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Full Length Article

Effect of Dietary Enrichment with Canola Oil on Glucose Tolerance, Tissue Glycogen Content and Viscera in *Coturnix cortunix Japonica*

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Abstract

This study determined the effects of enriching a standard diet with canola oil on growth, glucose tolerance (GT), mass of viscera and tissue glycogen content in Japanese quail. Five-week old quail were randomly allocated to the control (STD; n=20) and test (HFD; n = 18) diet. After 5 weeks half the number of birds on each diet was subjected to an oral glucose tolerance test and the other half to an intravenous glucose tolerance test after which the birds were maintained on their respective diets for a week before the tests were repeated. After re-feeding for another week the fasting and fed-state blood glucose concentration (BGC) was then determined. Following euthanasia, viscera masses and tissue glycogen content were determined. There were no differences in the growth performance of quail on the two diets. Post-gavage, the BGC of male quail on the STD returned to basal concentration 90 min earlier compared to their counterparts on the HFD. The BGC of the female quail on the HFD at 30 min post-infusion was higher (P = 0.0476) compared to that of their counterparts on the STD. Pooled results showed similarity in oral and intravenous glucose tolerance between quail on the control and test diet. Despite similarities in the abdominal fat pad (AFPM), liver masses and liver, thigh and breast glycogen content between the groups, female quail had heavier (P = 0.0001) liver masses compared to their male counterparts. Male quail on either diet had heavier (P = 0.001) AFPM compared to females. While the HFD had gender related effects on GT, pooled results showed that supplementing the diet with canola oil did not elicit glucose intolerance, neither did affect growth and tissue glycogen content.

Keywords: Japanese quail; Growth performance; Glucose tolerance; Tissue glycogen content

Introduction

The Japanese quail, *Cortunix cortunix japonica*, family *Phasidae* (Vali, 2008) has potential both as a research animal and as a source of table protein (Kayang *et al.,* 2004). The quail complements conventional poultry sources of protein for humans (Nishibori *et al.,* 2002). Quail mature sexually at 5 weeks of age (Tuleun *et al.,* 2011), have a mature body mass of 80-300 g (Vali, 2008) and each female lays 200-300 eggs annually (NRC, 1991). The early sexual maturity, short generation interval, and minimum space requirements (Wechsler and Schmid, 1998; Mosleh *et al*., 2012), resistance to most poultry diseases (Vali, 2008) make quail an ideal bird for smallholder production. Due to its high protein to lipid ratio (Mareko *et al.,* 2006), the consumption of quail meat is reported to reduce the risk of the development of cardiovascular diseases (Chizzolini *et al.,* 1999).

Increasing the fat content in poultry feeds has been observed to improve the feed conversion efficiency (Bryant *et al.,* 2005; Atapattu and Senevirathne, 2013) and to promote intramuscular fat accretion by the birds without abdominal fat deposition (Crespo and Esteve-Garcia, 2000). In mammals, high fat diets have been demonstrated to cause metabolic dysfunction with several abnormalities including glucose intolerance (Gollisch *et al.,* 2009). Tissue glycogen content is one of the indicators of meat quality (Allen *et al.,* 1998); its depletion in slaughter stock leads to the production of dry and firm meat (Sanz *et al.,* 1996). While the effect of high fat diets on glucose tolerance in other poultry species and some mammals have been interrogated (Crespo and Esteve-Garcia, 2000; Gollisch *et al.,* 2009), there is a dearth of information on the effects of a high fat diet on growth performance, glucose handling and glycogen storage by the Japanese quail hence our study sought to determine the effects of supplementing a standard quail diet with canola oil on the growth performance, glucose tolerance and fasting glycogen storage in the tissues of the Japanese quail.

Materials and Methods

Dietary Treatments

Two diets were used during the experiment: a control and

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test diet. The control diet (STD) was a standard commercial poultry feed [Epol®, Centurion, South Africa (protein 170 g kg^{-1}, moisture 120g kg^{-1}, fat 25 g kg^{-1}, fibre 70 g kg^{-1}, calcium 25 g kg^{-1}, phosphorus 6 g kg^{-1} and total lysine 6.5 g kg^{-1})]. The test diet, a high fat diet (HFD), was made up by enriching the STD with canola oil (Cansa Southern Oil Ltd, Swellendam, South Africa) at 10% w/w.

Ethical Clearance for the Study and Experimental Animals

The Animal Ethics Screening Committee (AESC) of the University of the Witwatersrand approved the study (AESC approval number 2011/07/03). Thirty-eight 5-week old mixed gender Japanese quail used in the study were obtained from a commercial supplier in peri-urban Johannesburg, South Africa.

Adaptation Period: Housing, Feeding and General Health

The quail were housed in the Central Animal Services unit of the University of the Witwatersrand under a deep litter system. A 12-h light cycle regime with lights off from 1900 to 0700 h was used. Infra-red lighting provided extra heating. On arrival the birds were given a week-long adaptation period during which they had *ad libitum* access to the control diet (STD) and clean drinking water. The birds were also routinely dewormed with piperizine [Kyron Laboratories (PVT) Ltd, Johannesburg, South Africa] at 1ml L^{-1} of drinking water.

Experimental Design and Experimentation

Immediately following the adaption period, the birds were randomly divided into two groups: group 1 (n = 20) was assigned to the control (STD) diet while group 2 (n = 18) was assigned to the test (high fat; HFD) diet. The experimental period/feeding trial was carried out under similar housing and lighting regime as during the adaptation period. The birds were kept on their respective diets for the duration of the experiment.

Body Mass Measurement

Each quail was weighed twice weekly on an electronic scale (Presica 310 M, Laser, Johannesburg, South Africa) by placing them in a pre-weighed box. In order to accustom the birds to handling and prevent handling-induced hyperglycaemia, each bird was handled for a couple of minutes (before weighing) twice weekly before the onset of experimental interventions.

