Effect of dietary red grape pomace on growth performance, hematology, serum biochemistry, and meat quality parameters in Hy-line Silver Brown cockerels

Ontiretse Jonathan1, Caven Mguvane Mnisi1,2*, Cebisa Kumanda3, Victor Mlambo4

1 Faculty of Natural and Agricultural Science, Department of Animal Science, North-West University, Mafikeng, South Africa, 2 Faculty of Natural and Agricultural Science, Food Security and Safety Niche area, North-West University, Mafikeng, South Africa, 3 Department of Animal Sciences, University of Pretoria, Pretoria, South Africa, 4 Faculty of Agriculture and Natural Sciences, School of Agricultural Sciences, University of Mpumalanga, Nelspruit, South Africa

* mnisiecm@gmail.com

Abstract

Red grape (Vitis vinifera L.) pomace’s (RGP) beneficial bioactive compounds could improve growth and meat quality traits in chickens and thus valorize RGP waste that is usually disposed in landfills to the detriment of the environment. This study investigated the effect of RGP inclusion in diets of Hy-line Silver Brown cockerels on physiological and meat quality responses. Five isonitrogenous and isocaloric diets were formulated by mixing a standard grower diet with RGP at 0 (G0), 15 (G15), 30 (G30), 45 (G45) and 60 g/kg (G60). A total of 250, 5-week-old cockerels (304.6 ± 6.57 g live-weight) were evenly allocated to 25 pens replicated 5 times per experimental diet. No linear and quadratic trends (P > 0.05) were observed for overall feed intake, body weight gain, feed conversion ratio, and meat quality traits as dietary RGP levels increased. Erythrocytes linearly decreased (P < 0.05), whereas mean corpuscular hemoglobin and urea linearly increased (P < 0.05) with RGP levels. There were significant quadratic effects for glucose, phosphorus, total protein, albumin, globulin, and cholesterol, from which a maximum RGP inclusion level was calculated to be 43 g/kg. In conclusion, dietary red grape pomace had no adverse effect on physiological parameters and meat quality traits of Hy-line Silver Brown cockerels. However, including red grape pomace beyond 43 g/kg could compromise serum biochemical parameters of the birds.

Introduction

In rural communities, indigenous chickens play significant nutritional and socio-economic roles as major sources of animal protein and income for various households [1]. These birds are characterized by a long-life span, tolerance to harsh nutritional environment, strong adaptability, natural scavenging, and nesting habits [2,3]. These attributes have resulted in renewed
efforts to preserve the genetic pool of indigenous chicken breeds [4], by developing hybrid chicken strains. Naturally, indigenous chickens are slow-growing birds with poor feed conversion ratios, low egg production and high mortality rates [4] compared to the conventional broiler and layer chickens. It is for this reason that improved chicken strains such as the Hy-line Silver Brown (HSB) are currently attracting research interest as alternative sources of dietary protein for human consumption. In comparison to indigenous birds, the improved chicken strains have better feed utilization efficiency, growth rates and high resistance against several poultry diseases [5]. To ensure sustainable intensification of improved chicken strains, there is a dire need to identify and evaluate locally available feed resources that have nutraceutical properties and have no direct food value for humans. Non-conventional feed sources such as red grape pomace (RGP) have the potential to improve growth rates, health, and feed utilization [6] while reducing feeding costs.

Red grape pomace is a by-product of grape wine-making process and is generally discarded in landfills as a waste product despite its abundance in polyphenolic compounds with potential nutritional and health benefits [7,8]. According to Spradling [9], the disposal of RGP generates serious environmental pollution, thus alternative uses of RGP need to be identified to facilitate proper management and/or utilization of this by-product. Nicodemus et al. [10] stated that RGP is a rich source of beneficial bioactive compounds with antioxidant, antimicrobial, growth-stimulating and meat-enhancing properties. Thus, the use of RGP in chicken feeds could significantly reduce the waste at wineries, while ensuring the production of healthier poultry products. Indeed, consumers are more attracted to meat that is organically produced, free of antibiotic residues and synthetic antioxidants [11]. It is important for animal producers to be able to modify the nutritional value of animal products to positively impact on human health and disease, while reducing feed costs and protecting the environment. The use of RGP in chicken diets has the potential to meet the requirements of health-conscious consumers when it comes to poultry products, because its bioactive compounds (phenolic acids, flavonoids and proanthocyanidins) are known to have health benefits [12]. Nonetheless, the presence of tannins as well as structural carbohydrates in RGP may interfere with the digestibility and utilization of nutrients in chickens [8]. In addition, too much research attention has focused on the effect of RGP in broiler chickens, with limited studies investigating its feed value in indigenous chicken diets. It is, therefore, crucial to establish the optimum dietary inclusion level of RGP that would not compromise the nutritional and health status of indigenous birds. From this viewpoint, the study was designed to determine the effect of varying inclusion levels of RGP on growth performance, haemato-biochemical indices, carcass characteristics, size of internal organs, and meat quality traits of HSB cockerels. We hypothesized that dietary RGP would improve the physiological and meat quality responses of HSB cockerels.

Materials and methods

Animal rights statement

The study was approved by the North-West University Animal Production Sciences Research Ethics Committee (approval number: NWU-00482-18-S5) and conformed to the guidelines for Use and Care of Research Animals.

Research site and ingredients

The study was conducted in summer at Molelwane Research farm (25° 28’0” S, 22° 28’0” E) of the North-West University, South Africa. Sun dried red grape (Vitis vinifera L. var. Shiraz) pomace was procured from Blaauwklippen wine estate (Western Cape, South Africa) as
described by Kumanda et al. [7]. The RGP was ground (Polymix PX-MFC 90 D, Kinematica AG, Switzerland) to pass through a 1-mm mesh screen before blending with the other feed ingredients.

