Effects of Allium mongolicum Total Flavonoids on Antioxidant Capacity in Meat Sheep (Postprint)

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Date: 2017-10-10T00:00:00+00:00

Abstract

This experiment aimed to investigate the effects of dietary supplementation with total flavonoids of Allium mongolicum on total antioxidant capacity (T-AOC), total superoxide dismutase (T-SOD), catalase (CAT), glutathione peroxidase (GSH-PX) activities, and malondialdehyde (MDA) content in meat sheep, so as to elucidate the antioxidant effects of total flavonoids of Allium mongolicum on meat sheep and determine the optimal supplementation level in the diet. Sixty Small-tailed Han sheep aged approximately 6 months with a body weight of (39.9±3.2) kg were selected as experimental animals and randomly divided into 4 groups according to the principle of similar birth month and body weight, with 15 sheep per group. The control group was fed the basal diet, while the three experimental groups were supplemented with 11, 22, and 33 mg/kg of total flavonoids of Allium mongolicum in the basal diet, respectively. The preliminary period lasted for 15 d, and the formal experimental period lasted for 60 d. On days 0, 15, 30, 45, and 60 of the experimental period, blood samples were collected from the jugular vein after fasting, and serum was separated. At the end of the experimental period, 3 sheep were randomly selected from each group for slaughter, and liver and spleen samples were collected to determine antioxidant indices in serum and tissues. The results showed: 1) Dietary supplementation with 11–33 mg/kg total flavonoids of Allium mongolicum significantly increased serum (starting from day 45) and liver T-AOC ($P<0.05$), but had no significant effect on spleen T-AOC ($P>0.05$). 2) Dietary supplementation with 11–33 mg/kg total flavonoids of Allium mongolicum showed certain improvements in serum, liver, and spleen T-SOD activities, serum CAT activity, and serum and spleen GSH-PX activities, with the strongest effect observed at 33 mg/kg, and the effect in serum became apparent after day 30; however, it had no significant effects on liver and spleen CAT activities or liver GSH-PX activity ($P>0.05$). 3) Dietary supplementation with 11–33 mg/kg total flavonoids of Allium mongolicum decreased serum and liver MDA content, with the effect in

serum becoming apparent after day 15, and had no significant effect on spleen MDA content (P>0.05). The results suggest that dietary supplementation with 11–33 mg/kg total flavonoids of Allium mongolicum can significantly improve antioxidant indices in meat sheep, and its effects are time- and dose-dependent, with the antioxidant capacity beginning to exert its effects after 30 days of feeding.

Full Text

Effects of Flavonoids from *Allium mongolicum* Regel on Antioxidant Capacity of Meat Sheep

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Abstract: This experiment was conducted to investigate the effects of dietary supplementation of flavonoids from *Allium mongolicum* Regel on total antioxidant capacity (T-AOC), activities of total superoxide dismutase (T-SOD), catalase (CAT), and glutathione peroxidase (GSH-PX), and malondialdehyde (MDA) content in meat sheep, and to determine the optimal supplementation level. Sixty 6-month-old Small-tailed Han sheep with similar body weight [(39.9±3.2) kg] were randomly assigned to 4 groups (n=15). The control group received a basal diet, while three experimental groups received the basal diet supplemented with 11, 22, or 33 mg/kg flavonoids from *Allium mongolicum* Regel. The preliminary feeding period lasted 15 days, followed by a 60-day experimental period. Blood samples were collected via jugular venipuncture on days 0, 15, 30, 45, and 60, and serum was harvested. At the end of the experiment, three sheep from each group were randomly selected for slaughter, and liver and spleen samples were collected for antioxidant index determination. The results showed: (1) Dietary supplementation with 11–33 mg/kg flavonoids significantly increased T-AOC in serum (from day 45) and liver (P<0.05), but had no significant effect on spleen T-AOC (P>0.05). (2) Supplementation with 11–33 mg/kg flavonoids enhanced T-SOD activity in serum, liver, and spleen; CAT activity in serum; and GSH-PX activity in serum and spleen, with the 33 mg/kg group showing the strongest effects that became evident after day 30 in serum. However, no significant effects were observed on CAT activity in liver and spleen or GSH-PX activity in liver (P>0.05). (3) Flavonoid supplementation reduced MDA content in serum and liver, with effects becoming apparent after day 15 in serum, but had no significant effect on spleen MDA content (P>0.05). These results indicate that dietary supplementation with 11–33 mg/kg flavonoids from *Allium mongolicum* Regel significantly improves antioxidant indices in meat sheep in both time-dependent and dose-dependent manners, with antioxidant effects becoming evident after 30 days of feeding.

