Immunomodulatory Effect of *Pinus Armandi Franch* Seed Oil

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Abstract: *Pinus armandi franch is a special local plant in China and its seed oil is a nice resource of linoleic acid (LA) and linolenic acid. This study investigated the effects of enriching the mouse diet with the ω-6 and ω-3 polyunsaturated fatty acids (PUFA), LA (18:2 ω-6) and α-linolenic acid (18:3 ω-3) different ratios (ω-6/ω-3) on total lipids in Pinus armandi franch seed oil (PAFO), and immune evaluation indexes in plasma, lymph nodes, and spleen from isolated immune cells. Kunming mice were fed a commercial chow. PAFO with a certain ratio (ω-6/ω-3) could promote the proliferation of spleen lymphocytes induced by ConA in mice and the optimal ratio was 4 or 6. PAFO with different ratios (ω-6/ω-3) could increase the spleen index and thymus index and there were significant differences between PAFO group and control group ($p < 0.01$). The results indicate that the ratio of ω-6 PUFA: ω-3 PUFA is 4 or 6 improve some of the indices of the mice immune status and that a moderate increase in the level of ω-6 PUFA in the mice diet does not suppress the immune system.*

Keywords: *Pinus armandi franch, Seed oil, ω-6 PUFA, ω-3 PUFA, Immunocompetence.*

Introduction

In recent years there has been increased interest in the impacts of polyunsaturated fatty acids (PUFA) on the immune responses of humans and animals [25, 27]. It has been reported that feeding rats or mice high levels of *ω*-3 PUFA, docosahexaenoic acid (DHA), and eicosapentaenoic acid (EPA) resulted in marked suppression of in lymph node, thymus, vitro spleen, and peripheral blood lymphocyte proliferation. Feeding mice or rats high levels of *ω*-3 PUFA as linolenic acid (LNA) also restrained *in vitro* lymphocyte proliferation. An increase in the concentration of dietary fat, particularly of the *ω*-6 PUFA, has been shown to restrain the immune status in several animal models [7, 13, 23]. Effect of dietary *ω*-6 PUFA on the human immune status is not well defined. In a recent study managed with the rats, we found that a moderate increase in the level of *ω*-6 PUFA did not restrain several indices of the immune status tested [19, 24].

Humoral immune response is another important situation of the immunity and can be assessed by measuring antibody production and activity. Dietary supplementation of *ω*-3 PUFA derived from fish oil decreased antibody production in mice, rats, and humans [2, 22]. It has also been reported that *ω*-3 PUFA from fish oil or linseed oil had no impact on antibody

production in rats and rabbits. In chickens, feeding ω-3 PUFA from fish oil produced more antibodies to sheep red blood cells than feeding saturated fat (lard) and ω-6 PUFA rich oil [3, 4]. Antibody production developed more rapidly, accomplished a higher level, and was more persistent in the chicks fed lower levels of ω-6 to ω-3 PUFA (linoleic acid (LA) to LNA) ratio. Lymphocytes, with several phenotypes, play significant roles in determining an immune response. Alteration of the proportion of a lymphocyte subset could change the functions of the immune system. The manufacture of IgG is enhanced when rats are fed high levels of ω-3 PUFA (DHA and EPA) compared with those fed high levels of saturated fatty acids [5, 6].

The contradictory results obtained from mammalian studies on the effects of dietary ω-6 and ω-3 PUFA on antibody production and immune cell subset populations might be due to different species, basal diet, fat amount, and ratio of ω-6 to ω-3 PUFA and various fatty acids of ω-3 PUFA used for disparate studies [1, 20]. Immune responses have been found to impact mice nutrient utilization and performance [18]. Modulation of the immune status of mice may produce salutary effects and provide a new avenue in improving poultry production. For example, the reduction of the suppressive effect of inflammation by adding fish oil to chicken diets improved the growth and feed efficiency [8]. Therefore, it is significant to know the effects and principles of dietary fatty acids on mice immune responses.

