



Increased IgA and IgG serum levels using a novel yam–boxthorn noodle in a BALB/c mouse model

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Abstract

To determine whether yam–boxthorn noodle, a newly developed functional noodle, has immunomodulatory effects in vivo, we measured the changes in visceral organ weight, immunoglobulin (Ig) A, IgE, IgG, IgM serum level and IgA level in the intestinal lavage fluid of female BALB/c mice after continuously consuming the test diet for 5 weeks. We found that body weights and absolute and relative organ weights (lung, heart, liver, spleen and kidney) in female BALB/c mice did not significantly change compared with those from the control group. The IgA and IgG serum levels in the experimental group significantly increased in a dose-dependent manner when the yam–boxthorn noodle concentration in the AIN 76 diet rose from 3% to 30%. However, the IgE and IgM level in the serum and the IgA level in the intestinal lavage fluid did not significantly change. These experiments demonstrate that the functional noodle, yam–boxthorn noodle, exhibits immunomodulatory effects in vivo with increasing serum antibody levels, especially in IgA and IgG. These results are valuable for future nutraceutical and immuno-pharmacological use.

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1. Introduction

Oriental noodles have been developed for several centuries and play an important role in the diet of many Asians (Hou, 2001). However, most traditional noodles are made from simple ingredients, i.e., wheat flour, water, salt, and alkali (Baik and Lee, 2003). We have developed a novel noodle, yam–boxthorn noodle, which is developed from enriched yam (*Dioscorea alata* L.) and boxthorn (*Lycium barbarum*). The yam–boxthorn noodle promotes health, especially immunomodulatory and antioxidant effects (Liou, 2003). It was proven that the yam–boxthorn noodle demonstrates both hypolipidaemic and anti-oxidant effects on female BALB/c mice

through a five week feeding trial (Lin et al., 2005, 2006). However, the immunomodulatory function of the yam–boxthorn noodle has not yet been evaluated. The functional products with specific health benefits from immunological activity have been the fastest-growing sector, moving swiftly into the mainstream. Therefore, it becomes so urgent to study the effects of the yam–boxthorn noodle on immunomodulation in vivo.

Herbal medicines used for immunomodulation have gained rapid development in recent years around the globe. Most Chinese medicinal ingredients promote cellular or humoral immune responses by promoting lymphocyte proliferation and serum antibody titer after vaccination in chicken (Kong et al., 2004). *Ginkgo biloba* extract, EGb 761, has a markedly immuno-stimulating activity on the nonspecific and humoral immune responses of hypothalamic-pituitary-adrenal axis activation model in stressed rats (Villasenor-Garcia et al.,

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2004). *Aeginetia indica* Roxbert extracts used in Thailand to treat diabetes and dermal swelling has a T cell stimulatory activity (Auttachoat et al., 2004a) and immunomodulatory activities in humoral immunity, innate immunity and hematology in vivo (Auttachoat et al., 2004b). *Amaranthus spinosus* (thorny amaranth) water extracts have traditionally been used for treating diabetes in Taiwan to directly stimulate proliferation of B lymphocytes in vitro and increase humoral immunity in vivo (Lin et al., 2005, 2006). Mucopolysaccharide of yam (*Dioscorea batatas* Decne) increases immunomodulatory activities on splenocytes and peritoneal macrophages in vitro (Choi et al., 2004). A kind of homogeneous glycoconjugate, LbGp2, isolated from the fruit of *Lycium barbarum* L. (boxthorn) is shown with good immunoactivity and anti-oxidative activity in vitro (Peng et al., 2001). Though extensive studies of selected functional foods or ingredients have been conducted in biochemistry, immunology and clinical trials, the scientific evidences for most traditional herbs or their medical food products in vivo remain almost unknown (Xu, 2001).

Humoral immunity, especially the antibody secretion from different compartments in body, plays an important part to defend infectious diseases and to maintain the memory as the same infectious agents revisit in vivo. The gut mucosa is the major site of antibody production in human, including secretory immunoglobulin (Ig) A and local IgG, and the IgA⁺ cells in the gut lamina propria are generated and isotype-switched in situ from B220⁺ IgM⁺ lymphocytes (Golby and Spencer, 2002). Three different antiviral functions of IgA in epithelium, including immune exclusion, intracellular neutralization and virus excretion, suggest that total or specific IgA antibody reacted to the particular viral component is an important indicator of the magnitude of the antiviral effect (Yan et al., 2002). Antibodies to human rotavirus (HRV) (IgM, IgA or IgG) are detected in serum and intestinal contents of pigs after they are challenged with the virus, and the antibody titers in intestinal contents, although lower, show similar trends to the serum responses (To et al., 1998). However, the serum IgA is also contributed to intestinal lymph IgA, and vice versa, in a porcine model (Vaerman et al., 1997). Both IgG and IgA antibodies are also implicated in host defenses against bacterial infections, such as *Neisseria meningitidis* serogroup B, in human (Vidarsson et al., 2001). If non-specific or specific antibodies, including IgM, IgA, IgG, are moderately induced, they could play an important role for protective immunity. Even though oral tolerance within the T cell system may represent an important regulatory mechanism for normal immunity to ingested foods in healthy subjects (Husby, 2000), some functional foods produced from probiotics (bacteria from the genera *Bifidobacterium* and *Lactobacillus*, and yeast) or prebiotics (traditionally grains, particularly whole grains, used in nutrition as

