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RESEARCH

The Effect of Plant Quercetin on Immune Function of *Microtus Fortis*

Yuting An[1] · Dongmei Yang[1] · Bo Ma[1] · Dongming Liu[2] · Kaidong Deng[3] · Shuanglun Tao[1] · Huijuan Cai[1] · Yangjing Ou[1] · Junnian Li[1]

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Abstract

Quercetin, a widely occurring phytogenic flavonoid, is the primary secondary metabolite in the diet of *Microtus fortis*. This study investigated the effects of dietary quercetin on immune organ development and cytokine levels in *Microtus fortis*. *Microtus fortis* were fed a standard diet containing 0, 1.5%, 3%, 5%, or 8% quercetin for 20 consecutive days. The results show that, compared to the control group, treatment with 5% quercetin increased the spleen and thymus indices by 14.47% and 11.67%, respectively. The proliferation of T lymphocytes, B lymphocytes and macrophages in *Microtus fortis* varied significantly cross groups, depending on the concentrations of quercetin in the culture medium. Additionally, IL-2 and IL-10 secretion levels increased by 8.90% and 14.99%, while TNF-α and IL-1β decreased by 11.13% and 17.40%. However, at an 8% quercetin concentration, compared with the 5% treatment, the indices of spleen and thymus decreased by 9.09% and 7.79%, respectively Similarly, IL-2 and IL-10 secretion levels decreased by 8.86% and 5.34%, while TNF-α and IL-1β secretion increased by 10.7% and 6.22%, respectively. These findings suggest that low concentrations of quercetin promote immune organ development and cytokine secretion in *Microtus fortis*, whereas higher concentrations exert inhibitory effects.

Keywords Quercetin · Co-evolution · Immune function · *Microtus fortis*

Introduction

The co-evolution of animal-plant systems is one of the most important areas of research in modern ecology (Hattas et al. 2011). This process demonstrates how plants have developed various toxic secondary metabolites (PSMs) as a defense mechanism against herbivory. These toxic metabolites limit the feeding of phytophagous animals, thereby enhancing the plants'survival and competitive advantage. Although the toxicity of PSMs plays a critical role in animal survival, the potential beneficial effects of these compounds remain largely unexplored. Additionally, few studies have investigated the effects of PSMs on the digestive immune system of foragers. Understanding the role of PSMs in animal-plant co-evolution, particularly their effects on the immune system of mammalian herbivores, could provide valuable insights into the self-regulation of population density.

Quercetin, a widely distributed photogenic flavonoid, is present in various plant structures, such as flowers, leaves, and fruits (Mlcek et al. 2016). Freeland and Janzen proposed the secondary metabolite hypothesis from an evolutionary perspective, suggesting that PSMs could inhibit digestion in animals, promoting them to avoid eating plants containing these metabolites (Freeland and Janzen 1974). PSMs have been shown to impair energy and protein utilization in herbivores (Hartmann 2007; Marsh et al. 2007; Villalba and Provenza 2005; Loke et al. 2008), damage liver function, and compromise cell membrane integrity (Bergeron et al. 1987). They also inhibit enzyme activity (Mansoori et al. 2007) and negatively affect, growth, reproduction, and survival (Lindroth and Batzli 1984; Ye et al. 2016, Tanase et al. 2019). However, PSMs also exhibit beneficial effects on herbivorous mammals (Corbin et al. 2008). For example, plant flavonoids posses anti-allergic and anti-anaphylaxis properties (Mlcek et al. 2016), as well as anti-inflammatory (Lee et al. 2008; Shaik et al. 2006), anti-viral (Neznanov et al. 2008),

✉ Junnian Li
junnianl@163.com

[1] College of Biological Resources and Environmental Sciences, Jishou University, Jishou 416000, China

[2] Department of Human Nutrition, Foods, and Exercise, Virginia Tech, Blacksburg, VA 24061, USA

[3] College of Animal Science and Food Engineering, Jinling University of Science and Technology, Nanjing 210089, China

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antioxidant, and cardiovascular protective effects (Perez-Vizcaino et al. 2006; Loke et al. 2008). In addition, PSMs can effectively prevent lipid peroxidation and inhibit oxidase, thus protecting against free radical damage. Animals with mechanisms to detoxify PSMs must assess whether the energy and nutrient intake from the diet overweighs the metabolic cost of detoxification. If the concentration of PSMs in the diet exceeds the animal's tolerance level, the herbivore will cease eating the food to avoid poisoning.

The immune system's function directly influences an organism's resistance to disease (Chen et al. 2007). Indicators sych as the thymic index, cytokine (CK) levels, and the spleen index are primary markers of immune function. Certain pathological processes are often accompanied by abnormal CK production, which is directly or indirectly involved in the inflammatory responsed to resist bacterial, viral, and other pathogenic microorganisms. A well-regulated inflammatory response depends on the balance between anti-inflammatory factors (e.g., IL-2, IL-4, and IL-10) and pro-inflammatory factors (e.g., IL—1 beta, TNF alpha, IL—6). This equilibrium prevents excessive inflammation while minimizing various types of pathological damage (Bierhaus et al. 2003). Quercetin has been shown to influence the immune organs of mice, but research on its optimal concentration and role in immune function remains limited.

This study aimed to determine the effect of quercetin on the immune function of *Microtus fortis* by incorporating different concentrations of quercetin into their diet. The immune parameters of *Microtus fortis* were measured cross diets with varying concentrations of quercetin, providing new scientific insights into the co-evolution of herbivorous and plants.

Materials and Methods

Experimental Animals and Breeding Management

Microtus fortis were purchased from the Institute of the Ecology of Subtropical Agriculture in Changsha, Chinese Academy of Sciences. The male and female pairs were housed in a stainless steel mesh covered, transparent polypropylene cage (dimensions: 464 mm × 314 mm × 200 mm), and the inside of the cage was covered with wood chips as bedding. Experimental diet for *Microtus fortis* was supplied by Hunan Slack Landscape of Experimental Animal Company, and its nutritional composition is detailed in Table 1. *Microtus fortis* had ad libitum access to drinking water. The animal room was well ventilated, maintained at a temperature between 18 to 26℃ with a relative humidity of 60 ~ 70%, and operated on a 12 h light–dark cycle.