**Diet formulation and analyses**

A feed formulation software was used to formulate five isonitrogenous and isocaloric experimental diets (Table 1). The diets were formulated using a standard chicken grower diet mixed with 0 (G0), 15 (G15), 30 (G30), 45 (G45) and 60 g/kg (G60) RGP. The maximum inclusion

| Ingredients                        | G0     | G15    | G30    | G45    | G60    |
|------------------------------------|--------|--------|--------|--------|--------|
| Red grape pomace                   | 0.0    | 15.0   | 30.0   | 45.0   | 60.0   |
| Maize yellow                       | 704.0  | 678.0  | 651.0  | 621.0  | 591.0  |
| Soya oilcake (46.5%)               | 199.0  | 161.0  | 124.0  | 85.0   | 47.0   |
| Full-fat soya                      | 42.0   | 90.0   | 138.0  | 193.0  | 249.0  |
| Gluten 60                          | 21.00  | 22.00  | 24.00  | 22.00  | 20.00  |
| Feed lime (50:50 mix)              | 14.50  | 14.30  | 14.00  | 13.60  | 13.30  |
| Monodicalcium phosphate            | 7.20   | 7.30   | 7.40   | 7.50   | 7.50   |
| Lysine (sint 78%)                  | 2.91   | 2.88   | 2.86   | 2.72   | 2.58   |
| Fine salt                          | 3.12   | 3.16   | 3.20   | 3.29   | 3.37   |
| Sodium bicarbonate                 | 1.83   | 1.77   | 1.71   | 1.59   | 1.47   |
| Methionine (DL 98%)                | 1.90   | 1.81   | 1.73   | 1.68   | 1.62   |
| Threonine (98%)                    | 0.34   | 0.32   | 0.30   | 0.28   | 0.25   |
| Phytase                            | 0.10   | 0.10   | 0.10   | 0.10   | 0.10   |
| Choline chloride (60%)             | 0.80   | 0.80   | 0.80   | 0.80   | 0.80   |
| Anticoccidial drug                 | 0.50   | 0.50   | 0.50   | 0.50   | 0.50   |
| Antibiotic mix                     | 0.40   | 0.40   | 0.40   | 0.40   | 0.40   |
| Premix                             | 0.50   | 0.50   | 0.50   | 0.50   | 0.50   |

**Nutritional composition**

| Nutrients                          | G0     | G15    | G30    | G45    | G60    |
|------------------------------------|--------|--------|--------|--------|--------|
| Dry matter                         | 895.9  | 898.4  | 901.0  | 903.7  | 906.3  |
| Ash                                | 48.47  | 48.91  | 49.28  | 49.84  | 50.41  |
| Metabolisable energy (MJ/kg)       | 12.14  | 12.14  | 12.14  | 12.14  | 12.14  |
| Crude protein                      | 179.7  | 179.4  | 179.9  | 179.6  | 179.7  |
| Crude fat                          | 38.71  | 47.11  | 55.44  | 65.07  | 74.80  |
| Crude fibre                        | 25.30  | 33.24  | 41.17  | 49.40  | 57.63  |
| Calcium                            | 8.18   | 8.21   | 8.21   | 8.20   | 8.19   |
| Phosphorus                         | 4.95   | 4.91   | 4.88   | 4.85   | 4.79   |
| Potassium                          | 7.12   | 7.25   | 7.39   | 7.61   | 7.84   |
| Sodium                             | 1.80   | 1.80   | 1.80   | 1.80   | 1.80   |
| Chloride                           | 3.00   | 3.00   | 3.00   | 3.01   | 3.00   |

1Diet: G0 = a standard chicken grower diet without red grape pomace; G15 = a standard chicken grower diet mixed with 15 g/kg red grape pomace; G30 = a standard chicken grower diet mixed with 30 g/kg red grape pomace; G45 = a standard chicken grower diet mixed with 45 g/kg red grape pomace; G60 = a standard chicken grower diet mixed with 60 g/kg red grape pomace.

2Ingredients: Phytase = Axtra phytase (100 g/t sk); Anticoccidial drug = salinomycin (12%); Antibiotic mix = olaquindox (10%); Premix: vitamin A (11000 IU), vitamin D3 (2500 IU), vitamin E (25 IU), vitamin K3 (2.0 mg), vitamin B1 (2.5 mg), vitamin B2 (4.5 mg), vitamin B6 (5.1 mg), niacin (30 mg), pantothenic acid (10 mg), folic acid (0.7 mg), biotin (0.12 g), copper sulphate (8.0 mg), potassium iodide (0.34 mg), ferrous sulphate (80 mg), magnesium sulphate (100 mg), sodium selenite (0.25 mg), and zinc sulphate (79 mg).

https://doi.org/10.1371/journal.pone.0259630.t001
level was based on results from our previous study with broiler chickens where dietary RGP at 100 g/kg compromised growth performance. The chemical composition of the RGP and the experimental diets were analyzed as described in our previous studies [7,8]. The RGP used in this study contained 966.1 g/kg dry matter (DM), 898.8 g/kg DM organic matter, 113.7 g/kg DM crude protein, 409.3 g/kg DM neutral detergent fibre, 323.3 g/kg DM acid detergent fibre, 182.3 g/kg acid detergent lignin, 70.99 g/kg DM ether extract, 1.21 AU soluble condensed tannins, and 16.43 g TAE/kg total soluble phenolics.

Feeding trial and design
Two hundred and fifty, four-week-old Hy-Line Silver Brown male chicks were bought from Quality Breeders (PTY) LTD located in Valhalla (−25.8001° S; 28.1554° E) in Centurion, South Africa. The birds were evenly and randomly housed in 25 pens (3.5 m L x 1.0 m W x 1.85 m H), designated as the experimental units, each carrying 10 birds. The five experimental diets were randomly allocated to the pens such that each treatment was replicated 5 times. The birds were allowed to acclimatize to the pens and adapt to experimental diets for one week before commencement of measurements from week 5 to week 16 of age. The wire-mesh pens had slatted floors that required no bedding. Feed was offered using poultry feed tubers and water was provided using normal poultry drinkers. The birds had free access to the diets and water, and rearing was conducted under natural lighting. At week 5 of age, the birds were weighed to obtain initial body weight (304.6 ± 6.57 g live-weight), and subsequently weighed weekly to determine average body weight gain (BWG). Feed was provided daily and refusals were collected before the next feeding and weighed to calculate average feed intake (FI) per bird. The BWG and FI data were used to calculate feed conversion ratio (FCR).

