Protease treatment of canola meal-containing Japanese quail diets: Effect on physiological parameters and meat quality traits

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ABSTRACT
The utility of canola meal as protein source for quails may be limited by trypsin inhibitors that interfere with protein digestion. This study was, therefore, designed to assess the effect of including a heat-stable protease mono-enzyme (75′000 PROT/g; EC/IUB no. 3.4.21) in canola meal-containing diets on physiological and meat quality traits of adult female Japanese quails. A total of 240, five-week old quails (163.9 ± 9.56 g live-weight) were distributed into 30 replicate pens, to which five isocaloric and isonitrogenous diets were randomly allocated. The diets were: control diet (CON; a commercial grower mash with no canola meal (CM) inclusion); control diet in which 17.5% of soybean ingredients were replaced with CM (CM0), and CM0 diet treated with 0.01%, 0.02% or 0.03% of protease (CM01, CM02 and CM03, respectively). Quails were slaughtered at 10 weeks of age. There was no week × diet interaction effect on average weekly feed intake (AWFI), average weekly weight gain (AWG) and feed conversion efficiency (FCE). Protease inclusion had no effect on haemo-biochemical parameters, overall growth performance, internal organs, carcass characteristics, and meat quality parameters. It was concluded that inclusion of protease does not improve the utilization of CM-containing diets in adult female Japanese quails.

Introduction
The growth of Japanese quail industry to complement the existing poultry species is driven by the high demand of dietary animal protein to feed a fast-growing human population. Quails have superior qualities over other poultry birds such as faster growth rates, early sexual maturity, short generation intervals and low space requirements (Puspamitra et al. 2014; Mnisi et al. 2017). These attributes have resulted in widespread production of quails under high-input, intensive production systems. In these systems, the provision of high quality protein is critical to meet the nutritional requirements of quails and optimize their productivity. Soybean is one of the vegetable protein sources used during feed formulations by food and feed manufacturing industries because of its high protein content and a relatively well-balanced amino acid profile (Cromwell et al. 1999). Currently, the use of soybean meal (SBM) is limited by its high market prices, which have resulted in high feed costs for quail producers (Mnisi and Mlambo 2018). This has prompted the search for alternative and inexpensive protein sources such as canola meal (CM). Canola meal is a protein source that has the potential to wholly or partially replace soy-based protein in poultry diets (Mushtaq et al. 2007) but has not been adequately evaluated for use in quail diets. This by-product of oil extraction is currently not being used directly as a source of food for humans thus is available at much lower prices compared to soybean. As a result, use of CM in quail diets has the potential to reduce feed costs and increase flexibility in feed formulation.

Despite the fact that CM protein is a potential ingredient for use in the feed industry, its utilization is limited by the presence of some undesirable compounds such as protease inhibitors, glucosinolates, phytates, phenols, non-starch polysaccharides and high dietary fibre. These antinutrients may reduce nutrient bioavailability and eventually decrease growth performance of animals (Aider and Barbana 2011; Singh et al. 2017). The presence of protease/trypsin inhibitors (17.7 TIU/mg) (Hussain 2015) reduces the utilization of CM, this is because trypsin inhibitors are known to bind to trypsin and chymotrypsin enzymes thereby reducing their digestive activities. Indeed, inclusion levels of CM more than 125 g/kg in place of SBM is reported to reduce voluntary feed intake in Japanese quails (Mnisi and Mlambo 2018).