Table 1 Basic feed formula

Materials	Proportion (%)
Water	10
Crude protein	18
Ether extract	≥ 4
Crude fiber	≤ 5
Crude ash	8
Calcium	1.8
Phosphorus	1.2
Lysine, methionine	≥ 0.82
Cystine	≥ 0.53

Food Treatment

Based on the standard basal feed, the experimental diets were prepared by adding quercetin (95% purity) to the crushed pellet feed at concentrations of 1.5% (T2), 3% (T3), 5% (T4), or 8% (T5), respectively. A feed pellet machine (Guangzhou Good Machinery Company) was used to produce pelletized feed measuring 2 ~ 3 cm in length and 12 mm in diameter. For the health selection test, 40 adult rats aged 5–8 months old with similar body weights were randomly divided into 5 groups using a random number table. The were housed in cages (290 mm × 178 mm × 178 mm) and fed one of the experimental diets (T1-T5) for a 20-day experimental period.

Determination Parameters

Evaluation of Immune Organ Index

During the 20-day experimental period, blood samples were collected via orbital puncture. Serum was extracted from blood, transferred to 1.5 mL anticoagulant EP tubes, and stored at −20℃in a cryogenic refrigerator for future analysis. Following blood collection, animals were euthanized by cervical dislocation. The spleen and thymus were then carefully excised, with any attached tissue removed, and the organs were washed with chilled PBS. The immune organ index was calculated using the following formula:

$$\text{Calculation formula}: \text{immune organ index (mg/g)} = \text{immune organ fresh weight (mg)/pre} - \text{dissected fasting weight (g)}$$

Immunocyte Collection

Immediately following authorization of the *Microtus fortis*, 8–10 mL of RPMI-1640 medium was injected

intraperitoneally. The abdominal cavity was then gently massaged for 2 min, and the peritoneal lavage fluid was collected with 1 mL syringe. This was followed by additional irrigation with 1 mL of RPMI-1640 medium, repeated 3 times. The collected lavage solution was centrifuged and the supernatant discarded. The precipitated cells were washed once with 3 mL D hank's solution and centrifuged at 1500 r/min for 10 min. The cells were then resuspended in RPMI-1640 medium containing 5% fetal bovine serum. A 4% Trypan Blue dye was used to adjust the cell concentration to 2× 106 PCS/mL. For macrophage adherence, 2 mL of the prepared suspension was added to a 6-well cell culture plate and incubated at 37 °C in 5% CO_2 for 3 h. Non-adherent cells were removed by washing and collected for the following procedure.

Next, 200 µL/well of the macrophage suspension was seeded into a sterile 96-well plate, and incubated in 5% CO_2 at 37℃. After incubation for 3 h, all non-dherent cells were removed, and 100 µL of fresh medium was added to each well. To activate macrophages, 20 µLof PHA (30 µg/mL) was added per well, followed by adding 100 µL/well of neutral red dye at a final concentration of 0.1%. After reaction for 3 h, the supernatant was discarded, and cells were washed with PBS 3 times. For cell lysis, 200 µL/well of a mixture of ethanol and ice acetic acid (1:1) was added, and the plate was left overnight. The optical density (OD) was measured at 520 nm using a microplate reader.

Lymphocyte Separation and Purification

The separation and purification of lymphocytes were performed based on the method established by Gao Wei (Wei et al. 2007), involving the preparation of a nylon cotton column, density gradient centrifugation, and column separation. The nylon cotton column was mounted vertically over the collection tube and washed with 15 mL of pre-warmed culture medium containing serum at a flow rate of approximately one drop per second, discarding dead cells. The T cells in the eluate were collected, while the cotton column was further rinsed with pre-cooled culture medium to isolate B lymphocytes in the final eluate. T and B lymphocytes were resuspended in medium containing 5% FBS, and cell concentrations were djusted to 2× 10^6 cells/mL using Trypan blue staining. The cells were cultured at 37℃ with 5% CO_2 and saturated humidity for 24 h, 48 h, and 72 h, respectively.

Cell Stimulation and Quercetin Treatment

T cells, B cells, and macrophages were stimulated with concanavalin A (ConA), lipopolysaccharide (LPS), and polyhydroxyalkanoates (PHA), respectively. The cells were then treated with vehicle or quercetin at 1.25, 2.5, 5, 10, 15, 20, or 30 µmol/mL. Four hours before the end of the experiment, 20 µl of MTT solution was added to each well. After incubation, the supernatant was discarded, and 100µL of dimethyl sulphone solution was added. The plate was shken to dissolve the formazan crystals, and the optical density was measured immediately at 570 nm using a microplate reader.

Flow Cytometry

On the 20th day of the experimental period, blood samples were collected via orbital puncture from the *Microtus fortis* in each group. Whole blood treated with heparin sodium anticoagulant was used for the detection of CD4 + and CD8 + cells. The samples were divided into five groups (a, b, c, d, and e) for antibody staining as follows: Tube a (Blank control): 500 µL of anticoagulant whole blood; tube b: 500 µL of anticoagulant whole blood + 1 µL CD3-FITC; tube c: 500 µL of anticoagulant whole blood + 1 µL CD3-FITC + 1 µL CD4-PE; tube d: 500 µL of anticoagulant whole blood + 1 µL CD3-FITC + 1 µL CD8a-eFluor 450; tube e: 500 µL of anticoagulant whole blood + 1 µL CD3-FITC + 1 µL CD4-PE + 1 µL CD8a-eFluor 450. The samples were gently vortexed for 30 s and incubated at room temperature in the dark for 15 min. Following incubation, a cell lysate and fixative solution were added, and the samples were incubated for an additional 20 min. Afterward, the samples were washed, centrifuged, and resuspended in 500 µL of PBS buffer. The prepared samples were analyzed using flow cytometry.

Cytokine Levels

Anticoagulant whole blood samples were left at room temperature for 2 h or centrifuged at 1000 × g at 4℃ for 20 min. TNF-alpha, IL-beta, IL-2, and IL-4 were measured by using ELISA kits according to the manufacturer's instructions (Elabscience Biotechnology Company, LTD.).

