Journal homepage: http://www.sasas.co.za/journals

South African Journal of Animal Science 2018, 48 (No. 6)

Effect of quercetin supplementation on the growth, feed efficiency and serum hormone levels of New Zealand White rabbits

M.K. North[1], A. Dalle Zotte[2] & L.C. Hoffman[1#]

[1] Department of Animal Sciences, Stellenbosch University, Private Bag X1, Matieland, Stellenbosch 7602, South Africa
[2] Department of Animal Medicine, Production and Health, University of Padova, Agripolis, Viale dell'Università, 16, 35020 Legnaro, Padova, Italy

(Received 20 July 2018; Accepted 8 November 2018; First published online 7January 2019)

Abstract

Meat rabbits could provide a new avenue for agricultural development in South Africa, and flavonoids may be able to improve their live performance. This study investigated the effects of quercetin dihydrate (0 and 2 g/kg feed) on the growth parameters and serum-free triiodothyronine (fT3), free thyroxine, somatotropin, and cortisol levels of growing rabbits. It also provides the first growth and feed conversion ratio (FCR) data for South African New Zealand Whites. Control (Ctrl) and quercetin-supplemented (Qrc) diets were provided to 34 (16 male, 18 female) and 32 (15 male, 17 female) rabbits, respectively, from 5 to 12 weeks old. Live performance was measured weekly, and serum hormone levels were determined at 11 weeks old. Overall, the rabbits performed well for live weight and growth rate, growing from 1052 ± 13.4 g to 3192 ± 45.3 g (5 - 12 weeks). Females had higher cortisol levels than males, as found for other species (female = 10.9 ng/mL, male = 3.89 ng/mL). Qrc rabbits tended to have a higher overall FCR than Ctrl rabbits (Ctrl = 3.83, Qrc = 4.01), possibly due to lower feed digestibility, and had smaller sex differences in growth and FCR than Ctrl rabbits, possibly owing to xenoestrogenic activity. They also tended to have higher fT3 levels than Ctrl rabbits (Ctrl = 3.62 ng/mL, Qrc = 4.98 ng/mL), possibly owing to the inhibition of binding to transthyretin. However, it does not seem that the provision of quercetin is commercially justified for improving the live performance of rabbits.

Keywords: Cortisol, feed conversion ratio, flavonoid, growth, rabbit, somatotropin, thyroid hormones
[#] Corresponding author: lch@sun.ac.za

Introduction

Rabbits show great potential in South Africa, not only for large-scale commercial meat production systems, but also for smaller rural development projects (Oseni, 2012). They can be farmed intensively on small areas of land, are highly prolific, and reach slaughter weight in a short period, resulting in rapid returns on capital investment (Abu, 2008; Oseni, 2012). Rabbits also grow efficiently on diets with a high-fibre, low grain content, reducing the direct competition with people for feed resources (Finzi, 2000; Abu, 2008). From a subsistence farmer's perspective, the small size of rabbits allows easy handling by women and children, and reduces the need for cold-storage at slaughter. Relatively simple unsophisticated farming systems can be used, and expensive commercial feeds can be supplemented with locally available forages (Finzi, 2000; Abu, 2008).

However, as for all intensive livestock industries, rabbit farming faces challenges in terms of shrinking profit margins – which necessitate increasing the efficiency of production – and demands by consumers to produce not only a high quality healthy product, but also one that is environmentally and socially responsible (Verbeke & Viaene, 2000; Dalle Zotte & Szendrő, 2011). This has resulted in bans on the use of antibiotics as growth promotors in animal feeds in Europe, and recommendations from the World Health Organisation that the non-therapeutic use of antibiotics in animals should be limited worldwide. Similar guidelines have been released by authorities in the USA, Canada and Australia (European Commission, 2005; Serratosa *et al.*, 2006; Landers *et al.*, 2012; FDA Centre for Veterinary Medicine, 2013). These policies of restriction,

URL: http://www.sasas.co.za
ISSN 0375-1589 (print), ISSN 2221-4062 (online)
Publisher: South African Society for Animal Science
http://dx.doi.org/10.4314/sajas.v48i6.17

control, and surveillance also apply to all animal products imported into these regions, and thus affect production in exporting nations as well (Johnson, 2010). It is therefore necessary for agricultural researchers to investigate alternative natural options, such as flavonoids.

Flavonoids are naturally occurring polyphenolic compounds that are produced by plants as secondary metabolites, and have long been recognised as having extensive pharmacological actions (Havsteen, 2002; Erlund, 2004; Tapas *et al*., 2008). Some, which have prominent antimicrobial and antioxidant activities, have been brought to the attention of the livestock industry as potential growth promotors (Erlund, 2004; Tapas *et al*., 2008). However, owing to the wide range of bioactivities possessed by these compounds, including mutagenic effects and interactions with hormone systems, the risks of negative effects of supplementation must be considered (Dakora, 1995; Havsteen, 2002; Dos Santos *et al*., 2011).

Quercetin is one of the best studied and characterized flavonoids, and is the most widely occurring dietary flavonol (Erlund, 2004). Livestock feed resources containing quercetin include white clover flowers (Schittko *et al*., 1999), trefoil (Reynaud & Lussignol, 2005), sainfoin (Thill *et al*., 2012), members of the Brassicaceae family (Cartea *et al*., 2011), sweet potatoes (Park *et al*., 2016), apple pomace (Lu & Foo, 1997), cottonseed meal (Blouin *et al*., 1981) and sunflower meal (Karamać *et al*., 2012). The biochemical activities of quercetin have been found to be extensive. However, in many cases the exact mechanisms of action have yet to be elucidated (Erlund, 2004). Livestock studies are relatively limited, and studies on poultry have had varying results, with Liu *et al*. (2014) finding that intermediate levels (0.2 - 4 g/kg feed) of supplementation improved feed efficiency and laying rates in hens, but higher levels (0.6 g/kg feed) had a negative effect. Similarly, Goliomytis *et al*. (2014) found relatively high inclusion levels caused a non-significant increase in the feed conversion ratio (FCR) of broiler chickens. While no studies have examined the effects of quercetin on meat rabbit growth parameters, there is evidence of effects on rabbit doe reproductive traits (Naseer *et al*., 2017), bone histology (Babosová *et al*., 2016) and thyrotropin secretion (Kováčik *et al*., 2015).

The aim of this study was therefore to provide the first data on the effects of quercetin supplementation on the growth and feed efficiency of growing rabbits, and to investigate its impact on the serum levels of several key hormones. The choice of hormones was based on their relative importance to growth and development and previous findings on the effects of flavonoids on their metabolism. Cortisol is well established as an indicator of chronic stress levels (Möstl & Palme, 2002), and the antioxidant effects of flavonoids have been suggested to aid in ameliorating the effects of stress (Onderci *et al*., 2004; Tuzcu *et al*., 2008). Flavonoids have also been found to interact with the thyroid hormone system (Narayana *et al*., 2001), and thyroid hormones and somatotropin are important for physiological development (Spencer, 1985).

In addition, this study provides the first reported growth data for South African New Zealand White rabbits, which have been genetically isolated from the rest of the world for approximately 32 years owing to a ban on the importation of rabbits.

Materials and Methods

Ethical clearance for this study was obtained from Stellenbosch University Animal Care and Use Committee (Protocol number SU-ACUD16-00094).