carriers of macro- and micro-elements, proteins, fiber, and vitamins), potentially result in faster isotype switching within IgA and IgM antibodies and are accompanied by the growth of cells producing IgM antibodies (Tomasik and Tomasik, 2003). The yam–boxthorn noodle, a newly developed, medical plant-based functional product, enriched with cereal constitutes such as yam (*Dioscorea alata* L.) and boxthorn (*Lycium barbarum*), may be regarded as a prebiotics and useful to increase our protective immunity daily.

It is difficult to wholly evaluate both the cellular and humoral immune responses in vivo. Actually, both male and female animals were administrated into different animal models. Male and female animals have similar immune responses in many situations. However, we found that immune sensitivities of female BALB/c mice were higher than those of male BALB/c mice according to our preliminary data and other published reports. The female BALB/c mouse model was one of the most popular animal models to investigate immune responses in vivo for the moment. Thus, only female BALB/c mice were used to evaluate the effects of yam–boxthorn noodles on immune responses in vivo to moderately reduce the use amount of animals in this study. The aims of this study were designed to examine both the antibody titers from serum and intestinal lavage fluid from female BALB/c mice through a five week feeding trial of the yam–boxthorn noodle.

2. Material and methods

2.1. Preparation of yam–boxthorn noodles

The yam–boxthorn noodle, a newly developed noodle in Taiwan, enriched with yam (*Dioscorea alata* L.) and boxthorn (the fruit of *Lycium barbarum*) was prepared as described by Lin et al. (2005, 2006). In brief, a new variety of *D. alata* L. (Tainong No. 2) and boxthorn (*L. barbarum*) were treated, lyophilized, milled into powder and then passed through a 200 mesh sieve, respectively. The yam–boxthorn noodle was prepared from the patent flour, yam powder, boxthorn powder, sodium chloride and water in 600, 330, 70, 20 and 320 g proportions, respectively. The ingredients were then mixed to form dough (Liou, 2003) and finally cut into strips about 1.40 mm × 30 cm. The yam–boxthorn noodles were freshly prepared and then placed into plastic bags and stored at 4 °C until use (Baik and Lee, 2003; Liou, 2003). The moisture, protein, and mineral (ash) content of the yam–boxthorn noodles were determined according to Approved Methods 44-15A, 46-30, and 08-01 (AACC, 2000; Park et al., 2003). The protein, mineral, lipid, crude fiber, and carbohydrate content of the yam–boxthorn noodle, based on the dry weight, were 14.9%, 3.28%, 0.22%, 28.5%, and 53.2%, respectively.

2.2. Experimental animals and yam–boxthorn noodle diets

The female BALB/c mice (6 weeks old) were obtained from the National Laboratory Animal Center, National Applied Research Laboratories, National Science Council in Taipei, ROC and maintained in the Department of Food Science at National Chung Hsing University College of Agriculture and Natural Resources in Taichung, Taiwan, ROC. The mice were housed and kept on a chow diet (lab standard diet) for 2 weeks before feeding the experimental diet. The animal room was kept on a 12-h-light and 12-h-dark cycle with constant temperature ($25 \pm 2^\circ\text{C}$) and humidity. After the acclimation period, the mice were divided into four groups ($n = 9$). Each group was fed with one of the yam–boxthorn noodle diets for 5 consecutive weeks ad libitum. The specified experimental diets consisted of AIN 76 semi-purified diets that were partially replaced, respectively, by 0%, 3%, 12%, and 30% dry yam–boxthorn noodle powder. The chemical analyses of ingredients and experimental feeds were described as Lin et al. (2005, 2006). The composition of the yam–boxthorn noodle diets is given in Table 1. The contents included casein, corn starch, cellulose, soybean oil, and mineral mix in AIN 76 formula adjusted using the protein, carbohydrate, crude fiber, lipid, and ash content of the dry noodle powder to maintain the nutritional balance in the specified AIN 76 experimental feeds. Mice food intake and body weight were measured every 3 days during the experimental period.