Statistical Analysis

SPSS 20.0 software was used for statistical analysis, with initial weight as the covariate in a covariance analysis. The effects of quercetin on weight gain in *Microtus fortis* were analyzed using one-way ANOVA, specifically assessing different concentrations of quercetin on *Microtus fortis*, immune organ weight, and cytokine levels. The Duncan method was applied for multiple comparisons. Data were expressed as Mean ± SE. A $P < 0.05$ indicated a significant difference, and $P < 0.01$ indicated a highly significant difference. To further analyze the relationship between quercetin concentration and cytokines, the levels of quercetin, TNF-α, IL-1β, IL-2, and IL-10 were analyzed individually. Quercetin

content was defined as Cq, while TNF-α, IL-1β, IL-2, and IL-10 were defined as Cα, C1, C2, and C10, respectively.

Results

Development of Immune Organs

Compared to the control group, quercetin treatment significantly increased the spleen index of *Microtus fortis* in a dose-dependent manner, with 1.5%, 3%, 5%, and 8% concentrations resulting in increases of 8.15%, 11.60%, 14.47%, and 6.5%, respectively ($P < 0.05$, Fig. 1). Similarly, quercetin treatment at these doses increased thymus index by 6.93%, 8.76%, 11.67%, and 7.18%, respectively ($P < 0.05$, Fig. 2).

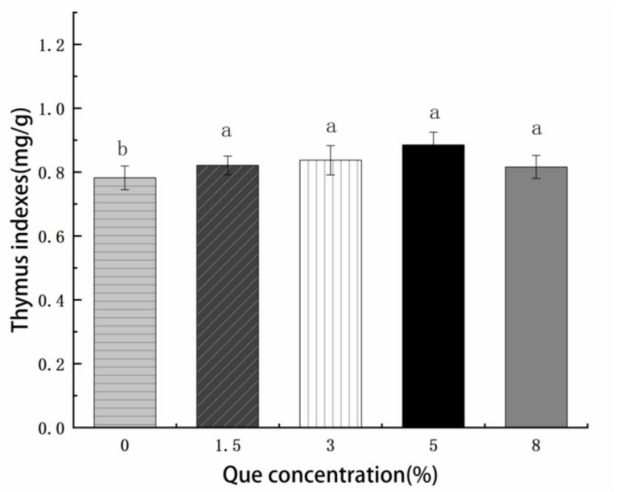

Fig. 2 Thymus indexes of *Microtus fortis* fed a control (0) or quercetin (Que) containing diet for 20 days. Different letters on the bar graphs indicate significant differences ($P < 0.05$)

Effects of Quercetin on Immune Cells

Proliferation of B Lymphocytes

Compared to the LPS group, the proliferative activity of B lymphocytes was significantly increased at a quercetin concentration below 2.5 μmol/mL after 24 h of culture ($P < 0.01$). However, the proliferative activity of B lymphocytes was significantly decreased at a quercetin concentration of 20 μmol/mL ($P < 0.01$, Fig. 3). After 48 h of incubation, the proliferation activity of B lymphocytes was significantly increased at a quercetin concentration of 1.25 μmol/mL ($P < 0.01$) but significantly reduced at quercetin concentrations above 15 μmol/mL ($P < 0.01$). Additionally, after 72 h of culture, the proliferation activity of B lymphocytes was significantly lower than that of LSP group When quercetin concentration was 10 μmol/mL. When the quercetin concentration exceeded 15 μmol/mL, a significant reduction in proliferative activity was also observed at 48 h compared to the LPS group.

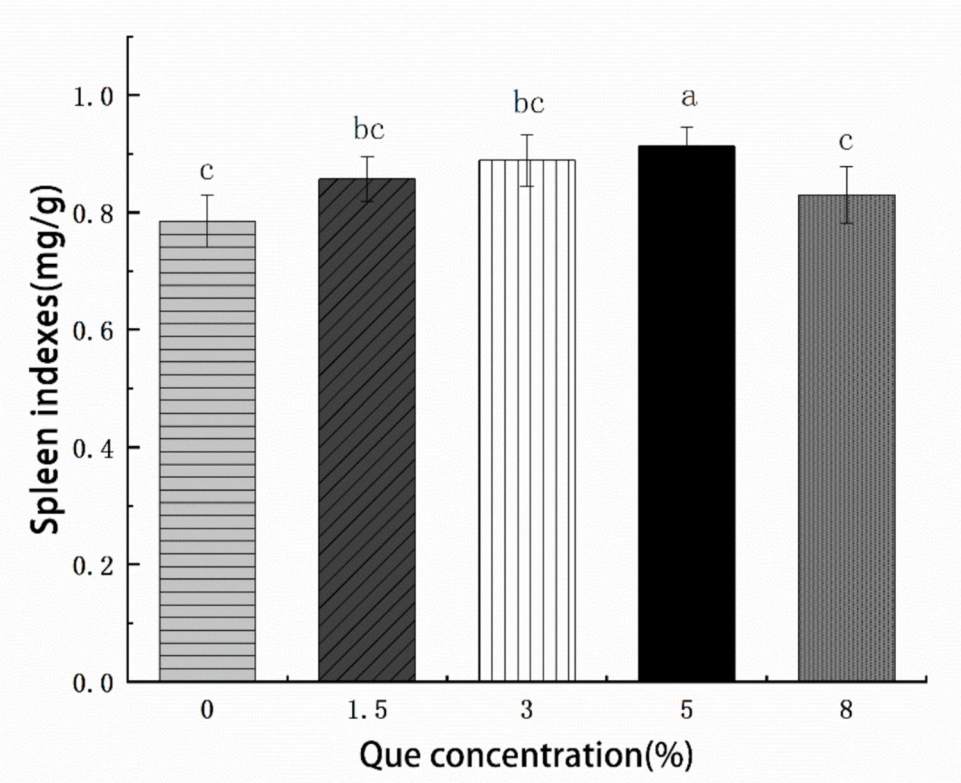

Fig. 1 Spleen indexes of *Microtus fortis* fed a control (0) or quercetin (Que) containing diet for 20 days. Different letters on the bar graphs indicate significant differences ($P < 0.05$)

