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

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A R T I C L E   I N F O

Article history:
Received 26 July 2017
Received in revised form 19 December 2017
Accepted 8 January 2018
Available online 31 January 2018

Keywords:
Broiler
Digestibility
Growth
Immunity
Meat quality
Grape pomace

A B S T R A C T

This experiment was conducted to explore the efficacy of grape pomace (Vitis vinifera) on growth performance, apparent total tract digestibility of nutrients, blood profile, and meat quality in commercial broilers. Four hundred broiler chicks (3-d-old) were randomly allotted to 4 dietary treatments for 28 d. Each treatment had 5 replicates with 20 birds per replicate. The dietary treatments were 1) control, 2) 5 g/kg grape pomace (GP), 3) 7.5 g/kg GP, and 4) 10 g/kg GP supplemented in diets after drying. Supplementation of GP did not show linear effects (P > 0.05) on body weight (BW) gain, however, quadratic effects (P < 0.05) on BW gain were observed during d 0 to 7 and d 8 to 14. Body weight gain, feed intake, and feed conversion ratio remained unaffected during d 22 to 28 and overall period. The nutrient digestibility studies conducted at the end of the feeding trial did not show (P > 0.05) any effect due to GP supplementation, except a quadratic trend (P = 0.07) for digestibility of ash was observed. Serum levels of glucose, triglyceride, and high-density lipoprotein cholesterol were not affected (P > 0.05), however, total cholesterol and serum immunoglobulin G levels showed quadratic effects (P < 0.05) due to GP supplementation. The thio-barbituric acid reactive substances values in breast meat linearly decreased (P < 0.01) in supplemented groups at 0, 5, and 10 d of storage showing linear effects due to GP supplementation, and quadratic effects were also observed at 5 and 10 d of storage. The meat color value such as redness was also decreased (P < 0.05) in supplemented groups showing both linear and quadratic effects. Overall, it could be concluded that GP supplementation showed quadratic effects on BW gain during early growth stages and was effective in reducing serum cholesterol level and improving meat quality parameters in broilers.

1. Introduction

Food and Agriculture Organization of the United Nations has reported that grape (Vitis vinifera) is one of the most abundant fruits in the world with production of 61 × 10^6 t in 2001 and 66 × 10^6 t in 2007 (FAOSTAT, 2002; FAOSTAT, 2008). Grape produce by-products such as stem, seed, and skin from juice extraction process. Its by-product is called grape pomace (GP). Grape pomace contains phytochemicals which are rich in flavonoids such as catechin, anthocyanin, and epicatechin (Saito et al., 1998; Djilas et al., 2009; Turner, 2009).

Anthocyanins of flavonoids have been known having antioxidant capacity and preventing cells from oxidative damage (Pandey and Rizvi, 2009). Total anthocyanin content in GP is 1,134 mg/kg (Khanal et al., 2009). Anthocyanins act as an antioxidant source by giving electron to free radicals (Pandey and Rizvi, 2009). It has been reported that they are able to improve antioxidative enzyme in cells or tissues (Wu and Prior, 2008). Recent trend of using phytochemicals as a natural antioxidant source is more acceptable than synthetic antioxidants as synthetic oxidants may cause mutagenic
and toxic effects on the body (Djilas et al., 2009; Sen et al., 2010). Synthetic antioxidants such as butylated hydroxytoluene when added in feeds for chicken are retained in the meat and eventually reach the food chain (Nieuw-Echevarria et al., 2015).

The beneficial effects of GP feeding in poultry have been proved in prior experiments (Kara et al., 2016; Kasapidou et al., 2016). Supplementation of GP as an antioxidant source as high as 30 g/kg (Brenes et al., 2007) and 60 g/kg (Goni et al., 2006) decreased lipid oxidation in meat of broiler chicks. Furthermore, Viveros et al. (2010) reported that GP increased population of beneficial bacteria in ileum of broiler chicks. The large quantities available and the amount of bioactive compounds present in GP make it a suitable candidate to be used in animal nutrition. However, systematic studies are lacking in terms of performance and digestibility as well as its effect on meat quality. Hence, this study was carried out to elucidate the effects of GP on growth performance, apparent total tract digestibility of nutrients, blood profile, carcass traits, and meat quality of broilers.

