy (SpectraStar XL, Unity Scientific, Australia). The concentration of phosphorus (P), calcium (Ca), sodium (Na), chloride (Cl), and potassium (K) were analysed according to the Agri-Laboratory Association of Southern Africa (AgriLASA, 1998).

Table 1 Dietary ingredients (g/kg, *as is* basis) of basal grower and finisher diets supplemented with incremental levels of red grape pomace

	Grower (14–28 d)				Finisher (29–42 d)			
Ingredients	**GP0**	**GP15**	**GP30**	**GP50**	**GP0**	**GP15**	**GP30**	**GP50**
Grape pomace	0	15	30	50	0	15	30	50
Soya oilcake (46.5%)	199	161	124	72	168	133	98	52
Full-fat soya	42	90	138	212	55	97	140	196
Gluten 60	21	22	24	21	0	0	0	0
Lysine (sint 78%)	2.91	2.88	2.86	2.67	1.93	1.88	1.83	1.76
Methionine (dL 98%)	1.9	1.81	1.73	1.66	1.51	1.43	1.35	1.25
Threonine (98%)	0.34	0.32	0.3	0.27	0.1	0.08	0.06	0.03
Maize yellow	704	678	651	611	751	729	706	677
Feed Lime (50:50 mix)	14.5	14.3	14	13.5	12.5	12.3	12.2	11.9
MDCP (ws >70%)	7.2	7.3	7.4	7.5	2.2	2.3	2.3	2.3
Salt (fine)	3.12	3.16	3.2	3.31	2.78	2.82	2.87	2.94
Sodium bicarbonate	1.83	1.77	1.71	1.55	1.91	1.82	1.72	1.6
AXTRA PHY 10000 P (100g/t sk)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Zinc bacitracin (15%)	0	0	0	0	0.5	0.5	0.5	0.5
Choline Cl (60%)	0.8	0.8	0.8	0.8	0	0	0	0
Salinomycin (12%)	0.5	0.5	0.5	0.5	0	0	0	0
Olaquindox (10%)	0.4	0.4	0.4	0.4	0.2	0.2	0.2	0.2
Premix no spec	0.5	0.5	0.5	0.5	0	0	0	0
Premix no spec + choline chloride	0	0	0	0	2.5	2.5	2.5	2.5

GP0 = commercial broiler diet without GP; GP15 = commercial broiler diet containing 1.5% GP; GP30 = commercial broiler diet containing 3% GP; GP50 = commercial broiler diet containing 5% GP

Table 2 Chemical composition (g/kg, unless stated otherwise) and metabolizable energy values (MJ/kg) of experimental grower and finisher diets

	Grower (14–28 d)				Finisher (29–42 d)			
Parameters	**GP0**	**GP15**	**GP30**	**GP50**	**GP0**	**GP15**	**GP30**	**GP50**
Dry matter	891.6	893.4	896.2	899.2	889.9	890.8	893.3	896.4
[2]ME (MJ/kg)	12.0	11.9	12.0	12.0	12.1	12.1	12.2	12.2
Crude protein	170	170	170.1	170	160	161	161	160
Crude fat	34.21	34.96	35.4	39.21	41.95	49.62	53.2	58.62
Crude fibre	25.0	31.41	38.55	43.12	34.2	40.12	47.1	54.25
Organic matter	822.2	832.4	847	851.21	846.12	844.33	852	861.2
Calcium	7.99	8.0	8.1	8.0	6.65	6.6	6.6	6.0
Phosphorus	4.99	4.99	4.91	4.86	3.3	3.29	3.33	3.33
Sodium	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8
Chloride	2.88	3.1	3.1	3.1	2.5	2.5	2.5	2.5
Potassium	7.21	7.0	7.1	7.0	6.65	6.62	6.6	6.6

GP0 = commercial broiler diet without GP; GP15 = commercial broiler diet containing 15 g/kg GP; GP30 = commercial broiler diet containing 30 g/kg GP; GP50 = commercial broiler diet containing 50 g/kg GP
[1]ME = metabolizable energy