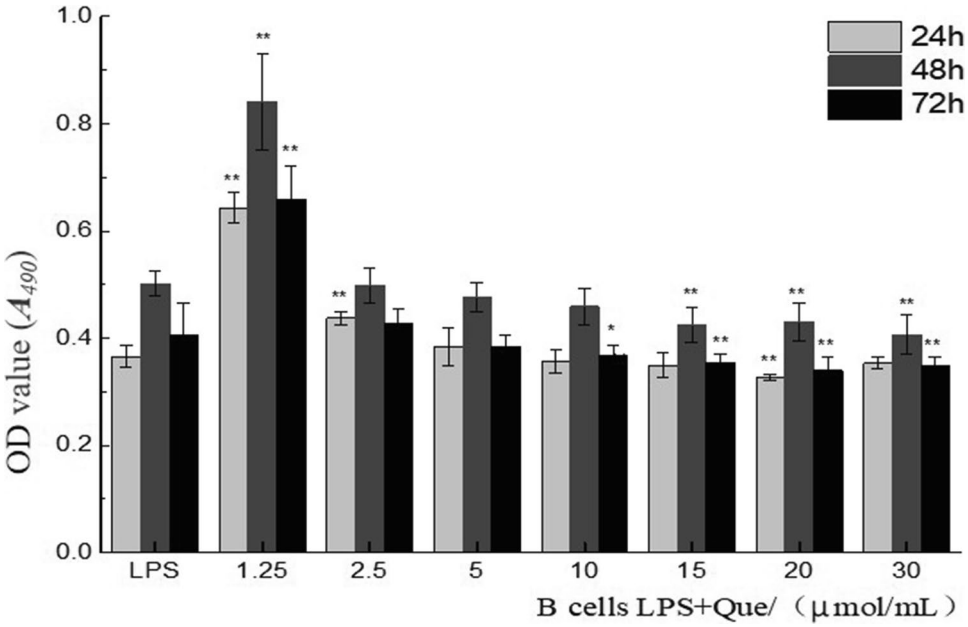

Fig. 3 The effect of quercetin on the proliferation of B lymphocytes. B cells isolated from microtus fortis were treated with LSP in the presence or absence of quercetin (Que) at indicated concentrations of quercetin (Que) for 24 h, 48 h, or 72 h Cell proliferation was measured by MTT assay. * and **, $P < 0.05$ and $P < 0.01$ vs. LPS group

Proliferation of T Lymphocyte

Compared to the ConA stimulation group, the proliferative activity of T lymphocytes was significantly enhanced at quercetin concentrations below 2.5 μmol/mL ($P < 0.01$) but significantly decreased at concentrations above 15 μmol/mL ($P < 0.05$). When cultured for 48 h, the proliferative activity of T lymphocytes was significantly enhanced at a quercetin concentration below 15 μmol/mL ($P < 0.05$). At 72 h, quercetin concentrations below 10 μmol/mL significantly enhanced T lymphocyte proliferation compared to the stimulation group ($P < 0.01$). Conversely, at quercetin concentrations above 20 μmol/mL, the proliferative activity of T lymphocytes was significantly reduced compared to that of the stimulation group ($P < 0.01$, Fig. 4).

Proliferation of Macrophage

Compared to the PHA-stimulated group, the proliferation activity of macrophages was significantly enhanced at quercetin concentrations below 15 μmol/mL after 24 h of culture ($P < 0.01$). At quercetin concentration below 5 μmol/mL for

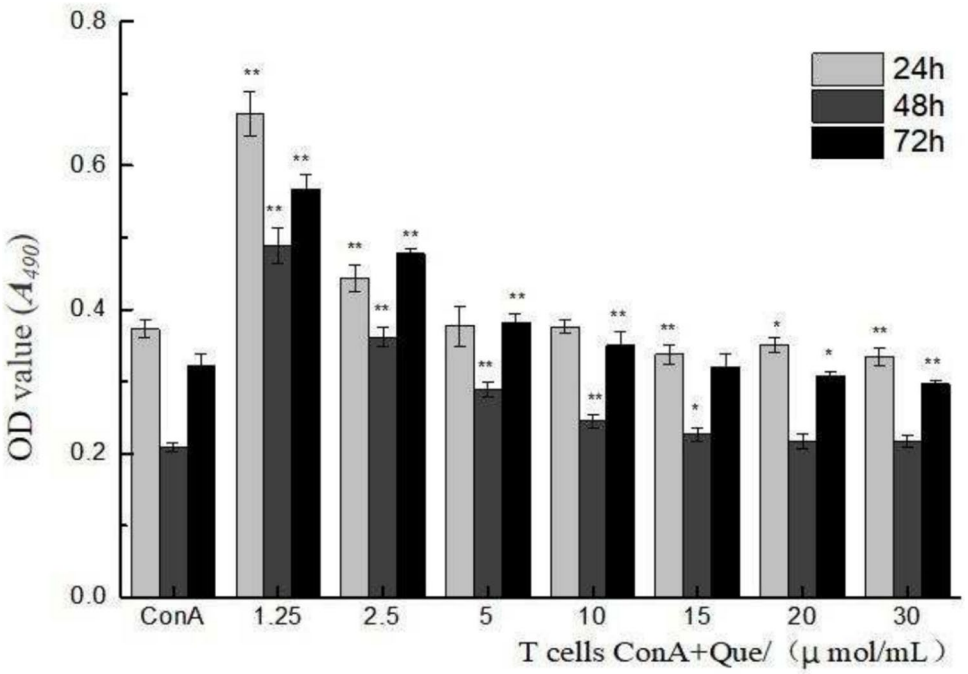

Fig. 4 The effect of quercetin on the proliferation of T lymphocytes isolated from Microtus fortis. T lymphocytes were treated with ConA in the presence or absence of different concentrations of quercetin (Que) for 24, 48, and 72 h. The cell proliferation was measured by MTT assay and expressed as OD. *$P < 0.05$ vs. LPS group; **$P < 0.01$, vs. ConA-alone group

