Effects of Mulberry Leaf Powder on Immune and Antioxidant Functions and Muscle Flavor in New Zealand White Rabbits[1]Postprint

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Abstract

This experiment aimed to investigate the effects of dietary supplementation with different levels of mulberry leaf powder on immune function, antioxidant capacity, and muscle flavor in New Zealand white rabbits. A total of 120 35-day-old weaned New Zealand white rabbits were randomly assigned to 4 groups with 3 replicates each, with 10 rabbits per replicate (half male and half female). The four groups were fed experimental diets containing mulberry leaf powder at levels of 0% (control), 15% (Test Group I), 20% (Test Group II), and 25% (Test Group III) for a 35-day experimental period. The results showed that serum acid phosphatase (ACP) activity and interleukin-6 (IL-6) content in Test Groups I and II were significantly higher than those in the control group ($P<0.05$). Serum lysozyme (LMZ) activity exhibited an upward trend with increasing mulberry leaf powder supplementation, with Test Groups II and III showing significant differences from the control group ($P<0.05$). Serum catalase (CAT) activity in Test Group I was extremely significantly higher than that in both the control group and Test Group III ($P<0.01$). Serum malondialdehyde (MDA) content showed no significant differences among the test groups ($P>0.05$), but all test groups were significantly lower than the control group ($P<0.05$). Serum total antioxidant capacity (T-AOC) displayed an upward trend with increasing mulberry leaf powder supplementation, with all test groups being significantly higher than the control group ($P<0.05$). Inosine monophosphate (IMP) content in the longissimus dorsi muscle was significantly higher in Test Groups I and II compared to the control group ($P<0.05$), while IMP content in the leg muscle was significantly higher in Test Groups II and III ($P<0.05$). The contents of essential amino acids, umami amino acids, total amino acids, and polyunsaturated fatty acids in both the longissimus dorsi and leg muscles were higher in Test Groups I and II than in the control group ($P>0.05$). It is concluded

that mulberry leaf powder effectively improves immune function, antioxidant capacity, and muscle flavor in New Zealand white rabbits, with supplementation levels of 15%~20% demonstrating optimal efficacy.

Full Text

Effects of Mulberry Leaf Powder on Immune and Antioxidant Functions and Muscle Flavor of New Zealand White Rabbits

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Abstract: This study investigated the effects of dietary supplementation with different levels of mulberry leaf powder on immune and antioxidant functions and muscle flavor in New Zealand white rabbits. A total of 120 weaned New Zealand white rabbits (35 days old) were randomly allocated into four groups with three replicates per group (10 rabbits per replicate, half male and half female). The four groups were fed experimental diets containing 0% (control), 15% (Trial Group I), 20% (Trial Group II), and 25% (Trial Group III) mulberry leaf powder for 35 days. The results showed that serum acid phosphatase (ACP) activity and interleukin-6 (IL-6) content in Trial Groups I and II were significantly higher than those in the control group ($P < 0.05$). Serum lysozyme (LMZ) activity increased with mulberry leaf powder supplementation, with significant differences observed between Trial Groups II and III and the control group ($P < 0.05$). Serum catalase (CAT) activity in Trial Group I was significantly higher than in the control group ($P < 0.01$). While no significant differences in serum malondialdehyde (MDA) content were found among treatment groups ($P > 0.05$), all supplemented groups showed significantly lower MDA levels than the control ($P < 0.05$). Serum total antioxidant capacity (T-AOC) increased with supplementation level and was significantly higher in all treatment groups compared to the control ($P < 0.05$). Inosine monophosphate (IMP) content in the longissimus dorsi muscle was significantly higher in Trial Groups I and II than in the control ($P < 0.05$), while IMP content in leg muscle was significantly higher in Trial Groups II and III ($P < 0.05$). The contents of essential amino acids, flavor amino acids, total amino acids, and polyunsaturated fatty acids in both longissimus dorsi and leg muscles were higher in Trial Groups I and II than in the control ($P > 0.05$). These findings demonstrate that mulberry leaf powder effectively improves immune and antioxidant functions and muscle flavor in New Zealand white rabbits, with optimal supplementation levels of 15–20%.

