

# Effect of long-term oral administration of the flavonoid quercetin on the antioxidant profile of young and adult reproductive rabbit does

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## Abstract

In industrialized farms, rabbit does undergo intensive production rhythms which overlap lactation and gestation, leading to a high energy mobilization and increasing oxidative stress. Accordingly, we hypothesize that administration of the flavonoid quercetin (QR) may improve the antioxidant status of young and adult rabbit reproductive females. In this study, the effect of daily oral administration of 300 mg/kg QR for 8 weeks was assessed on the antioxidant profile of 24 New Zealand × Californian rabbit does, assigned to 4 experimental groups: rearing young (8–16 weeks old) and adult does at the end of their reproductive life (12–14 months old, with at least 3–4 reproductive cycles) treated (YQ and AQ) or not (YC and AC) with QR, respectively. Plasma glutathione (GSH), as well as serum superoxide dismutase (SOD) and malondialdehyde (MDA) were measured during the experimental period. To assess the health status of the animals, a physical examination was also performed. GSH plasma concentrations were significantly higher in young does at weeks 1 and 4, but not at week 8 of the experiment, irrespectively of QR administration. An increase in GSH plasma concentration was observed during the 8-week experiment in both AQ and AC groups. Furthermore, QR administration did not alter either SOD or MDA serum activity and concentration in any group during the experimental period. Physical examination revealed no differences between the experimental groups. In conclusion, under our experimental conditions, QR did not modify the general clinical or the antioxidant profile of young and adult reproductive rabbit females.

## KEYWORDS

glutathione, malondialdehyde, physiological status, quercetin, reproductive rabbit does, superoxide dismutase

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## 1 | INTRODUCTION

In rabbit livestock systems, does are raised under intensive reproductive rhythms where artificial insemination is performed in the early post-partum period. This leads to overlapping gestation and lactation during their productive life, which requires a high energy mobilization, causing a negative energy balance and increasing reactive oxygen species (ROS). ROS decrease doe reproductive efficiency and shorten their lifespan (Lorenzo et al., 2014), subsequently increasing culling rates (Rosell & De La Fuente, 2009).

An optimum antioxidant status in reproductive females improves reproductive efficiency. It has been shown that Quercetin (QR, 3, 5,7,30,40-pentahydroxyflavone), a strong flavonoid with antioxidant properties (Li et al., 2016), improves reproductive outcomes in adult rabbit does when administered for 20 days under heat stress (Naseer et al., 2017). However, literature data are lacking concerning the effect of QR administration at the beginning of the reproductive life, or whether QR administration can improve the antioxidant status of young and adult rabbit does under standard environmental conditions.

In this study, we aimed to determine if the long-term administration of QR may improve doe antioxidant status without affecting their physiological status. Therefore, we administered 300 mg/kg QR orally for 8 weeks, measuring the antioxidant profile by measuring glutathione [GSH], superoxide dismutase [SOD] and malondialdehyde [MDA] of reproductive rabbit does at the beginning (young: 8 weeks old) and at the end (adult: 12 months old) of their productive life. Physiological status was assessed by physical examination of clinical signs.

## 2 | MATERIALS AND METHODS

### 2.1 | Experimental design

Quercetin monohydrate (QR) (Merck-SIGMA) was administered orally (PO) at 300 mg/kg once a day for 8 weeks, mixed with 2 mL commercial orange juice. A total of 24 rabbit does were divided into 4 experimental groups: young (from 8 to 16 weeks) and adult (from 12 to 14 months) does treated (YQ and AQ) or not (YC and AC) with QR, respectively. It has been previously reported that 20% of the total QR administered orally is absorbed in gut (Manta et al., 2020). Thus, the expected effective dose in our experimental groups was 60 mg/kg. Blood sampling and physical examination of each doe were performed at weeks 1, 4 and 8 of treatment.

### 2.2 | Animals

New Zealand White × California rabbit does were allocated in the experimental farm of the Technical University of Madrid (UPM), in enriched individual cages (50 cm long × 90 cm wide × 63 cm high), including elevated platforms, plastic footrests and wooden objects,

with free access to water and fed with a commercial diet (16% crude protein, 37% crude fibre, 3.7% fat and 2400 kcal/kg of digestible energy; NANTA, Spain). Multiparous adult does (12 months old, 3–4 parturitions) had been subject to a semi-intensive reproductive rhythm (AI at 11 days post-partum) in the experimental farm before the study and were restricted to 150 g feed/day, while young does were all nulliparous (8 weeks old) and fed ad libitum. Temperature (20–25°C), humidity (60%–75%), ventilation and light/darkness (16:8) were constant. During our experiment, neither young nor adult rabbit does were inseminated.