Some research showed a prominent increase in the activity of natural killer (NK) cells when the fat intake for a group of the rats was reduced [15, 26]. Furthermore the NK activity could be suppressed by increasing the fat content of the diet, and both the polyunsaturated and saturated dietary fats were equally valid in suppressing the NK activity [10]. The results from the rats researches dealing with the effect of total dietary fat on immune status are consistent with the results acquired from animal experiments. However, the limited results regarding the effects of ω-6 PUFA on the immune system in humans are at variance with the results acquired in animal researches where ω-6 PUFA has been found to be a more effective inhibitor of the immune system than the monounsaturated and saturated fatty acids [9, 14].

The lipid composition of leucocytes is determined by the fatty acid composition of dietary oils. In mammals, these cells acquire essential fatty acids from circulating blood plasma and are dependent on the hepatic desaturase and elongase enzymes to afford long-chain PUFA which are required to sustain normal cellular function, Thus, factors which influence the activity of these enzymes can influence the content of PUFA. Several health organizations have recommended a reduction in the intake of total fat calories to improve the cardiovascnlar and cerebrovascular diseases [17, 21, 28]. The impact of such dietary recommendations on the immune system is currently unknown. In light of the results obtained from animal studies, an increased intake of PUFA may restrain the immune system which may not be desirable in healthy individuals. It is therefore important to regulate the effects of both the compression and the type of dietary fat on the human immune system.

Pinus armandi franch seed oil (PAFO) is an inexpensive source of several polyunsaturated fatty acid types, thus it could be widely used for isolation and purification of PUFA [11, 12]. PAFO was tested in this work and evaluated them for potential use as a new oil crop to provide a dietary supplement by investigating the effects of the seed oil in the diet on enhancing the immune response of rats. The objective of the current study is to examine the effects of different dietary ratios of ω-6 to ω-3 PUFA and different ω-3 fatty acids on mouse immune responses including in peritoneal macrophages phagocytosis function, lymphocyte subset proportion, and vitro lymphocyte proliferation.

Materials and methods

Materials

Pinus armandi franch seeds were obtained from Shanxi Agricultural Seed Station (Taiyuan, People's Republic of China). All biochemical reagents and assay kits for biochemical variables were obtained from Shanghai Mingdian Biological Engineering Co. Ltd, China. All other reagents are analytical grade from Sinopharm Chemical Reagent Co. Ltd. *Pinus armandi franch* seed oil was prepared by means of solvent extraction method and LA was purified from *Pinus armandi franch* seed oil by means of molecular distillation.

Mice were grouped and fed

Kunming mice with an initial body weight of about 100 g were purchased from Shanxi Experimental Animal Center, China. Animals were housed in normal conditions (temperature 22 ± 2 ℃, a minimum relative humidity of 40%, 12 h light/dark cycle) and had free access to water and diet in an approved animal house facility at Shanxi Experimental Animal Center, China. Kunming mice were randomly divided into 6 groups with 10 mice in each group. They were divided into blank control group and PUFAs drug group. Soybean oil was used as solvent and the blank group was given equal amount of soybean oil. Oral gavage was administered once a day for 28 days. Mice in each group ate freely.

Establishment and administration of animal models

Mice were randomly divided into three groups: normal control group, treatment group and negative control group. The treatment group was divided into: low-dose group 5 mg/kg bw; medium dose group, 20 mg/kg bw; high-dose group, 80 mg/kg bw. The normal and negative control groups were given the same amount of soybean oil. Oral gavage was administrated once a day for 28 days.