2. Material and methods

2.1. Preparation of grape pomace

Grape pomace was procured from Manna Grape Company in Chuncheon-si, Gangwon-do, Korea. For preparing it as a feed supplement, GP was dried at 65 °C for 4 d. The dried GP was further used for this experiment.

2.2. Broiler management

A total of 400, 3-d-old male broilers (ROSS 308, average 75 g BW) were procured from local commercial hatchery and housed for 4 wk in floored pens with rice husk as bedding material. Birds were randomly allocated to 4 dietary treatments for 4 wk. Each treatment had 5 replicate pens with 20 birds per pen with the size of 2 m x 1 m. The GP was supplemented at 0 (Control), 5 (GP1), 7.5 (GP2), and 10 g/kg (GP3) in the diets to 4 dietary treatments according to a randomized complete block design, respectively. Grape pomace was dried and then required amount was mixed with 5 kg corn before being added to mix final. Different diets were formulated for starter phase (d 1 to 14 of trial) and finisher (d 15 to 28 of trial) as shown in Table 1. The diets either meet or exceed the National Research Council (1994) requirements for broilers. Feed and water were available for ad libitum consumption. Furthermore, feed intake and BW were measured at d 7, 14, 21, and 28 of feeding trial. The experimental protocol was approved by Animal Research and Ethics Committee of Kangwon National University, Chuncheon.

The room temperature was maintained at 33 °C for the d 3 and then reduced gradually to 22 °C. Air ventilation was arranged by automatic ventilator to manage room humidity.

2.3. Sample collection and analysis

At the end of trial, 10 chicks from each treatment were selected that represented the treatment (2 chicks per replication). Blood was taken by cardiac puncture and placed into vacutainer tubes (Becton Dickinson, Franklin Lakes, New Jersey, USA). Before the blood samples were centrifuged at 3,000 × g for 15 min, the samples were refrigerated at 4 °C. Serum was separated and stored at −80 °C for further analysis of total cholesterol, triglyceride, glucose, high-density lipoprotein cholesterol, and immunoglobulin G (IgG). The serum biochemical measurements were determined by using kits (Asan Pharmaceutical Co. Ltd., Seoul, Korea) and spectrophotometer (Optizen 3220 UV, Mecasy Co. Ltd., Daejon, Korea), except IgG which was analyzed by using kit (Bethyl Laboratories, Montgomery, AL, USA), and microplate spectrophotometer (Powerwave XS, BioTek Instruments Korea Ltd., Seoul, Korea).

After blood collection, birds were slaughtered. After evisceration, carcass and organ weights including abdominal fat, liver, heart, spleen, bursa of Fabricius, and thymus were measured. After the weighing, breast meat was collected and packaged, then kept at −4 °C until time course analysis for thiobarbituric acid reactive substances (TBARS) and meat color values at 0, 5, and 10 d of storage. Before measurement of TBARS and color values, breast meat samples were thawed at room temperature for 1 h. The TBARS value was analyzed according to procedure of Sinnhuber and Yu (1977) and meat color for lightness, redness, and yellowness was measured by using color difference meter (Yasuda Seiko Co., Minolta, Japan).

Digestibility studies were done after 28 d of feeding trial. Forty birds (10 birds per treatment; 2 birds that represented pen) were placed individually in separate cages (0.2 m x 0.2 m). After acclimatization for 4 d, excreta from each cage were collected for further 72 h of trial (Sales and Janssens, 2003). Afterward, excreta was dried in dry air oven at 65 °C for 4 d and stored for further chemical analysis.

The diets and excreta were analyzed for moisture by oven drying (930.15), ash by incineration (942.05), protein by Kjeldahl (984.13), and ether extract by Soxhlet fat analysis (920.39), as described by the AOAC International (2000). Gross energy was analyzed using bomb calorimeter (Parr 1281, Parr Instrument Co., Moline, IL, USA). The proximate composition of the diets and excreta was used to calculate apparent total tract digestibility (ATTD) of DM, crude protein, ether extract, and crude ash according to the standard formula for the total collection method (Maynard and Loosli, 1969):