According to Nir et al. (1993), birds are known to produce proteolytic enzymes that are important for protein utilization, however, a substantial amount of dietary protein has been observed to pass the gastro-intestinal tract without being completely digested (Wang and Parsons 1998; Lemme et al. 2004). This is also likely to occur when CM is fed due to the presence of protease/trypsin inhibitors (Berot et al. 2005; Hussain 2015) that interfere with the proteolytic activities of pepsin or trypsin and hinder protein/amine acid digestibility (Sariçicçek

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et al. 2005). In order to include higher levels of CM in quail diets, the application of an exogenous mono-component protease enzyme may be required to disrupt the starch-protein matrix in order to liberate digestible amino acids (Zanella et al. 1999; Ding et al. 2016). Indeed, the incorporation of enzymes in poultry diets has been reported to increase nutrient utilization and thereby enhancing weight gain (Romero et al. 2013; Cowieson and Roos 2016; Stefanello et al. 2016). Exogenous proteases in canola-based diets were reported to improve feed efficiency, protein utilization and performance in broilers (Ghazi et al. 2003; Liu et al. 2015). To our knowledge, the effectiveness of exogenous proteases in improving the utilization of canola-containing quail diets has not been investigated. This study was, therefore, designed to investigate the effect of treating canola-containing diets with graded levels of protease on growth performance, haemo-biochemical parameters, carcass and meat quality characteristics of adult female Japanese quails fed a canola-containing diet. We hypothesized that adding a mono-component protease enzyme (75’000 PROT/g; EC/IUB no. 3.4.21) will improve growth performance, haemo-biochemical parameters, carcass and meat quality characteristics of Japanese quails fed diets in which 17.5% soybean ingredients were replaced with CM.

Materials and methods

Statement of ethics and description of study site

The experiment was reviewed and approved by the Animal Research Ethics Committee, North-West University (AREC-MC); approval number: NWU-00521-16-A9. The study was conducted at the North-West University Molelwane farm (25°40.459’S, 26°10.563’E), located in the semi-arid region of the North West province of South Africa with an altitude of 1224 m above sea level. The study was conducted in the spring season, and the ambient temperatures ranged between 19°C and 25°C. The annual rainfall ranges between 300 and 600 mm.

Feed ingredients and diet formulation

Canola meal was purchased from Southern Oil (PTY) LTD (Western Cape, South Africa) while, SBM and all other ingredients were supplied by Optifeeds (PTY) LTD (Lichtenburg, South Africa). According to the suppliers, both CM and SBM were by-products of solvent extraction of oil, but the extraction process ensures that no considerable amounts of solvent remain in the by-products. The process of oil extraction from canola did not involve application of heat while heat treatment was used for soybean oil extraction. The protease enzyme (75’000 PROT/g; EC/IUB 3.4.21) was received from A-Feeds (PTY) LTD (Gauteng, South Africa). Five isoenergetic and isonitrogenous dietary treatments were formulated using Format® software (Optifeeds (PTY) LTD, Lichtenburg, South Africa) as follows: CON = control diet (a commercial grower mash with no CM inclusion), CM0 = control diet in which 17.5% of soybean ingredients were replaced with CM, CM01 = CM diet treated with 0.01% of protease enzyme, CM02 = CM diet treated with 0.02% of protease enzyme, and CM03 = CM diet treated with 0.03% of protease enzyme, as shown in Table 1.

Chemical analyses

The formulated dietary treatments (CON, CM0, CM01, CM02 and CM03) were analyzed using the methods of AOAC International (AOAC 2005) for laboratory dry matter (DM; AOAC method no. 930.15). Crude protein was determined following the standard macro-Kjeldahl method (N; AOAC method no. 984.13). Crude fibre was determined using the ANKOM2200 Fibre analyser (ANKOM Technology, New York) with 0.255 N crude fibre acid solution and then with 0.313 N crude fibre base solution. Crude fat and metabolizable energy (ME) content were determined using models from the near infrared reflectance spectroscopy SpectraStar XL (Unity Scientific, Australia). Minerals were analyzed following the Agri Laboratory Association of Southern Africa guidelines (AgriLASA 1988).