Effect of PAFO on proliferation of spleen lymphocytes in mice

The spleens of mice were taken in a sterile environment and placed in a small dish with appropriate amount of PBS liquid. The spleen was gently torn up with tweezers and filtered through a screen with an aperture of 0.074 mm. Lymphocyte isolation solution was used for separation. Cell layers rich in lymphocytes were absorbed and washed with Hanks solution for 3 times, centrifugation for 10 min (1000 r/min) each time. The cells were then suspended in 2 ml of complete culture medium. Trypan blue staining was used and the number of living cells (should be over 95%) was counted. The cell concentration was adjusted to 2×10^5 cells/mL. The cell suspension was divided into two wells and added to the 24-well culture plate with LML for each well. Add 50 uL ConA to one well (equivalent to 5 ug/ml). The other well was cultured in 5% CO_2 at 37 ℃ for 72 h. Before the end of culture, 0.7 mL of supernatant was gently sucked out of each well, and 0.7 mL of RPMI-1640 medium without calf serum was added, and 50 μL of MTT (5 mg/mL) was added to each well for further culture for 4 h. At the end of the culture, isopropanol was added to each well and mixed with an oscillator until the purple crystals were completely dissolved. Then they were divided into 96-well culture plates, and 6 wells were divided into each well as parallel samples. An enzyme-linked immunoassay was used to determine the optical density at 570 nm.

Thymus and spleen index

After the last gavage for 4 h, the mice were weighed and sacrificed for cervical dislocation, thymus and spleen were stripped, the surface blood stains were sucked dry with filter paper, and the thymus and spleen indexes were calculated by analytical balance weighing.

Determination of phagocytosis of mouse mononuclear macrophages

After the last gavage for 24 h, Indian ink diluted 10 times was injected into the tail vein of mice. The time was measured immediately after the ink was injected. After injection of ink, 20 L of blood was taken from the orbit at 2 min and 10 min, respectively, and it was immediately added to 2 mL 0.1% Na_2CO_3 solution. After blending, the lateral absorbance value (OD) at 600 nm was taken from Na_2CO_3 as blank control. The mice were sacrificed, the liver and spleen were taken, the blood stains on the surface of the viscera were sucked up with filter paper and weighed respectively. Phagocytic index was used to represent the carbon clearance ability of mice.

Determination of the degree of toe swelling (DTH) in mice caused by SRBC

After the subjects were fed on the 16th day, 2% SRBC cell suspension was taken, and each mouse was intraperitoneally injected with 0.2 mL, followed by gavage. After the immunization, the thickness of the left plant foot was measured for 4 days, and then 20% SRBC cell suspension was subcutaneously injected at the measured site. After the injection, the thickness of the left foot plantar was measured for 24 h. The same site was measured for three times, and the average value was taken. The degree of DTH is indicated by the difference in the thickness of the foot plantar before and after the attack.

Statistical analysis

Results are represented as means ± standard deviation for each group. One way ANOVA was used for analyzing the data followed by a post hoc Tukey test to compare the control and treatment groups. The *p*-values less than 0.05 were considered as statistically significant. The statistical analysis was performed on SPSS Statistics 22.0.

Results and discussion

Effects of PUFA on the proliferation of splenocytes

Effects of different ratios of PUFA on proliferation of splenic lymphocytes in mice. The spleen is the place where all kinds of immune cells live, and it is also an important part for producing immune response to antigens and immune effector substances. The degree of proliferation of splenic lymphocytes induced by ConA can reflect the cellular immunity of the body. The test results are shown in Table 1.

Table 1. Effects of different ratios of PUFA on the proliferation of splenocytes

Group	ω-6/ω-3	OD, (630 nm)
NG	-	0.177 ± 0.009
PAFOG	2	0.189 ± 0.014*
	4	0.264 ± 0.013**
	6	0.259 ± 0.017**
	8	0.223 ± 0.014*
	10	0.212 ± 0.019*

Note: * – the result is significantly different from that of the blank control group ($p < 0.05$); ** – the result is highly significantly different from that of the blank control group ($p < 0.01$); values represent means ± S.D. for $n = 10$ rats; NG – normal group; PAFOG – diet with different ω-6/ω-3 ratios of PAFO.