Experimental Interventions: Glucose Tolerance Test

After 5 weeks of feeding, half the number birds on each diet were subjected to an oral glucose tolerance test (OGTT), while the other half was subjected to an intravenous glucose tolerance test (IVGTT). The birds, for both the OGTT and IVGTT, were subjected to a 12-h overnight fasting period before the glucose tolerance tests. For the OGTT, fasting blood glucose concentrations were determined (time interval 0) on each quail using a glucometer calibrated according to manufacturer's instructions (Ascentia, Elite™, Bayer Corporation, Mishawaka, USA). Blood for the fasting blood glucose concentration determination and after an oral glucose challenge was taken via a pin prick of the wing vein after sterilisation of the area to be pricked with a cotton gauze swab impregnated with alcohol (Loxham *et al.*, 2007). Following the determination of the fasting blood glucose concentrations, quail were gavaged via orogastric intubation with 5 g kg^{-1} body mass of sterile 50% w/v glucose solution [Merck Chemicals (Pty) Ltd, Johannesburg, South Africa]. Post-gavage blood glucose concentrations at time intervals 15, 30, 60 and 120 minutes, were then determined (Sinsigalli *et al.*, 1987). For the IVGTT, fasting blood glucose concentrations were determined (time interval 0) as previously described by Sinsigalli *et al.* (1987) after which each quail was infused via a wing vein with 2 mL kg^{-1} body mass of sterile 50% w/v glucose solution (Intramed, Port Elizabeth, South Africa). Post-infusion blood glucose concentrations were then determined from blood droplets collected by vein puncture of the opposite wing to that infused at time intervals 10, 30, 60 and 120 minutes as described by Sinsigalli *et al.* (1987).

Following the first set of the OGTT and IVGTT, the birds were maintained on their respective diets and were given a week-long recovery period. Thereafter the tests (OGTT and IVGTT) were reversed such that birds that were initially subjected to an OGTT were then subjected to an IVGTT and vice versa. Similar protocols to those used for the initial OGTT and IVGTT were followed during the repeat tests.

Terminal Procedures: Tissue Harvesting

After the repeat glucose tolerances tests, the birds were given a week to recover on their respective diets. Half the number of quail on the STD and HFD (n = 10 and n = 9, respectively) were then fasted overnight while their counterparts (n = 10, n = 9, respectively) were allowed access to feed overnight. Blood glucose concentrations were then measured in the fasted and fed quail using a glucose meter (Ascentia Elite™, Bayer Corporation, Mishawaka, USA) according to the manufacturer's instructions. The quail were then euthanized by intra-peritoneal injection of 200 mg kg^{-1} body mass of sodium pentobarbital (Euthanaze, Centaur Labs, South Africa). The abdominal fat pad, the liver, and thigh and breast muscles were then dissected out. The abdominal fat pad and liver of each bird were weighed using an electronic balance (Presica 310 M, Laser,

Johannesburg, South Africa). The presence of testicles and ovaries was used to confirm the sex of each bird. The liver and breast and thigh muscles were placed in separate sealable polythene bags and stored in a freezer at -20°C pending glycogen analysis.

Liver and Muscle Glycogen Determination

The glycogen content of the liver, thigh and breast muscle samples of each bird were determined by indirect acid hydrolysis as described by Passonneau and Lauderdale (1974). Briefly, 0.1 g of the liver or 1 g of thigh or breast muscle was placed in a test tube to which 1 mL of 0.03 M HCl was added followed by homogenisation using an ultra-turrax homogeniser (Janke and Kunkel, Ika-Werk, Germany). Immediately following homogenisation, 1 mL of 1 M HCl was added to the test tube to hydrolyse glycogen in the sample. The test tubes with the samples sealed with aluminium foil to minimise evaporation were then placed in a water bath with boiling water for 2 h to ensure total hydrolysis of glycogen. Thereafter 1 mL of 1M NaOH was added to each sample in the test tube in order to neutralise the acid. The glucose concentration of each hydrolysate was then measured using a glucometer (Ascentia Elite™, Bayer Corporation, Mishawaka, USA) according to the manufacturer's instructions.

Data Analysis

Data were analyzed using Graphpad Prism software (Graph-pad Software Inc., San Diego, USA). Data for weekly body mass, OGTT and IVGTT were analyzed using a repeated measures analysis of variance. Weekly mean body mass between the groups and the mean blood glucose concentration at different time periods after oral glucose gavage or intravenous glucose infusion were compared using the Bonferroni post hoc test. Data for blood glucose concentration in the birds at termination (fed vs fasted), abdominal fat pad and liver masses and tissue glycogen concentrations between the groups were analyzed using a two-way analysis of variance with the student t-test being used to compared the group means.

The model used for the analysis of variance for weekly body mass and glucose tolerance tests was:

$$Y_{ijk} = \mu + T_i + B_j + C_k + e_{ijk},$$

Where, Y_{ijk} = blood glucose concentration at time C post-gavage/post-infusion.

μ = mean common to all observations.

T_i = Treatment (diet) effect (n = 1,2).

B_j = fixed individual bird effect (= 1,2,3 .....19).

C_k = fixed effect of sampling time on blood glucose concentration (n = 1.2...5).

e_{ijk} = random residual error.

The model used for analysis of variance for variables determined at study termination [blood glucose (fed versus fasted, tissue glycogen content, and viscera masses) was:

$$Y_{ijk} = \mu + T_i + B_j + e_{ijk}$$

Where, Y_{ijk} = response variable of interest

μ = mean common to all observations.

T_i = treatment (diet) effect (n = 1, 2).