Keywords: *Allium mongolicum* Regel; flavonoids; meat sheep; antioxidant

capacity

Introduction

Allium mongolicum Regel, belonging to the family Liliaceae of Angiospermae, is a species of *Allium* with numerous biological activities, also known as Mongolian chive. As a characteristic plant of desert grasslands and dune regions, it possesses strong drought and cold resistance and is widely distributed across Xinjiang, Qinghai, Gansu, and western Inner Mongolia in China. Particularly in Ordos, Alxa, and Xilingol, *A. mongolicum* is a well-known delicacy with unique flavor and rich nutritional value, representing a distinctive wild vegetable in Inner Mongolia. The plant is rich in protein, amino acids, lipids, minerals, trace elements, polysaccharides, and flavonoids, and has been reported to lower blood pressure, improve appetite, enhance immunity, and exhibit antioxidant, anti-aging, and antimicrobial activities, earning it the reputation of "Ganoderma among vegetables."

All living organisms produce free oxygen and oxidative radicals during physiological processes, which are directly associated with pathological changes and disease development. Currently, synthetic drugs such as butylated hydroxytoluene (BHT) and butyl hydroxy anisole (BHA) are commonly used for radical scavenging. Flavonoids, as natural polyphenolic compounds, exhibit potent pharmacological and biological activities, with studies demonstrating strong free radical scavenging and antioxidant properties comparable to or better than BHT and BHA. As an important component of *A. mongolicum*, flavonoids have been shown by Zhao Chunyan to enhance antioxidant capacity in mice. The antioxidant activity of flavonoids is closely related to their unique chemical structure, particularly the multiple phenolic hydroxyl groups and C2-C3 double bonds that can donate oxygen and electrons and chelate metal ions, thereby reducing their pro-oxidant effects.

Previous research demonstrated that in vitro supplementation of 16–21 mg/kg flavonoids from *A. mongolicum* improved rumen microbial fermentation parameters including gas production, pH, ammonia nitrogen, microbial protein, and volatile fatty acids in sheep. While extraction processes, purification, structural identification, and biological activities of these flavonoids have been investigated through in vitro studies, no reports exist on their effects on antioxidant capacity in meat sheep. Based on preliminary in vitro results from Bao Lingling, this experiment selected supplementation levels of 11, 22, and 33 mg/kg flavonoids to determine the optimal range and investigate their effects on antioxidant capacity in serum, liver, and spleen of meat sheep, providing a scientific basis for application as a natural feed additive.

Materials and Methods

Preparation of Flavonoids from *Allium mongolicum* Regel

Flavonoids used in this experiment were prepared in our laboratory using the optimal extraction method described by Sa Ruli. Ultrasonic extraction was performed for 15 minutes at 40°C with 75% ethanol at a solid-liquid ratio of 1:30, yielding 12.85 mg flavonoids per gram of *A. mongolicum* powder. The product was a yellow powder with low water solubility. Structural identification by Sa Ruli revealed the presence of monosaccharides, 3' ,4' -epoxy-7-O-5-methoxyflavonol, 7-O-5,4' -dimethoxy-3-oxyhydroxyflavone, sugars, rutin, luteolin-5' -O-glucosyl-4-hydroxyphenylpropionic acid, isoquercitrin sugars, acacetin, and other flavonoid compounds.

Reagents and Instruments

Assay kits for total antioxidant capacity (T-AOC), total superoxide dismutase (T-SOD), catalase (CAT), malondialdehyde (MDA), and glutathione peroxidase (GSH-PX) were purchased from Nanjing Jiancheng Bioengineering Institute. Major equipment included a multifunctional microplate reader (A-5082, TECAN, Austria), a 37°C incubator (HH-400, Beijing Hengxing Laiguangying Medical Equipment Factory), and an adjustable temperature water bath.