As shown in Table 1, PAFO with different ω-6/ω-3 ratios could promote the proliferation of splenic lymphocytes to a certain extent. The ω-6/ω-3 ratio was 4 or 6, which had the most significant effect compared with the control group ($p < 0.01$). When the ω-6/ω-3 ratio is less than 6, the promoting effect enlarged with increase of the ratio. However, when the ω-6/ω-3 ratio is greater than 6, its promoting effect decreased with the increase of the ratio.

Lymphocyte proliferation is an important indicator of cellular immunity. The results of this study showed that certain ω-6/ω-3 ratio of PAFO could promote the proliferation of splenic lymphocytes induced by ConA in mice, and the optimal effect ratio was 4 or 6.

Effect of PUFAs on the index of immune organs and DTH

The weight gain of the thymus and spleen meaned the proliferation of lymphocytes, which directly reflected the strength of the immune response, as shown in Table 2.

Table 2. Effects of PUFA on immune organ index and DTH in normal mice

Group	ω-6/ω-3	Spleen index, (g/10g·bw)	Thymus index, (g/10g·bw)	Toe swelling, (mm)
NG	-	36.19 ± 1.71	26.24 ± 2.41	0.78 ± 0.031
PAFOG	2	42.39 ± 1.21*	31.49 ± 1.92*	1.24 ± 0.033**
	4	47.01 ± 2.82*	36.04 ± 2.17*	1.22 ± 0.038**
	6	47.51 ± 2.74*	38.14 ± 1.42*	1.23 ± 0.057**
	8	46.54 ± 3.13*	35.02 ± 1.71*	1.21 ± 0.043*
	10	40.59 ± 1.33*	29.23 ± 1.42	1.12 ± 0.024*

Table 2 showed that PAFO with different ω-6/ω-3 ratios could increase the spleen index and thymus index, with significant difference compared with the control group ($p < 0.01$). When the ω-6/ω-3 ratio was 6, the promotion effect was the most obvious in the experimental group. When the ω-6/ω-3 ratio was 10, the effect of PAFO on the thymus index of mice was not significant compared with the control group.

Different ω-6/ω-3 ratios of PAFO could enhance DTH in mice, and the ω-6/ω-3 ratios of 2, 4 and 6 could significantly ($p < 0.01$) improve the immunity of the body after secondary sensitization, and enhance the delayed type hypersensitivity. This study showed that certain ω-6/ω-3 ratio of PAFO can improve the immunity of the body after secondary sensitization, and significantly enhance the delayed type hypersensitivity.

The spleen is the largest immune organ in the animal body, accounting for 25% of the total lymphoid tissue. The spleen contains a large number of lymphocytes and macrophages and is the center of cellular and humoral immunity. The weight gain of the thymus and spleen often means the proliferation of lymphocytes, which directly reflects the strength of the immune response.

Effects of PAFO on the phagocytosis of mononuclear macrophages

Phagocytosis of granular foreign bodies is an important function of the mononuclear macrophage system. The liver and spleen were the main organs to perform this function. The liver cells accounted for 90% of the phagocytosis, and the spleen cells accounted for about 10%. The experimental results are shown in Table 3.

The HC_{50} values were shown in Table 3. When the ω-6/ω-3 ratio of PAFO was 4 or 6, the HC_{50} values of mice could be increased. Compared with the control group, there was significant difference ($p < 0.05$).When the ω-6/ω-3 ratio was 2 and 8, there was no significant difference compared with the control group. The optimum ω-6/ω-3 ratio is 4 and 6. The results showed that certain ω-6/ω-3 ratio of PAFO could significantly increase the serum hemolysin content and promote the specific humoral immune function of mice.