Two diets were tested, namely a control (Ctrl) and a quercetin dihydrate-supplemented (Qrc) treatment diet. The Ctrl diet was formulated to be a complete nutritionally balanced rabbit grower feed (Table 1). The Qrc diet was produced from this Ctrl diet through the addition of 2 g/kg quercetin dihydrate (*Sophorae japonica* flower extract, from Chengdu Okay Plant and Chemical Co., Ltd, Qionglai, China). The quercetin dihydrate was added to the feed during initial mixing, prior to pelleting. Neither of the feeds contained coccidiostats, and both feeds were manufactured by Pennville (Pty) Ltd (Pretoria, South Africa).

The total flavonoid content of the two diets was determined colorimetrically. Flavonoid extracts were prepared by sonicating (Power Sonic 405, United Scientific, Cape Town, South Africa) 0.4 g of feed with 10 ml 1% formic acid in 50% methanol for two 15-minute periods, with a 10-min break. The extracts were then centrifuged (Sigma 2-16 K, Wirsam Scientific, Cape Town, South Africa) at 2000 × *g* for 10 minutes, and the supernatant was filtered using a PES (prefilter) 0.22 µm, 33 mm syringe filter (Agela Technologies, Wilmington, USA). The extract was stored at -20 °C until analysis. The colorimetric assay was performed as described by Herald *et al*. (2012), with adaptations. Briefly, 100 µL distilled water, 10 µL 5% sodium nitrite and 10 µL extract or standard were combined in a 2 mL microcentrifuge tube and vortexed. After incubating at room temperature for 5 minutes, 15 µL 10% aluminium chloride was added, and the tube was vortexed and incubated at room temperature for a further 6 minutes. Thereafter, 50 µL 1 M sodium hydroxide and 50 µL distilled water were added, and the tubes were vortexed, prior to centrifuging at 3220 × g for 5 minutes. A 200 µL aliquot of the supernatant was transferred to a clear 96 well microplate (Greiner Cellstar 96-well flat-bottom plate, Sigma-Aldrich, St Louis, USA) and the absorbance at 510 nm was measured (Spectrostar Nano, BMG Labtech, Ortenberg, Germany). The total flavonoid content was determined relative

to a quercetin (Sigma-Aldrich, Steinheim, Germany) standard (25–500 µg/mL; y = 0.0007x + 0.0569; R^2 = 0.997) and is expressed as milligrams quercetin equivalents per gram dry matter.

Table 1 Ingredients and chemical composition of the rabbit grower control (Ctrl) diet and the total flavonoid content of the Ctrl diet and the diet supplemented with 2 g quercetin dihydrate/kg feed (Qrc)

Ingredients	**g/kg**
Alfalfa	362
Wheat bran	356
Sunflower meal	126
Wheat	50.0
Molasses	50.0
Soya hulls	31.0
Soybean oil	11.0
Vitamin and mineral premix*	5.00
Limestone	3.70
Mono dicalcium phosphate 21%	2.10
L-lysine	1.40
Salt	1.00
DL-methionine	0.80
Chemical composition	g/kg
Dry matter	883
Crude protein	185
Ether extract	37.1
Ash	83.1
Crude fibre	190
Neutral detergent fibre (NDF)	317
Acid detergent fibre (ADF)	177
Acid detergent lignin (ADL)	61.3
Total flavonoid content	(mg Qrc eq/g DM)
Ctrl feed	5.03
, Qrc feed	5.25

*Vitamins and minerals provided per kg of diet: 17 500 IU vitamin A, 7 500 IU vitamin D_3, 583 mg choline, 250 mg vitamin C, 208 mg antioxidant, 200 mg vitamin B_5, 183 mg manganese, 167 mg zinc, 100 mg vitamin E, 83 mg niacin, 67 mg iron, 17 mg copper, 8.3 mg vitamin B_2, 4.8 mg vitamin B_6, 3.3 mg vitamin K_3, 3.3 mg vitamin B_1, 3.3 mg folic acid, 3.3 mg iodine, 0.75 mg cobalt, 0.5 mg selenium, 0.15 mg biotin, 0.015 mg vitamin B_{12}
DM: dry matter; Qrc eq: quercetin equivalents

The growth trial was carried out at Mariendahl Experimental Farm outside Stellenbosch in the Western Cape, South Africa (33°51'02.9"S 18°49'35.2"E) from August to October 2017, using 66 purebred New Zealand White rabbits. The 31 male and 35 female rabbits were assigned to the two dietary treatment groups according to live weight and litter, such that the litters were split between the treatments and each treatment had a similar average starting weight (Ctrl males 1022 ± 32 g, Ctrl females 1074 ± 24 g, Qrc males 1069 ± 25 g, Qrc females 1042 ± 21 g). The sexes were distributed more or less equally between the dietary treatments (16 Ctrl males, 18 Ctrl females, 15 Qrc males, 17 Qrc females).

At weaning at five weeks old the rabbits were transferred from doe cages in the breeder house to individual grower cages in two grower rooms. To take into account any possible environmental differences between the two grower rooms, the dietary treatments and sexes were distributed evenly in and between the

two rooms. The grower cages were constructed from welded wire mesh and were 60 x 60 cm and 50 cm high, with the floors of the cages 1.5 m off the ground. Wood shavings were spread below the cages to absorb urine and were changed whenever necessary.

To reduce the risk of digestive disturbances, the rabbits were provided with a commercial multi-strain powdered probiotic product (Protexin Soluble, Kyron Laboratories Pty Ltd., Johannesburg, South Africa), which was sprinkled on their feed (3 g/kg) from one week prior to weaning until one week after weaning. The experimental feeds were provided ad libitum from the day of weaning until slaughter at 12 weeks old, and fresh water was freely available at all times via automatic water lines supplemented with bottle-based nipple drinkers. Daily feed intake (FI) was determined weekly for each rabbit/cage by assigning a set weight of feed to the cage at the beginning of the week and weighing remaining, refused or spilled feed at the end of the week. All rabbits were weighed individually at the same time each week to determine live weight (LW) and calculate average daily gain (ADG). Feed was not withheld prior to weighing, and LW values thus included the weight of the full gastrointestinal tract. Daily FI and ADG data were used to calculate the (FCR) each week.

A 12L : 12D light regime was used throughout the trial, and artificial ventilation was provided via fans in room A and an air-conditioning system in room B. Temperature and relative humidity were recorded in the two rooms throughout the trial using automatic temperature loggers (LogTag humidity and temperature recorder, Model HAX0-8), with average recorded temperature and humidity in rooms A and B during the growth period being 14.9 ± 0.21 ºC, 72.3 ± 0.96% and 16.4 ± 0.14 ºC, 63.4 ± 0.73%, respectively.