The feeding trial was carried out at the North-West University (NWU) Experimental Farm (26° 41'36" S, 27°05'35" E) and was approved (NWU-02006-20-A5) by the NWU Animal Production Research Ethics Committee (Mafikeng, South Africa). The trial was conducted during spring season with temperatures ranging from 7 to 28 °C. A total of 720, day-old male chicks were randomly and evenly placed in 24 replicate pens with a floor space of 1.32 m^2 (1 m L × 1.2 m W × 1.55 H) excluding space occupied by 5 L Poltek drinkers and Poltek tube feeders. The 30 birds per pen (22.7 birds/m^2)

was higher than the industry recommended maximum stocking density of 15 broiler birds/m^2. The chicks were raised on a standard commercial starter diet from days 1–10. On the 11^{th} day, the chicks were adapted to the experimental diets for 3 d. Throughout the first 2 w of the feeding trial, the house temperature was maintained at 35 °C using infrared electric bulbs. Measurements were taken from days 14–42. Throughout the feeding trial, the birds had unrestricted access to feed and clean water. Every morning, feed intake (FI) was calculated as the difference between feed given and feed refused. The starting body weights (300 ± 9.30 g live weight) of the birds were measured at two weeks of age and thereafter, body weights were taken at weekly intervals to determine the average weekly body weight gain (BWG). The gain-to-feed ratio (G:F) was calculated as a ratio of weight gain to feed intake.

Blood samples were collected from two randomly selected birds/pens on day 41 before feeding. Blood samples were taken from the branchial vein under the wing using disposable needles (23 gauge) and syringes (5 mL) and transferred into labelled tubes for whole blood and serum. The automated IDEXX LaserCyte Haematology Analyzer and Vet Test Chemistry Analyzer instruments (IDEXX Laboratories SA (Pty) Ltd, Gauteng, South Africa) were used to measure the haematological and serum biochemical parameters. The final body weight (FBW) was measured at day 42 for all the birds.

On day 42, two birds were randomly selected from each pen for the latency-to-lie test (LTL). The test focuses on how the broiler interacts with water as described by Berg & Sanotra (2003). Each bird was placed in a plastic tray with water (32 °C) at a depth of 3 cm. The length of time it took the bird to sit in the water was recorded. The latency-to-lie test is based on the assumption that birds whose legs are healthy will avoid sitting in the water for as long as possible. If a bird remained standing for 10 minutes, the test was terminated and the bird's legs were judged to be healthy (Weimer *et al*., 2020; Paneru *et al*., 2023).

Feather score was determined using two randomly selected birds per pen, as described by Gyles *et al*. (1962). The palm of the hand was gently moved over the breastbone in a forward to backward direction, and each bird was graded on a three-point scale (1 = no visible skin, complete feather cover; 2 = relatively small amount of skin showing; and 3 = relatively large amount of skin showing) based on the amount of flesh that could be seen through the pressed feathers. Gait score was measured on two randomly picked birds/pen based on their ability to walk using a scale of 0 (normal gait, walking freely) to 5 (bird unable to walk), as described by Kestin *et al*. (1992).

At day 42, all the birds were taken to a commercial abattoir (Rooigrond, North West, South Africa), where they were stunned and slaughtered by cutting their jugular veins. Immediately after bleeding and plucking out of feathers, the birds were eviscerated to determine internal organ sizes (gizzard, liver, spleen, proventriculus, duodenum, jejunum, and caecum), carcass weights, and carcass portions (breast, wing, thigh, and drumstick).

Breast meat pH and temperature were both measured immediately after slaughter and 24 h post-slaughter using an electrode instrument with a sharp spear-shape mounted on a Corning Model 4 pH–temperature meter (Corning Glass Works, Medfield, MA, USA). After every 20 samples, the pH meter was re-calibrated using standard solutions of pH 4, 7, and 10. A colour spectrophotometer (BYK-Gardener GmbH, Geretsried, Germany) was used to measure breast meat colour coordinates (a^* = redness, b^* = yellowness, and L^* = lightness) both after slaughter and 24 h post-slaughter. The measurement area was 20 mm in diameter, and the illumination was D65 day light at a 10° observation angle. The colour coordinates, a^* and b^*, were used to calculate the hue angle and chroma values (Priolo *et al*., 2002). The filter-paper press method developed by Grau & Hamm (1957), was used to measure the water holding capacity (WHC) in duplicate samples of breast meat. Drip loss and cooking loss were determined using the breast meat sample following the method of Honikel *et al*. (1998). Shear force (N) measurements were taken using samples of raw breast meat (Lee *et al*., 2008).