Blood collection and analyses
At week 16 of age (2 days before slaughter), blood samples were collected from 10 birds randomly selected per treatment group. The blood samples were collected (2 ml) from a punctured wing vein using a 5 ml disposable syringe fitted with a 23-gauge disposable needle, where 1 ml of blood was immediately transferred to sterilized hematological tubes containing ethylene diamine tetra acetic acid, and another 1 ml transferred to serum biochemical tubes following the guidelines by Washington & van Hoosier [13]. Hematological and serum biochemical parameters were determined using an automated IDEXX LaserCyte Hematology and an automated IDEXX Vet Test Chemistry Analyzers (IDEXX Laboratories, Inc., Gauteng, South Africa), respectively.

Carcass yield, cuts, and internal organ weights
At 16 weeks of age, all the cockerels were electrically stunned and slaughtered in a locally registered abattoir under strict hygiene. After slaughtering, carcasses from the different dietary treatments were used to determine carcass yields, hot carcass weight (HCW), and cold carcass weight (CCW). Weights of carcass cuts and internal organs were weighed using an electronic weighing scale (Explorer® EX224, OHAUS Corporation, NJ, US) and expressed as a proportion of HCW (g /100 g HCW).

Determination of meat quality parameters
According to the Commission International De l’ Eclairage [14], breast meat color coordinates: lightness (L*), redness (a*) and yellowness (b*) were measured 24 h post-mortem using a Spectrophotometer CM 2500c color-guide (Konika Minolta, Japan) set and calibrated as...
prescribed by the manufacturer. The color coordinates were used to calculate hue angle and chroma values as guided by Priolo et al. [15]. Simultaneously, breast meat pH was measured using a portable meat pH meter fitted spear-type electrode manufactured by Corning Glass Works (Medfield, MA, USA). The pH meter was calibrated every after 10 measurements using the standard pH solutions provided by the supplier.

For cooking losses, pieces of the breast meat samples were individually weighed and cooked until they reached an internal temperature of 75˚C following the method by Honikel [16]. The cooked breast meat samples were then sheared using a Warner-Batzlzer blade mounted on a Texture Analyzer (TA.XT plus, Stable Micro Systems, Surrey, UK) to determine shear force (N), a measure of meat tenderness. Breast water holding capacity (WHC) was determined using the filter-paper press method developed by Grau & Hamm [17] and calculated as follows:

\[
WHC(\%) = 100 - \left(\frac{\text{Initial weight} - \text{Weight after pressing}}{\text{Initial weight}}\right) \times 100
\]

Statistical analysis

Overall feed intake, physiological responses, carcass characteristics, internal organs and meat quality data were analyzed using one-way ANOVA by means of the general linear model procedure of SAS version 9.4 [18], where diet was the only factor. Dose-related responses to incremental levels of RGP were evaluated using polynomial contrasts (RSREG PROC, [18]). The quadratic equation: \(y = ax^2 + bx + c\), where \(y\) is the response variable; \(c\) is the intercept; \(a\) and \(b\) are the coefficients of the quadratic equation; \(x\) is RGP level (g/kg), was used to determine the optimal inclusion level of RGP as \(\frac{b}{2a}\). Significant trends derived from statistically similar RGP level means (based on the GLM procedure) were disregarded. For all statistical tests, significance was set at \(P < 0.05\) and least squares means were compared using the probability of difference option in SAS.

Results

Growth performance and blood indices

Regression results showed that there were no linear or quadratic trends \((P > 0.05)\) for overall BWG, FI, FCR and slaughter weights as dietary RGP levels increased (Table 2). Similarly, no dietary influences \((P > 0.05)\) were observed on overall BWG, FI, FCR and slaughter weights of the cockerels.

### Table 2. Effect of red grape pomace-containing diets on overall body weight gain, overall feed intake, overall feed conversion ratio, and slaughter weight of Hy-Line Silver Brown cockerels.

| Parameters       | G0  | G15 | G30  | G45  | G60  | SEM  | Linear | Quadratic |
|------------------|-----|-----|------|------|------|------|--------|-----------|
| Overall BWG (g)  | 1285.1 | 1285.9 | 1278.9 | 1237.4 | 1349.8 | 43.91 | 0.742   | 0.168     |
| Overall FI (g)   | 5108.0 | 5460.0 | 5574.7 | 5378.9 | 5709.9 | 195.0 | 0.335   | 0.810     |
| Overall FCR      | 3.98  | 4.29  | 4.37  | 4.17  | 4.33  | 0.157 | 0.300   | 0.291     |
| Slaughter weight (g) | 1599.0 | 1605.3 | 1577.7 | 1561.0 | 1569.8 | 52.16 | 0.831   | 0.124     |

1 Diets: G0 = a standard chicken grower diet without red grape pomace; G15 = a standard chicken grower diet mixed with 15 g/kg red grape pomace; G30 = a standard chicken grower diet mixed with 30 g/kg red grape pomace; G45 = a standard chicken grower diet mixed with 45 g/kg red grape pomace; G60 = a standard chicken grower diet mixed with 60 g/kg red grape pomace.

2 Parameters: Overall BWG = overall body weight gain; Overall FI = overall feed intake; Overall FCR = overall feed conversion ratio.

https://doi.org/10.1371/journal.pone.0259630.t002
Table 3 shows that mean corpuscular hemoglobin (MCH) linearly increased \( y = 38.5 (\pm 6.27) + 0.772 (\pm 0.478) x \); \( R^2 = 0.499, P = 0.006 \) in response to RGP levels. There were dietary effects \((P > 0.05)\) on MCH and eosinophils. Cockerels on the control diet G0 had lower MCH \((38.08 \text{ pg})\) compared to those on G45 and G60 diets, which were similar \((P > 0.05)\). Similarly, diet G0 promoted lower eosinophils \((0.655 \times 10^9/\text{L})\) than diets G15 and G45, which did not differ \((P > 0.05)\).

There were quadratic trends \((P > 0.05)\) for glucose, symmetric dimethylarginine (SDMA), phosphorus, total protein, albumin, globulin, and cholesterol in response to dietary RGP levels (Table 4). Serum urea linearly increased \([y = 0.76 (\pm 0.025) + 0.001 (\pm 0.002) x; R^2 = 0.272, P = 0.028]\) as RGP levels increased.