At 11 weeks old 18 rabbits from each dietary treatment (Ctrl and Qrc), consisting of both sexes and distributed throughout the two grower rooms, were selected for blood sampling (8 Ctrl males, 10 Ctrl females, 9 Qrc males, 9 Qrc females). Blood (*ca*. 5 mL per rabbit) was collected by a trained animal technician from the central ear artery, using Healthcare™ IV Cannula 24 G needles with catheters (Harsoria Healthcare Pvt. Ltd., Haryana, India) and sterile 5 mL syringes (Avacare Health, Johannesburg, South Africa). After withdrawal, the blood was transferred to BD Vacutainer® SST™ II Advance Plus blood collection tubes (Becton Dickinson & Company, New Jersey, USA) for serum separation. To reduce stress and improve the ease of blood withdrawal *ca*. 200 µL Neurotranq (Virbac RSA (Pty) Ltd, Gauteng, South Africa) was injected into the caudal ear artery using an insulin syringe (Becton Dickinson & Company, New Jersey, USA) prior to blood withdrawal. A Techniplast rabbit restrainer (Labotec, Cape Town, South Africa) was used during the procedure.

The collected blood was allowed to clot at room temperature for at least 30 minutes, before centrifuging at 1000 × *g* for 15 minutes and transferring the separated serum into 2 mL graduated microtubes (Scientific Specialities Inc., California, USA). The serum was stored at -20 ºC until further analysis.

The free thyroxine (fT4), free triiodothyronine (fT3) and somatotropin (GH) concentrations of the serum samples were determined in duplicate using commercial ELISA (enzyme-linked immunosorbent assay) kits from Elabscience (fT4-E-EL-RB2049, fT3-E-EL-RB0919 and GH-E-EL-RB2030) purchased from Biocom Africa (Pty) Ltd (Centurion, South Africa).

The standard curves used for quantification all had correlation coefficient values above 99.7%.

The cortisol concentration of the samples was determined using ultra-performance convergence chromatography tandem mass spectrometry (UPC^2-MS/MS), as described by Quanson *et al*. (2016) and Needham *et al*. (2018). Briefly, 500 µL serum was combined with 50 µL 0.3 ng/µL cortisol D-4internal standard (Cambridge Isotope Laboratories, Andover, MA, USA) in glass tubes, 1.5 mL *tert*-methyl butyl ether (MTBE, Sigma-Aldrich, Steinheim, Germany) was added, and the tubes were shaken at 1000 RPM for 10 minutes. After shaking, the tubes were frozen at −80 ºC for 1 hour to remove the aqueous phase and the organic phase was transferred to clean glass tubes. The MTBE was evaporated at 55 ºC under a flow of nitrogen gas before re-suspending in 150 µL 50% methanol (ROMIL, Cambridge, England) and transferring into HPLC vials. The extract was frozen at −20 ºC until analysis.

Chromatographic separation and identification of the extracted compounds was performed as described by Quanson *et al*. (2016), using an Acquity UPC^2 system with an Acquity UPC^2 BEH 2-EP (3 mm x 100 mm, 1.7 um) column (Waters Corporation, USA). A cortisol external standard curve ($y = 0.04x + 0.007$; $R^2 = 0.996$) with concentrations from 0.05 to 250 ng/mL was used for the quantification of the cortisol content.

The trial used a randomized block experimental design to take into account any environmental differences between the two grower rooms, and the main effects of interest – namely diet, sex and age – were combined in a three-factor factorial structure. Because the rabbits were housed individually, each rabbit served as an experimental unit.

The data were analysed using Statistica version 13 software, with normality being tested with normal probability plots and homoscedasticity assessed with Levene's test. The variance, estimation, precision and comparison (VEPAC) mixed-model function was used with the restricted maximum likelihood (REML)

estimation method to determine the significance of the blocks, main effects and second- and third-order interactions for the live performance data. Weaning weight was included as a covariate. In addition to the basic analysis, simple linear regression lines were fitted for each diet-sex treatment group for the live performance data, and the intercept and slope parameters were compared across the diet-sex treatment groups to determine whether significant differences were present.

The R lm package was used to test the significance of the blocks, main effects and interactions for the hormone data, with LW at 11 weeks as a covariate. Pearson's and Spearman's correlation coefficients were calculated for the relationships between the concentrations of the hormones and the daily FI, ADG and FCR at 11 weeks old. The LSD post hoc test was used to compare the individual values of the treatment groups if second- and third-order interactions were found to be significant, for both the live performance and hormone data.

Main effects and interactions with $P \leq 0.05$ were considered significant, whereas those with $P \leq 0.10$ were reported as trends. Values are reported as the LS mean ± standard error of the mean (SEM).

Results

The statistical analysis of the data found that the block effect was relatively limited, with only a single variable, the serum-free T3 concentration ($P = 0.01$), differing between the blocks.

The rabbits increased ($P < 0.001$) in weight almost linearly during the growth period, from 1052 ± 13.4 g at weaning at 5 weeks to 3192 ± 45.3 g at 12 weeks (Figure 1). There was no significant difference in LW between the diet-sex treatment groups at any point, although the variation within and between the groups increased with age. The similarity of growth between the treatment groups is demonstrated by the regression parameters in Table 2.

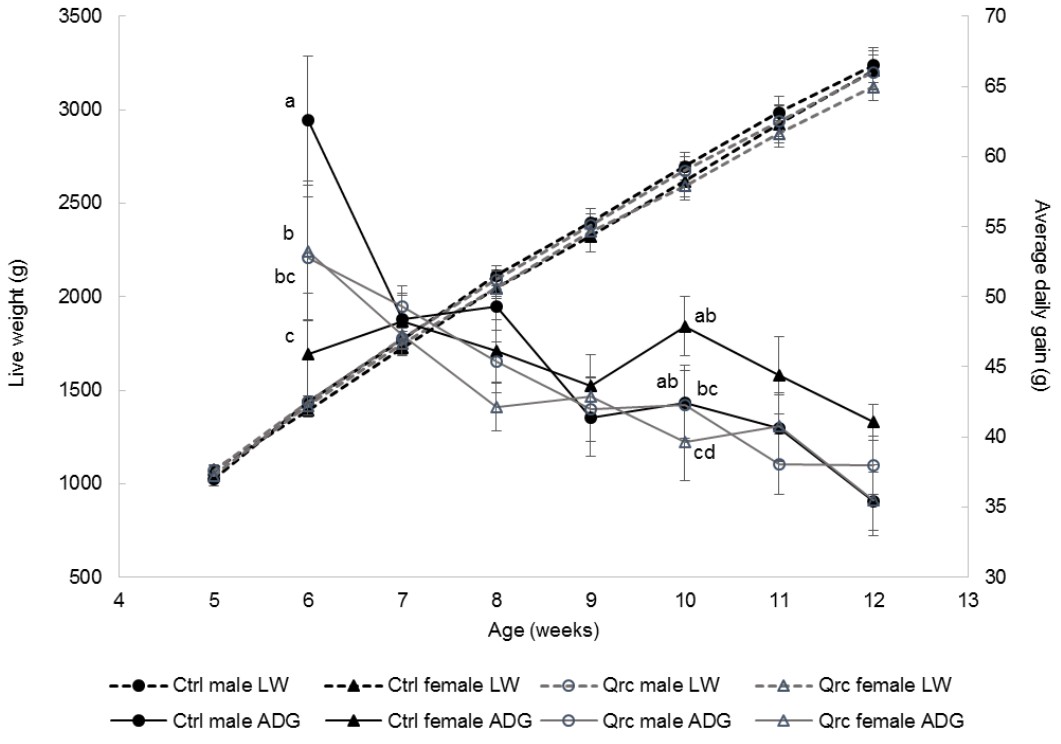

Figure 1 Live weight (LW) and average daily gain (ADG) from 5 to 12 weeks old of male and female New Zealand White rabbits fed diets with (Qrc) quercetin dihydrate supplemented at 2 g/kg or without (Ctrl)
For ease of interpretation, significant differences ($P \leq 0.05$) between the diet-sex treatment groups are shown weekly, as indicated by the significance letters adjacent to the data points. Error bars show the standard errors of the means