2.3. Sample collection and analytical methods

2.3.1. Serum collection

On day 35, after 14 h of fasting, the animals were weighed and anaesthetized with an ether. The animals were immediately exsanguinated by retro-orbital venous plexus puncture and the blood was collected into a

1.5 ml vial. The blood was allowed to stand for 2 h at room temperature, then centrifuged at 12,000g for 15 min at 4°C to separate the serum. The serum were collected and stored at -30°C for later analysis.

2.3.2. Tissue analysis

Immediately after the serum collection, the animals were sacrificed using CO_2 inhalation for tissue studies. The thoracic and abdominal cavities were opened aseptically, and the organs such as heart, lung, liver, spleen, and kidney were removed and weighted. To evaluate the possible effects of yam–boxthorn noodle diets on the body, organ and relative organ weights, the visceral organs weights in each experimental group were expressed as absolute and relative organ weights, respectively.

2.3.3. Intestinal IgA collection

Immediately after the visceral organs of mice were excised and weighted, the small intestine was removed from the duodenum to the terminal ileum and flushed with 10 mL calcium- and magnesium-free Hank's balanced salt solution (HBSS) (Biochrom AG, Berlin) to remove intestinal contents. The contents were collected and stored at -30°C for later analysis of intestinal IgA levels by ELISA (Ikeda et al., 2003).

2.3.4. Antibody quantification by ELISA

Serum antibody levels of IgA, IgE, IgG, and IgM and intestinal IgA were analyzed using the mouse IgA, IgE, IgG, and IgM ELISA Quantitation Kit (catalog number: E90-103, E90-115, E90-131, and E90-101, Bethyl Laboratories, Inc., TX, USA). Briefly, IgA, IgE, IgG, and IgM levels in serum and IgA secretion in intestinal lavage were measured in a sandwich enzyme-linked immunoabsorbent assay (ELISA). Microtitre plate wells (flat bottom; high binding; Costar, Corning, NY, USA) were coated with 100 μL goat antimouse IgA-, IgE-, IgG-, and IgM-affinity purified antibody (Bethyl Laboratories, Inc.) at a concentration of 10 $\mu\text{g}/\text{mL}$ carbonate buffer (0.05 M sodium carbonate, pH 9.6) for 60 min at room temperature. After washing two times in wash buffer (50 mM Tris, pH 8.0, 0.1 M NaCl, 0.05% Tween 20), 200 μL blocking solution (1% BSA, 50 mM Tris, pH 8.0, 0.15 M NaCl) were post-coated to each well for 30 min. After washing two times in wash buffer (50 mM Tris, pH 8.0, 0.1 M NaCl, 0.05% Tween 20), 100 μL purified mouse IgA, IgE, IgG, and IgM standards (Bethyl Laboratories, Inc.) serially diluted in diluent (1% BSA, 50 mM Tris, pH 8.0, 0.15 M NaCl, 0.05% Tween 20) was added to each well and the plate was incubated for 60 min at room temperature. After washing two times again, the plate was further incubated with 100 μL horseradish peroxidase-conjugated goat antimouse IgA, IgE, IgG, and IgM antibody (Bethyl Laboratories, Inc.) per well for 60 min. After washing three times again The plate was developed by adding 100 μL

Table 1
Composition of the semi-purified yam–boxthorn noodle diets

Ingredients (g/kg feed)	Yam–boxthorn noodles in AIN 76 diet			
	0%	3%	12%	30%
Casein	200	195.5	182.2	155.4
DL-methionine	3	3	3	3
Corn starch	325	309	261.2	165.5
Sucrose	325	325	325	325
Cellulose	50	41.5	15.8	–
Soybean oil	50	49.9	49.7	49.3
AIN76 mineral mix	35	34.02	31.06	25.16
AIN76 vitamin mix	10	10	10	10
Choline bitartrate	2	2	2	2
Dry noodle powder ^a	–	30	120	300

^a Dry noodle powder was obtained from fresh yam–boxthorn noodle air dried in oven (50°C) overnight, and contained 14.9% crude protein, 3.28% ash, 0.22% crude fat, 28.5% crude fiber and 53.2% carbohydrate.

substrate solution containing 3,3',5,5'-tetramethyl benzidine (TMB) per well and incubated for 5–30 min (Johnson et al., 2003). The reaction was stopped by adding 100 μ L, 2 M sulfuric acid per well and the absorbance at 450 nm was measured with an ELISA reader (ASYS Hitech GmbH, Austria).