Experimental Animals and Design

Sixty 6-month-old Small-tailed Han sheep weighing (39.9±3.2) kg were used as experimental animals. A single-factor multi-level complete block design was employed, with animals randomly divided into 4 groups (n=15 each) based on similar birth month and body weight: control group, Test Group I, Test Group II, and Test Group III.

Flavonoid Supplementation and Diet Composition

The control group received a basal diet, while experimental groups received the basal diet supplemented with 11, 22, or 33 mg/kg flavonoids from *A. mongolicum* Regel. The supplementation levels were selected based on previous rumen stability studies showing that 22 mg/kg produced optimal effects in the rumen. The composition and nutrient levels of the basal diet are presented in Table 1 .

Table 1 Composition and nutrient levels of the basal diet (air-dry basis) %

Items	Content
Ingredients	
Chinese wildrye	
Alfalfa	
Corn	

Items	Content
Wheat bran	
Sunflower seed meal	
Pea stalk	
Pomace	
CaHPO	
NaCl	
Premix	
Total	
Nutrient levels	
DE/(MJ/kg)	
CP	
NDF	
ADF	
Ca	

Note: 1) The premix provided per kg of diet: Fe (as ferrous sulfate) 25 mg, Zn (as zinc sulfate) 29 mg, Cu (as copper sulfate) 8 mg, Mn (as manganese sulfate) 30 mg, I (as potassium iodide) 0.04 mg, Co (as cobalt sulfate) 0.1 mg, VA 3,200 IU, VD 1,200 IU, VE 20 IU. 2) DE was a calculated value, while others were measured values.

Animal Management

The experimental period lasted 75 days, including a 15-day preliminary period for deworming, disinfection, and vaccination. During the 60-day formal experimental period, animals were fed at 06:00 and 18:00 daily with roughage first followed by concentrate, with free access to water.

Sample Collection and Preparation

On days 0, 15, 30, 45, and 60 of the formal period, 5 mL blood samples were collected from the jugular vein of 15 sheep per group before morning feeding using non-anticoagulant tubes. After standing for 40 minutes for natural coagulation and serum separation, samples were centrifuged at 800×g for 10 minutes. Serum was collected in dry EP tubes for immediate use or stored at -20°C. At the end of the experiment, three sheep from each group were selected for slaughter based on similar daily feed intake and average daily gain. Liver tissue (0.1-0.2 g) was collected, washed with precooled physiological saline to remove surface blood and residues, blotted dry with filter paper, weighed accurately, and placed in 5 mL cryotubes. For analysis, tissues were ground and homogenized in homogenization medium to prepare 10% liver homogenates.

Measurement Methods

Serum, liver, and spleen samples were analyzed for T-AOC, T-SOD, CAT, GSH-PX activities, and MDA content. All procedures and calculations strictly followed the instructions provided in the assay kits from Nanjing Jiancheng Bioengineering Institute. For tissue antioxidant indices, protein content was first determined using the protein quantification kit from the same institute, followed by antioxidant measurements according to kit protocols. Dietary nutrient levels were determined as follows: crude protein by Kjeldahl method, neutral detergent fiber (NDF) and acid detergent fiber (ADF) by Van Soest method, calcium by potassium permanganate titration, and phosphorus by molybdenum yellow colorimetry.

Statistical Analysis

Data were analyzed using SAS 9.0 software. Differences among groups were evaluated by one-way ANOVA. Results are expressed as "mean ± standard deviation." $P<0.05$ was considered statistically significant, while $P>0.05$ indicated no significant difference.

Results

Serum T-AOC

As shown in Table 2 , on day 0, Test Group I showed relatively higher T-AOC compared to other groups, while Test Groups II and III were higher than the control, but differences were not significant ($P>0.05$). On day 15, T-AOC in the control, Test Group I, and Test Group II showed increasing trends compared to day 0, but differences remained non-significant ($P>0.05$). The control group was higher than all test groups, but without significant differences ($P>0.05$). Test Group III showed a decrease from day 0, but the difference was not significant ($P>0.05$). On day 30, all test groups showed elevated T-AOC compared to the control, though differences were not significant ($P>0.05$), with Test Group II showing the highest value. On day 45, Test Groups I, II, and III exhibited significantly higher T-AOC than the control ($P<0.05$), with Test Group III showing the highest activity, followed by Test Groups II and I, though differences among test groups were not significant ($P>0.05$). On day 60, all three test groups had significantly higher T-AOC than the control ($P<0.05$), but no significant differences existed among test groups ($P>0.05$). Compared to day 0, both control and test groups showed significant increases in T-AOC on day 60 ($P<0.05$). These results indicate that dietary flavonoid supplementation began to affect serum T-AOC after 30 days of feeding.