Response surface regression analysis (Proc RSREG; SAS, 2010) was used to estimate the GP inclusion levels that maximized (GP_{max}) or minimized (GP_{min}) response parameters. The data were examined for linear and quadratic effects using polynomial contrasts. Weekly FI, BWG, and G:F data were analysed using repeated measures analysis in the general linear model procedure of SAS (2010). The data was further analysed for dietary differences using one-way analysis of variance by means of PROC GLM. The probability of difference was used to compare least squares means. The Kruskal–Wallis test was used to explore statistical differences between treatment groups for gait and feather scores. For all statistical tests, significance was also declared at P <0.05.

Results and Discussion

Repeated measures analysis showed that there were no time (in weeks) and diet interaction effects on FI (P = 0.579), BWG (P = 0.687), and G:F (P = 0.438). Table 3 indicates that there were no linear and quadratic trends for overall FI, overall BWG, and overall G:F. Similar results were observed in studies by Aditya *et al*. (2018) and Ebrahimzadeh *et al*. (2018), who reported that the inclusion rate of GP between 5 and 10 g/kg diet did not affect BWG or G:F for the duration of the feeding trial. The condensed tannin content of GP is approximately 15% DM (Kumanda *et al*., 2019), which could reduce feed intake and weight gain in birds. Indeed, feeding broiler chicks with diets containing high-tannin sorghum, mimosa tannins, and fava beans has been shown to reduce growth rate (Brufau *et al*.,1998; Kumar *et al*., 2005). Contrary to our results, Rabie *et al*. (2021) reported that feeding GP-containing diets positively affected body weight gain and G:F of chicks when compared to the control group. Studies involving the use of grape by-products have shown inconsistent results in terms of growth performance of chickens. This could be due to variation in phenolic content of GP, which is influenced by a number of factors including grape variety, processing methods, and growing conditions (Hassan *et al*., 2019).

Table 3 The effect of incremental levels of red grape pomace (GP) on overall feed intake (g/bird), body weight gain (g/bird), and gain-to-feed ratio of broilers raised under high stocking density

	[1]Dietary treatments					Significance		
[2]Parameters	GP0	GP15	GP30	GP50	SEM	*P*-value	Linear	Quadratic
Overall FI (g/bird)	3272.43	3240.45	3216.25	3205.7	69.92	0.909	0.486	0.814
Overall BWG (g/bird)	1386.1	1378.0	1436.18	1285.5	45.62	0.163	0.205	0.101
Overall G:F (g:g)	0.42	0.42	0.44	0.4	0.01	0.354	0.519	0.142
Final body weight (g/bird)	1686.5	1673.8	1734.1	1593.7	45.87	0.218	0.250	0.173

[1]Dietary treatments: GP0 = commercial broiler diet without GP; GP15 = commercial broiler diet containing 15 g/kg GP; GP30 = commercial broiler diet containing 30 g/kg GP; GP50 = commercial broiler diet containing 50 g/kg GP
[2]Parameters: Overall FI = overall feed intake; Overall BWG = overall body weight gain; Overall G:F = overall gain-to-feed ratio

Blood can be utilized as a reliable indicator of an animal's physiological status and overall health. Table 4 shows that there were linear and quadratic trends for neutrophils [y = 16.80 (± 1.722) – 7.26 (± 1.889) x + 1.15 (± 0.362) x^2] in response to different levels of GP. Neutrophils are extremely efficient phagocytes, which multiply in response to an infection or stress. In this study, the GP0 group had the highest number of neutrophils, which can be an indication that those birds endured more stress when reared under high stocking density compared to birds on GP-containing diets. Broiler chickens fed GP30 showed the least number of neutrophils, suggesting that antimicrobial activity at this level of GP inclusion reduced pathogenic challenge in these birds, hence they did not need to produce high levels of neutrophils. This could be attributed to the antimicrobial activity of phenolics such as resveratrol, hydroxytyrosol, quercetin, and several phenolic acids (Aziz *et al*., 1998). It has been determined that several phenolic chemicals may act as antibacterial agents by suppressing pathogenic microorganisms in the gut (Hervert-Hernandez *et al*., 2009; Jonathan *et al*., 2021). The antimicrobial effect of grape seed extract has also been demonstrated using *in vitro* studies (Ganan *et al*., 2009; Hervert-Hernandez *et al*., 2009), as well as rats (Dolara *et al*., 2005) and chickens (McDougald *et al*., 2008). However, the current findings are in conflict with Jonathan *et al*. (2021), who reported that neutrophils were not affected by different levels of dietary GP in Hy-line Silver Brown cockerels. The discrepancy could be because in this previous study, the cockerels were not reared under high stocking density and thus were not physiologically stressed. There are, however, few reports on the effect of dietary GP on plasma biochemical indices of broiler chickens (Erinle & Adewole, 2022).