Table 5 shows that there were no dietary effects on creatinine, calcium, alkaline phosphatase (ALKP), gamma glutamyl transferase (GGT), and total bilirubin. Cockerels on diet G60 had

| Parameters | Regression equations | \( R^2 \) | \( P \) value | Optimum |
|-----------|----------------------|----------|-------------|---------|
| Glucose   | \( y = 20.7 (\pm1.15)− 0.32 (\pm0.110) x + 0.004 (\pm0.0006) x^2 \) | 0.366 | 0.008 | 40.0 |
| SDMA      | \( y = 103.1 (\pm8.21)− 2.64 (\pm0.051) x + 0.036 (\pm0.009) x^2 \) | 0.571 | 0.001 | 36.7 |
| Phosphorus| \( y = 3.30 (\pm0.250)− 0.043 (\pm0.018) x + 0.001 (\pm0.0002) x^2 \) | 0.273 | 0.038 | 43.0 |
| Total protein | \( y = 82.6 (\pm0.689)− 0.945 (\pm0.488) x + 0.017 (\pm0.0073) x^2 \) | 0.353 | 0.027 | 27.8 |
| Albumin   | \( y = 24.7 (\pm1.15)− 0.296 (\pm0.075) x + 0.004 (\pm0.0016) x^2 \) | 0.325 | 0.014 | 37.0 |
| Globulin  | \( y = 55.4 (\pm5.40)− 0.690 (\pm0.382) x + 0.015 (\pm0.006) x^2 \) | 0.472 | 0.021 | 23.0 |
| Cholesterol | \( y = 5.18 (\pm0.313)− 0.057 (\pm0.022) x + 0.001 (\pm0.0003) x^2 \) | 0.341 | 0.013 | 28.5 |

\( ^1 \text{SDMA} = \text{symmetric dimethylarginine.} \)

https://doi.org/10.1371/journal.pone.0259630.t004
higher glucose (19.98 mmol/L) than those on diets G30 and G45, which did not differ ($P > 0.05$). Diet G0 promoted higher SDMA (85.63 μg/dL) than diets G30 and G45, which were similar ($P > 0.05$). Nonetheless, birds on diet G0 had the same ($P > 0.05$) SDMA as those in diets G15 and G60. Serum urea was lowest (0.69 mmol/L) in the control group than in diets G15, G45 and G60, which did not differ ($P > 0.05$). Diet G30 promoted the least phosphorus (2.23 mmol/L) than diet G15 (3.08 mmol/L) but had similar ($P > 0.05$) phosphorus levels as the cockerels on the other treatments. Similarly, diet G30 promoted the lower total protein (68.20 g/L) than diet G60 (91.30 g/L) but had the same ($P > 0.05$) total protein as the cockerels on the other treatments.

Cockerels on diet G60 had higher globulin (67.9 g/L) than diets G15 and G30, which were similar ($P > 0.05$). Diet G60 promoted higher albumin (23.4 g/L) than diets G30 and G45, which did not differ ($P > 0.05$). Diet G15 had higher alanine transaminase (ALT) (34.70 U/L) than G45 diets (15.7 U/L). Cockerels on diet G60 had higher cholesterol (5.24 mmol/L) than diets G30 and G45, which were similar ($P > 0.05$). Nonetheless, the control diet G0 promoted similar ($P > 0.05$) glucose, phosphorus, total protein, albumin, globulin, ALT, and cholesterol levels as the RGP-containing diets.

### Carcass, visceral organs, and meat quality

Neither quadratic nor linear effects ($P > 0.05$) were observed for all carcass characteristics and internal organs as RGP levels increased (Table 6). Likewise, there were no significant dietary influences on carcass traits and visceral organs, except on proventriculus and jejunum. Cockerels on the control diet G0 had similar ($P > 0.05$) proventriculus and jejunum as those in diets G15, G30 and G60. However, diet G15 promoted the lighter ($P < 0.05$) proventriculus and jejunum weights compared to diet G45.

### Table 5. Effect of red grape pomace-containing diets on hematological parameters of 16-week-old Hy-Line Silver Brown cockerels.

| Parameters       | G0           | G15          | G30          | G45          | G60          | SEM | Linear | Quadratic |
|------------------|--------------|--------------|--------------|--------------|--------------|-----|--------|-----------|
| Glucose (mmol/L) | 16.64<sup>a</sup> | 19.23<sup>b</sup> | 15.02<sup>a</sup> | 15.48<sup>a</sup> | 19.98<sup>b</sup> | 1.105 | 0.915  | 0.008     |
| SDMA (μg/dL)     | 85.63<sup>c</sup> | 80.20<sup>c</sup> | 48.50<sup>a</sup> | 53.20<sup>b</sup> | 77.30<sup>c</sup> | 6.960 | 0.071  | 0.001     |
| Creatinine (μmol/L) | 12.13       | 12.50        | 9.60         | 14.90        | 18.50        | 2.286 | 0.156  | 0.083     |
| Urea (mmol/L)    | 0.69<sup>a</sup> | 0.80<sup>b</sup> | 0.78<sup>ab</sup> | 0.82<sup>b</sup> | 0.83<sup>b</sup> | 0.023 | 0.028  | 0.766     |
| Phosphorus (mmol/L) | 2.63<sup>b</sup> | 3.08<sup>b</sup> | 2.23<sup>a</sup> | 2.47<sup>ab</sup> | 2.93<sup>ab</sup> | 0.192 | 0.352  | 0.038     |
| Calcium (mmol/L) | 3.34         | 3.21         | 2.98         | 3.01         | 3.64         | 0.210 | 0.970  | 0.004     |
| Total protein (g/L) | 73.25<sup>a</sup> | 73.20<sup>a</sup> | 68.20<sup>a</sup> | 72.40<sup>b</sup> | 91.30<sup>c</sup> | 5.400 | 0.117  | 0.027     |
| Albumin (g/L)    | 21.00<sup>b</sup> | 22.60<sup>b</sup> | 19.30<sup>a</sup> | 19.00<sup>a</sup> | 23.40<sup>b</sup> | 1.145 | 0.720  | 0.014     |
| Globulin (g/L)   | 52.38<sup>ab</sup> | 46.40<sup>a</sup> | 48.90<sup>a</sup> | 52.80<sup>ab</sup> | 67.90<sup>b</sup> | 4.124 | 0.014  | 0.021     |
| ALT (U/L)        | 25.88<sup>b</sup> | 34.70<sup>b</sup> | 25.30<sup>ab</sup> | 15.70<sup>a</sup> | 20.0<sup>ab</sup> | 4.391 | 0.059  | 0.965     |
| ALKP (U/L)       | 266.8        | 342.9        | 399.7        | 431.5        | 373.4        | 102.8 | 0.489  | 0.657     |
| GGT (U/L)        | 49.00        | 35.60        | 43.00        | 43.70        | 49.50        | 5.041 | 0.312  | 0.311     |
| Total bilirubin (μmol/L) | 8.50     | 18.80        | 10.20        | 7.20         | 12.30        | 5.171 | 0.502  | 0.722     |
| Cholesterol (mmol/L) | 4.77<sup>b</sup> | 4.63<sup>ab</sup> | 4.32<sup>a</sup> | 4.26<sup>a</sup> | 5.24<sup>b</sup> | 0.224 | 0.531  | 0.013     |

<sup>a,b,c</sup> In a row, means with common superscripts do not differ ($P < 0.05$).