Experimental design

A total of 400 three-week old mixed gender Japanese quails were purchased from a farm called Quail Breeders (Gauteng, South Africa). In an environmentally controlled house, the

### Table 1. Ingredient and chemical composition (g/kg) of canola meal-containing diets treated with a mono-component protease enzyme.

| Ingredients                  | CON   | CM0   | CM01  | CM02  | CM03  |
|------------------------------|-------|-------|-------|-------|-------|
| Protease                     |       |       |       |       |       |
| Canola oil cake              | 0     | 0     | 0.10  | 0.20  | 0.30  |
| Fine yellow maize            | 175.0 | 175.0 | 175.0 | 175.0 |       |
| Prime gluten 60              |       |       |       |       |       |
| Full fat soya meal           | 50.7  | 174.0 | 174.0 | 174.0 | 174.0 |
| Soybean meal                 | 196.7 | 0.0   | 0.0   | 0.0   | 0.0   |
| Limestone powder             | 14.5  | 12.2  | 12.2  | 12.2  | 12.2  |
| Mono calcium phosphate       | 7.2   | 5.6   | 5.6   | 5.6   | 5.6   |
| Fine salt                    | 3.2   | 3.2   | 3.2   | 3.2   | 3.2   |
| Sodium bicarbonate           | 1.7   | 1.6   | 1.6   | 1.6   | 1.6   |
| Choline powder               | 0.8   | 0.8   | 0.8   | 0.8   | 0.8   |
| L-Threonine                  | 2.8   | 2.9   | 2.9   | 2.9   | 2.9   |
| Methionine                   | 0.4   | 0.0   | 0.0   | 0.0   | 0.0   |
| Grower – phytase             | 1.9   | 1.8   | 1.8   | 1.8   | 1.8   |
| Coxistac                     | 0.5   | 0.5   | 0.5   | 0.5   | 0.5   |
| Olaquindox                   | 0.4   | 0.4   | 0.4   | 0.4   | 0.4   |
| ME (MJ/kg)                   | 12.10 | 11.80 | 11.50 | 11.30 | 11.00 |
| Dry matter                   | 88.65 | 89.06 |       |       |       |
| Crude protein                | 18.00 | 18.94 |       |       |       |
| Crude fat                    | 4.162 | 6.244 |       |       |       |
| Crude fibre                  | 2.315 | 4.176 |       |       |       |
| Calcium                      | 0.850 | 0.850 |       |       |       |
| Phosphorus                   | 0.497 | 0.563 |       |       |       |
| Sodium                       | 0.180 | 0.180 |       |       |       |
| Chlorine                     | 0.300 | 0.300 |       |       |       |
| Potassium                    | 0.763 | 0.733 |       |       |       |

Diets: CON = control diet (a commercial grower mash with no canola meal inclusion), CM0 = control diet in which 17.5% of soy-based protein was replaced with canola meal, CM01 = CM diet treated with 0.01% of protease enzyme, CM02 = CM diet treated with 0.02% of protease enzyme and CM03 = CM diet treated with 0.03% of protease enzyme. ME: metabolizable energy.

The inclusion levels of the protease were determined based on the recommended level (0.2 g/kg inclusion rate) prescribed by the supplier for chickens. Thus, a total of 3 inclusion levels: one level below and another above the recommended inclusion level were investigated for quails.
quails were reared until 5 weeks of age to allow gender differentiation using a commercial grower-mash diet purchased from Optifeeds (PTY) LTD (Lichtenburg, North West province). At 5 weeks of age, a total of 240 female Japanese quails were attained upon gender sexing. The female quails were randomly allocated to 30 pens. The experimental unit was a pen holding 8 quails each, which was replicated 6 times per dietary treatment. The pens were in a form of standing cages with 4 partitions (pens). The size of each pen was 100 cm long × 60 cm wide × 30 cm high. The five dietary treatments (CON, CM0, CM01, CM02 and CM03) were randomly allocated to the pens and quails were reared until they were 10 weeks old. Quails were allowed to adapt to dietary treatments for a week before measurements commenced.