Table 3. Effects of PUFA on the phagocytotic functions and antibody production capacity in normal mice

Group	ω-6/ω-3	Phagocytic index	HC_{50}
NG	-	5.12 ± 0.19	113.01 ± 16.02
PAFOG	2	$7.59 \pm 0.44^{**}$	118.27 ± 16.71
	4	$7.21 \pm 0.31^{**}$	$146.08 \pm 19.39^{*}$
	6	$7.29 \pm 0.24^{**}$	$149.09 \pm 21.04^{*}$
	8	$6.91 \pm 0.33^{*}$	119.02 ± 18.04
	10	$6.22 \pm 0.38^{*}$	124.39 ± 22.42

The phagocytosis ability of macrophages in normal mice was improved to varying degrees after PAFO with different ω-6/ω-3 ratios. Compared with the control group, the ratio of 2, 4 and 6 PAFO could significantly ($p < 0.01$) promote the phagocytosis of normal mouse macrophages. The phagocytic capacity of macrophages decreased with the increase of the dose. When the ratio was 10, the promotion effect was the least, and the difference was significant compared with the control group ($p < 0.05$).

Effects of PAFO on immune organs in immunocompromised mice

The thymus and spleen are the main immune organs of the body. Immunosuppressants can suppress or atrophy them. The experimental results are shown in Table 4.

Table 4. Effects of PUFA on immune organs index and DTH in immunocompromised mice

Group	ω-6/ω-3	Spleen index, (g/10g·bw)	Thymus index, (g/10g·bw)	Toe swelling, (mm)
NG	-	40.91 ± 3.68	34.01 ± 2.81	0.74 ± 0.13
IG	-	$21.01 \pm 1.19^{**}$	$24.02 \pm 2.71^{*}$	$0.39 \pm 0.31^{*}$
PAFOG	2	$51.04 \pm 3.29^{\#\#}$	26.03 ± 2.29	0.49 ± 0.02
	4	$43.03 \pm 2.24^{\#\#}$	$38.41 \pm 3.51^{\#\#}$	$0.59 \pm 0.61^{\#}$
	6	$51.07 \pm 3.39^{*\#\#}$	$39.64 \pm 12.24^{\#}$	$0.62 \pm 0.27^{\#}$

Note: IG – immunocompromised group; $^{\#}$ – the result is significantly different from that of the immunocompromised group ($p < 0.05$); $^{\#\#}$ – the result is highly significantly different from the immunocompromised group ($p < 0.01$).

Immunoenhancers can increase the weight of thymus and spleen. Organ index can indirectly reflect the degree of organ damage and is a relatively simple detection method. In this experimental study, the results of organ indexes of mice in each group showed that the spleen index and thymus index of the immunocompromised group were significantly ($p < 0.01$) and significantly ($p < 0.05$) lower than those of the blank control group, respectively, indicating that the modeling was successful. PAFO with ω-6/ω-3 ratios of 2, 4, and 6 significantly ($p < 0.01$) increased the spleen index of immunocompromised mice induced by cyclophosphamide. When the ratio was 6, it was significantly ($p < 0.05$) higher than that of blank control group.

PAFO with ω-6/ω-3 ratio of 4 could significantly ($p < 0.01$) increase the thymus index of immunocompromised mice. Compared with the immunocompromised group, the ratio of 2 and 6 in PAFO group was also significantly different ($p < 0.05$). However, there was no significant difference in thymus index between the PAFO group with ω-6/ω-3 ratio of 2 and the blank control group. The experimental results showed that certain ω-6/ω-3 ratio of PAFO could increase the thymus and spleen indexes of immunocompromised mice and enhance their immune ability.

Effects of PAFO on the phagocytosis of mononuclear macrophages in immunocompromised mice

The carbon clearance ability of immunocompromised mice was significantly lower ($p < 0.01$) than that of normal mice. However, after feeding PAFO for 20 days, the phagocytosis ability of peritoneal macrophages in immunocompromised mice was improved to varying degrees, and there was no significant difference from that of normal mice. Among them, the ω-6/ω-3 ratio of 4 and 6 PAFO groups could significantly ($p < 0.01$) improve the phagocytic capacity of peritoneal macrophages in immunocompromised mice. The experimental results are shown in Table 5.