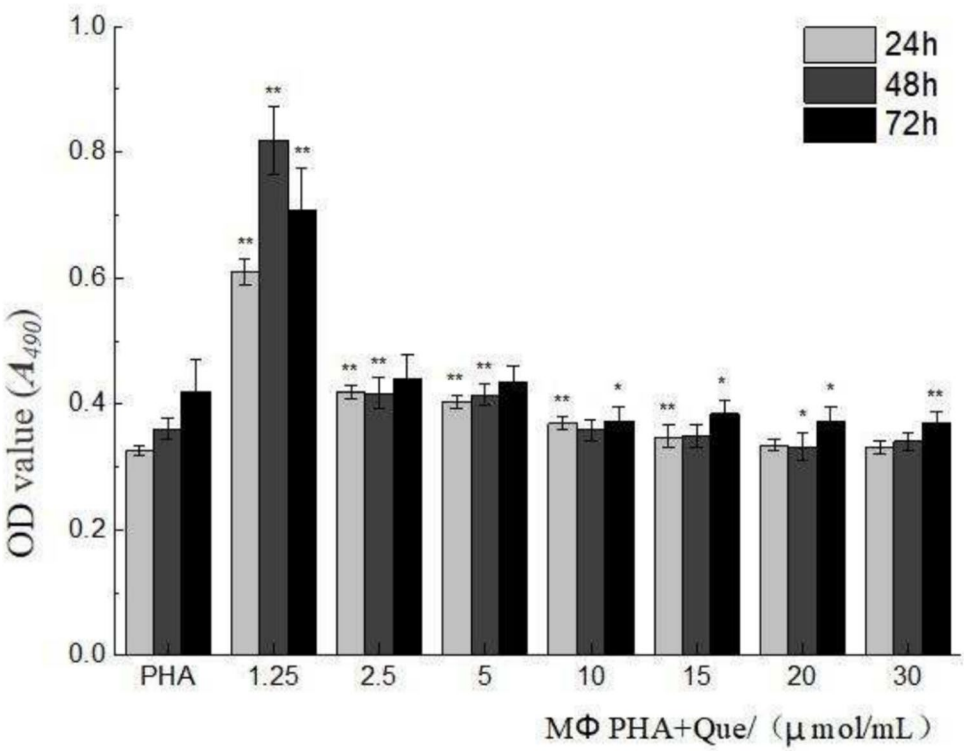

Fig. 5 The effect of quercetin on the proliferation of macrophagocytes isolated from *Microtus fortis*. The cells were treated with PHA in the presence or absence of different concentrations of quercetin (Que) for 24, 48, and 72 h. The cell proliferation was measured by MTT assay and expressed as OD. $^{*}P < 0.05$ vs. LPS group; $^{**}P < 0.01$ vs. PHA-alone group

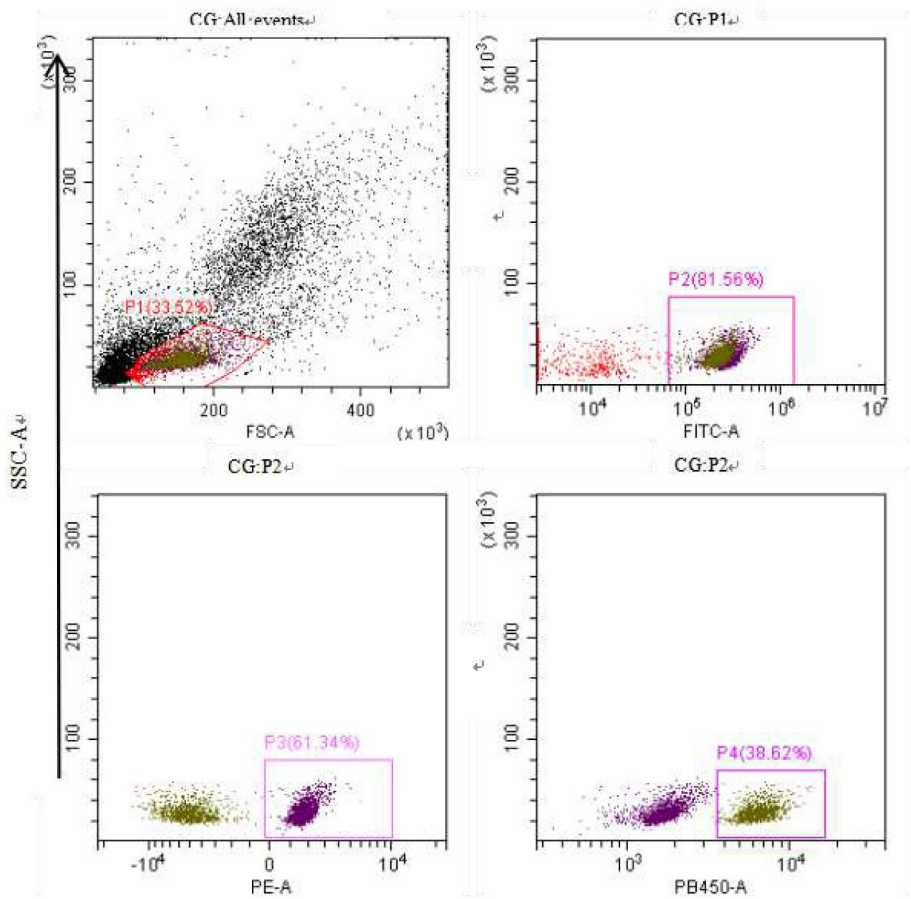

Fig. 6 The distribution of lymphocytes in *Microtus fortis*. All Events represent the distribution of lymphocytes. P1, P2, P3, and P4 represents T lymphocyte population, CD3 + cells (FITC fluorescence), CD4 + cells (PE fluorescence), and P4 represents CD8 + cells (PB450 fluorescence), respectively