2.4. Statistical analysis

The yam–boxthorn noodle diet effect was assayed using unpaired Student's *t*-test with two tails to test if significant differences were present from the control group. Values are expressed as group averages and standard deviations ($n = 9$). Differences were considered statistically significant if $P \leq 0.05$.

3. Results

3.1. Feed intake, body weight gain, absolute visceral, and relative visceral weights

Feed intake, body weight gain, absolute visceral and relative visceral weights of female BALB/c mice fed with yam–boxthorn noodle diets through the 5 weeks of experimental period are shown in Table 2. The mean feed intake in different groups of feeding yam–boxthorn noodle diets through the experimental period was 4.05 ± 0.79 , 4.44 ± 0.18 , 4.23 ± 0.16 and 4.32 ± 0.27 , respectively. There was not significantly different among the four diet groups. The increases in body weight gained over the 35 days of feeding for different concentrations of yam–boxthorn noodle diets was from $21.19 \pm$

13.16% to $24.77 \pm 9.22\%$. However, there were also no significant differences in body weight gain among control (0%), low dose (3%), medium dose (12%), and high dose (30%) of yam–boxthorn noodle diets over the 5 weeks of feeding. Among lung, heart, liver, spleen and kidney in the female BALB/c mice, the absolute and relative weights of the liver was maximum and that for the spleen was minimum. However, we found that there were no significant differences between individual visceral organs in both absolute and relative organ weights after the 5 weeks of feeding of yam–boxthorn noodle diets. These data suggest that the formula of yam–boxthorn noodle feeds as shown in Table 1 is safe and has no toxic side effects on body and visceral organ weights.

3.2. Serum IgG antibody levels

The changes of serum total IgG levels of the mice fed the yam–boxthorn noodle diets are given in Fig. 1. The serum IgG concentrations were increased in the yam–boxthorn noodle-fed mice at all experimental concentrations used in a dose–response manner. The serum levels of total IgG in groups of control, low, medium and high dose of yam–boxthorn noodle diets were 1486 ± 670 , 1638 ± 720 , 2312 ± 531 and 2722 ± 399 μ g/ml, respectively, at the fifth weeks after continuously feeding. The mice fed the yam–boxthorn noodle diet at the concentrations of 12% (medium concentration) and 30% (high concentration) had significantly higher levels of IgG compared with the mice fed the AIN76 diet ($P = 0.010$ and 0.0002 , respectively) after continuously consuming the test diets for 5 weeks. It was also found that the mice fed all the concentrations of 0%, 3%,

Table 2

Effects of yam–boxthorn noodle diets on feed intake, body weight gain, absolute and relative visceral organ weights of female BALB/c mice through five week consecutive feeding

Feeding group (conc. of yam–boxthorn noodles)	Control (0%)	Low (3%)	Medium (12%)	High (30%)
Feed intake (g/day)	4.05 ± 0.79	4.44 ± 0.18	4.23 ± 0.16	4.32 ± 0.27
<i>Body weight</i>				
Initial (g)	19.53 ± 1.88	18.68 ± 1.43	19.31 ± 1.67	19.16 ± 1.95
Final (g)	23.50 ± 1.44	23.52 ± 1.86	23.97 ± 0.81	23.43 ± 1.75
Increased (%)	21.19 ± 13.16	24.54 ± 8.18	24.77 ± 9.22	22.81 ± 6.84
<i>Absolute organ weight (g)</i>				
Lung	0.157 ± 0.012	0.144 ± 0.020	0.150 ± 0.006	0.145 ± 0.015
Heart	0.130 ± 0.011	0.129 ± 0.010	0.137 ± 0.006	0.135 ± 0.017
Liver	0.526 ± 0.025	0.528 ± 0.023	0.543 ± 0.022	0.531 ± 0.025
Spleen	0.117 ± 0.018	0.109 ± 0.007	0.120 ± 0.019	0.123 ± 0.016
Kidney	0.335 ± 0.048	0.312 ± 0.022	0.335 ± 0.019	0.327 ± 0.043
<i>Relative organ weight (%)</i>				
Lung	0.670 ± 0.053	0.658 ± 0.044	0.626 ± 0.031	0.620 ± 0.070
Heart	0.551 ± 0.025	0.597 ± 0.081	0.571 ± 0.035	0.574 ± 0.039
Liver	2.243 ± 0.161	2.454 ± 0.311	2.269 ± 0.112	2.283 ± 0.275
Spleen	0.502 ± 0.087	0.466 ± 0.034	0.500 ± 0.085	0.521 ± 0.045
Kidney	1.419 ± 0.134	1.452 ± 0.219	1.400 ± 0.079	1.391 ± 0.112

Data are expressed as averages $\pm$ SD ($n = 9$), and there were no statistical difference between control and feeding groups of yam–boxthorn noodle diets.