Table 2 Effect of flavonoids from *Allium mongolicum* Regel on T-AOC in serum of meat sheep (n=15)

Items	Day 0	Day 15	Day 30	Day 45	Day 60
Control group	3.19±0.96C	3.31±1.05D	3.29±0.87C	3.29±0.98C	3.69±0.85BC
Test group I	3.77±0.83BC	4.15±0.65bB	4.90±0.83bA	3.49±0.61DC	4.05±0.69BC
Test group II	4.99±0.86aB	6.19±1.33aA	3.42±0.85C	3.26±0.79C	4.51±1.04B
Test group III	4.36±0.87B	5.03±0.71aB	6.62±1.43aA	5.13±1.59aB	6.99±1.61aA

Note: In the same row, values with the same or no capital letter superscripts mean no significant difference (P>0.05), while different capital letter superscripts mean significant difference (P<0.05); in the same column, values with the same or no small letter superscripts mean no significant difference (P>0.05), while different small letter superscripts mean significant difference (P<0.05). The same as below.

Serum T-SOD Activity

As shown in Table 3 , on day 0, Test Group II showed the highest serum T-SOD activity, but no significant differences existed among groups (P>0.05). On day 15, T-SOD activity increased in both control and test groups, with no significant intergroup differences (P>0.05), though Test Group II showed the highest value. On day 30, all three test groups were significantly higher than the control (P<0.05), but no significant differences were observed among test groups (P>0.05). On day 45, the control group showed decreased T-SOD activity, while test groups maintained an upward trend and were significantly higher than the control (P<0.05). Test Group III was higher than other test groups, but differences were not significant (P>0.05). On day 60, Test Group III showed the highest activity, significantly higher than the control, Test Group I, and Test Group II (P<0.05), while Test Groups I and II were significantly higher than the control (P<0.05). These results demonstrate that the effect of flavonoids on serum T-SOD activity increased with dosage and became evident after 30 days of feeding.

Table 3 Effect of flavonoids from *Allium mongolicum* Regel on T-SOD activity in serum of meat sheep (n=15)

Items	Day 0	Day 15	Day 30	Day 45	Day 60
Control group	75.07±5.27A	75.79±3.47A	75.09±3.21bA	74.44±7.15bA	75.17±4.18cA
Test group I	74.43±5.68C	75.49±3.98C	79.22±3.72aB	86.78±3.17aA	87.76±3.14bA

Items	Day 0	Day 15	Day 30	Day 45	Day 60
Test group II	75.72±4.47C	76.39±7.02C	80.44±3.46aB	87.00±3.67aA	88.37±3.29bA
Test group III	75.70±2.97C	75.93±4.40C	81.46±4.33aB	88.39±4.09aA	91.08±3.99aA

Serum CAT Activity

As shown in Table 4 , CAT activity showed an increasing trend with higher flavonoid supplementation levels, and time was also a factor affecting CAT activity. On day 0, no significant differences in CAT activity were observed among groups (P>0.05), though Test Group II showed the highest value. On day 15, Test Group III exhibited significantly higher CAT activity than the control (P<0.05) and was higher than Test Groups I and II, but differences were not significant (P>0.05). On day 30, all three test groups were significantly higher than the control (P<0.05), but no significant differences existed among test groups (P>0.05). On day 45, all test groups were significantly higher than the control (P<0.05), with Test Group II showing the highest value among test groups, though intergroup differences were not significant (P>0.05). On day 60, all three test groups were significantly higher than the control (P<0.05), with no significant differences among test groups (P>0.05). These results indicate that dietary flavonoid supplementation enhanced serum CAT activity, with effects becoming evident after 45 days of feeding.