### Table 1

| Item | Starter (d 0 to 14) | Finisher (d 15 to 28) |
|------|-------------------|---------------------|
| Ingredient, g/kg | | |
| Corn | 400.8 | 395.1 |
| Wheat flour | 0.2 | 0 |
| Wheat | 150 | 150 |
| Corn gluten | 20 | 15.2 |
| Distiller’s dried grains with solubles | 40 | 40 |
| Full fat soybean | 80 | 90 |
| Soybean meal | 205.6 | 177.6 |
| Rapeseed meal | 20 | 20 |
| Rye flour | 15 | 14.8 |
| Monocalcium phosphate | 11.2 | 8.8 |
| Sodium chloride | 2.8 | 2.8 |
| Yellow grease | 42.4 | 32 |
| Refined beef tallow | 0 | 12.8 |
| Choline chloride (50%) | 1.6 | 1.9 |
| DL-methionine (98%) | 3.1 | 2.0 |
| L-lysine (98%) | 4.4 | 3.8 |
| Threonine (98%) | 0.5 | 0.5 |
| Vitamin premix \(^1\) | 1 | 1 |
| Mineral premix \(^2\) | 1 | 1 |
| Clincac | 0.5 | 0 |
| Maduraminic | 0 | 0.5 |
| Chemical composition, g/kg | | |
| Crude protein | 210 | 200 |
| Crude fat | 66.1 | 90.4 |
| Ash | 55.7 | 51.6 |
| Metabolizable energy, MJ/kg | 13.18 | 13.40 |
| Lysine | 13.5 | 12.5 |
| Methionine + Cysteine | 10 | 9.5 |

\(^1\) Vitamin per kilogram of diet: Vitamin A 14,000 IU, Vitamin D 3,000 IU, Vitamin E 40 mg, Vitamin K\(_2\) 2.4 mg, thiamine 1.2 mg, riboflavin 5 mg, pyridoxine 3 mg, cobalamin 0.02 mg, niacin 40 mg, pantothenic acid 10 mg, folic acid 0.5 mg, biotin 0.07 mg.

\(^2\) Minerals per kilogram of diet: Mn 72 mg, Fe 48 mg, Cu 5 mg, Zn 60 mg, Se 0.18 mg, Co 0.24 mg, I 11 mg.
There was no linear and quadratic effects (\(P > 0.05\)) during d 8 to 14 showing quadratic effects on BW gain (\(P < 0.05\)) (Table 3). Dietary supplementation of GP at different levels had no effect on overall feed conversion ratio (FCR) although linear effect (\(P < 0.01\)) was found at d 15 to 21. Also, supplemental GP levels showed quadratic effects (\(P < 0.05\)) on FCR during d 0 to 7, and d 15 to 21.

The ATTD of crude ash showed quadratic trend (\(P = 0.07\)) but ATTD of other nutrients did not show any linear or quadratic effects, thereby not influenced by GP supplementation (Table 4).

Serum total cholesterol levels were lower in GP supplemented groups showing a quadratic effect (\(P < 0.05\)) (Table 5). In addition, IgG values also showed a quadratic effect (\(P < 0.05\)) due to GP supplementation. However, other serum biochemicals such as glucose, high-density lipoprotein (HDL)-cholesterol, and triglyceride did not show any difference among treatments (Table 5).

Supplementation of GP in diets did not show any effect on carcass traits such as carcass percent, abdominal fat, bursa of Fabricius, heart, liver, spleen, and thymus (Table 6).

The TBARS value was decreased (\(P < 0.01\)) in supplemented groups at 0, 5, and 10 d of storage showing a linear effect (Table 7). There were also quadratic effects (\(P < 0.01\)) in TBARS value on 5 and 10 d of storage. The meat color value for lightness showed a quadratic effect (\(P < 0.01\)) at 10 d of storage but there was no effect at 5 d of storage (Table 7). The redness value of breast meat in GP supplemented groups decreased (\(P < 0.01\)) showing a linear effect at both 5 and 10 d of storage, but GP levels did not affect yellowness values at 5 d of storage. However, at 10 d, yellowness values were influenced by GP supplementation showing both linear and quadratic effects (\(P < 0.01\)).

2.4. Statistical analysis

All the data collected were statistically analyzed using SPSS 19.0 software version. Experiment was a randomized complete block design and appropriate linear and quadratic components of the treatment effects were determined. Pens were the experimental unit for all analysis. Linear and quadratic effects due to levels of GP were measured. An alpha level of 0.05 was used to determine statistical significance and differences among means with 0.05 < \(P < 0.10\) was accepted as representing tendencies to differences.