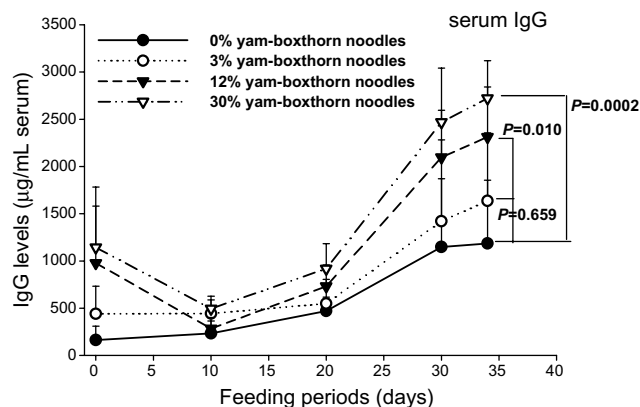


Fig. 1. Effects of yam-boxthorn noodle diets on serum IgG levels from female BALB/c mice through 5 weeks consecutive feeding: (a) data are expressed as averages $\pm$ SD ($n=9$); (b) mean values were significantly different from the control group (0% yam-boxthorn noodle diet) if $P \leq 0.05$ assayed by unpaired Student's *t*-test.

12% and 30% yam-boxthorn noodle diets increased the serum levels of IgG as feeding time prolonged. However, this increase in serum IgG antibody titers demonstrated yam-boxthorn noodle diet concentrations as: high concentration (30%) > medium concentration (12%) > low concentration (3%) > control (0%).

3.3. Serum IgA antibody levels

The changes in total serum IgA levels in the mice fed the yam-boxthorn noodle diets are given in Fig. 2. The serum IgA concentrations were significantly increased in the yam-boxthorn noodle-fed mice at all experimental concentrations used in a dose-response manner. The total IgA serum levels in groups of control, low, medium and high dose of yam-boxthorn noodle diets were 686 ± 186 , 1076 ± 193 , 1633 ± 271 and 1973 ± 275 µg/ml, respectively, at the fifth weeks after continuously

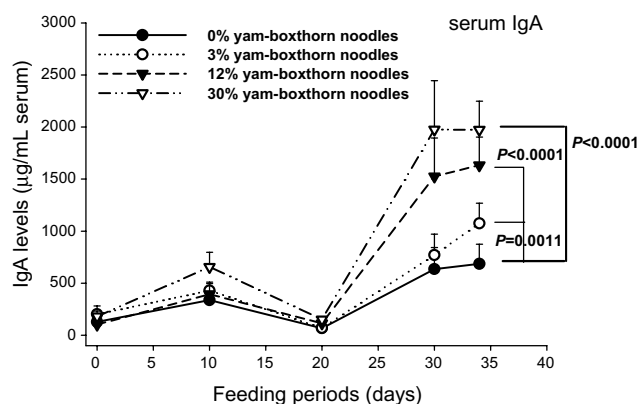


Fig. 2. Effects of yam-boxthorn noodle diets on serum IgA levels from female BALB/c mice through 5 weeks consecutive feeding: (a) data are expressed as averages $\pm$ SD ($n=9$); (b) mean values were significantly different from the control group (0% yam-boxthorn noodle diet) if $P \leq 0.05$ assayed using unpaired Student's *t*-test.

feeding. The mice fed the yam-boxthorn noodle diet at the concentrations of 3%, 12%, and 30% had significantly higher IgA levels compared with the mice fed the AIN76 diet ($P=0.0011$, $P \leq 0.0001$ and $P \leq 0.0001$, respectively) after continuously consuming the test diets for 5 weeks. It was also found that the mice fed all concentrations of 0%, 3%, 12% and 30% yam-boxthorn noodle diets increased the serum levels of IgA as feeding time prolonged. However, this increase in serum IgA antibody titers demonstrated yam-boxthorn noodle diet concentrations as: high concentration (30%) > medium concentration (12%) > low concentration (3%) > control (0%).

3.4. Serum IgM antibody levels

The changes in total serum IgM levels in the mice fed the yam-boxthorn noodle diets are given in Fig. 3. The serum IgM concentrations were not significantly changed in the yam-boxthorn noodle-fed mice at all experimental concentrations used. The serum levels of total IgM in groups of control, low, medium and high dose of yam-boxthorn noodle diets were 334 ± 19 , 334 ± 16 , 353 ± 29 and 352 ± 29 µg/ml, respectively, at the fifth weeks after continuously feeding. It was also found that the mice fed all concentrations; 0%, 3%, 12% and 30% yam-boxthorn noodle diets increased the IgM serum levels as feeding time prolonged. However, this increase in serum IgM antibody titers did not significantly change among all experimental concentrations used.