Table 4 Effect of flavonoids from *Allium mongolicum* Regel on CAT activity in serum of meat sheep (n=15)

Items	Day 0	Day 15	Day 30	Day 45	Day 60
Control group	3.21±0.41B	3.10±0.41B	3.60±0.64C	3.54±0.59C	2.95±0.61bB
Test group I	3.42±1.43abB	3.72±0.73abC	3.79±1.24aBC	3.07±0.44bB	3.98±0.55aB
Test group II	4.20±0.64aB	4.04±0.52aBC	3.50±0.43bA	4.23±0.76aA	4.38±0.75aAB
Test group III	4.41±0.56aB	3.29±0.38bAB	4.44±1.12aAB	4.74±1.25aA	5.17±0.93aA

Serum GSH-PX Activity

As shown in Table 5 , serum GSH-PX activity increased significantly after 15 days of flavonoid supplementation (P<0.05) and continued to strengthen over time. On day 0, no significant differences existed among groups (P>0.05). On day 15, Test Group I was significantly higher than the control (P<0.05), while Test Groups II and III were significantly higher than both the control and Test

Group I (P<0.05). On day 30, all three test groups were significantly higher than the control (P<0.05), with Test Group III showing the highest activity, which was also significantly higher than Test Group I and the control (P<0.05). On day 45, all three test groups were significantly higher than the control (P<0.05). On day 60, Test Groups II and III were significantly higher than both the control and Test Group I (P<0.05), while Test Group I was significantly higher than the control (P<0.05). These results demonstrate that flavonoids from *A. mongolicum* significantly affect serum GSH-PX activity in meat sheep, with effects strengthening over time.

Table 5 Effect of flavonoids from *Allium mongolicum* Regel on GSH-PX activity in serum of meat sheep (n=15) (U/mL)

Items	Day 0	Day 15	Day 30	Day 45	Day 60
Control group	84.92±2.83B	84.72±2.37cB	84.94±2.84cB	89.50±9.67cA	89.66±5.765cA
Test group I	84.14±2.52E	95.17±3.30bD	118.97±8.74bC	173.82±11.41aB	183.76±11.96bA
Test group II	84.84±3.00E	110.93±6.41aD	118.43±9.84aC	165.37±9.00bB	203.16±9.80aA
Test group III	84.23±2.73E	110.94±4.98aD	124.08±7.12aC	166.73±8.89ab	203.16±7.33aA

Serum MDA Content

As shown in Table 6 , on day 0, no significant differences in serum MDA content were observed among groups (P>0.05), though Test Group I showed relatively higher content. On day 15, all three test groups had significantly lower MDA content than the control (P<0.05), with no significant differences among test groups (P>0.05). The MDA content on day 15 was significantly lower than on day 0 (P<0.05). On day 30, Test Groups II and III maintained a decreasing trend, with significantly lower content than the control (P<0.05), while Test Group I was lower than the control but not significantly (P>0.05). On day 45, all three test groups were significantly lower than the control (P<0.05), with no significant differences among test groups (P>0.05). On day 60, all three test groups were significantly lower than the control (P<0.05), with Test Group III significantly lower than both the control and Test Group II (P<0.05), and Test Groups I and II significantly lower than the control (P<0.05). These results indicate that flavonoids from *A. mongolicum* inhibit serum MDA content, with effects becoming evident after 15 days.

Table 6 Effect of flavonoids from *Allium mongolicum* Regel on MDA content in serum of meat sheep (n=15) (nmol/mL)

Items	Day 0	Day 15	Day 30	Day 45	Day 60
Control group	5.93±1.77	6.14±2.14A	5.84±1.80A	5.53±0.81a	5.37±1.79a
Test group I	5.08±3.06a	4.71±0.82a	3.81±4.23abB	3.14±1.56bB	2.32±0.74bB
Test group II	5.86±1.18A	3.47±3.61bB	2.24±2.30bBC	2.15±0.69bBC	1.77±0.44cdC
Test group III	2.35±1.81bB	2.37±1.46bB	1.94±1.48bcB	2.06±0.97bB	2.28±1.83bB

Liver Antioxidant Capacity

As shown in Table 7 , liver T-AOC in all three test groups was significantly higher than in the control (P<0.05), with no significant differences among test groups (P>0.05). For liver T-SOD activity, Test Group III was significantly higher than the control and other test groups (P<0.05), while Test Group II was higher than the control and Test Group I, but not significantly (P>0.05). Flavonoid supplementation had no significant effect on liver CAT activity (P>0.05). For GSH-PX activity, all test groups were higher than the control, but differences were not significant (P>0.05). Regarding liver MDA content, Test Groups I and II were lower than the control, but not significantly (P>0.05), while Test Group III was significantly lower than the control (P<0.05), with no significant differences among test groups (P>0.05).