B_j = fixed individual bird effect (= 1, 2, 3 .....19).

e_{ij} = random residual error.

Results

Fig. 1 shows the growth profile of the two dietary groups of quail over the 8-week experimental period. While the quail grew significantly during each week compared to their induction body mass (Fig. 1), the body mass gain of the quail on the HFD was not significantly different to the control group. Fig. 2a to c show glucose handling by the quail on the two diets following an oral glucose challenge, while Fig. 3a to c show glucose handling by quail following an intra-venous glucose challenge. Enriching a standard commercial quail diet with 10% canola oil (w/w) had no effect on oral (P = 0.9038; pooled results) and intravenous (P = 0.4365; pooled results) glucose handling by quail (Fig. 2a and 3a, respectively).

For the OGTT, blood glucose concentration peaked 15 min post-gavage in both the STD-fed and HFD-fed group (pooled data; Fig. 2a) and was significantly higher (P<0.0001) in both groups compared to the basal (fasting) blood glucose concentration. The blood glucose concentration returned to basal concentration 60 min post-gavage in the STD-fed quail and at 120 min in the HFD-fed quail (Fig. 2a). The blood glucose concentration of the male quail on both the STD and HFD peaked 15 min post-gavage, the peak concentration was significantly higher (P<0.0001) than the basal glucose concentration (Fig. 2b). Similarly, the blood glucose concentration of female quail on the STD and HFD also significantly peaked (P = 0.0057, quail on STD; P = 0.0394 quail on HFD) at 15 min post-gavage (Fig. 2c). While the blood glucose concentration of the male quail on the STD diet returned to basal concentration at 30 min post-gavage, the blood glucose concentration of their counterparts on the HFD returned to basal concentration at 120 min post-gavage (Fig. 2b). The blood glucose concentration of the female quail on both the STD and HFD returned to basal concentration at 60 min post-gavage (Fig. 2c).

In the IVGTT, while diet had no effect on blood glucose concentration across the sampling time periods (Fig. 3a; pooled data) blood glucose concentration peaked at 10 min post-infusion for quail on both the STD and HFD (Fig. 3a) with the peaks representing 114% and 153% higher blood glucose concentration for quail on the STD and HFD, respectively, when compared to basal glucose concentration. The blood glucose concentration of quail on the two diets

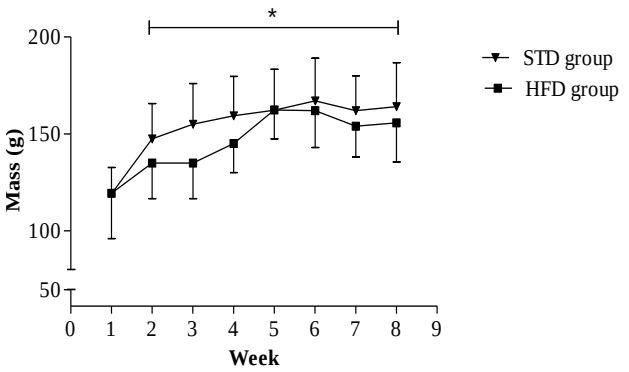

Fig. 1: Effect of supplementing a standard commercial diet with 10% canola oil on weekly body masses of Japanese quail. Quail on both the control (STD) and test (HFD) significantly gained weight (P <0.05) compared to mean induction body weight, however there were no differences (P> 0.05) in weekly body masses (weeks 2 to 8) between quail on the control and test diet. Data presented as means ± SD. n=20 for STD; n = 18 for HFD. STD = standard commercial diet (control). HFD = test [STD + 10% canola oil (w/w)] diet

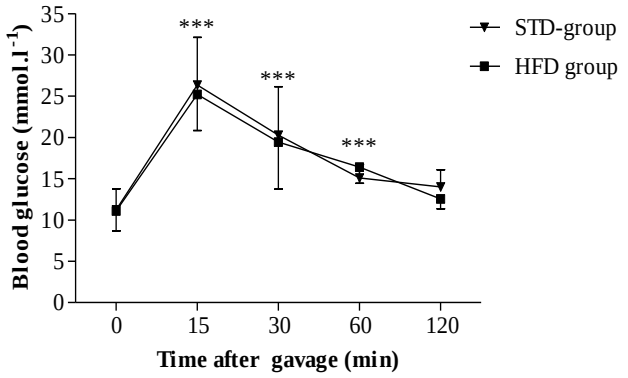

Fig. 2a: Effect of supplementing a standard commercial diet with 10% canola oil on oral glucose tolerance in Japanese quail. Quail on the STD and HFD had similar (P>0.05) blood glucose concentration at time periods 15 to 120 min post-gavage. Blood glucose concentrations of quail on both dietary groups peaked 15 min post-gavage and were significantly higher (P < 0.0001) compared to basal concentration. While the blood glucose concentration of quail on the STD and HFD was significantly higher (P<0.0001) compared to basal 30 min post-gavage, at 60 min post-gavage, only the blood glucose concentration of quail on the HFD was still significantly higher (P < 0.0001) compared to basal. Data presented as means ± SD. n=10 for STD; n = 9 for HFD. STD = standard commercial diet (control). HFD = test [STD + 10% canola oil (w/w)] diet

(STD and HFD) returned to basal concentration at 60 min post-infusion (Fig. 3a). On analyzing the IVGTT of the different genders, the blood glucose concentration of the male quail on both the STD and HFD significantly peaked at 10 min post-infusion (P<0.0001, quail on STD; P=0.0057, quail on HFD) (Fig. 3b). Similarly the blood glucose concentration of female quail on the STD and HFD also significantly peaked (P = 0.0101, quail on STD; P=0.0057 quail on HFD) at 10 min post-infusion (Fig. 3c). At 30 min post-infusion, the blood glucose concentration