Average daily gain decreased ($P < 0.001$) over time, and showed far more variation from week to week and between the treatment groups than LW, as demonstrated by the diet-sex-week third-order interaction ($P = 0.001$). To simplify the interpretation of Figure 1, significance has been indicated between the treatment groups only within each time point, as seen at week 6 and 10. The ADG during the first week post-weaning was the most variable, with male Ctrl rabbits growing fastest, female Ctrl rabbits slowest, and Qrc rabbits

having similar, intermediate gains. Thereafter, ADG stabilized across the treatment groups and remained relatively similar until 10 weeks old, at which point Ctrl females were growing fastest, Qrc females slowest and the males were intermediate. In terms of the general pattern of ADG, it appeared that the Qrc groups showed less sex-related variation over the growth period, with the latter also being demonstrated by the similar slopes and intercepts of the regression lines for the male and female rabbits in the Qrc group (Table 2). While the very low R^2 values must be taken into account when interpreting these regression parameters, the poor fit is caused by generally high variation in the data rather than by a non-linear relationship; therefore the parameters probably still have some merit.

Table 2 Intercepts, slopes and coefficients of determination (R^2) of linear regression lines fitted to live weight, average daily gain, voluntary feed intake and feed conversion ratio data for male and female New Zealand White rabbits fed a diet supplemented with quercetin dihydrate (Qrc) (2 g/kg), or without (Ctrl), from 5 to 12 weeks old

		Control (Ctrl)		Quercetin (Qrc)	
		Male	**Female**	**Male**	**Female**
Live weight	N	128	144	120	136
	Intercept	-473.5	-410.4	-367.5	-354.5
	Slope	313.5	304.2	302.8	293.7
	R^2	0.94	0.91	0.95	0.95
Average daily gain	N	108	124	105	117
	Intercept	80.00^{a}	51.51^{c}	65.97^{ab}	65.75^{b}
	Slope	-3.73^{ab}	-0.74^{a}	-2.49^{b}	-2.51^{b}
	R^2	0.56	0.14	0.46	0.42
Voluntary feed intake	N	108	124	105	117
	Intercept	70.56	62.25	69.01	75.43
	Slope	10.53	11.15	10.69	9.26
	R^2	0.58	0.67	0.63	0.65
Feed conversion ratio	N	108	124	105	117
	Intercept	-0.72^{b}	1.53^{a}	0.01^{ab}	0.06^{ab}
	Slope	0.51^{a}	0.25^{b}	0.44^{a}	0.44^{a}
	R^2	0.81	0.46	0.77	0.55

^{abc} Means in the same row with different superscript letters differ significantly ($P \leq 0.05$)

Voluntary feed intake (VFI) and FCR both increased ($P < 0.001$) with age (Figure 2). Similarly to the LW, there was no difference between the groups in VFI, or the changes in VFI, over the growth period (Table 2). However, there was a tendency for a third-order interaction for the FCR ($P = 0.08$), and the LSD test found that the treatment groups differed significantly at 6, 10 and 12 weeks old. The variation at 6 weeks was probably simply a reflection of the differences in ADG, as seen in Figure 1, with the high ADG of the Ctrl males resulting in a low FCR, and vice versa for the Ctrl females. This was also the case at 10 weeks old. At 12 weeks, the continued high ADG of the Ctrl females resulted in a lower FCR for this group, while the Ctrl males and Qrc groups retained similar higher FCR values. As was observed for the ADG, the sex-effect on the FCR over the growth period seemed less for the Qrc than for the Ctrl groups (Figure 2, Table 2).

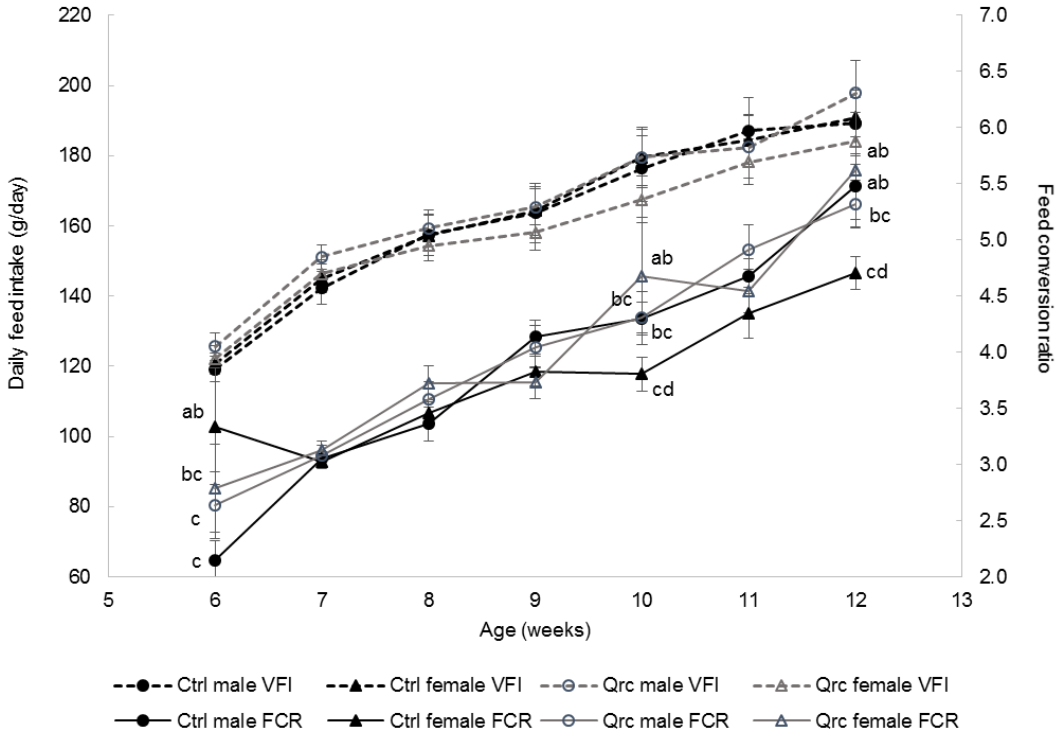

Figure 2 Daily voluntary feed intake (VFI) and feed conversion ratio (FCR) from 6 to 12 weeks old of male and female New Zealand White rabbits fed diets supplemented at 2 g/kg with quercetin dehydrate (Qrc) or without (Ctrl)

For ease of interpretation, significant differences ($P \leq 0.05$) between the diet-sex treatment groups are shown weekly, as indicated by the significance letters adjacent to the data points. Error bars show the standard errors of the means

There were no significant diet-sex interactions for the hormone data and the two main effects were consequently examined individually (Table 3). Only fT3 levels showed any sign of a dietary influence (P = 0.06), with the Qrc rabbits having higher fT3 concentrations in the serum than the Ctrl rabbits. The cortisol concentration showed a large sex-effect (P <0.01), with female rabbits having much higher serum levels than males. None of the hormones correlated significantly with any of the 11 week live-performance data (Table 4). However, the Pearson's correlation coefficient between the VFI and serum cortisol concentration tended towards significance (P = 0.08), suggesting a negative relationship between cortisol and feed intake.