Table 5 shows that there was no dietary influence (P >0.05) on serum biochemical parameters in broilers chickens fed with the diets containing GP. There were neither linear nor quadratic effects (P >0.05) for serum biochemical parameters observed in broiler chickens. Serum biochemistry is a clinical tool that is highly reliable and is widely utilised to monitor any abnormal changes in response to both exogenous and endogenous factors (Toghyani *et al*., 2010). Serum biochemical parameters in the present study were not influenced by GP-containing diets. A similar result was reported by Kumanda *et al*. (2019) who observed that the inclusion of GP in broiler diets had no effect on all serum biochemistry parameters. In addition, Pascariu *et al*. (2017) showed that none of the serum indicators of antioxidant status were affected when broiler chickens were fed GP-containing diets.

Table 4 The effects of incremental levels of red grape pomace (GP) diets on haematological parameters of Ross 308 chickens under high stocking density

[2]Parameters	**[1]Dietary treatments**				**SEM**	**Significance**		
	GP0	**GP15**	**GP30**	**GP50**		***P*-value**	**Linear**	**Quadratic**
Erythrocytes (×10^9/L)	1.9	2.96	2.53	2.86	0.300	0.081	0.167	0.400
Haematocrits (%)	11.31	17.2	12.75	15.56	2.358	0.309	0.577	0.795
Haemoglobin (g/dL)	8.8	9.13	7.66	9.13	0.619	0.322	0.887	0.206
MCV (fL)	49.23	58.41	45.3	51.18	7.029	0.614	0.744	0.890
MCH (pg)	42.95	34.08	21.73	31.46	7.701	0.306	0.221	0.139
RDW (×10^9/L)	22.75	27.73	20.18	26.25	2.843	0.260	0.900	0.524
Reticulocytes (×10^9/L)	348.23	571.83	315.38	376.46	90.065	0.212	0.550	0.781
WBC (×10^9/L)	243.51	228.23	162.03	186.98	41.102	0.494	0.216	0.417
Neutrophils (×10^9/L)	17.25[b]	9.85[ab]	5.96[a]	9.16[a]	1.913	0.003	0.007	0.004
Lymphocytes (×10^9/L)	300.93	255.71	194.88	264.88	68.823	0.748	0.641	0.323
Monocytes (×10^9/L)	9.46	5.46	6.8	7.83	2.200	0.627	0.860	0.331
Eosinophils(×10^9/L)	1.61	0.91	1.33	1.10	0.274	0.326	0.501	0.610
Basophils(×10^9/L)	0.20	0.28	0.48	0.16	0.199	0.624	0.982	0.244
Platelets (×10^9/L)	1153.58	920.83	448.31	716.91	353.548	0.552	0.291	0.334
MVP (fL)	2.81	6.06	5.91	3.78	1.216	0.185	0.845	0.046

[1]Dietary treatments: GP0 = commercial broiler diet without GP; GP15 = commercial broiler diet containing 15 g/kg GP; GP30 = commercial broiler diet containing 30 g/kg GP; GP50 = commercial broiler diet containing 50 g/kg GP
[2]Parameters: MCV = mean corpuscular volume; MCH = mean corpuscular haemoglobin; RDW = red blood cell distribution width; WBC = white blood cells; MVP = mean platelet volume

