Effects of Different Levels of Pomelo Leaf Diet on Serum Biochemical Parameters, Digestive and Immune Functions in Growing Meat Rabbits (Postprint)

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Abstract

This experiment aimed to investigate the effects of diets containing different levels of pomelo leaves on the physiological functions of growing meat rabbits, and to further evaluate the feasibility of pomelo leaves as a feed ingredient for rabbits. A total of 120 healthy 35-day-old French Ira commercial rabbits with the same genetic background were selected. After a 7-day acclimation period, they were randomly divided into 4 groups according to the principle of similar body weight [(1,179.25 ± 53.74) g], with 10 replicates per group and 3 rabbits per replicate (cage). The control group was fed a basal diet without pomelo leaves, while the experimental groups were fed experimental diets containing 6% (Group I), 12% (Group II), and 18% (Group III) pomelo leaves, respectively. The four diets were essentially equal in energy, nitrogen, and fiber content. The experimental period lasted 28 days. The results showed: 1) Except that serum globulin content in Group III was significantly higher than that in the control group and Group II ($P < 0.05$), and the albumin/globulin ratio in Group III was significantly lower than that in Group II ($P < 0.05$), there were no significant differences among groups in other serum biochemical indices ($P > 0.05$). 2) Dietary supplementation with pomelo leaves had no significant effect on digestive organ development indices in growing meat rabbits ($P > 0.05$). Except that the propionic acid proportion in the later stage of the experimental groups and the pH and ammonia nitrogen concentration of cecal contents in the middle stage of Group III were significantly lower than those in the control group ($P < 0.05$), and the pH in the middle stage of Group II was significantly higher than that of Group III ($P < 0.05$), there were no significant differences among groups in other cecal fermentation parameters ($P > 0.05$). 3) Except that serum immunoglobulin A level in Group III was significantly lower than

that in the control group and Group I ($P < 0.05$), and serum immunoglobulin A level in Group I was significantly higher than that in the control group and Group II ($P < 0.05$), there were no significant differences among groups in other immune indices ($P > 0.05$). It was concluded that ad libitum feeding of diets containing 6%–18% pomelo leaves to growing meat rabbits for 4 weeks had no significant negative effects on serum biochemical indices, digestive function, and immune function, further suggesting that pomelo leaves can be used as a feed ingredient for rabbits.

Full Text

Effects of Diets Containing Different Levels of Pummelo Leaves on Serum Biochemical Indices, Digestion and Immune Functions of Growing Rabbits

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Abstract

This study investigated the effects of diets containing different levels of pummelo leaves on the physiological function of growing rabbits to evaluate the feasibility of using pummelo leaves as a feed ingredient. One hundred twenty healthy 35-day-old French Ira rabbits with identical genetic backgrounds were used. After a 7-day adaptation period, the rabbits were randomly allocated into four groups (ten replicates per group, three rabbits per replicate) based on similar body weight [(1179.25±53.74) g]. The control group received a basal diet without pummelo leaves, while the experimental groups received diets containing 6% (Group I), 12% (Group II), and 18% (Group III) pummelo leaves, respectively. All four diets were formulated to have similar energy, nitrogen, and fiber contents. The experimental period lasted 28 days.

The results showed: (1) Serum globulin content in Group III was significantly higher than in the control and Group II ($P<0.05$), and the albumin/globulin ratio in Group II was significantly higher than in Group III ($P<0.05$). No significant differences were observed in other serum biochemical indices among groups ($P>0.05$). (2) Dietary pummelo leaf supplementation had no significant effect on digestive organ development indices ($P>0.05$). The propionic acid ratio in the later stage and the pH and ammonia nitrogen concentration of cecal contents in Group III during the mid-stage were significantly lower than in the control group ($P<0.05$), while the pH in Group II during the mid-stage

was significantly higher than in Group III (P<0.05). Other cecal fermentation parameters showed no significant differences among groups (P>0.05). (3) Serum immunoglobulin A (IgA) level in Group III was significantly lower than in the control and Group I (P<0.05), while IgA level in Group I was significantly higher than in the control and Group II (P<0.05). No significant differences were found in other immune indices among groups (P>0.05). In conclusion, feeding growing rabbits diets containing 6%–18% pummelo leaves for four weeks did not negatively affect serum biochemical indices, digestive function, or immune function, suggesting that pummelo leaves can be used as a feed ingredient for rabbits.