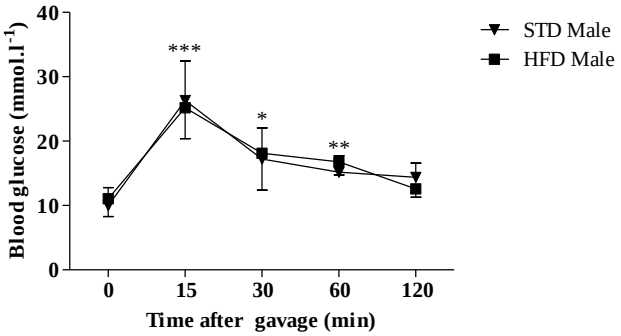

Fig. 2b: Effect of supplementing a standard commercial diet with 10% canola oil on oral glucose tolerance in male Japanese quail. Male quail on the STD and HFD had similar (P > 0.05) blood glucose concentration at time periods 15 to 120 min post-gavage. The blood glucose concentration significantly peaked (P < 0.0001) at 15 min post-gavage compared to basal concentration in quail on either diet. The blood glucose concentration of quail on the STD returned to basal concentration at 30 min post-gavage, but the blood glucose concentration of quail on the HFD remained significantly higher at 30 min (P = 0.0263) and 60 min (P = 0.0019) post-gavage compared to basal concentrations. Data presented as means ± SD. n = 5 for STD; n = 7 for HFD. STD = standard commercial diet (control). HFD = test [STD + 10% canola oil (w/w)] diet

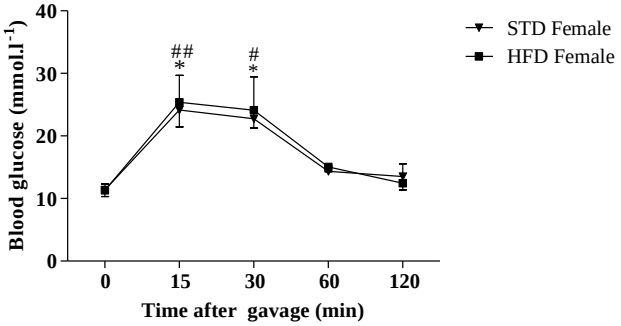

Fig. 2c: Effect of supplementing a standard commercial diet with 10% canola oil on oral glucose tolerance in female Japanese quail. Female quail on the STD and HFD had similar (P > 0.05) blood glucose concentration at time periods 15 to 120 min post-gavage. The blood glucose concentration of both groups significantly peaked at 15 min ($^{\#\#}$P = 0.0057; quail on STD and *P = 0.0394; quail on HFD) post-gavage compared to basal and remained significantly high ($^{\#}$P = 0.0132; quail on STD and *P=0.0263; quail on HFD) at 30 min post-gavage compared to basal. Data presented as means ± SD. n =5 for STD; n = 3 for HFD. STD = standard commercial diet (control). HFD = test [STD + 10% canola oil (w/w)] diet

of the female quail on the HFD was significantly higher (P=0.0476) compared to that of female quail on the STD (Fig. 3c). While the blood glucose concentration of the female quail on the STD returned to basal concentrations at 30 min post-infusion, the blood glucose concentration of their counterparts on the HFD returned to basal

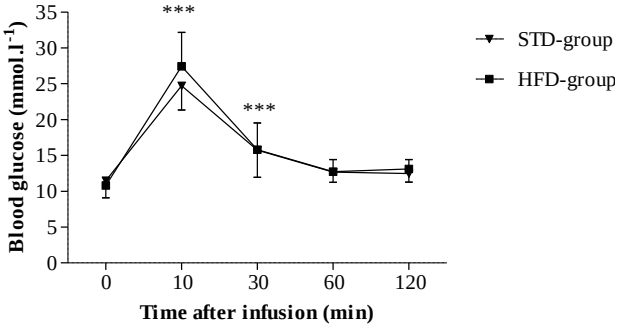

Fig. 3a: Effect of supplementing a standard commercial diet with 10% canola oil on intravenous glucose tolerance in Japanese quail. Quail on STD and HFD had similar (P > 0.05) blood glucose concentration at time periods 10 to 120 min post-infusion. Blood glucose concentrations of quail on both dietary groups peaked at 10 min post-infusion and were significantly higher (P < 0.0001) up to 30 min post-infusion compared to basal concentration. Blood glucose concentrations returned to basal concentration for quail on both diets 60 min post-infusion. Data presented as means ± SD. n=10 for STD; n = 9 for HFD. STD = standard commercial diet (control). HFD = test [STD + 10% canola oil (w/w)] diet

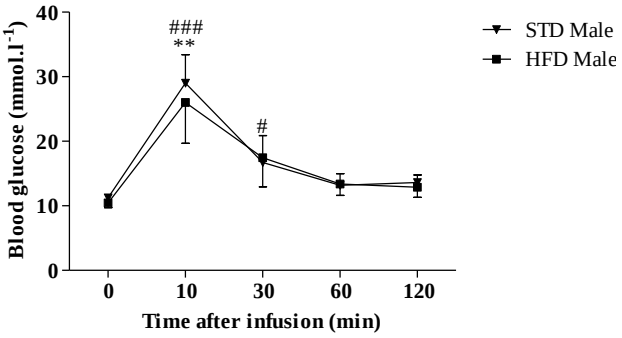

Fig. 3b: Effect of supplementing a standard commercial diet with 10% canola oil on intravenous glucose tolerance in male Japanese quail. Male quail on the STD and HFD had similar (P > 0.05) blood glucose concentration at time periods 10 to 120 min post-infusion. The blood glucose concentration of both groups significantly peaked at 10 min ($^{###}$P < 0.0001; quail on STD and **P = 0.0057; quail on HFD) post-infusion. The blood glucose concentration of quail on the STD remained significantly high ($^{\#}$P = 0.0106) compared to basal concentration at 30 min post-infusion while the blood glucose concentration of quail on the HFD returned to basal concentration at 30 min post-infusion. Data presented as means ± SD. n =5 for STD; n = 7 for HFD. STD = standard commercial diet (control). HFD=test [STD + 10% canola oil (w/w)] diet

concentration 60 min post-gavage (Fig. 3c).