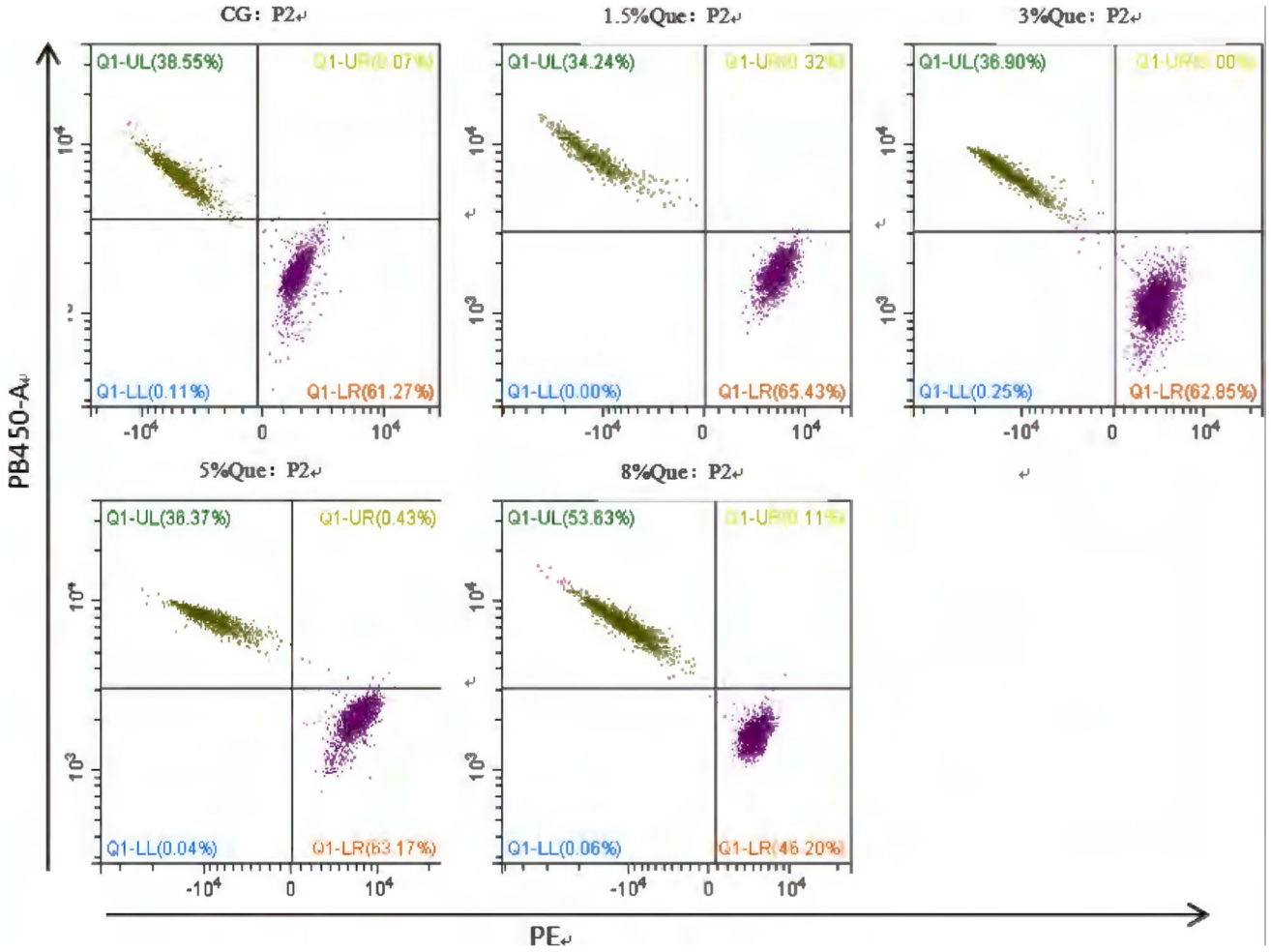

Fig. 7 The distribution of CD4 + and CD8 + cells in Reed voles fed a diet with various concentrations of quercetin. All Events represent the distribution of lymphocytes. P1, P2, P3, and P4 represents T lymphocyte population, CD3 + cells (FITC fluorescence), CD4 + cells (PE fluorescence), and P4 represents CD8 + cells (PB450 fluorescence), respectively. Q1-UL:CD8 + cells, and Q1-LR: CD4 + cells

48 h, the proliferation activity of macrophages was also significantly increased ($P < 0.01$). However, at 20 µmol/mL of quercetin, proliferation activity of macrophages was significantly decreased ($P < 0.05$). After 72 h of culture, macrophage proliferation was significantly increased at a quercetin concentration of 1.25 µmol/mL compared with the stimulation group ($P < 0.01$), while quercetin concentration above 10 µmol/mL led to a significant reduction in macrophage proliferation relative to the stimulation group ($P < 0.01$, Fig. 5).

CD4 + and CD8 + Cells

The distribution of lymphocytes, as analyzed by flow cytometry, is shown in Fig. 6. In the control group, T lymphocytes accounted for 33.52%, CD3 + cells accounted for 81.56%, CD4 + cells accounted for 61.34% and CD8 + cells accounted for 38.62% of CD3 + cells. The ratio of CD4 + and CD8 + cells is shown in Fig. 7. The CD4 +/CD8 + ratio in the control group was 1.57, compared to 1.91 in the 1.5% quercetin group, 1.70 in the 3% group, 1.73 in the 5% group, and 0.86 in the 8% group. There was no significant difference in the CD4 +/CD8 + ratio between the control group and the 1.5%, 3%, and 5% quercetin groups. However, the CD4 +/CD8 + ratio in the 8% quercetin group was significantly lower than that in the other groups. There were no significant differences between the control group and the 1.5%, 3%, and 5% quercetin group. However, the CD4 +/CD8 + ratio in the 8% quercetin group was significantly lower than that in all other groups.

Immunoactive Substance Levels

Immunoglobulin Levels

Serum levels of IgA, IgG, and IgM increased with increasing dietary quercetin concentrations (Fig. 8). At a quercetin