Key words: growing rabbits; pummelo leaves; serum biochemical indices; digestive function; immune function

Introduction

Annual pummelo leaf production in China is estimated to reach millions of tons, yet most leaves are burned or left to decompose naturally, wasting resources and polluting the environment. Research indicates that pummelo leaves have reasonable fiber composition and crude protein content similar to alfalfa meal. The flavonoid compounds such as naringin present in pummelo leaves can reduce blood lipids and enhance immunity, making them a potential feed ingredient for rabbits. Previous studies have shown that dietary composition affects blood physiological and biochemical indices, digestive organ development and function, and immune function in rabbits. A diet containing 12% pummelo leaves improved growth performance in growing rabbits, increasing average daily feed intake (ADFI), average daily gain (ADG), and feed conversion ratio by 5.16%, 9.53%, and 7.74%, respectively, compared to the control group. Furthermore, diets containing up to 18% pummelo leaves improved apparent total tract digestibility of nutrients and slaughter performance, with 12% pummelo leaves showing optimal results. However, the effects of pummelo leaf-containing diets on the physiological function of growing rabbits remain unreported. Therefore, this study investigated the effects of different dietary pummelo leaf levels on serum biochemical indices, digestive function, and immune function to further evaluate the feasibility of using pummelo leaves as a rabbit feed ingredient.

1.1 Experimental Design

A single-factor experimental design was employed. After a 7-day adaptation period, 120 healthy 35-day-old weaned French Ira rabbits with identical genetic backgrounds were randomly divided into four groups based on similar body weight [(1179.25±53.74) g], with ten replicates per group and three rabbits per replicate. The groups received diets containing 0% (control), 6% (Group I), 12% (Group II), and 18% (Group III) pummelo leaves, respectively. All four experimental diets had similar digestible energy (10.62, 10.72, 11.00, and

11.21 MJ/kg), crude protein (17.52%, 16.77%, 16.93%, and 17.04%), and crude fiber (11.96%, 12.93%, 11.94%, and 10.87%) contents. The experimental period lasted 28 days. The source and nutrient content of pummelo leaves and the composition and nutrient levels of experimental diets are described in reference [20]. All diets were pelleted with a diameter of 2.50 mm.

1.2 Animal Management

Rabbits were housed in metal mesh cages (60 cm × 60 cm × 45 cm) in the rabbit facility of the Animal Nutrition Institute teaching and research base at Sichuan Agricultural University. The ambient temperature was (22.93±2.47)°C, relative humidity was (75.53±9.12)%, with natural lighting and ventilation. Animals received routine immunization and management. Feed was provided four times daily (08:00, 12:00, 16:00, and 20:00) with free access to feed and water.

1.3 Sample Collection and Analysis

1.3.1 Sample Collection On day 15 of the experiment, rabbits were weighed at 08:00. Four rabbits from each group with body weights close to the group average were selected for cardiac blood collection (5 mL). Blood samples were centrifuged at 3000 r/min for 15 minutes, and serum was collected, aliquoted into EP tubes, and stored at -20°C for determination of routine serum biochemical indices and some immune indices. After blood collection, rabbits were euthanized by ear marginal vein injection of air, and samples were collected (stomach, stomach contents, small intestine, cecum, cecal contents, liver, spleen, and thymus) and weighed. Cecal content pH was measured immediately.

On day 29 at 08:00, another four rabbits per group were selected for sampling (without blood collection) using the same procedures.

1.3.2 Index Determination 1.3.2.1 Serum Biochemical Indices

Serum total protein (TP), globulin (GLB), albumin (ALB), urea nitrogen (UN), glucose (GLU), creatinine (CR), triglyceride (TG), total cholesterol (TC), high-density lipoprotein (HDL), low-density lipoprotein (LDL), and very low-density lipoprotein (VLDL) were determined by colorimetric methods. Serum alanine aminotransferase (ALT), aspartate transaminase (AST), and alkaline phosphatase (ALP) activities were determined by enzymatic colorimetric methods. These indices were measured using a Roche P800 modular biochemistry analyzer by Sichuan Ya' an People' s Hospital and Sichuan Agricultural University Animal Hospital.