Table 3 Concentrations (ng/mL) of free triiodothyronine, free thyroxine, somatotropin and cortisol in serum from 11-week-old male and female New Zealand White rabbits fed diets supplemented at 2 g/kg with quercetin dihydrate (Qrc) or without (Ctrl)

	Overall	Diet		Sex	
		Ctrl	Qrc	Male	Female
N	36	18	18	17	19
Free triiodothyronine	4.31 ± 0.375	3.62^{β} ± 0.420	4.98^{α} ± 0.599	4.31 ± 0.482	4.29 ± 0.577
Free thyroxine	6.72 ± 0.542	7.29 ± 0.769	6.19 ± 0.765	6.59 ± 0.709	6.88 ± 0.824
Somatotropin	1.45 ± 0.107	1.35 ± 0.147	1.56 ± 0.155	1.57 ± 0.160	1.34 ± 0.143
Cortisol	7.55 ± 1.03	6.13 ± 1.03	8.66 ± 1.76	3.89^{b} ± 0.53	10.90^{a} ± 1.55

[ab] Main effect means in the same row with different superscript letters differ significantly ($P \leq 0.05$)
[αβ] Main effect means in the same row with different capitalised superscript letters tend to differ ($P \leq 0.10$)

Table 4 Relationships between serum hormone concentrations and live performance parameters of 11-week-old male and female New Zealand White rabbits fed diets supplemented at 2 g/kg of quercetin dihydrate, or without, as indicated by Pearson's and Spearman's correlation coefficients

		Free triiodothyronine		Free thyroxine		Somatotropin		Cortisol	
		R	***P*-value**	**R**	***P*-value**	**R**	***P*-value**	**R**	***P*-value**
ADG	Pearson	-0.19	0.26	-0.10	0.56	0.04	0.81	-0.25	0.14
	Spearman	-0.19	0.26	-0.15	0.38	0.04	0.80	-0.06	0.72
FI	Pearson	-0.12	0.48	-0.08	0.63	-0.11	0.54	-0.29	0.08
	Spearman	-0.05	0.76	-0.11	0.52	-0.16	0.36	-0.16	0.36
FCR	Pearson	0.14	0.43	0.03	0.86	-0.15	0.38	0.02	0.91
	Spearman	0.20	0.23	0.02	0.90	-0.16	0.35	-0.11	0.53

ADG: average daily gain, FI: daily feed intake, FCR: feed conversion ratio, R: correlation coefficient

Discussion

The initial comparison of the growth data with values reported for New Zealand Whites in the literature suggests that the rabbits in this study performed well in terms of LW and ADG (Abdel-Samee, 1997; Anous, 1999; Marai *et al*., 1999; Ondruska *et al*., 2011; Nasr *et al*., 2017). However, their daily FI was similarly high, resulting in FCR values that were comparable with some literature (Marai *et al*., 1999; Ondruska *et al*., 2011) but much poorer than those reported for commercial European farms, by Maertens & Gidenne (2016). This may have been due to their wide use of hybrid-line rabbits rather than New Zealand Whites. However, the high growth rate and relatively high FCR may have been due to the fairly low temperatures during the growth period, as winter conditions improve growth, but increase FCR (Maertens & Gidenne, 2016). They may also have been linked to the individual housing conditions, as this has been found to increase growth and feed intake (Szendrő & Dalle Zotte, 2011). While the effects of feed composition on the FCR cannot be discounted, a comparison of the nutritional value of the feed used in this study (Table 1) with optimum acid detergent fibre (ADF) (16% - 17%), neutral detergent fibre (NDF) (31% - 33%), and acid detergent lignin (ADL) (>5%) values, and normally used total lipid contents (2% - 4%) seems to suggest that this was not a major contributing factor (Maertens & Gidenne, 2016). Although the ADF level (17.7%) was slightly above the recommended optimum level, the NDF level was within range (31.7%) and the lipid content was towards the higher end of the normal range (3.71%). However, it would be interesting to determine the nutrient digestibility of the feeds.

The patterns of change in the ADG and FCR were as expected, with the growth rate declining and the FCR values increasing as the rabbits matured, the effect on FCR being due to the increase in maintenance requirements and the shift towards fat deposition with age (Pascual *et al*., 2008; Maertens & Gidenne, 2016).

As the 6-week ADG represented the growth rate during the first week post-weaning, the significant differences between the diet-sex treatment groups at this age may have been due to variation between the groups in their response to the stress of the weaning process and adaptation to the new housing conditions. It is uncertain why the Ctrl males and females responded so differently to this. While a new relationship between the treatment groups had been established by 10 weeks old, with Ctrl females outperforming the other groups, there were no further differences in ADG up until slaughter. As one could expect, the FCR values followed a similar pattern to those of the ADG (Figure 2), with Ctrl females having the highest values for week 6, but the lowest for weeks 10 and 12.

Unlike the other growth parameters, FCR showed a tendency towards an effect of diet, with Qrc rabbits having a higher overall FCR than the Ctrl rabbits. This is detrimental from a production perspective, and appeared to be owing to a slightly higher ADG in the Ctrl than Qrc rabbits rather than any difference in VFI. Goliomytis *et al.* (2014) reported a similar negative effect of quercetin supplementation on the FCR of broiler chickens, and attributed this to nutrient dilution by the increasing quercetin content of the diet. Although this seems unlikely, since that their maximum inclusion level was only 1 g/kg feed, it is possible that it provides an explanation for the results of this study, particularly since a higher inclusion rate was used (2 g/kg). It is also possible that the effect on the FCR was because of a lower digestibility of the Qrc than Ctrl feed, owing to alterations of the composition of the caecal microbial population by the flavonoid. Quercetin is known to exhibit antimicrobial activity, and flavonoids have been found to influence the gut microbiome in human studies (Cushnie & Lamb, 2005; Tzounis *et al*., 2008). Alternatively, flavonoids have been found to reduce the *in vitro* digestibility of proteins and starch in bread through the formation of indigestible complexes

with the nutrients, and by inhibiting the activity of several digestive enzymes (Rohn *et al*., 2002; Świeca *et al*., 2013).

The apparent reduction of the effect of sex on ADG and FCR by the supplementation of quercetin is unusual, and does not seem to have been reported for quercetin or any other flavonoid in previous livestock trials. However, there is evidence that some flavonoids act as xenoestrogens, interacting with the sex hormone system by binding with oestrogen receptors and human plasma sex-hormone binding globulin (hSHBG), which is involved in the transport of steroid hormones in the body (Kuiper *et al*., 1998; Déchaud *et al*., 1999; Erlund, 2004). Flavonoids have also been found to influence the activity of some enzymes that are involved in oestrogen, androgen and progestin metabolism (Narayana *et al*., 2001). Naringenin, in particular, has been reported to exhibit some anti-oestrogenic activity (Ruh *et al*., 1995; Kuiper *et al*., 1998; Déchaud *et al*., 1999; Erlund, 2004). This may provide some explanation for the interaction observed in this trial. In a study on heat-stressed rabbit does, Naseer *et al*. (2017) found that the supplementation of quercetin had a positive effect on the number of ovarian follicles, and the quality of oocytes and granulosa cell apoptosis. However, this was ascribed to the antioxidant effects of the quercetin rather than to a direct effect on the sex-hormone system. Considerable further research is necessary not only to confirm the smaller sex differences in Qrc than Ctrl rabbits found in this study, but also to investigate the possible mechanism of action.