Feeding and quail management

Experimental diets and clean water were provided ad libitum during the feeding trial. Average weekly feed intake (AWFI) per bird was measured from 6 to 10 weeks of age by subtracting the weight of the feed refused from that of the feed offered and dividing the difference by the total number of quails in the pen. The initial live-weights of the quails were measured at the beginning of the experiment. Thereafter, average live-weight was measured weekly by weighing all the quails in each pen. These live-weights were used to calculate the average weekly weight gain (AWG) per bird as follows:

\[
\text{Average weekly weight gain (AWG)} = (W(T) - W(t_0)) / \text{bird},
\]

where \( t_0 = \) initial time (days); \( T = \) quail age in weeks; \( W(T) = \) weight/bird (g) at time \( T \); and \( W(t_0) = \) initial body weight/bird (g). Weekly feed conversion efficiency (FCE) was calculated as average weekly weight gained divided by average weekly feed consumption per bird.

Slaughter procedures and blood collection

After 10 weeks, all quails were taken to a local abattoir for slaughter. At the abattoir, all the quails were hung upside down by their feet on a rail and electrically stunned. Quails were then slaughtered by cutting the jugular vein with a sharp knife and they were left hanged until bleeding ended. At this point, about 4 mL of blood was collected from two quails, randomly selected from each pen, for blood analyses, using syringes into two sets of sterilized tubes. Tubes containing ethylene diamine tetra acetic acid as an anti-coagulant were used to collect blood for haematological analyses while tubes without anticoagulant were used to collect blood for serum biochemical analyses. Afterwards, quail carcasses were taken to the Animal Science laboratory for determination of internal organs, carcass characteristics and meat quality parameters.

Blood analyses

Blood for haematological analyses was stored in a cooler box with ice packs whereas for serum biochemical analyses blood samples were stored at room temperature for a maximum of 45 minutes to clot and thereafter refrigerated at 4°C (Washington and van Hoosier 2012). All analyses were done within 48 h of collection (Buetow et al. 1999). Haematological parameters (erythrocyte counts, haemoglobin, haematocrits, leucocyte counts, lymphocytes, monocytes and eosinophils) were determined using an automated IDEXX LaserCyte Haematology Analyser (IDEXX Laboratories, Inc.). Clotted blood was centrifuged in a macro-centrifuge (Hermle Labortechnik GmbH, Germany) at 1000 g for 15 minutes to generate serum for biochemical analysis according to Buetow et al. (1999). Total protein, albumin, bilirubin, creatinine and urea were analyzed using an automated IDEXX Vet Test Chemistry Analyser (IDEXX Laboratories, Inc.).

Internal organs and carcass characteristics

Weights of livers, cleaned gizzards, hearts were measured using a digital weighing scale (Explorer® EX2242, OHAUS Corp, US) and length of small intestines were determined using a measuring tape (NW Packaging, South Africa). Warm carcass weights (WCW) were recorded immediately after slaughter and then the carcasses were chilled for 24 h before being re-weighed to obtain the cold carcass weights (CCW). The dressing out percentage was calculated as the proportion of WCW on slaughter weight.

Meat quality parameters

Meat pH was recorded 24 h after slaughter on the breast muscle (central area of the breast) using a Corning Model 4 pH-temperature metre (Corning Glass Works, Medfield, MA) equipped with an Ingold spear-type electrode (Ingold Messtechnik AG, Udorf, Switzerland) according to Stanford et al. (2003). After every 20 measurements, the pH metre was calibrated with pH 4, pH 7 and pH 10 standard solutions (Ingold Messtechnik AG, Udorf, Switzerland) at a temperature of 2°C.

According to the Commission International de l’Eclairage (CIE) colour system guidelines (CIE 1976), colour of the meat \((L^* = \text{Lightness}, \ a^* = \text{Redness and } b^* = \text{Yellowness})\) was determined using a Minolta colour-guide (BYK-Gardener GmbH, Geretsried, Germany), with a 20 mm diameter measurement area and illuminant D65-day light, 10° observation angle. The colour metre was calibrated before measurements using the green standard. Colour recording was done in triplicate on the surface of a freshly cut slice of the breast muscle allowed to bloom for 1 hour on a polystyrene tray at 4°C. Hue angle was as \(\tan^{-1}(b/a)\), and chroma was calculated as the square root of \(a^2 + b^2\).