1.3.2.2 Digestive Organ Development Indices

Digestive organ ratio (%) = 100 × organ weight (g) / live body weight (g)
Stomach (cecal) content ratio (%) = 100 × stomach (cecal) content weight (g) / live body weight (g)

1.3.2.3 Cecal Fermentation Parameters

Cecal content pH was measured immediately after collection using a pH-3B acidity meter [21]. Total volatile fatty acid (TVFA) content was determined using a Varian CP-3800 gas chromatograph [22] after pretreatment of cecal contents. Ammonia nitrogen (NH -N) concentration was determined by colorimetry at 700 nm wavelength [23].

Acetic (propionic, butyric) acid ratio (%) = 100 × acetic (propionic, butyric) acid / (acetic acid + propionic acid + butyric acid)

1.3.2.4 Immune Indices

Serum immunoglobulin A (IgA), immunoglobulin G (IgG), and immunoglobulin M (IgM) levels were determined using enzyme-linked immunosorbent assay kits purchased from Nuoyuan Company.

Thymus (spleen) index (%) = 100 × thymus (spleen) weight (g) / live body weight (g)

1.4 Data Processing and Statistical Analysis

Data were processed using Excel 2003 and analyzed using SPSS 19.0 statistical software for variance analysis and Duncan' s multiple comparison test.

Results

2.1 Effects of Different Dietary Pummelo Leaf Levels on Serum Biochemical Indices

As shown in Table 1 , serum globulin content in Group III was significantly higher than in the control group and Group II ($P<0.05$), while the albumin/globulin ratio in Group II was significantly higher than in Group III ($P<0.05$). No significant differences were observed in other serum biochemical indices among groups ($P>0.05$). However, numerically, serum TG and VLDL contents in the experimental groups were lower, while TP, ALB, and HDL contents were higher than in the control group.

Table 1 Effects of diets containing different levels of pummelo leaves on serum biochemical indices of growing rabbits

Items	Control group	Group I	Group II	Group III
Total protein TP/(g/L)	53.78±3.45	56.85±1.12	54.78±3.20	58.20±1.43
Albumin ALB/(g/L)	33.45±3.19	35.30±0.42	35.53±2.74	34.78±0.69
Globulin GLB/(g/L)	20.33±1.08ab	21.55±1.42bc	19.25±1.87a	23.43±0.85c

Items	Control group	Group I	Group II	Group III
Albumin/Globulin A/G	1.65±0.17ab	1.64±0.17ab	1.86±0.23b	1.49±0.04a
Glucose GLU/(mmol/L)	7.26±0.26	7.71±0.79	6.93±0.46	7.68±0.79
Urea nitrogen UN/(mol/L)	3.75±2.35	2.56±0.25	4.02±2.30	2.76±0.62
Creatinine CR/(mol/L)	63.25±7.14	62.75±10.24	59.75±6.08	66.00±2.94
Total cholesterol TC/(mmol/L)	1.81±0.56	1.46±1.08	0.69±0.21	0.91±0.64
Triglyceride TG/(mmol/L)	1.94±0.24	1.86±0.51	1.71±0.22	1.05±0.15
High-density lipoprotein HDL/(mmol/L)	1.11±0.46	0.91±0.24	0.82±0.21	0.88±0.08
Low-density lipoprotein LDL/(mmol/L)	0.81±0.11	1.04±0.28	0.84±0.53	0.86±0.21
Very low-density lipoprotein VLDL/(mmol/L)	0.21±0.18	0.09±0.02	0.14±0.04	0.04±0.02
Alanine aminotransferase ALT/(U/L)	45.25±15.31	45.25±7.27	36.75±16.00	52.50±2.65
Aspartate transaminase AST/(U/L)	61.25±15.76	73.75±18.06	52.75±26.01	86.25±18.46
ALT/AST ratio AL/AS	0.80±0.41	0.64±0.17	0.77±0.24	0.63±0.15

Items	Control group	Group I	Group II	Group III
Alkaline phosphatase ALP/(U/L)	150.50±59.78	195.00±51.94	159.75±58.23	117.75±73.92