Table 7 Effect of flavonoids from *Allium mongolicum* Regel on antioxidant capacity in liver of meat sheep (n=3)

Items	T-AOC	T-SOD	CAT	GSH-PX	MDA
Control group	1.40±0.23b	32.17±0.29c	10.38±0.24	175.18±6.12	4.26±1.56a
Test group I	2.61±0.08a	32.11±1.11c	10.92±1.88	176.96±5.09	3.29±0.55ab
Test group II	2.54±0.63a	33.16±0.67bc	10.22±0.03	177.56±5.89	2.79±0.68ab
Test group III	2.79±0.89a	41.56±0.92a	10.22±0.09	177.48±5.68	2.49±0.53b

Spleen Antioxidant Capacity

As shown in Table 8 , spleen T-AOC in test groups was higher than in the control, but differences were not significant (P>0.05). For spleen T-SOD activity, Test Groups II and III were significantly higher than the control (P<0.05) and higher than Test Group I, but not significantly (P>0.05), with no significant difference between Test Groups III and II (P>0.05). Spleen CAT activity in test groups

was higher than the control, showing an upward trend, but differences were not significant (P>0.05). Test Group II showed the highest spleen GSH-PX activity, significantly higher than the control and other test groups (P<0.05), while Test Group I was significantly higher than the control and Test Group III (P<0.05). For spleen MDA content, flavonoids showed an inhibitory effect with a decreasing trend, but no significant differences were observed among groups (P>0.05).

Table 8 Effect of flavonoids from *Allium mongolicum* Regel on antioxidant capacity in spleen of meat sheep (n=3)

Items	T-AOC	T-SOD	CAT	GSH-PX	MDA
Control group	1.43±0.54	20.74±8.28b	11.21±0.31	257.22±4.13d	3.87±1.36
Test group I	1.47±0.39	25.17±2.26ab	11.38±0.31	285.11±2.90b	3.22±0.36
Test group II	1.86±0.16	30.81±1.60a	11.40±4.94	329.64±6.04a	3.05±0.59
Test group III	1.59±0.20	32.31±4.23a	11.93±0.81	271.88±8.87c	2.59±0.36

Discussion

Effects of Flavonoids on T-AOC in Serum and Tissues

T-AOC is one of the best indicators for measuring antioxidant capacity in living organisms, directly affecting health status and reflecting the metabolic state of free radicals in the antioxidant defense system. The collaborative action of antioxidants, scavenging of extracellular free radicals, and biological aging and disease can all be reflected through T-AOC. Flavonoids are natural polyphenolic compounds with high biological activity due to their multiple hydroxyl groups and conjugated aromatic system formed by three benzene rings. This unique structure can produce conjugation effects that stabilize intramolecular hydrogen bonds, thereby enhancing antioxidant capacity. Additionally, flavonoids from *A. mongolicum* may directly bind to free radicals in vivo to protect against oxidative damage.

Zhao Chunyan reported that feeding flavonoids from *A. mongolicum* to mice significantly increased serum T-AOC after 14 days and liver T-AOC after 7 days, suggesting that these flavonoids protect against lipid peroxidation by binding to free radicals or reactive oxygen species. While numerous studies have investigated flavonoids in small animals, research on antioxidant capacity in meat sheep remains limited, restricting the development of novel natural feed additives for livestock production. Therefore, investigating flavonoid effects on antioxidant

capacity in meat sheep provides a scientific foundation for livestock production and feed development.