### 2.3 | Physical examinations

General health status was determined by measuring lymph node size and texture, capillary refill time (CRT), mucous membrane appearance and abdominal palpation, as well as heart rate (HR) and respiratory rate (RR) by means of auscultation with a stethoscope (Pastor Meseguer, 2006).

### 2.4 | Antioxidant profile analysis

Four millilitre of blood were collected from the marginal ear vein. Plasma and serum were obtained in an EDTA tube and a blood collection tube, respectively, allowing them to clot for 30 min at 37°C. Then, samples were centrifuged at 700 × g for 20 min at 4°C. Blood plasma (for GSH determinations) and serum (for SOD and MDA determinations) were separated and stored at –80°C before analysis.

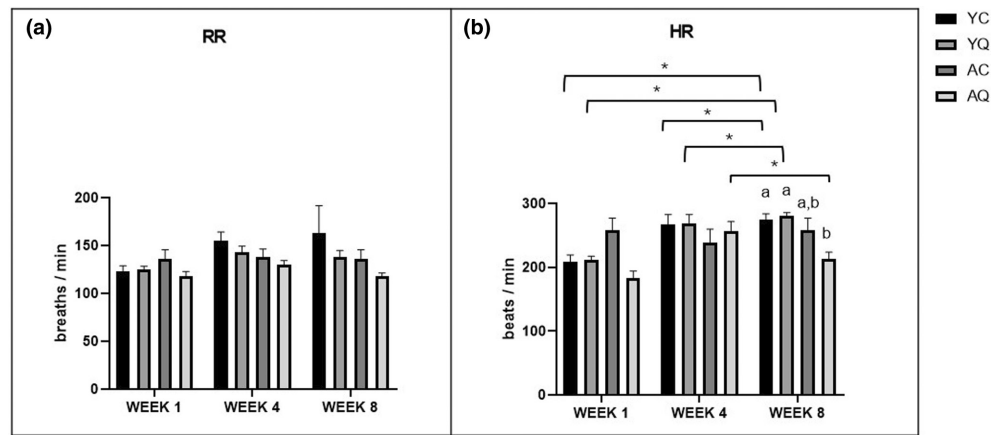
Commercially available kits for GSH and SOD (both from Merck-SIGMA) and MAD (LSBio) were used according to the manufacturer's instructions. A Multiskan GO plate reader (Thermo Scientific) was used for absorbance measurements at 412 nm for GSH and 450 nm for SOD and MDA.

### 2.5 | Statistical analysis

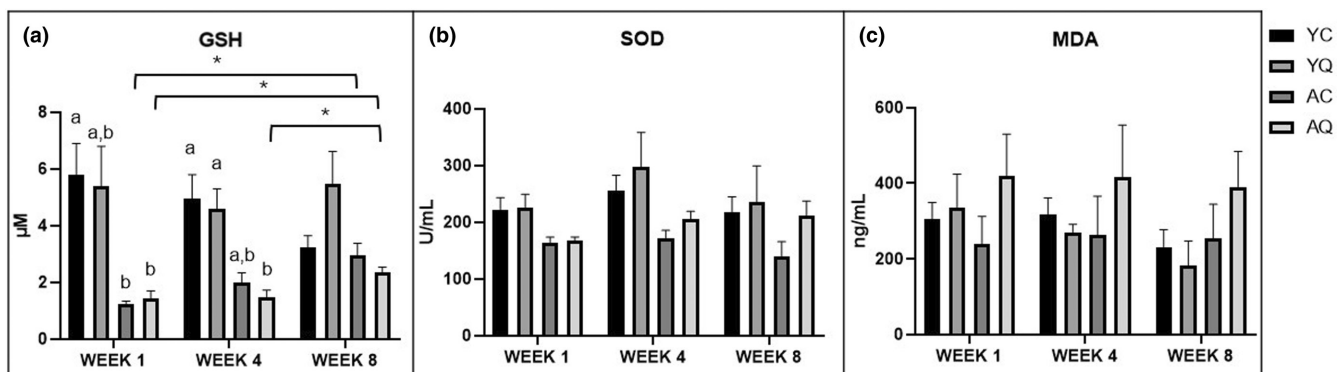
The results were tested for normality with the Shapiro–Wilk test and analysed by analysis of variance (ANOVA)—for normally distributed variables, or Kruskal–Walis—for non-normally distributed variables. Analysis was performed using R environment (v 4.3.1) through RStudio to assess the effect of rabbit doe age (young and adult), treatment (Control and QR) and length of QR administration (Weeks 1, 4 and 8). A post hoc Tukey or Dun test was applied to determine when the differences observed became significant at  $p < .05$ .