For determination of cooking losses, pre-weighed breast samples were placed in an oven set at 130°C for 20 min following the modified method of Honikel (1998). The losses were calculated as the difference between the final (cooked) and initial weights of the breasts and expressed as a proportion of initial weight. After cooking, cylindrical samples (12.5 mm core diameter) of breast muscle were sheared perpendicular to the fibre direction using a Meullenet-Owens Razor Shear Blade (A/MORS) mounted on a Texture analyser (TA XT plus, Stable Micro Systems, Surrey, UK). The reported value represented
the average Warner-Bratzler shear force measurements of each sample in Newtons.

**Statistical analysis**

All reported parameters were tested for normality using the NORMAL option in the Proc Univariate statement before being subjected to analysis of variance. Average weekly feed intake, AWG and FCE data were analyzed using the repeated measures analysis (SAS 2010).

Overall feed intake, overall weight gain, overall feed conversion efficiency, blood parameters, carcass characteristics and meat quality data were analyzed using the GLM procedure of SAS version 9.4 (SAS 2010) for the dietary treatments. The linear statistical model was as follows:

\[ Y_{ik} = \mu + D_i + E_{ik}, \]

where, \( Y_{ik} \) = dependant variable, \( \mu \) = population mean, \( D_i \) = effect of dietary treatments, and \( E_{ik} \) = random error associated with observation \( ik \), assumed to be normally and independently distributed. For all statistical tests, significance was declared at \( P < 0.05 \). Least squares means (LSMEANS) were compared using the probability of difference option in the LSMEANS statement of SAS.

**Results**

The chemical composition of the isoenergetic and isonitrogenous experimental diets on an as-fed basis is also shown in Table 1. The chemical composition of all the protease-treated CM-containing diets where the same as diet CM0. Repeated measures analysis indicated no significant week x diet interaction effect on AWFI, AWG and FCE. Table 2 shows that dietary treatments had no significant effect on overall feed intake, overall weight gain and overall FCE. Table 3 shows that diet had no significant effect on haematological and serum biochemical parameters of female Japanese quails.

There was no dietary influence \( (P > 0.05) \) in terms of internal organs, carcass characteristics and dressing out percentage. Weights of livers ranged from 4.45 to 4.72 g, hearts ranged from 2.08 to 2.25 g and gizzards ranged from 4.14 to 4.40 g. The length of small intestines ranged from 50.57 to 55.61 cm. Cold carcass weights ranged from 164.6 to 176.5 g, whereas the WCW ranged from 168.8 to 176.9 g. The dressing out percentage ranged from 67.92 to 69.97%.

Table 4 shows that experimental diets had no significant effect on meat pH and colour measured 24 h after slaughter of female Japanese quails. Dietary treatments had no influence \( (P > 0.05) \) on cooking losses and Warner-Bratzler shear force. Cooking losses ranged from 21.24 to 23.07%, while the Warner-Bratzler shear force ranged from 2.21 to 3.51 N.

**Discussion**

This study represents the first attempt to use an exogenous protease monoenzyme in adult female Japanese quail diets in which 17.5% of soybean ingredients had been replaced with CM. Repeated measures analyses showed no diet x week interaction effect on AWFI, AWG and FCE demonstrating that the effect of diets on growth performance did not depend on the age of the quails. In this study, inclusion of the protease monoenzyme did not improve the utilization of a CM-containing quail diet as indicated by the lack of differences between the untreated CM-containing diet and the protease-treated CM-containing diets in terms of feed intake, weight gain and FCE. These findings were in line with those of Marsman et al. (1997) who observed no effect on weight gain and feed conversion ratio of chicks fed exogenous protease-treated soy-based diets. Indeed, Sariççek et al. (2005) found that addition of CM reduced feed consumption, which can be due to the presence of other ANFs that are not substrates for the protease enzyme. Another possible explanation for the lack of enzyme effect on growth performance could be the use of the prescribed