Keywords: mulberry leaf powder; New Zealand white rabbits; inosine monophosphate; flavor

Introduction

China has the largest mulberry cultivation area in the world [1], representing a rich resource that has traditionally been used exclusively as silkworm feed, resulting in substantial underutilization. Recent research on resource development has revealed that mulberry leaves contain abundant amino acids, fatty acids, vitamins, trace elements, minerals, and bioactive compounds, offering exceptional nutritional value. Comparative analysis with alfalfa shows that mulberry leaves contain higher protein and carbohydrate levels and a greater variety of amino acids [2]. Both in vivo and in vitro studies have demonstrated that mulberry leaves exhibit high digestibility as animal feed, with livestock and poultry typically achieving digestibility rates of 70–90% [2]. Combined with characteristics such as high yield, wide geographic distribution, and good palatability [3], mulberry leaves show excellent potential for development as a high-quality protein feed for livestock and poultry.

Mulberry leaves contain numerous natural bioactive substances, including flavonoids, polysaccharides, sitosterol, isoquercitrin, γ-aminobutyric acid, 1-deoxynojirimycin, and superoxide dismutase, which exhibit hypotensive, hypoglycemic, anti-allergic, antioxidant, and anti-aging effects while enhancing stress tolerance and disease resistance [4]. Dietary supplementation with mulberry leaves can also improve meat quality and flavor. Chang [5] reported that mulberry leaf powder significantly improved chicken tenderness and umami taste, enhancing overall flavor quality. Wu et al. [6] found that supplementing diets with 3%, 5%, and 7% mulberry leaf powder significantly increased tyrosine, threonine, isoleucine, phenylalanine, and histidine contents in Huainan partridge chicken meat, thereby improving flavor. Guo et al. [7] demonstrated that fresh mulberry leaves in finishing pig diets increased muscle contents of inosine monophosphate, vitamin E, linoleic acid, total amino acids, and lysine while reducing cholesterol. Shi et al. [8] observed that mulberry leaf powder supplementation in rabbits produced more delicate meat with milder odor and better taste.

This experiment was designed to investigate the effects of different dietary levels of mulberry leaf powder on immune and antioxidant functions and on muscle flavor compounds (inosine monophosphate, fatty acids, and amino acids) in different muscle tissues of New Zealand white rabbits, and to identify optimal supplementation levels to provide theoretical guidance for mulberry leaf powder utilization.

Materials and Methods

1.1 Experimental Material Mulberry leaves were harvested in Zhenjiang City, Jiangsu Province, naturally sun-dried, and ground to 80 mesh to produce mulberry leaf powder (composition: 84.99% dry matter, 17.97% crude protein, 4.47% crude fat, 21.37% crude fiber, and 12.32% crude ash). The powder was stored at room temperature in a dry environment.

1.2 Experimental Animals and Diets One hundred twenty healthy New Zealand white rabbits (35 days old, similar body weight) were randomly divided into four groups with three replicates per group (10 rabbits per replicate, half male and half female). Experimental diets were formulated based on the nutritional requirements of New Zealand white rabbits and long-term feeding experience. All four diets contained a total of 32% alfalfa meal and mulberry leaf powder combined, with mulberry leaf powder levels of 0% (control), 15% (Trial Group I), 20% (Trial Group II), and 25% (Trial Group III). The experimental period lasted 35 days. Diet composition and nutrient levels are presented in Table 1 .

1.3 Animal Management All rabbits were housed in cages and fed pelleted diets twice daily with free access to water and feed. Conventional management and immunization procedures were applied consistently across all groups. The housing environment, including natural lighting and ventilation, was identical for all treatments, with daily cleaning of rabbit facilities.