Li Desheng et al. found that soybean flavonoids significantly increased serum T-AOC in lactating sows. Wang Wenjun et al. reported that aloe flavonoids significantly improved liver T-AOC in mice. Yang Xianyan demonstrated that seabuckthorn seed residue flavonoids enhanced T-AOC in serum and liver of menopausal rats. Our results showed that dietary supplementation with different levels of flavonoids from *A. mongolicum* for 60 days had varying effects on T-AOC in serum and tissues of meat sheep. Flavonoid supplementation significantly increased serum T-AOC in a time-dependent manner. Liver T-AOC was also significantly increased in all test groups compared to the control, while spleen T-AOC showed an upward trend but without significant effects. These findings are consistent with results from small animal studies. The enhancement of serum and liver T-AOC may be attributed to direct reaction of flavonoids with reactive oxygen species or free radicals, as well as the presence of key antioxidant functional groups such as 3' ,4' -epoxy-7-O-5-methoxyflavonol and 7-O-5,4' -dimethoxy-3-oxyhydroxyflavone in their structure.

Effects on T-SOD, CAT, and GSH-PX Activities in Serum and Tissues

Life activities continuously involve redox reactions maintained in balance by the antioxidant defense system, which includes enzymatic and non-enzymatic components. The enzymatic system comprises T-SOD, CAT, and GSH-PX, which are the primary enzymes and effective free radical scavengers. SOD catalyzes the conversion of superoxide anions (O) to hydrogen peroxide (H O), which is ultimately converted to water through synergistic action with other enzymes, reducing reactive oxygen damage. GSH-PX is a peroxide-decomposing enzyme that works with CAT and SOD to protect cell membrane structure and function.

Studies have shown that oral administration of poplar leaf flavonoids at 10, 20, and 30 mg/(kg · d) significantly increased T-SOD and GSH-PX activities in mouse serum and liver. Oral administration of *Eclipta prostrata* flavonoid extract (FEE) at 1.0 mg/mL significantly affected serum T-SOD and GSH-PX activities in mice, and at 5.0 mg/mL produced extremely significant effects, effectively preventing oxidative damage. Our results demonstrated that flavonoids from *A. mongolicum* affected serum T-SOD, CAT, and GSH-PX activities, with the 33 mg/kg group showing significant improvements that became evident after 30 days. Other test groups also enhanced these enzyme activities to varying degrees. However, no significant effects were observed on CAT activity in liver and spleen or GSH-PX activity in liver. The 33 mg/kg group significantly increased T-SOD activity in liver and spleen, while the 22 mg/kg group significantly enhanced spleen GSH-PX activity. Flavonoids may improve enzymatic defense capacity by regulating the expression of antioxidant enzyme-related genes, thereby promoting enzyme synthesis.

Effects of Flavonoids on MDA Content in Serum and Tissues

MDA is a lipid peroxide formed through lipid peroxidation reactions between free radicals and polyunsaturated fatty acids (PUFA) in biological membranes. MDA damages cell membrane integrity, induces cellular mutations, impairs the antioxidant defense system, and can cause disease or even death. Research has proven that MDA content is closely related to conditions such as atherosclerosis, as MDA binds to low-density lipoprotein (LDL) during cholesterol metabolism, causing intracellular cholesterol accumulation. MDA content directly reflects free radical levels and the degree of lipid peroxidation in vivo.

Studies on *Rhodomyrtus tomentosa* flavonoid extract in mice showed significant reduction in serum MDA content. Cai Shaofei et al. found that soybean isoflavones significantly decreased serum MDA content in ovariectomized rats. Our results indicated that flavonoids from *A. mongolicum* significantly reduced serum MDA content, with the 33 mg/kg group showing the most significant decrease compared to the control. The 33 mg/kg group also significantly reduced liver MDA content, while no significant effects were observed on spleen MDA content. The effective inhibition of MDA content by flavonoids is primarily attributed to their C6-C3-C6 structure, which can donate oxygen and electrons to effectively scavenge free radicals and protect against lipid peroxide damage.

In conclusion, dietary supplementation with 11–33 mg/kg flavonoids from *Allium mongolicum* Regel significantly improves antioxidant indices in meat sheep in both time-dependent and dose-dependent manners, with antioxidant effects becoming evident after 30 days of feeding.

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Note: Figure translations are in progress. See original paper for figures.