### Table 1. Effect of protease-treated canola meal-containing diets on haematological parameters of 10-week old Japanese quails.

| Parameters                  | Diets | CON  | CM0  | CM01 | CM02 | CM03 | SEM  | P-value |
|-----------------------------|-------|------|------|------|------|------|------|---------|
| Eosinophils (x10^3/L)       |       | 2.68 | 2.72 | 2.21 | 1.96 | 2.84 | 0.986| 0.941   |
| Erythrocyte count (x10^12/L)|       | 2.91 | 3.06 | 3.15 | 3.07 | 3.14 | 0.206| 0.854   |
| Haematocrit (%)             |       | 0.49 | 0.50 | 0.51 | 0.47 | 0.51 | 0.029| 0.838   |
| Haemoglobin (g/dL)          |       | 12.3 | 12.5 | 11.9 | 12.0 | 12.2 | 0.555| 0.904   |
| Leucocyte count (x10^9/L)   |       | 41.1 | 36.5 | 32.0 | 40.7 | 36.3 | 9.895| 0.936   |
| Monocytes                  |       | 37.8 | 31.5 | 27.4 | 25.0 | 31.2 | 7.974| 0.856   |

**Table 2. Overall effect of protease-treated canola meal-containing diets on feed intake (g/bird), weight gain (g/bird) and FCE of 10-week old Japanese quails.**

| Parameters                  | Diets   | CON  | CM0  | CM01 | CM02 | CM03 | SEM  | P-value |
|-----------------------------|---------|------|------|------|------|------|------|---------|
| Overall feed intake         |         | 753.7| 753.0| 753.1| 727.7| 726.4| 27.52| 0.045   |
| Overall weight gain         |         | 90.98| 81.73| 78.40| 88.59| 71.35| 8.993| 0.553   |
| Overall FCE                |         | 0.465| 0.403| 0.397| 0.462| 0.373| 0.045| 0.509   |

**Table 3. Effect of protease-treated canola meal-containing diets on serum biochemical parameters of 10-week old Japanese quails.**

| Parameters                  | Diets | CON  | CM0  | CM01 | CM02 | CM03 | SEM  | P-value |
|-----------------------------|-------|------|------|------|------|------|------|---------|
| Total protein (g/L)         |       | 37.3 | 32.8 | 32.4 | 35.3 | 36.7 | 5.30 | 0.901   |
| Albumin (g/L)               |       | 10.6 | 10.0 | 9.3  | 9.0  | 10.9 | 1.55 | 0.843   |
| Creatinine (µmol/L)         |       | 18.1 | 18.0 | 18.0 | 18.0 | 19.0 | 0.69 | 0.558   |
| Bilirubin (µmol/L)          |       | 0.63 | 0.67 | 5.15 | 8.38 | 1.07 | 3.83 | 0.442   |
| Urea (mmol/L)               |       | 0.41 | 0.50 | 0.52 | 0.59 | 0.60 | 0.08 | 0.456   |

**SEM:** standard error of the mean.
protease inclusion level (0.2 g/kg) that is meant for broilers. Even though three levels (0.1, 0.2 and 0.3 g/kg) of protease were used, no improvement in quail performance was observed, suggesting that, under these experimental conditions, these inclusion rates may possibly have been too low to improve nutrient utilization in adult quails.