Somatotropin plays an important part in the endocrine regulation of growth, stimulating the production of somatomedins, which act as hypertrophic and hyperplastic agents, thereby increasing tissue proliferation (Spencer, 1985). However, somatotropin exhibits catabolic behaviour, which can complicate the relationship between its serum levels and growth rates. This may explain the lack of effect of dietary treatment or sex on somatotropin levels, as well as the lack of correlation with the ADG.

In contrast to somatotropin, cortisol plays a purely catabolic role in the body, decreasing overall DNA synthesis, suppressing muscle protein synthesis, increasing muscle degradation rates and generally inhibiting growth (Spencer, 1985). In addition, the corticotropin-releasing factor system, which forms part of the hormonal stress response and ultimately stimulates the synthesis of cortisol, inhibits feed intake (Shibasaki *et al*., 1988; Richard *et al*., 2002). This concurs with the negative Pearson's correlation coefficient found for the relationship between cortisol levels and daily FI (Table 4), which suggested that intake tended to be lower when the serum cortisol level was higher.

The interpretation of serum cortisol levels is always complicated by the natural circadian variation in the secretion of this hormone (Möstl & Palme, 2002). Nonetheless, Gunn *et al*. (2016) found that women had consistently higher cortisol levels than men, throughout the circadian cycle, which supports the current findings that female rabbits had higher cortisol levels than males (Table 3). While this difference does not seem to have been reported in rabbits, female rats have been found to have higher baseline corticosterone levels and a number of studies have demonstrated differences in the stress response linked to sex in other species (Kirschbaum *et al*.,1992; Tilbrook *et al*., 2000; Green & McCormick, 2016). The sex-effect on the hypothalamo-pituitary-adrenal (HPA) axis has been linked to circulating androgen and oestrogen levels, with the former decreasing and the latter increasing the HPA axis function (Handa *et al*., 1994; Green & McCormick, 2016). It is interesting to note that this sex effect was already present at 11 weeks old in the rabbits in this study.

The only effect of diet on the hormone level was a tendency for Qrc rabbits to have higher concentrations of fT3 than Ctrl rabbits (Table 3). While this precise effect does not seem to have been found previously in rabbits, the effects of flavonoids, including quercetin, on thyroid hormone metabolism are widely reported (Narayana *et al*., 2001). This effect is generally considered anti-thyroid, with flavonoids inhibiting iodothyronine deiodinase isozymes, iodide organification and thyroid peroxidase enzyme activity, as well as down-regulating the expression of several genes involved in thyroid function, which makes the higher fT3 in Qrc than Ctrl rabbits seem counterintuitive (Formica & Regelson, 1995; Ferreira *et al*., 2002; Giuliani *et al*., 2014). However, flavonoids have been found to inhibit the binding of T3 and T4 to serum transthyretin, resulting in higher proportions of fT3 and fT4, which could provide an explanation for the findings of this study (Kohrle *et al*., 1989; Lueprasitsakul *et al*., 1990; Radović *et al*., 2006). While the difference in serum fT3 levels did not appear to cause significant differences in growth, it is possible that it may have been linked in some way to the higher FCR and smaller sex differences found for Qrc than Ctrl rabbits. Considering the important role that thyroid hormones play in both growth and feed intake, and the complex and dose-dependent catabolic and anabolic effects of these hormones (Spencer, 1985), further investigation of the total and bound levels of T3 and T4 may be warranted.

Although this study produced interesting results, it was limited in several ways. It would have been preferable, for statistical purposes, to have a larger number of replications for both the growth parameters and hormone levels. In addition, it would have been valuable for a range of inclusion levels of quercetin to be tested, to determine the dose-response of this flavonoid *in vivo*. Furthermore, determining the levels of other hormones, such as somatomedins and insulin, could have provided a clearer picture of the physiological

effects of the quercetin supplementation, as could the determination of the concentrations of total and bound T3 and T4.

Conclusion

From a production perspective, the conclusions of this study are extremely limited. While it is heartening to see the South African New Zealand Whites performing well in terms of growth, improved selection methods and the development of hybrid lines will be necessary to reach international standards of feed efficiency. In addition, the quercetin supplementation did not appear to have any immediately beneficial effects on production performance, and in fact had a detrimental effect on the FCR, possibly by lowering the digestibility of the feed. It therefore does not seem that the provision of quercetin is justified from a commercial perspective, at live performance level at least. Further research is necessary to identify possible causes for the effects found in this study, and to determine whether quercetin supplementation has any effect on nutrient digestibility *in vivo*.

However, some results may suggest interesting avenues for further investigation. Considering the possible diet effect on sex differences in growth and feed efficiency, it could be of interest to look at the influence of quercetin on the sex-hormone levels in breeding stock. Further work on the interaction of quercetin with thyroid hormone metabolism in rabbits may also be of interest.

In addition, this study has provided some evidence that previously reported sex-differences in the hypothalamo-pituitary-adrenal axis also exist in growing rabbits.

Acknowledgements

The authors would like to thank N. Markgraaff for performing the blood withdrawal necessary for this trial, and M. Kidd from the Centre for Statistical Consultation at Stellenbosch University for performing the statistical analysis of the data. The sampling and laboratory assistance provided by the staff and students of the Animal, Food Science and Biochemistry Departments of Stellenbosch University WERE also invaluable. This work is based on research supported by the South African Research Chairs Initiative of the Department of Science and Technology and the National Research Foundation of South Africa (UID: 84633). Any opinion, finding and conclusion or recommendation expressed in this material is that of the author(s) and the National Research Foundation does not accept any liability in this regard.

Authors' Contributions

All authors participated in the planning the experiments and writing and reviewing the manuscript. MKN was responsible for running the trial, performing laboratory analysis and data processing and interpretation.

Conflict of Interest Declaration

There are no conflicts of interest.

References

Abdel-Samee, A.M., 1997. Response of New Zealand White rabbits to thermal stress and its amelioration during winter and summer of North Sinai, Egypt. J. Arid Environ. 36, 333-342.

Abu, O.A., Onifade, A.A., Abanikannda, O.T.F. & Obiyan, R.I., 2008. Status and promotional strategies for rabbit production in Nigeria. In: Proceedings of the 9th World Rabbit Congress,10-13 June 2008, Verona, Italy, pp. 1499-1504.

Anous, M.R., 1999. Growth performance, slaughter and carcass compositional traits in rabbits of a local strain and New Zealand White breed raised in Burundi. Wrld Rabbit Sci. 7, 139-143.

Babosová, R., Omelka, R., Ďúranová, H., Kováčová, V., Lukáčová, M., Capcarová, M. & Martiniaková, M., 2016. Quercetin-induced changes in femoral bone microstructure of adult male rabbits. Potr. S. J. F. Sci. 10, 300-307.