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

2.2 Effects of Different Dietary Pummelo Leaf Levels on Digestive Organ Development and Cecal Fermentation Parameters

As shown in Table 2 , dietary pummelo leaf supplementation had no significant effect on digestive organ development indices of growing rabbits throughout the experimental period (P>0.05). Table 3 shows that the propionic acid ratio in the later stage (day 29) and the pH and ammonia nitrogen concentration of cecal contents in Group III during the mid-stage (day 15) were significantly lower than in the control group (P<0.05). Additionally, the pH of cecal contents in Group II during the mid-stage was significantly higher than in Group III (P<0.05). No significant differences were observed in other cecal fermentation parameters among groups (P>0.05).

Table 2 Effects of diets containing different levels of pummelo leaves on development indices of digestive organs of growing rabbits

Items	Control group	Group I	Group II	Group III
Stomach contents ratio	2.92±0.72	2.07±0.57	1.17±0.11	0.86±0.13
Stomach ratio	5.55±1.65	5.03±1.45	1.56±0.57	1.54±0.32
Caecal contents ratio	3.83±0.79	2.96±0.15	3.09±0.14	2.05±1.01
Caecal ratio	2.15±0.23	1.72±0.50	1.17±0.05	1.17±0.10
Small intestine ratio	1.18±0.07	0.97±0.12	0.79±0.28	1.02±0.07
Liver ratio	4.91±1.09	6.81±0.65	6.31±1.17	4.60±1.14
Spleen ratio	4.72±0.66	5.07±0.65	1.41±0.25	1.56±0.21

Items	Control group	Group I	Group II	Group III
Thymus ratio	1.98±0.24	1.21±0.10	1.30±0.21	1.33±0.27

Table 3 Effects of diets containing different levels of pummelo leaves on caecal fermentation parameters of growing rabbits

Items	Control group	Group I	Group II	Group III
pH	5.74±0.03b	5.54±0.15ab	5.61±0.28b	5.32±0.13a
NH - N/(mmol/L)	6.49±0.11	6.38±0.15	6.33±0.14	6.43±0.12
TVFA/(mmol/L)	29.17±6.39b	24.28±1.50ab	24.71±5.10ab	20.58±3.90a
Acetic acid ratio/%	28.39±7.05	28.78±1.35	23.83±1.39	26.21±6.54
Propionic acid ratio/%	42.53±3.26	55.91±6.56	43.58±18.62	58.52±14.85
Butyric acid ratio/%	45.95±2.59	45.20±10.97	48.14±7.27	46.80±10.82
Acetic acid/(propionic+butyric acid)	80.94±3.25	80.83±1.78	82.54±5.28	80.48±2.93
Mid-stage	80.52±1.22	81.90±3.08	80.90±4.05	80.30±0.86
Later stage	7.40±2.67	5.82±1.55	7.53±4.88	4.88±1.49
Mid-stage	8.97±1.25b	6.49±1.33a	6.24±0.30a	7.08±0.31a
Later stage	11.66±3.93	13.35±3.03	9.93±2.41	14.64±2.09
Mid-stage	10.52±2.12	11.61±1.83	12.86±3.75	12.62±0.99
Later stage	4.38±1.04	4.05±0.47	5.06±1.47	4.21±0.73
Mid-stage	4.15±0.31	4.67±1.16	4.42±1.15	4.08±0.22

2.3 Effects of Different Dietary Pummelo Leaf Levels on Immune Indices

As shown in Table 4 , serum IgA level in Group III was significantly lower than in the control group and Group I ($P<0.05$), while IgA level in Group

I was significantly higher than in the control group and Group II ($P<0.05$). No significant differences were observed in other immune indices among groups ($P>0.05$).