3. Results

For d 0 to 7, no linear effects (\(P > 0.05\)) on BW gain were found but there was a significant difference among treatments showing quadratic effect on BW gain (\(P < 0.01\)) (Table 3). Similar trend followed during d 8 to 14 showing quadratic effects on BW gain (\(P < 0.05\)). For d 15 to 21, 22 to 28, and overall (d 0 to 28) period, there was no linear and quadratic effects (\(P > 0.05\)) on BW gain of birds fed GP. The feed intake was not affected by dietary GP levels at any time period, except for d 8 to 14 where a quadratic trend (\(P = 0.06\)) was observed (Table 3). Dietary supplementation of GP at different levels had no effect on overall feed conversion ratio (FCR)

Table 2: Nutrient composition (g/kg) of grape pomace (as-fed basis).

| Nutrient composition | Content |
|----------------------|---------|
| Moisture             | 110     |
| Ash                  | 26.70   |
| Crude protein        | 95      |
| Crude fat            | 86.80   |
| Total polyphenol     | 8.34    |
| Total anthocyanin    | 1.05    |

1 Analyzed in triplicate.

Table 3: Effect of grape pomace on growth performance of broiler chicks.

| Item                  | Treatments2 | SEM | P-value |
|-----------------------|-------------|-----|---------|
|                      | GP0         | GP1 |         |
| Initial BW, g         | 72.6 ± 1.1  | 74.6 ± 1.6 |       |
| d 0 to 7              | 73.4 ± 1.1  | 73.6 ± 1.5 |       |
| BW gain, g/bird       | 167.58      | 177.46 | 178.95 |
| FCR                   | 1.75        | 1.51  | 1.67   |
| d 8 to 14             | 159.85      | 313.62 | 1.96   |
| BW gain, g/bird       | 279.02      | 320.90 | 311.00 |
| FCR                   | 487.36      | 515.40 | 533.60 |
| d 15 to 21            | 305.70      | 498.50 | 1.63   |
| BW gain, g/bird       | 558.56      | 543.70 | 536.50 |
| FCR                   | 747.52      | 759.10 | 787.80 |
| d 22 to 28            | 532.10      | 760.50 | 1.42   |
| BW gain, g/bird       | 13.4 ± 1.39 | 13.9   | 1.46   |
| FCR                   | 618.17      | 623.60 | 637.12 |
| Overall               | 627.33      | 834.80 | 1.33   |
| BW gain, g/bird       | 9.9         | 24.80  | 1.45   |
| FCR                   | 1,636.27    | 2,360.50 | 1.45   |

1 Each mean represents 5 replicates. NS = not significant.

4. Discussion

The growth performance data showed that the addition of GP at different levels did not significantly affect BW gain as well as feed intake and FCR during d 22 to 28 and overall period. The results are in agreement with Brenes et al. (2007) that inclusion of GP up to 60 g/kg did not affect growth performance but it reduced feed.
efficiency in 30 g/kg of GP level. However, a quadratic effect on BW gain was observed in the present study during d 0 to 7 and d 8 to 14. Goni et al. (2006) demonstrated that the addition of GP as high as 30 g/kg did not show any effect on growth performance either in BW gain, feed intake, or feed efficiency. A quadratic effect due to GP supplementation on FCR was observed in the present study during d 0 to 7 and d 15 to 21. Based on the observed data, the GP supplementation at 5 g/kg might have beneficial effect in improving BW gain during the first 2 weeks of study due to the polyphenols in the GP. It has been reported that condensed tannins content in GP is about 150 g/kg dry matter (Brenes et al., 2007). High tannin contents in diets generally reduce growth performance; and feeding reconstituted high-tannin sorghum, mimosa tannins, and fava beans also reduced growth rate in broiler chicks (Brufau et al., 1998; Kumar et al., 2005). In the current study, however, no negative effect of GP on the growth performance was observed, and this may be due to the low levels of GP that were used. Lau and King (2003) reported that broiler chicks fed diets with grape seed extract at different levels decreased growth performance and feed intake. However, in the present study feeding GP as a supplement did not show any negative effect on growth and the polyphenols and anthocyanins in the GP may have positive effects on growth during early life of broiler chicks.