1.4 Sample Collection 1.4.1 Blood Sampling
At the end of the feeding trial, 21 healthy rabbits per group (84 total) were selected for cardiac blood collection (10 mL per rabbit). Blood samples were left to stand for 30 minutes, then centrifuged at 3,500 rpm for 15 minutes. Serum was separated and stored at -20 °C for subsequent analysis.

1.4.2 Muscle Sampling
After the feeding trial, all rabbits were fasted for 12 hours, weighed, slaughtered, and exsanguinated. The longissimus dorsi and leg muscles were collected for flavor compound analysis.

1.5 Laboratory Analyses 1.5.1 Serum Immune Indices
Serum acid phosphatase (ACP) activity was measured by colorimetry; lysozyme (LMZ) activity by turbidimetry; and interleukin-2 (IL-2) and interleukin-6 (IL-6) contents by ELISA double-antibody one-step sandwich assay. All kits were purchased from Nanjing Jiancheng Bioengineering Institute and assays were performed according to manufacturer instructions.

1.5.2 Serum Antioxidant Indices
Superoxide dismutase (SOD) activity was determined by xanthine oxidase method; catalase (CAT) activity by visible light spectrophotometry; malondialdehyde (MDA) content by thiobarbituric acid (TBA) method; and total

antioxidant capacity (T-AOC) by colorimetry. All kits were from Nanjing Jiancheng Bioengineering Institute and used per manufacturer protocols.

1.6 Muscle Flavor Compound Analysis Inosine monophosphate content was measured by high-performance liquid chromatography. Fatty acid composition was determined by gas chromatography according to GB/T 9695.2-2008. Amino acid content was analyzed using an L-8900 amino acid analyzer according to GB/T 5009.124-2003.

1.7 Statistical Analysis Data were processed using Excel 2013 and expressed as mean ± standard deviation. Statistical analysis was performed using SPSS 21.0 software for variance analysis and multiple comparisons.

Results

2.1 Effects of Mulberry Leaf Powder on Serum Immune Indices As shown in Table 2 , serum ACP activity and IL-6 content were higher in all treatment groups than in the control, with significant differences observed in Trial Groups I and II ($P < 0.05$). Serum LMZ activity increased with mulberry leaf powder supplementation, with Trial Groups II and III showing significant differences from the control ($P < 0.05$). No significant differences in serum IL-2 content were detected among groups ($P > 0.05$).

2.2 Effects of Mulberry Leaf Powder on Serum Antioxidant Indices Table 3 shows that dietary mulberry leaf powder had no significant effect on serum SOD activity ($P > 0.05$). Serum CAT activity was higher in all treatment groups than in the control, with Trial Group I showing extremely significant differences ($P < 0.01$) and also being significantly higher than Trial Group III ($P < 0.01$). While no significant differences in serum MDA content were found among treatment groups ($P > 0.05$), all supplemented groups had significantly lower MDA levels than the control ($P < 0.05$). Serum T-AOC increased with supplementation level and was significantly higher in all treatment groups compared to the control ($P < 0.05$).

2.3 Effects of Mulberry Leaf Powder on Muscle Flavor Compounds
2.3.1 Inosine Monophosphate Content
Table 4 indicates that IMP content in longissimus dorsi muscle was highest in Trial Group II, with Trial Groups I and II showing significantly higher values than the control ($P < 0.05$). IMP content in leg muscle increased with supplementation level, with Trial Groups II and III being significantly higher than the control ($P < 0.05$).

2.3.2 Fatty Acid Composition
Tables 5 and 6 show that myristic acid and palmitoleic acid contents in both

muscles did not differ significantly among groups ($P > 0.05$), while arachidonic acid in leg muscle was significantly higher in all treatment groups than in the control ($P < 0.05$). Linolenic acid content in both muscles was significantly higher in Trial Group I than in the control ($P < 0.05$). Although polyunsaturated fatty acid contents in both muscles were not significantly different from the control ($P > 0.05$), numerical values were higher in all treatment groups.