Table 6 shows the effect GP on meat quality parameters of broilers raised at high stocking density. There was a decreasing linear trend for initial pH, where meat from GP50 birds had the lowest pH value. Furthermore, initial redness (*a**) [y = 2.69 (± 0.349) + 0.77 (± 0.338) x – 0.14 (± 0.063) x^2; R^2 = 0.196; P = 0.033; GP_{max} = 27.5 g/kg] and hue angle [y = 1.40 (± 0.025) – 0.06 (± 0.028) x – 0.01 (± 0.005) x^2; R^2 = 0.366; P = 0.045; GP_{max} = 30 g/kg] quadratically responded to incremental levels of GP. The dietary treatments had no effect on pH, temperature, lightness, yellowness, and chroma values of the breast meat. No quadratic trends were observed for all carcass traits; however, linear trends were observed for both WHC [y = 0.12 (± 0.19) + 0.10 (± 0.97) x; R^2 = 0.228; P = 0.020] and cooking loss [y = 0.13 (± 0.19) – 0.01 (± 1.01) x; R^2 = 0.019; P = 0.035]. Carcass performance is a crucial economic factor in the broiler industry (Nasr *et al*., 2017). Traits such as appearance and texture directly affect the willingness of consumers to purchase meat products.

Parameters such as pH value, colour, water loss rate, and shear force are widely used to evaluate the sensory characteristics of meat (Castellini *et al.*, 2002). Meat colour, texture, and general appearance are amongst the few factors that highlight meat quality change. Meat discoloration is associated with the processes of oxidation and enzymatic reduction of metmyoglobin levels in meat (Buckley *et al*., 1995; Shahidi & Wanasundara, 1996). In the current study, no meat quality parameters were influenced by dietary treatments. In contrast, Kumanda *et al*. (2019) showed an increase in broiler breast meat redness as the inclusion levels of GP increased, whereas the hue angle decreased with increasing levels of GP. These results are expected as the anthocyanin and free radicals in GP are known to improve the colour and quality of meat as part of their mode of action (Aditya *et al*., 2018). The lack of effect on shear force demonstrates that GP does not alter meat tenderness.

Table 5 The effects of incremental levels of red grape pomace (GP) diets on serum biochemical parameters of Ross 308 broiler chickens under high stocking density

[2]Parameters	[1]Dietary treatments				SEM	Significance		
	GP0	GP15	GP30	GP50		*P*-value	Linear	Quadratic
Glucose (mmol/L)	5.8	5.73	5.55	5.49	0.191	0.629	0.197	0.794
SDMA (μg/dL)	26.95	27.79	25.94	26.89	0.516	0.170	0.420	0.509
Creatinine (μmol/L)	9.05	9.02	9.03	9.1	0.045	0.608	0.333	0.328
Urea (mmol/L)	2.35	2.23	2.12	2.12	0.097	0.331	0.088	0.387
Phosphorus (mmol/L)	5.01	5.02	4.75	5.03	0.106	0.258	0.750	0.127
Calcium (mmol/L)	1.37	1.36	1.27	1.31	0.031	0.152	0.079	0.216
Total protein (g/L)	45.21	44.63	44.66	44.89	1.5	0.992	0.918	0.792
ALT (U/L)	129.17	139.17	133.5	136.33	4.5	0.483	0.581	0.622
ALKP (U/L)	281.67	304.81	309.95	316	10.869	0.207	0.063	0.381
GGT (U/L)	29.42	29.56	30.67	31.67	1.197	0.541	0.144	0.895
Cholesterol (mmol/L)	1.50	1.49	1.33	1.27	0.141	0.591	0.178	0.970
Amylase (U/L)	607.7	624.3	528.0	494.5	48.581	0.254	0.059	0.950
Lipase (U/L)	324.3	309.5	336.0	339.7	58.802	0.983	0.753	0.953

[1]Dietary treatments: GP0 = commercial broiler diet without GP; GP15 = commercial broiler diet containing 15 g/kg GP; GP30 = commercial broiler diet containing 30 g/kg GP; GP50 = commercial broiler diet containing 50 g/kg GP
[2]Parameters: SDMA = symmetric dimethylarginine; ALT = alanine transaminase; ALKP = alkaline phosphatase; GGT = gamma-glutamyl transferase

Table 6 The effects of incremental levels of red grape pomace on meat quality parameters of Ross 308 broiler chickens under high stocking density