The apparent total tract digestibility studies revealed that ash digestibility values showed quadratic trend due to GP supplementation. The reasons for these results remained obscure; however, the differences among GP levels in diets and tannins might have played roles in minerals bioavailability. The apparent total tract digestibility of other nutrients was not affected in this study due to GP levels in the diets. However, experiment on different animal species reported that GP up to 493 g/kg dry matter decreased digestibility of organic matter in sheep (Zalikarenab et al., 2007) in contrast with Bahrami et al. (2010) that utilization of GP as low as 50 g/kg increased digestibility of organic matter in lamb diets. Therefore, apparent total tract digestibility studies in both poultry and ruminants should be further researched.

Table 4
Effect of grape pomace on apparent total tract digestibility (%) of nutrients in experimental diets fed to broiler chicks.

| Item               | Treatments 2 | SEM       | P-value | Linear | Quadratic |
|--------------------|--------------|-----------|---------|--------|-----------|
| Dry matter         |               |           |         |        |           |
| GP0                | 73.33        | 77.57     | 77.21   | 72.8   | 1.35      |
| GP1                | 74.5         | 76.32     | 73.57   | 29.47  | 3.37      |
| GP2                | 54.67        | 59.0      | 59.57   | 54.2   | 1.95      |
| GP3                | 89.48        | 92.09     | 92.59   | 91.26  | 0.69      |
| Energy             | 77.25        | 81.73     | 79.42   | 77.08  | 1.19      |

SEM = standard error of means.
1 Each mean represents 5 replicates. NS = not significant.
2 GP0 = basal diet (BD), GP1 = BD containing 5 g grape pomace/kg diet, GP2 = BD containing 7.5 g grape pomace/kg diet, GP3 = BD containing 10 g grape pomace/kg diet.

In this study, serum total cholesterol values were decreased showing quadratic effects by adding GP in diets. This might have been caused by anthocyanin contents in GP. The results in the present study were almost similar to the result of Basuny et al. (2012) that eggplant peels as anthocyanin source decreased total cholesterol in male albino rats. Also, anthocyanin from Brassica campestris L. reduced total cholesterol in rats (Igarashi et al., 1989). No effect on serum triglyceride was reported in hens fed GP at 40 and 60 g/kg levels in accordance to our results (Kara et al., 2016). However, studies on effects on blood indices by using GP in poultry diets are still limited. Serum IgG values showed quadratic effects by feeding GP in this study. When total antioxidants are higher than free radicals, oxidative damage in the body decrease and in turn it increases the immunity (Turner, 2009). In this study, no differences were observed on other serum parameters such as triglyceride, glucose, and HDL cholesterol, demonstrating no role of GP on these serum indices.

The carcass percentage and organ weight were not affected by addition of GP as feed supplement to broiler diets. The results were almost similar to Brenes et al. (2007) that addition of GP up to 60 g/kg did not affect liver and pancreas weight but spleen weight in 15 g/kg level tended to be higher than other levels, and abdominal fat in GP groups tended to be decreased compared with the control in that study, however we did not find similar effects.

After measuring TBARS values in breast meat stored for 0, 5, and 10 d, it was revealed that supplementation of GP was able to retard lipid oxidation showing both linear and quadratic effects at the levels studied. These results are in agreement with Goni et al. (2006) that broiler chicks fed diets containing GP up to 30 g/kg decreased lipid oxidation in breast and thigh meat stored for 1, 4, and 7 d. Also, Brenes et al. (2007) demonstrated that GP added in diets up to 60 g/kg were able to inhibit lipid oxidation in breast meat of broiler chicks stored for 1, 4, and 7 d. These results indicated that anthocyanins in GP have role as an antioxidant against lipid oxidation in tissues. It is supported by Prior et al. (1998) and

Table 6
Effect of grape pomace on carcass traits [%] of broiler chicks.

| Item              | Treatments 2 | SEM       | P-value | Linear | Quadratic |
|-------------------|--------------|-----------|---------|--------|-----------|
| Carcass           |              |           |         |        |           |
| GP0               | 65.5         | 61.6      | 61.9    | 63.9   | 1.32      |
| GP1               | 1.53         | 1.52      | 1.65    | 1.72   | 0.09      |
| GP2               | 2.48         | 2.24      | 2.63    | 2.27   | 0.39      |
| GP3               | 0.95         | 1.04      | 0.93    | 0.9    | 0.05      |
| Abdominal fat     |              |           |         |        |           |
| Liver             |              |           |         |        |           |
| Spleen            |              |           |         |        |           |
| Heart             |              |           |         |        |           |
| Bursa of Fabricius|              |           |         |        |           |
| Thymus            |              |           |         |        |           |