<sup>1</sup>Diets: G0 = a standard chicken grower diet without red grape pomace; G15 = a standard chicken grower diet mixed with 15 g/kg red grape pomace; G30 = a standard chicken grower diet mixed with 30 g/kg red grape pomace; G45 = a standard chicken grower diet mixed with 45 g/kg red grape pomace; G60 = a standard chicken grower diet mixed with 60 g/kg red grape pomace.

<sup>2</sup>Parameters: ALT = alanine transaminase; ALKP = alkaline phosphatase; GGT = gamma glutamyl transferase; SDMA = symmetric dimethylarginine.

https://doi.org/10.1371/journal.pone.0259630.t005
Table 7 shows that there were no significant linear or quadratic trends for meat quality parameters in response to increasing dietary RGP levels. There were significant dietary effects only on WHC, where meat from cockerels in diet G0 had a lower WHC (93.39%) than meat from those in diet G45 (97.45%). However, the control diet G0 promoted similar (P > 0.05) WHC as diets G15, G30 and G60.

Table 6. Effect of red grape pomace-containing diets on carcass characteristics and visceral organ weights (g /100 g HCW) of Hy-Line Silver Brown cockerel.

| Parameters          | G0     | G15    | G30    | G45    | G60    | SEM   | Linear | Quadratic |
|---------------------|--------|--------|--------|--------|--------|-------|--------|-----------|
| Carcass yield (%)   | 70.10  | 70.52  | 72.44  | 71.23  | 70.24  | 1.233 | 0.194  | 0.625     |
| Warm carcass (g)    | 1120.4 | 1131.8 | 1141.5 | 1112.1 | 1100.8 | 36.99 | 0.940  | 0.658     |
| Cold carcass (g)    | 1078.7 | 1089.3 | 1105.2 | 1072.6 | 1074.6 | 35.19 | 0.664  | 0.359     |
| Breast              | 12.96  | 11.76  | 11.40  | 13.96  | 11.60  | 1.355 | 0.150  | 0.115     |
| Drumstick           | 7.49   | 7.63   | 7.80   | 7.80   | 7.72   | 0.102 | 0.633  | 0.208     |
| Thigh               | 6.96   | 7.01   | 7.08   | 7.35   | 7.34   | 0.149 | 0.007  | 0.828     |
| Wing                | 6.40   | 6.44   | 6.58   | 6.43   | 6.63   | 0.125 | 0.452  | 0.278     |
| Gizzard             | 3.06   | 3.17   | 3.24   | 3.04   | 3.10   | 0.058 | 0.544  | 0.081     |
| Liver               | 2.67   | 2.53   | 2.51   | 2.48   | 2.40   | 0.080 | 0.087  | 0.183     |
| Spleen              | 0.35   | 0.40   | 0.36   | 0.42   | 0.33   | 0.034 | 0.034  | 0.101     |
| Proventriculus      | 0.61<sup>a</sup> | 0.59<sup>a</sup> | 0.65<sup>b</sup> | 0.68<sup>b</sup> | 0.67<sup>b</sup> | 0.024 | 0.136  | 0.459     |
| Duodenum            | 1.43   | 1.52   | 1.50   | 1.74   | 1.46   | 0.089 | 0.739  | 0.172     |
| Jejunum             | 2.07<sup>b</sup> | 1.90<sup>a</sup> | 2.06<sup>b</sup> | 2.35<sup>b</sup> | 2.19<sup>b</sup> | 0.076 | 0.150  | 0.931     |
| Ileum               | 1.40   | 1.23   | 1.30   | 1.46   | 2.69   | 0.460 | 0.800  | 0.150     |
| Caecum              | 1.23   | 1.16   | 1.20   | 1.39   | 1.30   | 0.063 | 0.544  | 0.868     |

<sup>a,b</sup> In a row, means with common superscripts do not differ (P > 0.05).

1Diet: G0 = a standard chicken grower diet without red grape pomace; G15 = a standard chicken grower diet mixed with 15 g/kg red grape pomace; G30 = a standard chicken grower diet mixed with 30 g/kg red grape pomace; G45 = a standard chicken grower diet mixed with 45 g/kg red grape pomace; G60 = a standard chicken grower diet mixed with 60 g/kg red grape pomace.

https://doi.org/10.1371/journal.pone.0259630.t006

Table 7. Effect of red grape pomace-containing diets on meat quality traits of Hy-line Silver Brown cockerel.

| Parameters          | G0     | G15    | G30    | G45    | G60    | SEM   | Linear | Quadratic |
|---------------------|--------|--------|--------|--------|--------|-------|--------|-----------|
| pH                  | 6.46   | 6.58   | 6.54   | 6.58   | 6.46   | 0.101 | 0.748  | 0.397     |
| Lightness (L<sup>-</sup>) | 54.66  | 53.25  | 55.16  | 54.80  | 52.77  | 1.597 | 0.955  | 0.138     |
| Redness (a<sup>'</sup>) | 4.33   | 4.51   | 3.87   | 3.02   | 4.25   | 0.683 | 0.243  | 0.735     |
| Yellowness (b<sup>'</sup>) | 5.99   | 8.44   | 7.21   | 6.56   | 7.25   | 0.741 | 0.413  | 0.871     |
| Chroma              | 7.49   | 9.59   | 8.20   | 7.24   | 8.56   | 0.875 | 0.947  | 0.908     |
| Hue angle           | 0.97   | 1.07   | 1.08   | 1.13   | 1.06   | 0.058 | 0.043  | 0.668     |
| Shear force (N)     | 8.23   | 6.61   | 7.78   | 8.18   | 8.05   | 1.206 | 0.828  | 0.650     |
| Cooking loss (%)    | 30.76  | 30.03  | 33.95  | 31.92  | 30.90  | 1.503 | 0.316  | 0.325     |
| WHC (%)             | 93.39<sup>a</sup> | 96.14<sup>ab</sup> | 95.24<sup>ab</sup> | 97.45<sup>b</sup> | 96.29<sup>ab</sup> | 0.845 | 0.134  | 0.351     |

<sup>a,b</sup> In a row, means with common superscripts do not differ (P > 0.05).