3.5. Serum IgE antibody levels

The changes in total IgE serum levels in the mice fed the yam-boxthorn noodle diets are given in Fig. 4. The

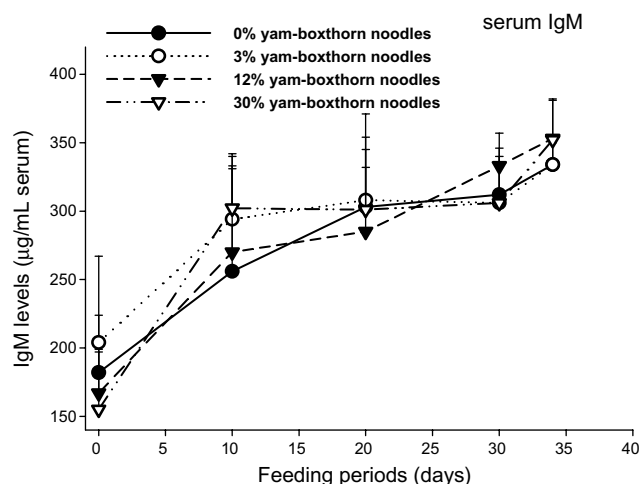


Fig. 3. Effects of yam-boxthorn noodle diets on serum IgM levels from female BALB/c mice through 5 weeks consecutive feeding: (a) data are expressed as averages $\pm$ SD ($n=9$); (b) no statistical difference between control and feeding groups of yam-boxthorn noodle diets.

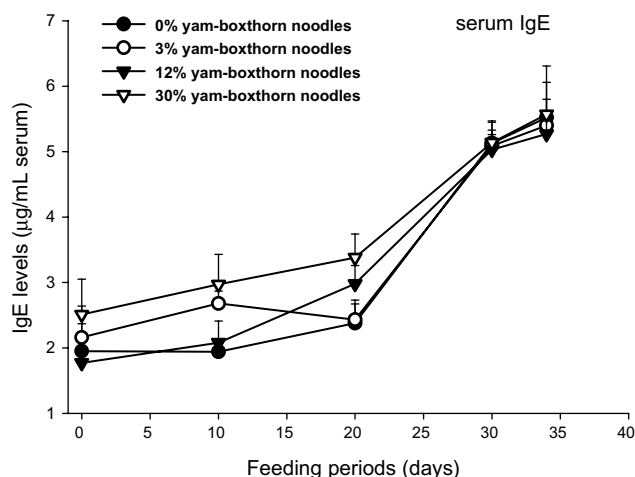


Fig. 4. Effects of yam–boxthorn noodle diets on serum IgE levels from female BALB/c mice through five week consecutive feeding: (a) data are expressed as averages $\pm$ SD ($n=9$); (b) no statistical difference between control and feeding groups of yam–boxthorn noodle diets.

serum IgE concentrations were not significantly changed in the yam–boxthorn noodle-fed mice at all experimental concentrations used. The total IgE serum levels in groups of control, low, medium and high dose of yam–boxthorn noodle diets were 5.519 ± 0.541 , 5.469 ± 0.395 , 5.274 ± 0.198 and 5.570 ± 0.743 $\mu\text{g/mL}$, respectively, at the fifth weeks after continuously feeding. It was also found that the mice fed all the concentrations of 0%, 3%, 12% and 30% yam–boxthorn noodle diets increased the IgE levels as feeding time prolonged. However, this increase in serum IgE antibody titers did not significantly change at all experimental concentrations used.

3.6. IgA antibody levels of intestinal lavage fluid

The changes of total intestinal IgA levels in mice fed the yam–boxthorn noodle diets are given in Fig. 5. The intestinal levels of total IgA in groups of control, low, medium and high dose of yam–boxthorn noodle diets were 864 ± 371 , 1411 ± 889 , 867 ± 366 and 997 ± 430 ng/mice, respectively, at the fifth weeks after continuously feeding. Even though the intestinal IgA levels in the mice fed low concentration of yam–boxthorn noodle diets slightly increased ($P=0.159$) as compared with that of control group, the intestinal IgA concentrations were not significantly changed in the yam–boxthorn noodle-fed mice at all experimental concentrations used.