2.3.3 Amino Acid Composition

Tables 7 and 8 present 17 amino acids detected in longissimus dorsi muscle. Contents of essential amino acids, flavor amino acids, and total amino acids were higher in all treatment groups than in the control, though differences were not significant ($P > 0.05$). In longissimus dorsi muscle, lysine content was significantly higher in Trial Groups I and II compared to the control and Trial Group III ($P < 0.01$). Aspartic acid content in leg muscle was significantly higher in Trial Groups I and II than in the control and Trial Group III ($P < 0.05$). Glycine content in longissimus dorsi muscle was highest in Trial Group I, significantly exceeding the control and Trial Group II ($P < 0.05$). In leg muscle, glycine content was highest in Trial Group II, showing extremely significant differences from all other groups ($P < 0.01$).

Discussion

3.1 Effects on Immune and Antioxidant Functions Mulberry leaves are rich in flavonoids, vitamin C, superoxide dismutase, and polysaccharides that can scavenge free radicals, hydrogen peroxide, and lipid peroxides, thereby inhibiting oxidative damage and protecting animal health. Doi et al. [9] isolated nine types of flavonols from mulberry leaves with primary functions of scavenging free radicals and lipid peroxides. Du et al. [10] demonstrated through DPPH radical scavenging assays that mulberry anthocyanins are potent antioxidants. This study measured serum ACP and LMZ activities, IL-2 and IL-6 contents, SOD and CAT activities, MDA content, and T-AOC to evaluate immune and antioxidant status. ACP, LMZ, IL-2, and IL-6 participate in specific and non-specific immune responses, maintaining physiological balance and serving as important immune function indicators [11]. SOD and CAT are key components of the antioxidant system, with higher activity indicating stronger oxidative capacity. T-AOC is a comprehensive indicator of antioxidant system function, reflecting the compensatory capacity of enzymatic and non-enzymatic systems under stress [12]. MDA, as an end product of lipid peroxidation, can damage cell membrane integrity and serves as another antioxidant function indicator [13]; lower MDA content indicates stronger antioxidant capacity.

The results demonstrate that mulberry leaf powder at various levels enhanced serum ACP and LMZ activities and significantly increased IL-6 content. All treatment groups showed increased SOD and CAT activities and T-AOC, with significantly reduced MDA content compared to the control. These effects were

particularly pronounced at 15–20% supplementation levels, indicating that mulberry leaf powder substantially enhances immune and antioxidant functions.

3.2 Effects on Muscle Flavor Research indicates that meat flavor is primarily determined by two compound classes: amino acids and nucleotides (including 5' -inosine monophosphate, 5' -xanthosine monophosphate, and 5' -guanosine monophosphate). Inosine monophosphate is the main component of umami taste, enhancing flavor while suppressing sour and bitter tastes, and is internationally recognized as a key indicator of meat quality [14,15]. Amino acid composition, content, and ratio significantly influence meat flavor [16], with glutamate, aspartate, glycine, alanine, and arginine being essential precursors for meat aroma [17]; higher contents correlate with better flavor. Fat content and distribution also affect muscle flavor [18], with fatty acid composition being a critical determinant of fat physicochemical properties and flavor quality [19,20]. Migdal et al. [21] reported that higher polyunsaturated fatty acid content in carcass fat improves meat flavor and nutritional value.

This study demonstrates that dietary supplementation with 15–20% mulberry leaf powder increased muscle IMP and unsaturated fatty acid contents while modifying amino acid composition and ratios, particularly increasing flavor amino acid contents. These results confirm that mulberry leaf powder effectively improves meat flavor and nutritional quality.

In conclusion, dietary supplementation with 15–20% mulberry leaf powder not only enhances immune and antioxidant functions in New Zealand white rabbits but also increases muscle inosine monophosphate content and modifies amino acid and fatty acid profiles, thereby improving rabbit meat flavor and nutritional value.

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