1Diet: G0 = a standard chicken grower diet without red grape pomace; G15 = a standard chicken grower diet mixed with 15 g/kg red grape pomace; G30 = a standard chicken grower diet mixed with 30 g/kg red grape pomace; G45 = a standard chicken grower diet mixed with 45 g/kg red grape pomace; G60 = a standard chicken grower diet mixed with 60 g/kg red grape pomace.

2WHC: Water holding capacity.

https://doi.org/10.1371/journal.pone.0259630.t007
Discussion

Red grape pomace can potentially be used as a functional feed ingredient in animal nutrition because of its high levels of bioactive compounds (phenolic acids, simple flavonoids, stilbenes, and proanthocyanidins) with beneficial antioxidant and antimicrobial properties [19]. However, the presence of structural carbohydrates and phenolic compounds in this by-product may limit its utilization in chicken diets [8,20]. Considering this, it is imperative to investigate the maximum tolerance level of RGP inclusion in diets of Hy-Line Silver Brown cockerels to optimize nutrient utilization, growth performance, health, and meat quality. In this study, neither linear nor quadratic trends were observed for overall feed intake of the cockerels in response to varying levels of RGP. These findings are similar to those of Francesch et al. [21] who demonstrated that inclusion of 50 g/kg of grape seed in the diet of Penedes chicken did not significantly affect feed intake. These results further corroborate the results by Alm El-Dein et al. [22] where dietary supplementation with up to 40 g/kg grape pomace did not affect feed intake in Inshas strain chickens. This suggests that the inclusion of RGP as a functional ingredient in chicken diets does not compromise the palatability of the diets. There were no significant dietary influences on overall BWG and FCR, which is consistent with the findings of Francesch et al. [21] and Alm El-Dein et al. [22] who reported no differences on growth performance of chickens fed grape pomace diets. Similarly, Lichovnikova et al. [23] reported that the diet containing RGP did not have any significant effect on growth or FCR of broiler chickens. In addition, the inclusion of RGP at 20 g/kg had no effect on FCR in molted layer hens [24].

Whole blood count is commonly used to monitor the pathophysiological and general health statuses of the birds in response to nutritional and environmental factors, infectious diseases, and stress. Erythrocyte, hemoglobin and MCH are indicators of the amount of oxygen received by body tissues [13]. Results from the current study show that there was a significant linear increase on MCH in response to dietary inclusion of RGP. This signifies the potential of RGP to increase the amount of hemoglobin per red blood cell and thus enhance oxygen transportation to tissues of the chicken. Indeed, chickens fed the RGP-containing diets had higher MCH when compared to those in the control group. It is, however, important to note that MCH values remained within the normal range for chickens [25]. Furthermore, cockerels on the control treatment had the lowest eosinophil levels while those fed the RGP-containing diets had the highest, which could have been an allergic response to some of the secondary metabolites present in RGP [26]. It is also worth noting that eosinophils are reported to exhibit diurnal changes. Leukocytes and neutrophils, which are reported to increase as a response mechanism towards infection and stress, were not significantly influenced by the inclusion of dietary RGP in the diets. Due to the presence of antinutritional compounds in RGP, it was expected that the whole blood count of the cockerels fed with the RGP-containing diets would be negatively affected. Nonetheless, the blood indices were observed to be within the normal range for healthy chickens implying that RGP had no negative post-ingestive feedback.

Serum biochemistry is a reliable clinical tool widely used to monitor any changes in response to internal and exogenous factors [27]. In this study, quadratic trends were observed for glucose, SDMA, phosphorus, total protein, albumin, globulin, and cholesterol in response to increasing levels of RGP levels. This confirms that higher inclusion levels of RGP beyond 43 g/kg can compromise the health status of the birds. Cockerels on the highest RGP inclusion level had high glucose levels, which was surprising because the high levels of cellulose and hemicellulose in the RGP was expected to reduce energy density and utilization, and thus causing a decline in glucose levels. Furthermore, RGP has a protective role in the β-cell functions of the pancreas, which is thought to reduce glucose levels due to the antioxidative effect of
proanthocyanidins in GP [7]. Moreover, research indicates that some phenolic compounds in grape seed may inhibit sodium-dependent glucose transport, increasing insulin resistance and reducing glucose absorption in the digestive tract [28]. Thus, the high levels of glucose could be a result of increased liver activity to detoxify amounts of secondary plant metabolites in RGP. These results were consistent with those of Khodayari and Shahriar [29], who reported that the inclusion of grape pomace at 60 g/kg in broiler diets elevated glucose levels due to high nitrogen-free extract (glucose and fructose) in the grape pulp. Urea levels were also high in cockerels fed with 60 g/kg of RGP diets when compared to those in the control group, which could have been influenced by the high total protein concentration. This is because there is a positive correlation between total protein and its constituents (urea, creatinine, bilirubin, and globulin). Similarly, no variation in total protein concentrations in the serum of chickens fed RGP-containing diets was reported [30]. High levels of liver enzymes (e.g., GGT, ALT and ALKP) above normal ranges signify hepatocellular degeneration [13]. In this study, the inclusion of dietary RGP did not alter the liver enzymes further demonstrating that phenolics and other antinutrients in RGP did not negatively affect the health status of the birds. Cockerels on diet 60 g/kg RGP had a high cholesterol level, in contrast to Khodayari and Shahriar [29] who reported a decline in cholesterol levels in broiler chickens fed diets with RGP. The fibre in RGP was expected to result in a reduction of the cholesterol level through the absorption of bile acids and various lipids [31].