Table 1 shows effects of the diets on the abdominal fat pad mass (AFPM) and liver mass of the male and female quail. Although the dietary treatments did not result in any significant differences in the liver mass (absolute and relative), the female quail had significantly (P<0.05) heavier livers (absolute and relative) compared to their male counterparts (Table 1). The absolute AFPM of female quail on the HFD was significantly lighter (P < 0.0001) compared

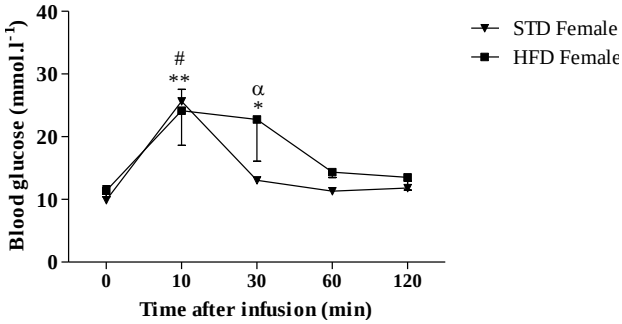

Fig. 3c: Effect of supplementing a standard commercial diet with 10% canola oil on intravenous glucose tolerance in female Japanese quail. Female quail on the STD and HFD diet had similar (P > 0.05) blood glucose concentration at time periods 10, 60 and 120 min post-infusion. The blood glucose concentration of both groups significantly peaked at 10 min ($^{\#}$P = 0.0101; quail on STD and **P = 0.0057); quail on HFD) post-infusion compared to basal concentration. The blood glucose concentration of quail on the STD returned to basal concentration at 30 min post-infusion but the blood glucose concentration of quail on the HFD was remained significantly higher (*P = 0.0132) compared to basal concentration and was also significantly higher ($^{\alpha}$P = 0.0476) compared to that of quail on the STD. Data presented as means ± SD. n =5 for STD; n = 3 for HFD. STD = standard commercial diet (control). HFD = test [STD + 10% canola oil (w/w)] diet

to that of their counterparts on the STD (Table 1). The male quail had significantly heavier (P < 0.05) AFPM (absolute and relative) compared to their female counterparts (Table 1). Between dietary treatments, there were no significant differences in the tissue (liver, breast and thigh) glycogen content of the quail on the STD and HFD (Table 2). Similarly, within dietary treatment there were no significant differences in the tissue (liver, breast and thigh) glycogen content of male and female quail (Table 2).

Discussion

Bryant *et al.* (2005) demonstrated that supplementing broiler chick diets with fat improved their feed conversion ratio. de Witt *et al.* (2009) demonstrated that dietary inclusion of saturated fatty acids (tallow) at 6% (w/w) resulted in the highest dressing percentage, breast weight and breast meat yield in broiler chickens. However in the current study supplementing a STD quail feed with 10% (w/w) canola oil had no effect on quail growth compared to birds on the control feed. The constituent fatty acids of canola oil are oleic acid (about 55 to 60 %), linoleic acid (21 to 25%) and alpha-linolenic acid (9 – 10%) (Dupont *et al.*, 1989; Kwon *et al.*, 1991; Ahmad *et al.*, 2013) thus, making it a polyunsaturated fatty acid (PUFA) rich oil. PUFA rich diets have been observed to reduce fat deposition in broiler chicken via preferential beta oxidation of the PUFA (with respect to saturated and monounsaturated fatty acids) (Crespo and Esteve-Garcia, 2002). According to Crespo and

Table 1: Effect of supplementing a standard commercial diet with 10% canola oil on the mass of the liver and abdominal fat pad of Japanese quail

Parameter	STD	HFD	P-value
Absolute Liver mass (g)			
Male	2.55±0.24[a]	2.29±0.43[a]	0.1622
Female	5.91±1.19[b]	6.41±1.15[b]	0.4019
Significance level	***	***	
Relative liver mass (%)			
Male	1.73±0.16[a]	1.53±0.30[a]	0.1233
Female	3.31±0.76[b]	3.58±0.70[b]	0.4715
Significance level	***	***	
Absolute Abdominal fat pad mass (g)			
Male	1.80±0.59[a]	1.96±0.99[a]	0.7038
Female	1.11±0.14[b, 1]	0.74±0.04[b, 2]	0.0001
Significance level	***	***	
Relative abdominal fat pad mass (%)			
Male	0.68±0.67[a]	0.95±0.69[a]	0.4118
Female	0.22±0.29[b]	0.20±0.28[b]	0.8736
Significance level	**	**	

[a,b]Within column means with different superscripts are significantly different at $P < 0.05$). [1,2]Within row means with different superscripts are significantly different. Quail on the STD and HFD diet had similar absolute and relative liver masses. The relative AFMP of quail on both diets was similar ($P > 0.05$), the absolute AFPM of the female quail on the HFD was significantly lighter ($P < 0.05$) compared to that of female quail on the STD. Within diet, female quail had significantly heavier ($P < 0.05$) liver masses compared to their male counterparts. Within diet, male quail had significantly heavier ($P < 0.05$) AFPM compared to their female counterparts. Data presented as means ± SD. n = 20 for STD split as 7 males and 13 females; n = 18 for HFD split as 12 males and 6 females. STD = standard commercial diet (control). HFD = test [STD + 10% canola oil (w/w)] diet. AFPM = abdominal fat pad mass. ***$P < 0.0001$; **$P<0.01$