## 3 | RESULTS

Physical examination revealed that doe general health status was alert, with no alterations found in lymph node size and texture;



**FIGURE 1** (a) Respiratory Rate (RR) and (b) Heart Rate (HR) for each experimental group at each sampled week (1, 4 and 8). Different letters indicate significant differences ( $p < .05$ ) between groups at the same sampled week. An asterisk (\*) indicates significant differences ( $p < .05$ ) for the marked group between weeks. AC, Adult Control; AQ, Adult Quercetin; YC, Young Control; YQ, Young Quercetin.



**FIGURE 2** (a) Glutathione (GSH) concentration ( $\mu\text{M}$ ); (b) Superoxide Dismutase (SOD) activity ( $\text{U/mL}$ ) and (c) Malondialdehyde (MDA) concentration ( $\text{ng/mL}$ ) for each experimental group at each sampled week (1, 4 and 8). Different letters indicate significant differences ( $p < .05$ ) between groups at the same sampled week. An asterisk (\*) indicates significant differences ( $p < .05$ ) for the marked group between weeks. AC, Adult Control; AQ, Adult Quercetin; YC, Young Control; YQ, Young Quercetin.

mucous membranes were pale pink with  $\text{CRT} < 2\text{s}$ , and abdominal palpation was soft, without discomfort in any rabbit does. The RR was above resting values (reference range: 30–60 breaths/min) (Figure 1a). No significant differences were found among groups or examination time points in any parameter, except HR. At week 8, HR was lower in AQ compared to both YC and YQ groups. In the AQ group, significant differences were found in HR between weeks 4 and 8, whereas in groups YC and YQ such differences were observed between weeks 1 and 8, and 4 and 8 (Figure 1b).

GSH concentrations at week 1 were significantly higher for females of group YC than for those of the adult groups (AC and AQ). YQ group showed intermediate GSH levels. At week 4, groups YC and YQ showed higher GSH levels compared to the AQ group (Figure 2a). In addition, significant differences were found for GSH concentrations in the AC group at weeks 1–8, and in the AQ group at weeks 1–8 and 4–8 (Figure 2a). No significant differences were found in SOD activity and MDA concentration between groups or experimental time points (Figure 2b,c, respectively).

## 4 | DISCUSSION

All parameters included in the physical examination, except RR, which was slightly increased in all experimental groups, were within rabbit physiological resting values (Pastor Meseguer, 2006). Interestingly, HR values increased during the study period, and differences between groups at the end of the experimental period were observed, showing that long-term handling of animals induces variations both in HR (although still within physiological values) and RR.

QR exerts its antioxidant effects by increasing intracellular GSH levels (Kang et al., 2013; Xu et al., 2019), although no information is available regarding potential differences between young and adult animals. In the current study, GSH plasma concentrations were lower in adults compared to young females in the first week of the experiment. This observation might be explained by the intensive production rhythm underwent by adult does during their productive life (Lorenzo et al., 2014), compared to young females, which had not started yet their reproductive life. This could explain why

after 8 weeks, GSH serum concentration increased in adult females, while it stayed stable in young females. It remains to be elucidated if those age-dependent plasma GSH concentration differences are also quantifiable in doe oocytes and how they will affect the reproductive outcome of the females.

No variations in serum SOD activity were observed in our study. However, it has been reported that short-term QUR administration increases neural expression of endogenous antioxidant enzymes such as SOD in gerbils (Chen et al., 2017).

Finally, MDA serum concentration seems to be highly stable in rabbit does, as QUR administration to rabbit does under heat stress did not modify MDA serum concentration (Naseer et al., 2017), as our results depict. Further studies are warranted to assess whether long-term QUR administration increases SOD and MDA expression in the rabbit doe reproductive tract.

In conclusion, our results revealed that long-term QUR administration did not modify the physical status or the antioxidant profile of reproductive rabbit females at the beginning (8 weeks old) and at the end (12 months old) of their productive lives. GSH plasma concentration indicates that rabbits undergo oxidative stress when subjected to semi-intensive production rhythms and that GSH returns to basal values when not undergoing production conditions, regardless of QUR consumption.

#### AUTHOR CONTRIBUTIONS

Experimental design: AVC, PGR, PLL, RMGG and MAA. Laboratory analysis: AVC, DJR, ECM, AGL, PGR, PLL, RMGG and MAA. Data analysis: AVC. Writing of the manuscript: AVC, RMGG, PLL, MAA. Funding acquisition: PGR, PLL, RMGG and MAA. All authors have revised and approved the final version of the manuscript.

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#### CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

#### DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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