4. Discussion

The results of this study exhibit that yam–boxthorn noodle, a newly developed noodle enriched with yam

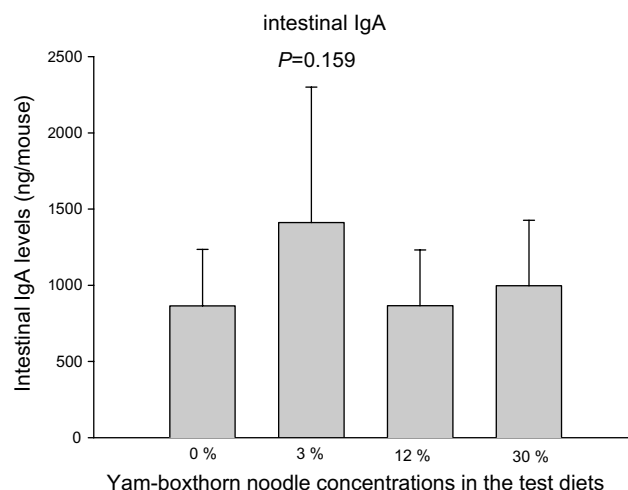


Fig. 5. Effects of yam–boxthorn noodle concentration in the test diets on intestinal IgA levels in the intestinal lavage fluid from female BALB/c mice through 5 weeks consecutive feeding: (a) data are expressed as averages $\pm$ SD ($n=9$); (b) no statistical difference between control and feeding groups of yam–boxthorn noodle diets.

(*Dioscorea alata* L.) and boxthorn (*Lycium barbarum*), is capable of increasing the IgG and IgA serum in an in vivo mouse model in a dose dependent manner (Figs. 1 and 2). However, the IgM and IgE serum did not significantly change with yam–boxthorn noodle diets for five weeks (Figs. 3 and 4). The hyper-IgG and -IgA effects in serum may be attributable to the ability of the immunoactive constituents from the fruit of *Lycium barbarum* L., such as glycoconjugates LbGp2 (Peng et al., 2001), LbGp3, LbGp4 and LbGp5 (Huang et al., 1998, 1999) and the mucopolysaccharide from yam (Choi et al., 2004). Pure *Lycium barbarum* polysaccharides have the best immunopharmacological effect when they are used at adequately lower doses (5–20 mg/kg/day) and show a remarkable effect on immunological enhancement (Luo et al., 1999). Polysaccharides from *Lycium barbarum* L. with main chain of α -1,4-D-polygalacturonans show an stronger immunomodulatory activity (Duan et al., 2001). A polysaccharide–protein complex from *Lycium barbarum* upregulates cytokine expression in human peripheral blood mononuclear cells (Gan et al., 2003). Choi and Hwang (2002) show that yam (*Dioscorea batatas*) mucopolysaccharide has the ability to induce cytokine production and cellular responses in murine peritoneal macrophages. Gaidamashvili et al. (2004) show that yam tuber storage proteins from *Dioscorea batatas* exhibit unique lectin activities. We assumed that lectin proteins and mucopolysaccharides also existed in Chinese yam (*Dioscorea alata* L.) and contributed to the immunomodulation in vivo. However, the immunoactive characteristic constituents from yam remains to be elucidated.

Contrary to our hypothesis, even though the mice fed all concentrations; 0%, 3%, 12% and 30% yam–

boxthorn noodle diets increased IgA serum levels as feeding time prolonged, serum IgM and IgE did not significantly change among different groups of yam–boxthorn noodle diets for five weeks. As the yam–boxthorn noodle could be regarded as a prebiotically functional product, it potentially enhanced the rate of isotype switching within the IgA and IgM antibodies and was accompanied by the growth of cells producing IgM antibodies in vivo (Tomasik and Tomasik, 2003). The overall results demonstrated the manifestation of hyper-IgA antibody in the serum and the maintenance of IgM antibody. B lymphocytes isotype switching in vivo and switching the immunoglobulin (Ig) class from IgM to IgG, IgE, or IgA isotype during an immune response (Kracker and Radbruch, 2004). Cytokines such as interleukin (IL)-4, IL-5, IL-6, and tumor necrosis factor alpha (TNF- α) (mostly T helper type 2 (Th2) cytokines) can direct murine class switching to IgG1 and IgE, and human class switching to IgG4 and IgE, while IL-2, interferon (IFN)- γ , IFN- α , and transforming growth factor- β (TGF- β) (mostly Th1 cytokines) promote murine IgG2a production but inhibit IgE production (Ayoub et al., 2003; Packard and Khan, 2003; Kracker and Radbruch, 2004). IgE production is critically dependent on the Th1/Th2 ratio in which Th1 (IL-2, IFN- γ) and Th2 (IL-4, IL-5) cytokines are elicited in vivo. Administration of *Lycium barbarum* polysaccharide–protein complex (LBP(3p)) increases the expression of IL-2 mRNA and protein levels in a dose-dependent manner in human peripheral blood mononuclear cells (Gan et al., 2003). We assumed that *Lycium barbarum* polysaccharide–protein complex in the yam–boxthorn noodle diets enhanced the secretion of Th1 cytokines, secondary inhibited the isotype switching to IgE, and maintained the lower serum IgE level in vivo. This finding also implicated that continuously feeding yam–boxthorn noodle diets can modulate the Th1/Th2 balance in favor of Th1, and demonstrate the possibility of protective immunity and anti-allergic activity.