Table 5. Effects of PUFA on the phagocytotic functions and antibody production capacity in immunocompromised mice

Group	ω-6/ω-3	Phagocytic index	HC_{50}
NG	-	2.93 ± 0.17	111.3 ± 10.4
IG	-	$2.22 \pm 0.17^{**}$	$79.7 \pm 10.8^{**}$
PAFOG	2	$2.63 \pm 0.17^{\#}$	$87.8 \pm 11.9^{**}$
	4	$2.84 \pm 0.59^{\#\#}$	$92.7 \pm 11.8^{**\#}$
	6	$2.83 \pm 0.08^{\#\#}$	$97.3 \pm 17.4^{*\#\#}$

Table 5 showed that certain ω-6/ω-3 ratio of PAFO can improve the phagocytic function of macrophages in mice and improve their non-specific immune function. However, the phagocytosis rate was different among the treatment groups with different ω-6/ω-3 ratios of PAFO. The phagocytosis rate was the highest in the 4 group, followed by the 6 group, and the lowest in the 2 group. This may be due to the fact that one of the fatty acids plays a major regulatory role when the ratio is 4, while the other one plays a major regulatory role when the ratio is 2.

The hemolysis value of half of immunocompromised group was significantly lower than that of blank control group ($p < 0.01$), indicating successful modeling. As the ω-6/ω-3 ratio increased, the immunocompromised mice showed an increased ability to produce antibodies. There was no significant difference in the ω-6/ω-3 ratio at 2 ($p < 0.05$) compared with the immunocompromised group. The ω-6/ω-3 ratio of 4 could significantly ($p < 0.05$) increase the ability of antibody production in immunocompromised mice. Among them, the ω-6/ω-3 ratio had the strongest ability to improve the half hemolytic value of immunocompromised mice, and the difference was extremely significant compared with the immunocompromised group ($p < 0.01$). But different ω-6/ω-3 ratios of PAFO did not increase the production of antibodies to normal levels in immunocompromised mice.

Determination of culture time of splenic lymphocytes in mice

Interferon (IFN) is a kind of low molecular weight, multifunctional protein, with a broad spectrum of antivirus, inhibit tumor growth and immune regulation of a variety of biological activities. ConA was selected as an IFN-γ inducer in this study, and the results were shown in Table 6.

Table 6. Effect of PAFO on the release of IFN-γ by mouse spleen lymphocyte *in vitro*

Time, h	OD, (450 nm)	IFN-γ concentration, (pg/mL)
12	0.703 ± 0.137	406.4 ± 19.3**
24	0.823 ± 0.157	487.4 ± 31.6**
48	0.709 ± 0.147	420.3 ± 24.8**
72	0.627 ± 0.143	362.7 ± 16.8**

As shown in Table 6, when the ω-6/ω-3 ratio was 4, there were extremely significant differences between the four groups and the blank control group ($p < 0.01$). The content of IFN-γ in the supernatant co-cultured with PAFO and splenic lymphocytes of mice increased with the extension of culture time within 24 h and began to decrease after 48 h. Therefore, 24 h was selected to conduct the following experiment. When the ω-6/ω-3 ratio was 4, IFN-γ content in the supernatant of PAFO and mouse spleen lymphocytes co-cultured at 24 h, compared with 48 h and 72 h, showed no significant difference ($p < 0.01$). Interferon is a kind of low molecular weight, multifunctional protein. IFN can be divided into leukocytes, lymphocyte-like interferon or α-interferon, fibroblast interferon or IFN-B, and immunointerferon or IFN-γ according to the producing cells, inducing conditions, antigen specificity and molecular structure. IFN-γ inducers include multiple mitotic sources, such as PHA, PWM, ConA, SPA, etc.