Blouin, F.A., Zarins, Z.M. & Cherry, J.P., 1981. Role of flavonoids in the production of color in biscuits prepared with wheat and cottonseed flours. J. Food Sci. 46, 266-271.

Cartea, M.E., Francisco, M., Soengas, P. & Velasco, P., 2011. Phenolic compounds in Brassica vegetables. Molecules 16, 251-280.

Cushnie, T.P.T. & Lamb, A.J., 2005. Antimicrobial activity of flavonoids. Int. J. Antimicrob. Agents 26, 343-356.

Dakora, F.D., 1995. Plant flavonoids: Biological molecules for useful exploitation. Aust. J. Plant Physiol. 22, 87-99.

Dalle Zotte, A. & Szendrő, Zs., 2011. The role of rabbit meat as functional food. Meat Sci. 88, 319-331.

Déchaud, H., Ravard, C., Claustrat, F., de la Perrière, A.B. & Pugeat, M., 1999. Xenoestrogen interaction with human sex hormone-binding globulin (hSHBG). Steroids 64, 328-334.

Dos Santos, M.C.S., Gonçalves, C.F.L., Vaisman, M., Ferreira, A.C.F. & de Carvalho, D.P., 2011. Impact of flavonoids on thyroid function. Food Chem. Toxicol. 49, 2495-2502.

Erlund, I., 2004. Review of the flavonoids quercetin, hesperetin, and naringenin. Dietary sources, bioactivities, bioavailability, and epidemiology. Nutr. Res. 24, 851-874.

European Commission, 2005. Ban on antibiotics as growth promoters in animal feed enters into effect. European Commission, Brussels, Belgium. Available at: http://europa.eu/rapid/press-release_IP-05-1687_en.htm.

Ferreira, A.C.F., Lisboa, P.C., Oliveira, K.J., Lima, L.P., Barros, I.A. & Carvalho, D.P., 2002. Inhibition of thyroid type 1 deiodinase activity by flavonoids. Food Chem. Toxicol. 40, 913-917.

Finzi, A., 2000. Raising rabbits for food security. In: Proceedings of the 7th World Rabbit Congress, 4-7 July 2000, Valencia, Spain, volume B. pp. 13-38.
Food and Drug Administration, Centre for Veterinary Medicine, 2013. Guidance for Industry #213: New animal drugs and new animal drug combination products administered in or on medicated feed or drinking water of food-producing animals: Recommendations for drug sponsors for voluntarily aligning product use conditions with GFI #209. US Department of Health and Human Services, Washington, DC, USA.
Formica, J.V. & Regelson, W., 1995. Review of the biology of quercetin and related bioflavonoids. Food Chem. Toxicol. 33, 1061-1080.
Giuliani, C., Bucci, I., Di Santo, S., Rossi, C., Grassadonia, A., Piantelli, M., Monaco, F. & Napolitano, G., 2014. The flavonoid quercetin inhibits thyroid-restricted genes expression and thyroid function. Food Chem. Toxicol. 66, 23-29.
Goliomytis, M., Tsoureki, D., Simitzis, P.E., Charismiadou, M.A., Hager-Theodorides, A.L. & Deligeorgis, S.G., 2014. The effects of quercetin dietary supplementation on broiler growth performance, meat quality, and oxidative stability. Poult. Sci. 93, 1957-1962.
Green, M.R. & McCormick, C.M., 2016. Sex and stress steroids in adolescence: Gonadal regulation of the hypothalamic-pituitary-adrenal axis in the rat. Gen. Comp. Endocrinol. 234, 110-116.
Gunn, P.J., Middleton, B., Davies, S.K., Revell, V.L. & Skene, D.J., 2016. Sex differences in the circadian profiles of melatonin and cortisol in plasma and urine matrices under constant routine conditions. Chronobiol. Int. 33, 39-50.
Handa, R.J., Burgess, L.H., Kerr, J.E. & O'Keefe, J.A., 1994. Gonadal steroid hormone receptors and sex differences in the hypothalamo-pituitary-adrenal axis. Horm. Behav. 28, 464-476.
Havsteen, B.H., 2002. The biochemistry and medical significance of the flavonoids. Pharmacol.Therapeut. 96, 67-202.
Herald, T.J., Gadgil, P. & Tilley, M., 2012. High-throughput micro plate assays for screening flavonoid content and DPPH-scavenging activity in sorghum bran and flour. J. Sci. Food Agric. 92, 2326-2331.
Johnson, R., 2010. Potential trade implications of restrictions on antimicrobial use in animal production. Congressional Research Service, Washington D.C., USA.
Karamać, M., Kosińska, A., Estrella, I., Hernández, T. & Duenas, M., 2012. Antioxidant activity of phenolic compounds identified in sunflower seeds. Eur. Food Res. Technol. 235, 221-230.
Kirschbaum, C., Wüst, S. & Hellhammer, D., 1992. Consistent sex differences in cortisol responses to psychological stress. Psychosom. Med. 54, 648-657.
Kohrle, J., Fang, S.L., Yang, Y., Irmscher, K., Hesch, R.D., Pino, S., Alex, S. & Braverman, L.E., 1989. Rapid effects of the flavonoid EMD 21388 on serum thyroid hormone binding and thyrotropin regulation in the rat. Endocrinology 125 (1), 532-537.
Kováčik, A., Packová, D. & Kolesárová, A., 2015. Impact of the T-2 toxin and quercetin on rabbit plasma levels of thyrotropin and thyroxine in vivo. J. Microbiol., Biotechnol. Food Sci. 4, 57-59.
Kuiper, G.G.J.M., Lemmen, J.G., Carlsson, B.O., Corton, J.C., Safe, S.H., Van der Saag, P.T., Van der Burg, B. & Gustafsson, J-A., 1998. Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor β. Endocrinology 139, 4252-4263.
Landers, T.F., Cohen, B., Wittum, T.E. & Larson, E.L., 2012. A review of antibiotic use in food animals: Perspective, policy, and potential. Public Health Rep. 127, 4-21.
Liu, H.N., Liu, Y., Hu, L.L., Suo, Y.L., Zhang, L., Jin, F., Feng, X.A., Teng, N. & Li, Y., 2014. Effects of dietary supplementation of quercetin on performance, egg quality, cecal microflora populations, and antioxidant status in laying hens. Poult. Sci. 93, 347-353.
Lu, Y. & Foo, L.Y., 1997. Identification and quantification of major polyphenols in apple pomace. Food Chem. 59 (2), 187-194.
Lueprasitsakul, W., Alex, S., Fang, S. L., Pino, S., Irmscher, K., Kohrle, J. & Braverman, L.E., 1990. Flavonoid administration immediately displaces thyroxine (T4) from serum transthyretin, increases serum free T4, and decreases serum thyrotropin in the rat. Endocrinology 126 (6), 2890-2895.
Maertens, L. & Gidenne, T., 2016. Feed efficiency in rabbit production: Nutritional, techno-economical and environmental aspects. In: Proceedings of the 11th World Rabbit Congress, 15-18 June 2016, Qingdao, China. pp. 337-351.
Marai, I.F.M., Ayyat, M.S., Gabr, H.A. & Abdel-Monem, U.M., 1999. Growth performance, some metabolites and carcass traits if New Zealand white broiler male rabbits as affected by heat stress and its alleviation, under Egyptian conditions. In: A. Testik & M. Baselga (eds). 2nd International Conference on Rabbit Production in Hot Climates. CIHEAM, Zaragoza. pp. 35-42.
Möstl, E. & Palme, R., 2002. Hormones as indicators of stress. Domest. Anim. Endocrinol. 23, 67-74.
Narayana, K.R., Reddy, M.S., Chaluvadi, M.R. & Krishna, D.R., 2001. Bioflavonoids classification, pharmacological, biochemical effects and therapeutic potential. Indian J. Pharmacol. 33, 2-16.
Naseer, Z., Ahmad, E., Epikmen, E.T., Uçan, U., Boyacioğlu, M. & Akosy, M., 2017. Quercetin supplemented diet improves follicular development, oocyte quality, and reduces ovarian apoptosis in rabbits during summer heat stress. Theriogenology 96, 136-141.
Nasr, M.A.F., Abd-Elhamid, T. & Hussein, M.A., 2017. Growth performance, carcass characteristics, meat quality and muscle amino-acid profile of different rabbit breeds and their crosses. Meat Sci. 134, 150-157.
Needham, T., Lambrechts, H. & Hoffman, L., 2018. Influence of Immunocastration vaccine administration interval on serum androgen concentrations and spermatogenic activity in ram lambs. Small Rumin. Res. Under Revision.
Onderci, M., Sahin, K., Sahin, N., Gursu, M.F., Doerge, D., Sarkar, F.H. & Kucuk, O., 2004. The effect of genistein supplementation on performance and antioxidant status of Japanese quail under heat stress. Arch. Anim. Nutri. 58 (6), 463-471.