Table 2: Effect of supplementing a standard commercial diet with 10% canola oil on Japanese quail tissue glycogen content expressed as glucose (mmol.l^{-1} homogenate) equivalent

Parameter	STD	HFD	P-value
Liver			
Male	2.36±0.97	1.62±1.25	0.1971
Female	1.22±0.58	1.53±0.58	0.2940
Overall	1.79±0.80	1.57±0.06	0.4982
Thigh muscle			
Male	1.88±0.61	1.78±0.46	0.6898
Female	1.73±0.56	1.85±0.47	0.6553
Overall	1.80±0.11	1.81±0.05	0.9561
Breast muscle			
Male	2.50±0.53	2.67±0.33	0.3975
Female	2.46±0.34	2.35±0.28	0.5002
Overall	2.48±0.03	2.51±0.23	0.8145

Data presented as means ± SD.n = 20 for STD split as 7 males and 13 females; n = 18 for HFD split as 12 males and 6 females. STD = standard commercial diet (control). HFD = test [STD + 10% canola oil (w/w)] diet

Esteve-Garcia (2002), the preferential oxidation of the PUFAs reduces the fatty acids available for fat deposition. Saturated fatty acid-rich diets increase fat accretion (Sanz *et al.*, 1999; Crespo and Esteve-Garcia, 2002) in broiler chickens, which (accreted fat) partially accounts for the increased body mass observed in chickens fed diets supplemented with oils rich in saturated fatty acids. Canola oil contains about 29 – 35% polyunsaturated fatty acids (Dupont *et al.,* 1989; Kwon *et al.,* 1991) thus, supplementing poultry diets with canola oil would increase their PUFA content. The increased dietary PUFA content would result in increased *in vivo* beta oxidation of the PUFA and hence reduced fat accretion. The preferential *in vivo* oxidation of PUFAs versus SFAs results in a shift from the oxidative to the lipogenic pathway (Wongsuthavas *et al.,* 2008), whereby the conversion of glucose into triglycerides is less efficient metabolically compared to the conversion of fatty acids into triglycerides (Newsholme and Leech, 1994). As a result, the feeding of diets rich in PUFAs in place of SFAs rich diets leads to less deposition of abdominal fat (Wongsuthavas *et al.,* 2008). Based on the observations by Crespo and Esteve-Garcia (2000) and the fatty acid content of canola oil (Dupont *et al.,* 1989; Kwon *et al.,* 1991), it could be speculated that enriching the standard quail diet with 10% (w/w) canola oil resulted in increased preferential beta oxidation of the PUFAs in the quail, that could have resulted in reduced deposition of abdominal fat, hence the observed similarity in the growth. Zanini *et al.* (2008) reported that supplementing broiler diets with canola oil resulted in a decrease in the lipid content of edible portions. Lipid accretion accounts for part of the body mass of animals and birds. It would therefore appear that in quail, canola oil could probably cause a decrease in lipid accretion of tissues which could explain the similarities in the growth performance of the quail on the STD and HFD.

In their work, Crespo and Esteve-Garcia (2003) noted that broilers fed diets rich in saturated fatty acids (SFAs) and monounsaturated fatty acids (MUFAs) had increased plasma insulin concentrations that were not accompanied by depletion in plasma glucose concentration compared to their counterparts fed diets rich in polyunsaturated fatty acids (PUFAs). This was inferred to suggest higher resistance to insulin and an imbalance of glucose insulin concentrations on birds fed SFAs and MUFAs rich diets (Crespo and Esteve-Garcia, 2003). The observations by Crespo and Esteve-Garcia (2003) suggest that the fatty acid profile of the oil used in enriching the diet has an effect on glucose metabolism with SFAs and MUFAs causing obesity the results in reduced insulin sensitivity and hence impaired glucose homeostasis, while PUFAs rich diets are associated with normal blood glucose homeostasis. Canola oil has about 29 – 35% of its constituent fatty acids as PUFAs and 59.7% as oleic acid (Dupont *et al.,* 1989; Kwon *et al.,* 1991). Polyunsaturated fatty acids have been shown to increase *in vivo* beta oxidation in broilers (Crespo and Esteve-Garcia 2003). Increased *in vivo* beta oxidation depletes the substrate that would normally lead to increased fat deposition that leads to obesity and subsequent reduced sensitivity to insulin. Dietary MUFAs, including oleic acid, have been reported to be effective in lowering blood cholesterol concentration (Huang *et al.,* 2008). In the current study, use of canola oil to enrich the diet could have resulted in an increase in PUFAs and MUFAs causing a synergist effect of increased *in vivo* beta oxidation that translated into

glucose homeostasis in quail fed the oil enriched diet thus explaining the observed similarities in glucose handling between quail on the STD and those on the HFD (Fig. 2a and 3a; pooled results).