Intestinal IgA, which is secreted from conventional B cells or from B-1 cells, plays a prominent role in the gut mucosal immune system, which serves to protect the body from the continuous threat of infection from intestinal bacteria or exogenous pathogenic bacteria (Kroese et al., 1996). Our results demonstrated that the intestinal levels of total IgA in groups of control, low, medium and high dose of yam–boxthorn noodle diets were varied at the fifth weeks after continuously feeding. The intestinal IgA levels in the mice fed low concentration of yam–boxthorn noodle diets increased 1.63 folds, however the intestinal IgA concentrations were not significantly changed ($P = 0.159$) as compared to that of the control group. The intestinal lymph IgA may contribute to serum IgA level, and vice versa (Vaerman et al., 1997). Both intestinal and serum

IgA antibodies are implicated in host defense against bacterial infections. Luo et al. (1999) shows that adequately lower doses (5–20 mg/kg/day) of pure *Lycium barbarum* polysaccharides have the best immunopharmacological effect. Our data also suggest that low dose (3%) of yam–boxthorn noodle diet for long-term feeding has the best immunomodulatory effect on the gut. Previous studies suggested that yam–boxthorn noodle, a functional noodle, may act as a staple food with immunological protection in the system or local intestine. Serum immunoglobulin levels represent a non-specific, relatively insensitive measure of immune function, however total serum immunoglobulin level might moderately reflect the actual humoral immunity in vivo. The yam–boxthorn noodle might provide a non-specific protective immunity daily, however the increase in IgG and IgA levels following treatment with yam–boxthorn noodle might be disadvantageous to autoimmune disease or hyper-immunity patients. More specific humoral and T cell mediated immune responses exerted by the yam–boxthorn noodle remain to be clarified.

Changes in body and visceral weight of BALB/c mice over the 35 days were indicators of nutritional status of feeding for different concentrations of yam–boxthorn noodle diets. There was not significantly different among the four diet groups in body weight gain, absolute and relative visceral organs after the 5 weeks of feeding of different concentrations of yam–boxthorn noodle diets (Table 2). However, the increases of body weight gained over the 35 days of BALB/c mice feeding for different concentrations of yam–boxthorn noodle diets was from $21.19 \pm 13.16\%$ to $24.77 \pm 9.22\%$. Constituents of *Lycium barbarum* polysaccharide (LBP-4) composed of six kinds of monosaccharides is reported to have the effects that can enhance food conversion rate and the content of zinc and iron in body of mice, and reduce the body weight on the growth of weanling mice (Zhang et al., 2002). However, Wanasundera and Ravindran (1994) suggest that the nutritional superiority of yams (*Dioscorea alata*, rich in Vitamin C and minerals but low in phytic acid contents) compared to other tropical root crops. The yam–boxthorn noodle was prepared from the patent flour, yam powder, boxthorn powder, sodium chloride and water in 600, 330, 70, 20 and 320 g proportions, respectively (Liou, 2003). Since the boxthorn powder was a minor constitute in the noodle as compared to that of yam powder, the overall results suggest that the formula of yam–boxthorn noodle feeds as shown in Table 1 was safe and has no toxic side effects on body and visceral organ weights. Previous studies support our purpose of developing the yam–boxthorn noodle to enhance humoral immunity. However, the effective compounds in yam–boxthorn noodles on systemic and local intestine immune response remain to be further elucidated.

5. Conclusions

This study showed that yam–boxthorn noodle diets significantly increased serum antibody levels especially in IgA and IgG. These results are valuable for future nutraceutical and immuno-pharmacological use. The yam–boxthorn noodle might provide a non-specific protective immunity daily, however the increase in IgG and IgA levels following treatment with yam–boxthorn noodle might be disadvantageous to autoimmune disease or hyper-immunity patients. This study has provided the evidences that yam–boxthorn noodle diets significantly increased serum antibody levels especially in IgA and IgG, however more specific humoral and T cell mediated immune responses exerted by the yam–boxthorn noodle remain to be clarified.

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