The ultimate measure of profitability in any poultry enterprise is the volume of meat of high quality. Nonetheless, the inclusion of dietary RGP had no effect on all carcass characteristics and some organs. These findings agreed with those of Aditya et al. [32], who reported that supplementation of RGP in diets of laying hens did not show any effect on carcass traits and internal organ sizes. However, the inclusion of dietary RGP linearly increased the size of the spleens, which could explain the linear increase observed for MCH. Spleen enlargement indicates an anatomical adaptation to fight against infectious or inflammatory diseases and stabilizes both red and white blood cells. These results are similar to those of Brenes et al. [19] who found that the addition of RGP up to 60 g/kg did not affect liver and pancreas weight but affected the spleen weights. Cockerels fed the control treatment had similar proventriculus, gizzard, and jejunum sizes as those in the RGP-containing diets. Theoretically, the size of the intestines was expected to be longer in the RGP cockerels as an adaptive mechanism to utilize fibre and enhance digestion.

In previous studies, the use of RGP in chicken feeds was reported to improve product quality [33,34]. Contrastingly, the inclusion of varying levels of RGP had no significant linear or quadratic effects on all meat quality parameters. This was surprising because the presence of anthocyanins in RGP [35] were expected to improve the color and appearance of the meat. Only water holding capacity was influenced by the inclusion of dietary RGP with meat from cockerels on the control diet retaining less water than meat from those in diet G45. Nonetheless, the control diet promoted similar WHC as diets G15, G30 and G60. It is, however, not clear what might have caused this variation on WHC, thus more research is required to understand the effect dietary RGP in chicken meat. Overall, dietary RGP did not compromise overall feed intake, growth performance, hematological parameters, carcass traits, internal organs, and meat quality responses of the chickens. However, levels beyond 43 g/kg of dietary RGP could negatively affect serum biochemical indicators of the birds.

Supporting information

S1 File.
(XLSX)
Author Contributions

Conceptualization: Caven Mguvane Mnisi, Victor Mlambo.
Data curation: Ontiretse Jonathan, Caven Mguvane Mnisi.
Formal analysis: Ontiretse Jonathan, Caven Mguvane Mnisi.
Investigation: Ontiretse Jonathan, Caven Mguvane Mnisi.
Methodology: Ontiretse Jonathan, Caven Mguvane Mnisi.
Project administration: Caven Mguvane Mnisi.
Resources: Caven Mguvane Mnisi.
Software: Caven Mguvane Mnisi.
Supervision: Caven Mguvane Mnisi, Victor Mlambo.
Validation: Caven Mguvane Mnisi, Victor Mlambo.
Visualization: Caven Mguvane Mnisi, Victor Mlambo.
Writing – original draft: Ontiretse Jonathan, Caven Mguvane Mnisi, Cebisa Kumanda, Victor Mlambo.
Writing – review & editing: Caven Mguvane Mnisi, Cebisa Kumanda, Victor Mlambo.