Table 4 Effects of diets containing different levels of pummelo leaves on immune indices of growing rabbits

Items	Control group	Group I	Group II	Group III
Thymus index	0.21±0.08	0.19±0.01	0.15±0.06	0.17±0.06
Spleen index	0.07±0.01	0.08±0.01	0.07±0.01	0.08±0.01
IgA/(g/mL)	68.24±11.58b	90.12±15.45c	51.56±16.63ab	41.30±9.67a
IgG/(g/mL)	1876.96±491.35	1458.97±835.15	1424.08±233.95	1998.61±657.42
IgM/(g/mL)	346.77±17.09	392.23±63.90	445.56±104.75	359.48±27.50

Discussion

3.1 Effects on Serum Biochemical Indices

Blood is a crucial component of the animal' s internal environment, and its composition changes can reflect metabolic status and health condition, influenced by factors such as dietary composition [8,24]. This study found no significant differences in most serum biochemical indices among groups. However, serum TG and VLDL contents in experimental groups were numerically lower, while HDL content was higher than in the control group, possibly related to naringin in pummelo leaves. Lan Song [25] reported that naringin content in pummelo leaves is approximately 1%, with higher levels in young leaves than old leaves. Seo et al. [5] reported that naringin significantly increased serum high-density lipoprotein cholesterol content and its proportion in total cholesterol while decreasing TG content in rats. In contrast, Yang Ying et al. [26] and Chanet et al. [27] found that naringin had no significant effect on blood lipid content in hyperlipidemic rats. This study also found that serum ALB and TP contents in experimental groups were higher than in the control group, similar to results reported for alfalfa meal supplementation [7]. Additionally, serum GLB content in the 18% pummelo leaf group was significantly higher than in the control and 12% groups, while serum ALT and AST activities showed no significant differences from the control group, suggesting that further research is needed to determine whether high-dose (18%) pummelo leaf supplementation affects liver function in growing rabbits. These results indicate that dietary pummelo leaf supplementation has no obvious negative effects on serum biochemical indices and may even promote protein metabolism and improve animal growth.

3.2 Effects on Digestive Organ Development and Function

The relative proportions of digestive organs and their contents are important indicators for evaluating digestive organ development in rabbits [8-9,28]. This study found that dietary pummelo leaf supplementation had no obvious adverse effects on digestive organ development in growing rabbits, consistent with reports on chicory pulp supplementation by Maertens et al. [29]. The cecum is one of the most important digestive organs in rabbits, and its internal environment significantly influences nutrient utilization and intestinal health [7-9,11-17]. Cecal content pH, TVFA content, and NH -N concentration are important indicators for evaluating cecal fermentation, affected by age, physiological status, and dietary composition [7-9,11-17]. This study found that dietary supplementation with 6%, 12%, and 18% pummelo leaves had no obvious adverse effects on cecal fermentation function and may even have exerted some beneficial effects. The specific reasons remain unclear but may be related to naringin in pummelo leaves and require further investigation. These results are similar to reports by Prasad et al. [14] and Volek et al. [12] that dietary mulberry leaves and chicory root increased cecal TVFA content and decreased pH in rabbits. Overall, dietary pummelo leaf supplementation had no adverse effects on digestive organ development and function in growing rabbits and did not affect their growth potential, consistent with previous findings that pummelo leaf supplementation did not significantly affect average daily gain or feed conversion ratio [20].

3.3 Effects on Immune Function

Immune organ indices and blood immunoglobulin levels are important indicators reflecting immune status [30-32]. This study found no significant differences in immune organ indices among groups, possibly because dietary fiber components and active ingredients were similar across groups. Chen Jihong [7] reported that thymus and spleen indices in rabbits increased with alfalfa meal supplementation. Chao et al. [10] and Tao Zhiyong [15] reported that thymus and spleen indices increased with dietary neutral detergent fiber and acid detergent fiber levels. This study also found that serum IgA level in the 6% pummelo leaf group was significantly higher than in the control group, suggesting that appropriate pummelo leaf supplementation may enhance immune function in growing rabbits. This is consistent with similar reports [33-34]. Liu Keyuan et al. [33] reported that serum IgA and IgG levels in rabbits increased with garlic stem supplementation. Zhu et al. [34] reported that serum IgG content decreased with decreasing dietary fiber/starch ratio. These results indicate that dietary pummelo leaf supplementation has no obvious adverse effects on immune function in growing rabbits.

In conclusion, under the conditions of this study, feeding growing rabbits diets containing 6%–18% pummelo leaves for four weeks did not negatively affect serum biochemical indices, digestive organ development and function, or immune function, and even exerted some beneficial effects. Combined with previous growth trial results, these findings further demonstrate that pummelo leaves

can be used as a feed ingredient for rabbits in practical production.

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