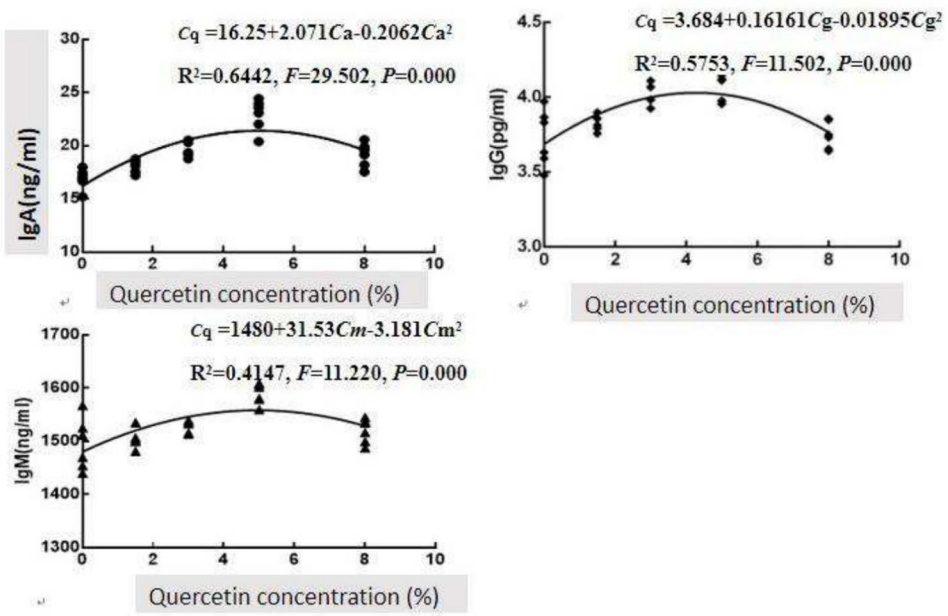

Fig. 8 Regressive relationships of different concentrations of quercetin and immune globulin levels of microtus fortis. The figure shows the logistic curve fitting of different immune proteins levels of microtus fortis. Quercetin at 5% is the zero cut-off point for the change of immune organ index, and there are significant differences

concentration of 5%, IgA, IgG, and IgM levels in serum increased by 32.00%, 9.07%, and 5.27%, respectively, compared to the control group ($P < 0.05$). However, at quercetin concentrations higher than 5%, serum levels of IgA, IgG and IgM were lower than those observed in 5% quercetin group ($P < 0.05$).

Cytokine Levels

Serum levels of IL-1β, IL-18, TNF-α, and IFN-γ decreased with increasing dietary quercetin concentrations (Fig. 9). At a quercetin concentration of 5%, serum levels of IL-1β, IL-18, TNF-α, and IFN-γ decreased by 17.40%, 19.27%, 11.13%, and 7.8%, respectively, compared to the control group ($P < 0.05$). At an 8% quercetin concentration, these levels remained lower than the control but were higher than those in the 5% quercetin group. Conversely, serum levels of IL-2, IL-4, and IL-10 increased with rising dietary quercetin concentrations. At 5% quercetin, IL-2, IL-4, and IL-10 levels rose by 8.9%, 10.38%, and 14.99%, respectively, compared to the control ($P < 0.05$). When the quercetin concentration reached 8%, serum levels of IL-2, IL-4, and IL-10 further increased; however, IL-2 and IL-10 levels were lower than those observed at the 5% concentration.

Discussion

Mammalian Herbivores have evolved diverse strategies to manage PSMs, ranging from physiological detoxification to adaptive foraging behavior (Lindroth & Batzli 1984; Marsh et al. 2006). Understanding the effects of PSM on mammalian herbivores have been a major research focus for over a century; however, there is limited research on the effects of PSMs on immune function. Our results show that moderate levels of quercetin improved immune function in *Microtus fortis*, while high concentrations inhibited immune function. This finding fills a critical research gap on the potential roles of PSMs in self-regulation of population density, and thereby enriches the theory of animal and plant co-evolution.

Our study found that low concentrations of quercetin promoted the proliferation of T lymphocytes, B lymphocytes, and macrophages, while high concentrations inhibited their proliferation, This differential response suggests that different immune cells showed varying sensitivities to quercetin concentrations, consistent with previous observations (Junfeng Li et al. 2002; Nan Wang et al. 2003). Alessio et al. collected the main food items of voles, extracted quercetin, and added it to the voles'food. After treating voles with 15% protein and 6% quercetin for 30 days, they observed a 23.6% decrease in spleen weight, a 29.3% decrease in thymus weight, and reductions of 17.6% and 22.1% in serum immunoglobulins compared to controls (Alessio et al. 2002).

The activation of immune cells by quercetin also varied significantly over time, likely due to difference in response mechanisms among different types of immune cells. Knight (Knight 2000) found that macrophage activation increases intracellular oxidation, leading to functional changes, while excessive oxidative stress subsequently weakens cellular functions (Alessio et al. 2002; Perez-Vizcaino et al. 2006). (Metodiewa et al. 1999) observed that high concentrations of quercetin can produce free radical semiquinones, which are cytotoxic and can lead to cell necrosis or apoptosis. In