[2]Parameters	[1]Dietary Treatments				SEM	Significance		
	GP0	GP15	GP30	GP50		*P*-value	Linear	Quadratic
pH_i	5.88	5.80	5.78	5.77	0.03	0.059	0.014	0.221
pH_{24}	5.88	5.83	5.82	5.83	0.035	0.564	0.312	0.302
$Temp_i$ (°C)	21.05	19.05	19.92	20.97	0.981	0.442	0.821	0.149
$Temp_{24}$ (°C)	11.52	10.10	11.27	10.92	0.559	0.329	0.823	0.441
L^*_i	59.42	58.60	58.35	60.42	1.061	0.523	0.494	0.184
L^*_{24}	61.93	62.43	61.38	62.50	0.580	0.509	0.751	0.540
a^*_i	2.78	3.27	3.93	2.87	0.361	0.131	0.704	0.033
a^*_{24}	2.60	2.68	3.07	2.45	0.223	0.276	0.836	0.113
b^*_i	15.07	14.33	15.87	14.30	0.631	0.279	0.727	0.454
b^*_{24}	13.77	13.18	14.33	13.25	0.396	0.175	0.760	0.464
Chroma	16.28	15.22	17.07	14.84	0.902	0.350	0.598	0.376
Hue angle	1.39	1.36	1.30	1.38	0.029	0.198	0.606	0.046
Breast (g)	315.63	300.87	316.79	296.30	12.148	0.548	0.423	0.778
Thigh (g)	104.55	96.79	101.06	99.96	2.929	0.338	0.510	0.310
Wing (g)	71.15	71.00	73.78	71.21	2.027	0.732	0.797	0.491
Drumstick (g)	85.00	83.70	87.33	82.52	2.117	0.436	0.628	0.376
Back length (cm)	18.60	18.63	18.40	17.09	1.145	0.748	0.331	0.595
WHC (%)	89.76	90.14	88.06	86.59	1.055	0.097	0.020	0.522
Drip Loss (%)	3.13	2.66	3.09	2.62	0.403	0.72	0.536	0.954
Cooking loss (%)	13.27	14.59	14.02	16.95	1.073	0.121	0.035	0.496
Shear force (N)	3.92	4.02	3.87	2.98	0.451	0.354	0.127	0.313

[1]Dietary treatments: GP0 = commercial broiler diet without GP; GP15 = commercial broiler diet containing 15 g/kg GP; GP30 = commercial broiler diet containing 30 g/kg GP; GP50 = commercial broiler diet containing 50 g/kg GP
[2]Parameters: Temp = temperature; *L** = lightness; *a** = redness; *b** = yellowness; WHC = water holding capacity

For internal organs, Table 7 shows that there were dietary effects (P <0.05) on the weights of the duodenum, ileum, small intestine, and colon. However, diets had no effect (P >0.05) on weights of liver, spleen, proventriculus, gizzard, duodenum, jejunum, cecum, and large intestines. Birds on GP30 had the highest duodenum weights (18.20 g). Quadratic responses were observed for liver [y = 49.94 (± 1.600) – 3.90 (± 1.549) x + 0.70 (± 0.293) x^2; GP_{max} = 27.9 g/kg; R^2 = 0.209; P = 0.026] and colon weights [y = 2.33 (± 0.154) – 0.14 (± 0.149) x + 0.06 (± 0.028) x^2; GP_{max} = 12.6 g/kg; R^2 = 0.116; P = 0.048] as dietary GP levels increased. There were linear decreases for ileum [y = 4.63 (± 1.74) – 0.68 (± 0.33) x; R^2 = 0.177; P = 0.029], cecum [y = 1.35 (± 0.71) – 0.18 (± 0.13) x; R^2 = 0.177; P = 0.038], large intestine [y = 0.70 (± 0.060) – 0.05 (± 0.11) x; R^2 = 0.219; P = 0.024], small intestine [y = 2.27 (± 1.69) – 0.18 (± 0.32) x; R^2 = 0.219; P = 0.024] and colon [y = 0.05 (± 0.0.02) – 0.15 (± 0.15) x; R^2 = 0.327; P = 0.002] weights in response to incremental levels of dietary GP.