Our results indicate that the HFD had gender related effects on OGTT and IVGTT (Fig. 2b and 3c, respectively). Magubane et al. (2013) observed that female quail on a standard diet supplemented with canola oil (10% w/w) had significantly higher ($P<0.05$) plasma triglycerides compared to their counterparts on the control diet. Thiébaud *et al.* (1982) observed that in humans, hyperlipidaemia caused a significant reduction in total glucose uptake that was associated with a concomitant decrease in both glucose oxidation and storage. The delayed decline in plasma glucose concentration in female quail on the HFD following an intravenous glucose challenge compared to their counterparts on the STD could have been due to hyperlipidaemia-induced reduced glucose uptake. Similarly, the delay to return to basal glucose concentration in male quail on a HFD could have been driven by hyperlipidaemia-induced reduced glucose uptake. On acquisition of the quail, the birds were not sexed consequently we did not have equal numbers of males and females. Thus when the quail were randomly assigned to the dietary treatments, the numbers of male and female quail on each dietary was not balanced. While pooled results (regardless of sex) of the current study point to similarities in glucose handling between the quail on the control and test diets, it has to be noted that when gender is taken into consideration, the sample size (number) of female quail on the HFD subjected to intravenous glucose challenge was too small. As such we recommend that our findings and discussion on glucose handling by female quail subjected to an intravenous glucose challenge be treated as preliminary.

In a study of the carcasses of quail, Banerjee (2010) reported that female quail had heavier liver masses (5.95 ± 0.40 g versus 2.91 ± 0.25 g) compared to that of male counterparts. In the current study, female quail on both the STD and HFD also had heavier livers (5.91 ± 1.19 g and 6.41 ± 1.15 g, respectively) compared to those of their male counterparts (2.55 ± 0.24 and 2.29 ± 0.43 g) on the STD and HFD, respectively. Our results thus show a trend similar to that reported by others. The heavier livers in the female quail could speculatively be ascribed to the differences in energy and metabolite demand of the females and males at sexual maturity. From the sixth week of the experiment the female quail, aged 11 weeks, started laying. Moran (1987) notes that, in hens, yolk proteins are almost exclusively synthesized in the liver and then transported to the developing follicles. The liver, would thus, during egg-lay, up-regulate metabolic processes in order to ensure a constant and adequate supply of the yolk proteins required for egg production. The up-regulation comes about as a result of the recruitment of more mitochondria and the cellular protein synthetic machinery; all which could possibly account for heavier livers in female birds, including quail, at the point of lay/sexual maturity.

Zanini *et al.* (2008) noted that in broilers dietary supplementation with canola oil resulted in a decrease in the lipid content of the liver and the gizzard (visceral organs). More importantly, Zanini *et al.* (2008) reported of a decrease in the SFAs and MUFAs content of the viscera. Wongsuthavas *et al.* (2008) noted that the preferential *in vivo* beta oxidation of PUFAs versus SFAs triggers the less efficient lipogenic pathway that is dependent on glucose in place of fatty acids thus resulting in a diminished abdominal fat deposition in broilers. It would appear that dietary supplementation of the quail feed with canola oil (high in PUFAs) had similar effects on quail as was the case in broilers, an inference, which could probably explain the similarities in the abdominal fat pad (and liver) masses of quail on the STD and their counterparts on a HFD (Table 1). Interestingly, in the current study, we observed that male quail, irrespective of the diet, had heavier abdominal fat pad masses compared to their female counterpart (Table 1); a finding similar to that reported by Barnejee (2010). The lower abdominal fat pad mass in female quail compared to their male counterparts could have been due to the probable mobilization of the visceral fat for energy to support the synthesis of metabolites required for egg production in the females.

Tissue glycogen content is dependent on dietary input and glucose homeostasis in the animal. The similarities in the liver and muscle (thigh and breast) of quail on the control and test diets were consistent with the similarities in glucose handling by the quail on the respective diets.

In conclusion, while the HFD had gender related effects on glucose tolerance, pooled results showed that supplementing the diet with canola oil (10% w/w) did not elicit glucose intolerance neither did it affect the growth performance, mass of viscera (liver and visceral fat) nor the concentration of glycogen in the liver and muscle (breast and thigh) of quail. Canola oil at 10% of the diet (w/w) cannot be used to improve the growth performance and meat quality of the Japanese quail.

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References

Ahmad, S., A.U. Haq, M. Yousaf, Z. Kamran, A.U. Rehman, M.U. Suhail and H.A. Samad, 2013. Effect of canola oil and vitamin a on egg characteristics and egg cholesterol in laying hens during hot summer months. *Pak. Vet. J.*, 33: 346–349

Allen, C.D., D.L.Fletcher, J.K. Northcutt and S.M. Russell, 1998. The relationship of broiler breast color to meat quality and shelf-life. *Poult. Sci.*, 77: 361–366

Atapattu, N.S.B.M. and T.S.M.S. Senevirathne, 2013. Effects of increasing levels of dietary cooked and uncooked banana meal on growth performance and carcass parameters of broiler chicken. *Pak. Vet. J*., 33: 179–182

Banerjee, S., 2010. Carcass studies of Japanese quails (*Cortunix cortunix japonica*) reared in hot humid climate of Eastern India. *World Appl. Sci. J*., 8: 174–176

Bryant, M., G. Wu and D.A. Roland, 2005. *Optimizing Dietary Energy for Profits and Performance of two Strains of White Leghorns*, p: 23. International Poultry Scientific Forum Abstracts, Atlanta, USA

Chizzolini, R., V. Zanardi and S. Ghidini, 1999. Calorific value and cholesterol content of normal and low-fat meat and meat products. *Trends Food Sci. Technol*., 10: 119–128

Crespo, N. and E. Esteve-Garcia, 2000. Dietary fatty acid profile modifies abdominal fat deposition in broiler chickens. *Poult. Sci*., 80: 71–78

Crespo, N. and E. Esteve-Garcia, 2002. Dietary polyunsaturated fatty acids decrease fat deposition in separable fat depots but not in the remainder carcass. *Poult. Sci*., 81: 512–518

Crespo, N. and E. Esteve-Garcia, 2003. Polyunsaturated fatty acids reduce insulin and very low density lipoprotein levels in broiler chickens. *Poult. Sci*., 82: 1134–1139

de Witt, F.H., S.P. Els, A. Hugo, H.J. van de Merwe and M.D. Fair, 2009. Influence of dietary lipid sources on carcass traits of broilers. *S. Afr. J. Anim. Sci*., 39: 49–52