References

1. Mtileni BJ, Muchadeyi FC, Mawase A, Groeneveld E, Groeneveld LF, Dzama K, et al. Genetic diversity and conservation of South African indigenous chicken populations. Journal of Animal Breeding and Genetics. 2011; 128:209–218. https://doi.org/10.1111/j.1439-0388.2010.00891.x PMID: 21554415
2. Research Hoffman I. and investment in poultry genetic resources—challenges and options for sustainable use. World’s Poultry Science Journal. 2005; 61:57–70. https://doi.org/10.1079/WPS200449
3. Van Marle-Koster E, Hefer CA, Groenen MAM. Genetic diversity and population structure of locally adapted South African chicken lines: Implications for conservation. South African Journal of Animal Science. 2008; 38:271–281.
4. Larbi A, Rymkiewicz P, Vasudev A, Low I, Shadan NB, Mustafah S, et al. The immune system in the elderly: A fair fight against diseases? Aging Health. 2013; 9:35–47. https://doi.org/10.2217/ahc.12.78
5. Atela J, Mlambo V, Mnisi CM. A multi-strain probiotic administered via drinking water enhances feed conversion efficiency and meat quality traits in indigenous chickens. Animal Nutrition. 2019; 5:179–184. https://doi.org/10.1016/j.aninu.2018.08.002 PMID: 31193861
6. Landy N, Ghalmakari G, Tohyani M. Performance, carcass characteristics, and immunity in broiler chickens fed dietary neem (Azadirachta indica) as alternative for an antibiotic growth promoter. Livestock Science. 2011; 142:305–309. https://doi.org/10.1016/j.livsci.2011.08.017
7. Kumanda C, Mlambo V, Mnisi CM. From landfills to the dinner table: Red grape pomace waste as a nutraceutical for broiler chickens. Sustainability. 2019; 11:1931. https://doi.org/10.3390/su11071931
8. Van Niekerk RF, Mnisi CM, Mlambo V. Polyethylene glycol inactivates red grape pomace condensed tannins for broiler chickens. British Poultry Science. 2020; 61:566–573. https://doi.org/10.1080/00071668.2020.1755014 PMID: 32290682
9. Spradling VB. Phenolics in red wine pomace and their potential application in animal and human health. University of Missouri–Columbia, Missouri, USA. 2008.
10. Nicodemus N, García J, Carabaño R, de Bias JC. Effect of substitution of a soybean hull and grape seed meal mixture for traditional fibre sources on digestion and performance of growing rabbits and lactating does. Journal of Animal Science. 2007; 85:181–187. https://doi.org/10.2527/jas.2005-365 PMID: 17179554
11. Kaluza J, Wolk A, Larsson SC. Red meat consumption and risk of stroke: A meta-analysis of prospective studies. Stroke 2012; 43:2556–2560. https://doi.org/10.1161/STROKEAHA.112.663286 PMID: 22851546
12. Chamorro S, Viveros A, Rebolé A, Rica BD, Arija I, Brenes A. Influence of dietary enzyme addition on polyphenol utilization and meat lipid oxidation of chicks fed grape pomace. Food Research International. 2015; 73:197–203. https://doi.org/10.1016/j.foodres.2014.11.054
13. Washington IM, van Hoosier G. Clinical Biochemistry and Hematology. University of Washington, Seattle, WA, USA. 2012. p. 59–91.
14. Commission Internationale de l’Eclairage (CIE). Recommendations on uniform color spaces-color. Di Erance equations, psychometric color terms; Supplement no. 2 to CIE Publication no. 15 (E-1.3.1.) 1978, 1971/(TC-1-3), Paris, France. 1976.
15. Priolo A, Miccol D, Agabriel J, Prache S, Dransfield E. Effect of grass or concentrate feeding systems on lamb carcass and meat quality. Meat Science. 2002; 61:179–185. https://doi.org/10.1016/s0309-1740(01)00244-3 PMID: 22061409
16. Honikel KO. Reference methods for the assessment of physical characteristics of meat. Meat Science. 1998; 49:447–570. https://doi.org/10.1016/s0309-1740(98)00034-5 PMID: 22060262
17. Grau R, Hamm R. About the water-binding capacity of the mammal muscle. II. Communication. The determination of water binding capacity of the muscle. Zeitschrift Lebensm Unters Forsch. 1957; 105:446–460. https://doi.org/10.1007/BF01126901
18. SAS. Users Guide: Statistics. Version 9.4. Statistical Analysis System Institute Inc. Carry, NC, USA. 2010.
19. Brenes A, Viveros A, Goni I, Centeno C, Sayago-Ayerdí SG, Arija I, et al. Effect of grape pomace concentrate and vitamin E on digestibility of polyphenols and antioxidant activity in chickens. Poultry Science. 2008; 87:307–316. https://doi.org/10.3382/ps.2007-00297 PMID: 18212374
20. Zaitkarenab L, Pirmohammadi R, Teimuriyansari A. Chemical composition and digestibility of dried white and red grape pomace for ruminants. Journal of Animal and Veterinary Advantages. 2007; 6:1107–1111.
21. Francesch A, Cartañá M. The effects of grape seed in the diet of the Penedes chicken, on growth and on the chemical composition and sensory profile of meat. British Poultry Science. 2015; 56:477–485. https://doi.org/10.1080/00071668.2015.1062842 PMID: 26081989
22. El-Dein AK, Rashed OS, Ouda MMM, Awaden NB, Ismail II, Mady MS. Comparative study between dietary supplementation of grape pomace and vitamin E as antioxidant on some productive, reproductive and physiological performance of male and female aged Inshas strain chickens. Egyptian Poultry Science Journal. 2017; 37:855–872. https://doi.org/10.21608/epsj.2017.7736
23. Lichovnikova M, Kalhotka L, Vojtech A, Borivoj K, Vojtech A. The effects of red grape pomace inclusion in grower diet on amino acid digestibility, intestinal microflora, and serum and liver antioxidant activity in broilers. Turkish Journal of Veterinary and Animal Sciences. 2015; 39:406–412. https://doi.org/10.3906/vet-1403-64
24. Kara K, Kocaoglu-Gucu B. The effects of different molting methods and supplementation of grape pomace to the diet of molted hens on postmolting performance, egg quality and peroxidation of egg lipids. Journal of Faculty of Veterinary Medicine, Erciyes University. 2012; 9:183–196.
25. Reece OW. Functional anatomy and physiology of domestic animals, 4th ed. Wiley-Blackwell: Ames, IA, USA. 2009. p. 49–78.
26. Kara K, Kocaoglu-Gucu B, Baytok E, Senturk M. Effects of grape pomace supplementation to laying hen diet on performance, egg quality, egg lipid peroxidation and some biochemical parameters. Journal of Applied Animal Research. 2016; 44:303–310. https://doi.org/10.1080/09712119.2015.1031758
27. Toghyani M, Toghyani M, Gheisari A, Ghalakmari G, Mohammadrezaei M. Growth performance, serum biochemistry and blood hematolgy of broiler chicks fed different levels of black seed (Nigella sativa) and peppermint (Mentha piperita). Livestock Science. 2010; 129:173–178. https://doi.org/10.1016/j. livestock.2010.01.021
28. Shanmuganayagam D, Warner TF, Krueger CG, Reed JD, Folts JD. Concord grape juice attenuates platelet aggregation, serum cholesterol and development of atheroma in hypercholesterolemic rabbits. Atherosclerosis. 2007; 190:135–142. https://doi.org/10.1016/j.atherosclerosis.2006.03.017 PMID: 16780846
29. Khodayari F, Shahria HA. The effect of red grape pomace on performance, lipid peroxidation (MDA) and some serum biochemical parameters in broiler. Advances in Bioresearch. 2014; 5:82–87.
30. Ebrahimzadeh SK, Navidshad B, Farhoormand P, Mirzaei Aghjehgheshlagh F. Effects of grape pomace and vitamin E on performance, antioxidant status, immune response, gut morphology and histopathological responses in broiler chickens. South African Journal of Animal Science. 2018; 48:324–336. https://doi.org/10.4314/sajas.v48i2.13
31. Evans RJ, Derkach V, Surprenant A. ATP mediates fast synaptic transmission in mammalian neurons. Nature. 1992; 357:503–505. https://doi.org/10.1038/357503a0 PMID: 1351659
32. Aditya S, Ohh SJ, Ahammed M, Lohakare J. Supplementation of grape pomace (Vitis vinifera) in broiler diets and its effect on growth performance, apparent total tract digestibility of nutrients, blood profile, and meat quality. Animal Nutrition. 2018; 4:210–214. https://doi.org/10.1016/j.aninu.2018.01.004 PMID: 30140761

33. Sáyago-Ayerdi SG, Brenes A, Gofii I. Effect of grape antioxidant dietary fiber on the lipid oxidation of raw and cooked chicken hamburgers. LWT-Food Science and Technology. 2009; 42:971–976. https://doi.org/10.1016/j.lwt.2008.12.006

34. Iqbal Z, Ali R, Sultan JI, Ali A, Kamran Z, Khan SA, et al. Impact of replacing grape polyphenol with vitamin E on growth performance, relative organs weight and antioxidant status of broilers. Journal of Animal and Plant Sciences. 2014; 13:1579–1583.

35. Távarez MA, Boler DD, Bess KN, Zhao J, Yan F, Dilger AC, et al. Effect of antioxidant inclusion and oil quality on broiler performance, meat quality, and lipid oxidation. Poultry Science. 2011; 90:922–930. https://doi.org/10.3382/ps.2010-01180 PMID: 21406381