In addition to trypsin inhibitors, CM also contains a range of other ANFs (glucosinolates, phytic acids, non-starch polysaccharides, phenolics, and erucic acids) that may be detrimental to the health of adult quails hence the need to monitor blood parameters. Haematological and serum biochemical parameters were also not influenced by the inclusion of protease but they fell within the normal ranges for quails as reported by Ali et al. (2012). Furthermore, the size of internal organs, carcass characteristics and dressing out percentage were not altered by the inclusion of exogenous protease, emphasizing the protease enzyme’s failure to trigger any measurable anatomical changes. It was surprising as canola has trypsin inhibitors (Hussain 2015), which are known to inhibit protein digestion by the enzyme trypsin resulting in protein amino acids unavailable to birds. Therefore, adding a protease enzyme was expected to alter blood parameters especially serum total protein as well as growth performance, carcass traits and meat quality. Meat quality can also be altered by enhanced protein utilization in protease-treated CM-containing diets. Dietary treatments had no significant effect on meat pH and colour values of female Japanese quails, further indicating that protease inclusion did not alter meat quality traits. However, the expectation was that these parameters would be altered by enhanced protein utilization in protease-treated CM-containing diets. In addition, secondary plant compounds such as phenolics, present in CM, are known to have in vivo anti-oxidative effects upon absorption and thus can affect meat quality parameters such as texture (Sur Arslan and Tatli Seven 2017), colour, pH and shelf-life. However, experimental diets also had no effect on meat cooking loss and Warner-Bratzler shear force values (meat texture), indicating that protease inclusion had no influence on these parameters. Meat pH, colour, cooking losses and Warner-Bratzler shear force values for female Japanese quails were within the normal ranges as reported by Mnisi and Mlambo (2018).

Considering the fact that several scholars reported improved feed utilization, increased growth rates and desirable FCE values when exogenous proteases were used (Ghazi et al. 2003; Stefanello et al. 2016), it was surprising that no such improvements were seen with adult female Japanese quails. A possible contributing factor to the discordance in findings across studies is the fact that the proteases used are not always fully described in terms of characteristics and activities. Discrepancies in the in vivo effectiveness of protease have been reported to be due to adverse responses to the enzyme, nutrient imbalances and failure of the enzyme to target its substrate (Cowieson and Ross 2016). Indeed, differences and inconsistencies relating to efficacy of various exogenous proteases have been revealed on a number of occasions. However, the differences are difficult to explain because of the limited details on the type of proteases applied (Kaczmarek et al. 2014; Mahmood et al. 2017).

Conclusions

The current study showed that adult female quails fed protease-treated diets had similar responses in terms of growth, haemobiochemical status and meat quality traits to those offered untreated canola-based diet. It was, therefore, concluded that the inclusion of exogenous protease enzyme does not improve the utilization of CM-containing diets in adult female quails.

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Disclosure statement

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Table 4. The effect of protease-treated canola meal-containing diets on meat quality parameters of 10-week old Japanese quails 24 h after slaughter.

| Parameters         | CON | CM0 | CM01 | CM02 | CM03 | SEM | P-value |
|--------------------|-----|-----|------|------|------|-----|---------|
| Meat pH            | 6.35| 6.24| 6.26 | 6.29 | 6.28 | 0.046| 0.508   |
| L*                 | 48.8| 47.3| 48.2 | 48.1 | 47.6 | 0.538| 0.400   |
| a*                 | 4.05| 5.06| 4.09 | 3.43 | 3.84 | 0.451| 0.170   |
| b*                 | 6.60| 6.86| 6.90 | 5.44 | 6.52 | 0.435| 0.150   |
| Chroma             | 7.77| 8.54| 8.05 | 6.47 | 7.58 | 0.560| 0.423   |
| Hue angle          | 1.03| 0.94| 1.04 | 1.02 | 1.05 | 0.038| 0.231   |

*Diets: CON = control diet (a commercial grower mash with no canola meal inclusion), CM0 = control diet in which 17.5% of soy-based protein was replaced with canola meal, CM01 = CM0 diet treated with 0.01% of protease enzyme, CM02 = CM0 diet treated with 0.02% of protease enzyme and CM03 = CM0 diet treated with 0.03% of protease enzyme.

bSEM: standard error of the mean.
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