The digestive system is crucial for nutrient digestion and absorption. It also acts as a selective barrier against pathogenic microbes and their metabolites. The integrity of the gut may be compromised by stress brought on by poor management practices, such as inadequate feed and water, high stocking density, and adverse weather conditions (Quinteiro-Filho *et al*., 2010; Laudadio *et al*., 2012). Loss of intestinal integrity and functionality can cause morbidity and, in some circumstances, mortality. It will also cause bacterial translocation, nutrient malabsorption, poor bird performance, and microbial contamination of poultry products. In the current study, dietary inclusion of GP at 30 g/kg had a positive effect on the gut of broiler chickens by increasing the duodenum and colon weights compared to the other three groups, which were statistically similar. Erinle & Adewole (2022) demonstrated that the addition of GP at 25 g/kg to broiler diets can maintain and improve gastrointestinal tract architecture in the absence of antibiotics. The current results disagree with those by Ebrahimzadeh *et al*. (2018), who reported no effect on sizes of internal organs when GP was added to broiler chicken diets at 50 and 75 g/kg. In the current study, the inclusion of GP up to 50 g/kg, resulted in linear reductions in weights of ileum, caecum, small intestines, large intestines, and colon of the birds.

Table 7 The effects of incremental levels of red grape pomace (GP) on internal organs of Ross 308 broiler chickens under high stocking density

	[1]Dietary treatments					Significance		
Parameters	**GP0**	**GP15**	**GP30**	**GP50**	**SEM**	***P*-value**	**Linear**	**Quadratic**
Liver (g)	49.79	46.10	44.17	48.06	1.690	0.136	0.438	0.026
Spleen (g)	2.62	2.41	2.71	2.46	0.221	0.751	0.840	0.867
Proventriculus (g)	7.78	7.34	8.06	7.28	0.340	0.336	0.575	0.569
Gizzard (g)	23.68	23.69	24.72	25.16	0.739	0.406	0.108	0.898
Duodenum (cm)	16.71^{ab}	15.64^{a}	18.20^{b}	15.86^{a}	0.523	0.010	0.885	0.23
Jejunum (cm)	24.30	24.51	25.34	25.10	0.803	0.777	0.389	0.704
Ileum (cm)	20.96	20.84	23.00	23.75	20.96	0.369	0.029	0.05
Cecum (cm)	10.32	10.90	13.18	12.18	0.734	0.051	0.038	0.197
Large intestine (cm)	12.96	13.18	14.85	14.79	0.636	0.085	0.024	0.629
Small intestine (cm)	60.59	60.44	67.00	66.07	1.697	0.018	0.011	0.577
Colon (g)	2.38	2.11	2.54	3.05	0.159	0.004	0.002	0.048

[1]Dietary treatments: GP0 = commercial broiler diet without GP; GP15 = commercial broiler diet containing 1.5% GP; GP30 = commercial broiler diet containing 3% GP; GP50 = commercial broiler diet containing 5% GP

With regards to welfare indicators, latency-to-lie (LTL) was not affected (P >0.05) by incremental levels of dietary GP in broilers raised in high stocking density. The group LTL medians were from 393.5–507.83 s. There were neither linear nor quadratic trends for LTL parameters observed in broiler chickens. A Kruskal–Wallis H test showed that there were no differences [H = 7.788, P = 0.051] between gait scores of treatment groups. The median gait score ranks were 17.8, 23.6, 24.7, and 32.0 for GP0, GP15, GP30, and GP50 groups, respectively. Inflammation of the foot pad substantially affects the welfare of chickens, productivity, and the selling price (Bendowski *et al*., 2022). There were no dietary effects (P >0.05) on feather scores [H = 1.136, P = 0.768]. The median feather score ranks were 26, 26, 22, and 24 for GP0, GP15, GP30, and GP50 groups, respectively.

Conclusion

The use of red grape pomace up to 50 g/kg did not improve productive performance, physiology, meat quality and welfare parameters, neither did it alleviate high stocking density-induced stress. Further research on the effectiveness of other phytochemicals, separately or in combination with GP are needed to enhance growth parameters of highly stocked Ross 308 broilers.

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Conflict of interest

No potential conflict of interest was reported by the authors.

Authors' Contributions

KKT, VM, and CMM conceptualised the study. KKT collected the data and conducted statistical analysis with VM and CMM. All authors read and approved the final version of the manuscript.

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