Ondruska, L., Rafay, J., Okab, A.B., Ayoub, M.A., Al-Haidary, A.A., Samara, E.M., Parkanyi, V., Chrastinova, L., Jurcik, R., Massanyi, P., Lukac, N. & Supuka, P., 2011. Influence of elevated ambient temperature upon some physiological measurements of New Zealand White rabbits. Vet. Med. 56, 180-186.

Oseni, S.O., 2012. Rabbit production in low-input systems in Africa–prospects, challenges and opportunities. In: Proceedings of the 10th World Rabbit Congress – September 3-6, 2012, Sharm El-Sheikh, Egypt. pp. 719-731.

Park, S.-Y., Lee, S.Y., Yang, J.W., Lee, J.-S., Oh, S.-D., Oh, S., Lee, S.M., et al., 2016. Comparative analysis of phytochemicals and polar metabolites from colored sweet potato (*Ipomoea batatas* L.) tubers. Food Sci. Biotechnol. 25 (1), 283-291.

Pascual, M., Pla, M. & Blasco, A., 2008. Relative growth of organs, tissues and retail cuts in rabbits selected for growth rate. In: Proceedings of the 9th World Rabbit Congress, 10-13 June 2008, Verona, Italy. pp. 211-216.

Quanson, J.L., Stander, M.A., Pretorius, E., Jenkinson, C., Taylor, A.E. & Storbeck, K-H., 2016. High-throughput analysis of 19 endogenous androgenic steroids by ultra-performance convergence chromatography tandem mass spectrometry. J. Chromatogr. B, 1031, 131-138.

Radović, B., Mentrup, B. & Köhrle, J., 2006. Genistein and other soya isoflavones are potent ligands for transthyretin in serum and cerebrospinal fluid. Br. J. Nutr. 95, 1171-1176.

Reynaud, J. & Lussignol, M., 2005. The flavonoids of *Lotus corniculatus*. Lotus Newsletter 35 (1), 75-82.

Richard, D., Lin, Q. & Timofeeva, E., 2002. The corticotropin-releasing factor family of peptides and CRF receptors: Their roles in the regulation of energy balance. Eur. J. Pharmacol. 440, 189-197.

Rohn, S., Rawel, H.M. & Kroll, J., 2002. Inhibitory effects of plant phenols on the activity of selected enzymes. J. Agric. Food Chem. 50, 3566-3571.

Ruh, M.F., Zacharewski, T., Connor, K., Howell, J., Chen, I. & Safe, S., 1995. Naringenin: a weakly estrogenic bioflavonoid that exhibits antiestrogenic activity. Biochem. Pharmacol. 50, 1485-1493.

Schittko, U., Burghardt, F., Fiedler, K., Wray, V. & Proksch, P., 1999. Sequestration and distribution of flavonoids in the common blue butterfly *Polyommatus icarus* reared on *Trifolium repens*. Phytochemistry 51, 609-614.

Serratosa, J., Blass, A., Rigau, B., Mongrell, B., Rigau, T., Tortadès, M., Tolosa, E., et al., 2006. Residues from veterinary medicinal products, growth promoters and performance enhancers in food-producing animals: a European Union perspective. Rev. Sci. Tech. Off. Int. Epiz. 25 (2), 637-653.

Shibasaki, T., Yamauchi, N., Kato, Y., Masuda, A., Imaki, T., Hotta, M., Demura, H., Oono, H., Ling, N. & Shizume, K., 1988. Involvement of corticotropin-releasing factor in restraint stress-induced anorexia and reversion of the anorexia by somatostatin in the rat. Life Sci. 43, 1103-1110.

Spencer, G.S.G., 1985. Hormonal systems regulating growth. A review. Livest. Prod. Sci. 12, 31-46.

Świeca, M., Gawlik-Dziki, U., Dziki, D., Baraniak, B. & Czyż, J., 2013. The influence of protein-flavonoid interactions on protein digestibility in vitro and the antioxidant quality of breads enriched with onion skin. Food Chem. 141, 451-458.

Szendrő, Zs. & Dalle Zotte, A., 2011. Effect of housing conditions on production and behaviour of growing meat rabbits: A review. Livest. Sci. 137, 296-303.

Tapas, A.R., Sakarkar, D.M. & Kakde, R.B., 2008. Flavonoids as neutraceuticals: A review. Trop. J. Pharm. Res. 7, 1089-1099.

Thill, J., Regos, I., Farag, M.A., Ahmad, A.F., Kusek, J., Castro, A., Schlangen, K., et al., 2012. Polyphenol metabolism provides a screening tool for beneficial effects of *Onobrychis viciifolia* (sainfoin). Phytochemistry 82, 67-80.

Tilbrook, A.J., Turner, A.I. & Clarke, I.J., 2000. Effects of stress on reproduction in non-rodent mammals: The role of glucocorticoids and sex differences. Rev. Reprod. 5, 105-113.

Tuzcu, M., Sahin, N., Karatepe, M., Cikim, G., Kilinc, U. & Sahin, K., 2008. Epigallocatechin-3-gallate supplementation can improve antioxidant status in stressed quail. Br. Poult. Sci. 49 (5), 643-648.

Tzounis, X., Vulevic, J., Kuhnle, G.G.C., George, T., Leonczak, J., Gibson, G.R., Kwik-Uribe, C. & Spencer, J.P.E., 2008. Flavanol monomer-induced changes to the human faecal microflora. Br. J. Nutr. 99, 782-92.

Verbeke, W.A.J. & Viaene, J., 2000. Ethical challenges for livestock production: Meeting consumer concerns about meat safety and animal welfare. J. Agric. Environ. Ethics 12, 141-151.