Dupont, J.P., J. White, K.M. Johnston, B.E. McDonald, S.M. Grundy and A. Bonanome, 1989. Food safety and health effects of canola oil. *J. Amer. Coll. Nutr*., 8: 360–375

Gollisch, K.S.C., J. Brandauer, N. Jessen, T. Toyoda, A. Nayer, M.F. Hirshman and L.J. Goodyear, 2009. Effects of exercise training on subcutaneous and visceral adipose tissue in normal- and high-fat diet fed rats. *J. Physiol. Endocrinol. Metabol*., 297: 495–504

Huang, Y., J.P. Schoonmaker, B.J. Bradford and D.C. Beitz, 2008. Response of milk fatty acid composition to dietary supplementation of soy oil, conjugated linoleic acid, or both. *J. Dairy Sci*., 91: 260–270

Kayang, B.B., A. Vignal, M. Inoue-Murayama, M. Miwa, J.L. Monvoisin, S. Ito and F. Minvielle, 2004. A first generation microsatellite linkage map of the Japanese quail. *Anim. Genetic*., 35: 195–200

Kwon, J.S., J.T. Snook, G.M. Wardlaw and D.H. Hwang, 1991. Effects of diets high in saturated fatty acids, canola oil, or safflower oil on platelet function, thromboxane B_2 formation and fatty acid composition of platelet phospholipids. *Amer. J. Clinl. Nutr*., 54: 351–358

Loxham, S.J., J., S.M. Teague, J. Poucher, A.V. De Schoolmester, Turnbull and F. Carey, 2007. Glucagon challenge in the rats: a robust method for the *in vivo* assessment of Glycogen phosphorylase inhibitor efficacy. *J. Pharmacol. Toxicol. Methods*, 55: 71–77

Magubane, M.M., B.W. Lembede, K.H. Erlwanger, E. Chivandi and J. Donaldson, 2013. Fat absorption and deposition in Japanese quail (*Coturnix coturnix japonica*) fed a high fat diet. *J. S. Afr. Vet.*, 84: in press

Mareko, M.H.D., S.J. Nsoso and K. Thibelang, 2006. Preliminary carcass and meat characteristics of guinea fowl (*Numidia melaeagris*) raised on concrete and earth floors in Botswana. *J. Food Technol*., 4: 313–317

Mosleh, N., S. Nazifi and A. Alaeddini, 2012. Changes in serum acute phase reactants, inflammatory mediators and gangliosides in Japanese quail (*Coturnix japonica*) with retained yolk sac. *Pak. Vet. J*., 32: 251–254

National Research Council (NRC), 1991. Quail. *In*: *Micro-livestock –Little Known Small Animals with a Promising Future*, pp: 147–155. Nation Academy Press, Washington DC

Newsholme, E.A. and A. Leech, 1994. *Biochemistry for the Medical Sciences*. John Wiley and Sons, New York, USA

Nishibori, M., M. Tsudzuki, T. Hayashi, Y. Yamamoto and H. Yasue, 2002. Complete nucleotide sequence of the *Coturnix chinensis* (blue-breasted quail) mitochondorial genome and a phylogenetic analysis with related species. *J. Hered.*, 93: 439–444

Passonneau, J.V. and V.R. Lauderdale, 1974. A comparison of three methods of glycogen measurement in tissues. *Anal. Biochem.*, 60: 405–412

Sanz, M.C., A. Flores, P. Perez de ayala and C.J. Lopez-bote, 1999. Higher lipid accumulation in broilers fed on saturated fats than in those fed on unsaturated fats. *Brit. Poult. Sci.*, 4: 95–101

Sanz, M.C., M.T. Verde, T. Siez and C. Saiiudob, 1996. Effect of Breed on the Muscle Glycogen Content and Dark Cutting Incidence in Stressed Young Bulls. *Meat Sci.*, 43: 31–42

Sinsigalli, N.A., J.P. McMurtry, J.A. Cherry and A.B. Siegel, 1987. Glucose tolerance, plasma insulin and immunoreactive glucagon in chickens selected for high and low body weight. *J. Nutr*., 117: 941–947

Thiébaud, D., R.A. DeFronzo, E. Jacot, A. Golay, K. Acheson, E. Meader, E. Jéquier and J. Felber, 1982. Effect of long chain triglycerides infusion on glucose metabolism in man. *Metabolism*, 31: 1128–1136

Tuleun, C.D., A.Y. Adenkola and T. Afele, 2011. Effect of dietary ascorbic acid supplementation on the performance of japanese (*Coturnix-coturnix japonica*) quails in a tropical environment. *J. Poult. Plant Sci*., 10: 1268–1275

Vali, N., 2008. The Japanese quail: A review. *Int. J. Poult. Sci.*, 7: 925–931

Wechsler, B. and I. Schmid, 1998. Aggressive pecking by males in breeding groups of Japanese quail (Coturnix japonica). *Brit. Poult. Sci*., 39: 333–339

Wongsuthavas, S., S. Terapuntuwat, W. Wongsrikeaw, S. Katawatin, C. Yuangklang and A.C. Beynen, 2008. Influence of amount and type of dietary fat on deposition, adipocyte count and iodine number of abdominal fat in broiler chickens. *J. Anim. Physiol. Anim. Nutr*., 92: 92–98

Zanini, S.F., G.L. Coinago, B.M.S. Pessoti, M.R. Bastos, F.P. Casegrande and V. R. Lima, 2006. Body fat of broiler chicken fed diets with two fat sources and conjugated linoleic acid. *Int. J. Poult. Sci*., 5: 241–246

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