IFN-γ can inhibit the proliferation of tumor cells and has direct effects on a variety of tumor cell lines and their primary cultures. IFN-γ can also enhance the activity of monocyte macrophages and promote their receptor expression by enhancing or activating host immune response to tumor cells, which is conducive to promoting monocyte macrophages to kill tumor cells through phagocytosis and antibody-dependent cell mediated cytotoxicity [9].

Dose effects of PAFO on the release of 1FN-γ by mouse spleen lymphocyte in vitro

IFN-γ can inhibit the proliferation of tumor cells and has direct effects on a variety of tumor cell lines and their primary cultures. After 24 h of culture, the content of IFN-γ in the supernatant of PAFO with different ω-6/ω-3 ratios co-cultured with mouse spleen lymphocytes increased with the increase of dose, as shown in Table 7.

IFN-γ interferes with the growth of most viruses. The mechanism is that IFN-γ binds to a receptor on the cell surface and induces the formation of an antiviral protein, thereby inhibiting viral replication. Compared with the blank control group, the ω-6/ω-3 ratio of 2 could significantly ($p < 0.05$) increase the IFN-γ concentration. When the ω-6/ω-3 ratio was 4 or 6, the IFN-γ concentration was significantly different from that of the blank control group ($p < 0.01$). It was found that PAFO with different ω-6/ω-3 ratios could promote the production of IFN-γ by mouse splenic lymphocytes *in vitro*.

Table 7. Effects of different ω-6/ω-3 ratios of PUFA on the release of IFN-γ by mouse spleen lymphocyte *in vitro*

Group	ω-6/ω-3	OD, (450 nm)	IFN-γ concentration, (pg/mL)
NG	-	0.193 ± 0.133	49.9 ± 9.4
PAFOG	2	0.214 ± 0.133	66.8 ± 9.2*
	4	0.282 ± 0.159	210.2 ± 17.8**
	6	0.408 ± 0.137	278.1 ± 20.3**
	8	0.509 ± 0.138	139.1 ± 33.8**
	10	0.703 ± 0.194	115.2 ± 32.2**

IFN-γ can activate NK cells to kill tumor cells, with various types of cells can also express the main immune antigen, amplifying the recognition stage of immune response. IFN-γ can also directly promote the differentiation of T and B cells and the maturation of CTL cells, and stimulate the secretion of antibodies by B cells. In addition, in recent years, there have been many reports that IFN-γ alone or in cooperation with TNF-α induces tumor cell apoptosis. It was found that PAFO with different ω-6/ω-3 ratios could promote the production of IFN-γ by mouse splenic lymphocytes *in vitro* [16].

Conclusion

Overall, results from the present investigation indicate a positive association between ω-6/ω-3 ratios of 4/1, and possibly 6/1, in addition to immune maturation, suggests ω-6 PUFA is needed when there is ω-3 PUFA supplemented. PAFO with different ω-6/ω-3 ratios had a significant effect on the induction of IL-2 in mice spleen lymphocytes stimulated by ConA. It could promote the production of IFN-γ by mouse splenic lymphocytes *in vitro*. Future investigations are aimed at further characterizing. Effects of fatty acids on cytokine secretion in mice to better understand health implications and intake requirements related to PUFA nutrition during early development.

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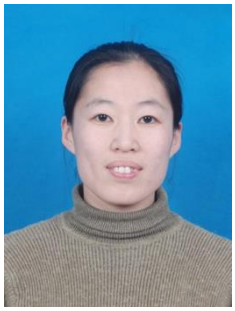

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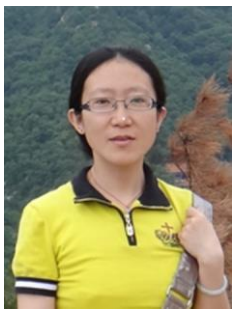

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