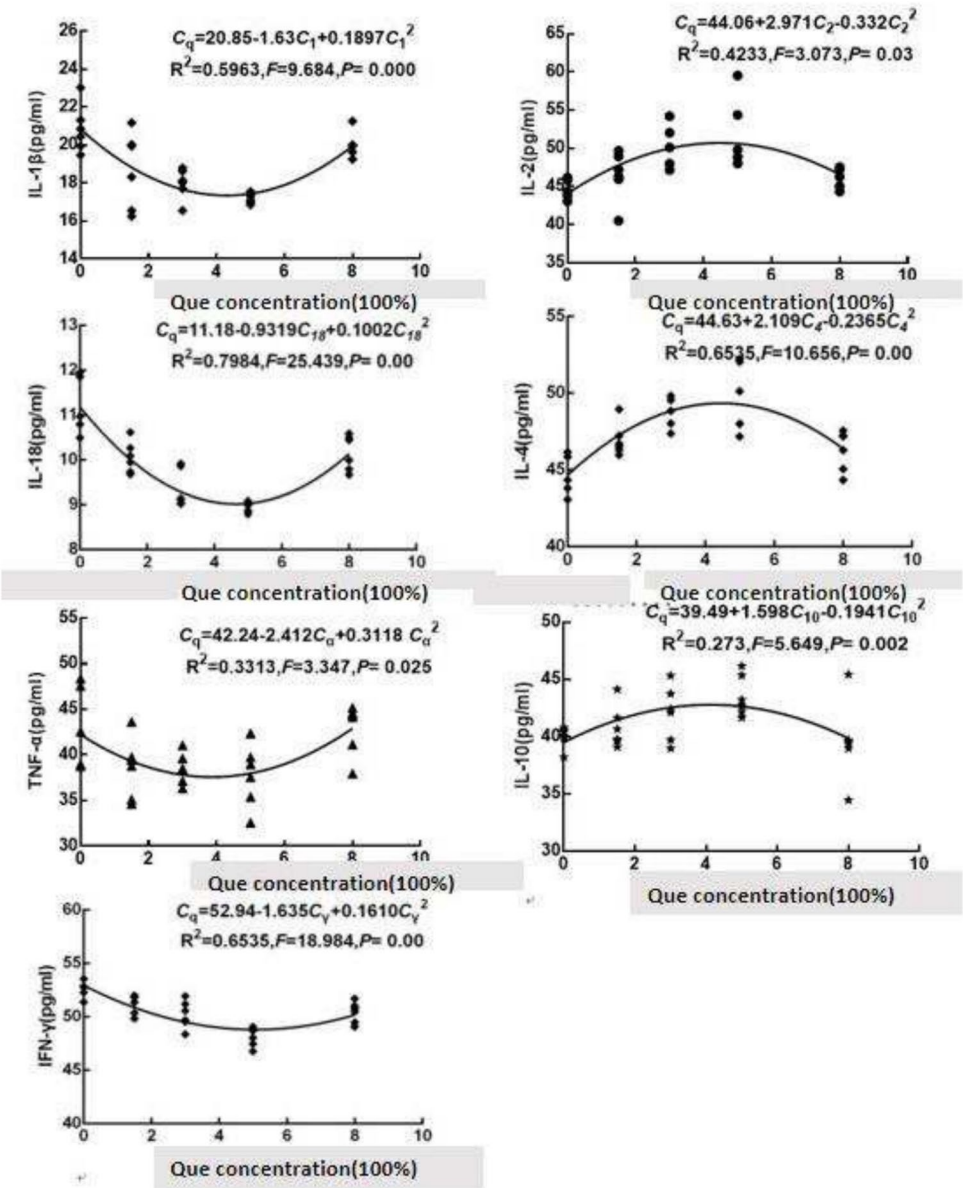

Fig. 9 Regressive relationships of different concentrations of quercetin and cytokines levels in microtus fortis. The figure shows the logistic curve fitting of different cytokines levels of microtus fortis, with 5% quercetin concentration used as zero cut-off point for the change of immune organ index, and there are significant differences

our study, high quercetin's inhibitory effect on immune cells might be linked to this cytotoxicity. Our findings also showed that serum levels of IgA, IgG, and IgM significantly increased at quercetin concentration below 5% ($P < 0.05$); however, when quercetin concentration reached 5%, serum IgA, IgG, and IgM levels were lower than those in the experimental group. This is consistent with the finding of Jin Zhong (Jin 2014), who reported that administration of sea buckthorn leaf flavonoid extract to rats significant increased IgA, IL-2, and IL-4 levels ($P < 0.05$), suggesting that moderate concentrations of quercetin promote B lymphocyte proliferation and increase immunoglobulin secretion (McLean and Duncan 2006).

In the present study, the CD4 +/CD8 + ratio was normal (1.4 to 2.0) in the control and quercetin groups with concentrations below 5%, which significantly increased the anti-inflammatory cytokines IL-2, IL-4, and IL-10 while decreasing pro-inflammatory cytokines IL-1β, IL-18, IFN-γ, TNF-α. However, at an 8% dose, anti-inflammatory cytokines were lower than at 5%, suggesting that quercetin at 8% concentration led to a reduction in CD4 + lymphocytes, impairing cellular and humoral immunity in *Microtus fortis*. Similarly, Tanaka and Taniuchi (2014) found that quercetin at below 160 μg/mL decreased IL-1β secretion from macrophages in a dose-dependent manner. (Park et al. 2009) demonstrated that high quercetin levels modulate T-bet and GATA-3 expression, altering the Th1/Th2 ratio in CD4 + cells by reducing Th2 cytokine IL-4 and increasing Th1 cytokine IFN-γ. Boesch-Saadatmandi et al. (2011) reported that biotin effectively inhibits

LPS-induced transcription and protein synthesis of TNF-α, IL-1, and macrophage inflammatory protein-1α in mouse macrophages. At 8% quercetin, the ratio of CD4 +/CD8 + dropped below 1.4, likely due to differential cytokine regulation in the microenvironment, which induces Th0 cell differentiation into various functional subsets (Ye et al. 2016). For instance, IL-12 promotes Th0 differentiation into Th1 cells, while IL-4 promotes Th2 cell development, similar to previous findings (Yunhui et al. 2024), indicating that quercetin might have therapeutic potential for immune-related diseases.

In conclusion, quercetin enhances immune function and increases survival at relatively low concentrations (< 5%) in *Microtus fortis*. At concentrations above 5%, however, quercetin intake may exceed the animal's detoxification capacity, leading to accumulation in tissues, thereby exerting toxic effects. These effects likely inhibit immune function possibly through mechanisms related to quercetin's prooxidant cytotoxicity at high concentrations.

Ecological Significance

Populations in natural environments have a capacity for self-regulation (Villar et al. 2014). When population density is low, small mammalian herbivores encounter abundant plant resources and tend to prefer plants with low levels of PSMs. Ingested PSMs at low levels improve immunity and survivability, thus supporting population growth. Conversely, when population density of mammalian herbivores is high, plant resources become scarce, forcing them to consume plants with higher PSM contents, including potentially toxic species (Karban and Myers 1989). High levels of PSM ingestion can impair immunity, increase mortality, and reduce population density. Thus, understanding the effects of quercetin on the immunity of small mammalian herbivores at various intake levels offers valuable insights into population density regulation mechanisms. This highlights the dual role of PSMs as both beneficial and limiting factors for the survival and regulation of phytophagous animal populations.

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Author Contributions Yuting An experimental operation, paper writing and data processing; Dongmei YANG Experimental operation and design, paper revision; BoMA experimental operation and data analysis; Dongming LIU and Kaidong DENG revised the paper; Data analysis by Shuanglun TAO; Huijuan CAI and Yangjing OU participated in the experimental operation, Junnian LI set the research direction and designed the experiment.

Data Availability No datasets were generated or analysed during the current study.

Declarations

Competing interests The authors declare no competing interests.

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