SEM = standard error of means.
1 Each mean represents 5 replicates. NS = not significant.
2 GP0 = basal diet (BD), GP1 = BD containing 5 g grape pomace/kg diet, GP2 = BD containing 7.5 g grape pomace/kg diet, GP3 = BD containing 10 g grape pomace/kg diet.
Shih et al. (2007) that anthocyanins act as a potent antioxidant by reducing free radicals. At 5 d after storage, no effect on lightness value was observed in the current study. But after 10 d of storage, lightness value showed a quadratic effect due to GP levels. Furthermore, they reported that redness and yellowness values did not differ as well by adding grape seed extract on raw pork patties. In addition, GP supplementation at 2.5, 5, or 10 g/kg feed did not affect the breast muscle color lightness and yellowness values (Kasapidou et al., 2016). In the current study, redness values were linearly decreased in supplemented groups at both 5 and 10 d of storage. These results are in accordance with that of Kasapidou et al. (2016) who reported GP affected redness and resulted in paler meat. Breast meat yellowness values showed both linear and quadratic effects due to GP supplementation at 10 d of storage in the present study. The effects of GP on meat quality showed the positive effects on TBARS values and linear and quadratic effects on meat color values were observed. With respect to meat quality it is concluded that supplementation of GP up to 10 g/kg in the diet of broilers decreased TBARS values in stored breast meat.

5. Conclusions

Based on the results of the present study, supplementation of GP up to 10 g/kg in the diet of broilers was effective in reducing serum cholesterol and improving meat quality parameters in broilers without affecting growth performance, nutrient digestibility, and carcass traits.

Conflicts of interest

The authors declare that there is no conflict of interest for this study.

Acknowledgement

Authors like to thank Laboratory of Feed Processing and Feed Biotechnology, Kangwon National University, Republic of Korea for supporting this research and Directorate General of Higher Education (DIKTI), Republic of Indonesia for its financial support to the first author for education in Korea.

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Table 7

Effect of grape pomace on thiobarbituric acid reactive substances (TBARS; μmol/g) and meat color values of broiler chicks.

| Item                              | SEM | P-value |
|-----------------------------------|-----|---------|
|                                   |     |         |
| d 0 of storage                    |     |         |
| TBARS                             |     |         |
| G0                                | 0.35| 0.30    |
| G1                                | 0.28| 0.27    |
| G2                                | 0.00| 0.01    |
| G3                                | 0.07|         |
| Lightness                         |     |         |
| G0                                | 0.50| 0.81    |
| G1                                | 0.44| 0.38    |
| G2                                | 0.11|         |
| G3                                | 0.01|         |
| Redness                           |     |         |
| G0                                | 1.84| 1.84    |
| G1                                | 0.30|         |
| G2                                | 0.01|         |
| G3                                | 0.01|         |
| Yellowness                        |     |         |
| G0                                | 8.41| NS      |
| G1                                | 0.11| NS      |
| G2                                | 0.01|         |
| G3                                | 0.01|         |
| d 10 of storage                   |     |         |
| TBARS                             |     |         |
| G0                                | 0.83| 0.54    |
| G1                                | 0.50| 0.46    |
| G2                                | 0.03|         |
| G3                                | 0.01|         |
| Lightness                         |     |         |
| G0                                | 0.62| NS      |
| G1                                | 0.62|         |
| G2                                | 0.01|         |
| G3                                | 0.01|         |
| Redness                           |     |         |
| G0                                | 1.23| 1.13    |
| G1                                | 0.17|         |
| G2                                | 0.01|         |
| G3                                | 0.05|         |
| Yellowness                        |     |         |
| G0                                | 9.06| 8.89    |
| G1                                | 0.14|         |
| G2                                | 0.01|         |
| G3                                | 0.01|         |

SEM = standard error of means.
1 Each mean represents 5 replicates. NS = not significant.
2 GP = basal diet (BD), GP1 = BD containing 5 g grape pomace/kg diet, GP2 = BD containing 7.5 g grape pomace/kg diet, GP3 = BD containing 10 g grape pomace/kg diet.

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