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Dietary effects of n-6:n-3 polyunsaturated fatty acid ratios on the antioxidant status of the liver in goslings*

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ABSTRACT

The objective of this study was to investigate the effects of dietary n-6:n-3 polyunsaturated fatty acid (PUFA) ratios on the oxidative stress of the liver in goslings. A total of 160 healthy Yangzhou geese was randomly divided into 4 groups, and each group was fed on diets with different n-6:n-3 PUFA ratios: A (12:1), B (9:1), C (6:1), and D (3:1), respectively. The liver was separated and antioxidant functions were evaluated on days 42, 56, and 70. The results showed that the activities of total superoxide dismutase, glutathione peroxidase, and catalase decreased when the geese aged, and were highest on d 42 in each group ($P<0.05$). The activities of these enzymes increased as the n-6:n-3 PUFA ratios decreased and were highest in the 3:1 group at all tested ages ($P<0.05$). It was also observed that the levels of H_2O_2, malondialdehyde (MDA), and nitric oxide (NO) in liver tissue tended to increase with age, and were relatively higher on d 70. Furthermore, the levels of H_2O_2, MDA, and NO decreased as n-6:n-3 PUFA ratios decreased and were lowest at 3:1 and 6:1 at all studied ages ($P<0.05$). Overall, diets containing comparatively low n-6:n-3 PUFA ratios, such as 3:1 or 6:1, could increase antioxidant function and decrease hepatocyte damage in liver tissue.

KEY WORDS: n-6:n-3 polyunsaturated fatty acid, liver, oxidative stress, goslings

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INTRODUCTION

Oxidative stress and damage to biomolecules by free radicals have been linked to aging and a variety of chronic diseases, particularly cardiovascular disease and cancer. As modern society develops, antioxidation, anti-aging, and suboptimal health are drawing increasingly more attention (Eder et al., 2002; Crane et al., 2005). Research (Liu, 2009) has suggested that polyunsaturated fatty acids (PUFA) can scavenge free radicals, improve the activities of superoxide dismutase (SOD) and other antioxidant enzymes, and may exert a preventive antioxidant role against free radical action. Animals can not covert n-6 PUFA into n-3 PUFA or *vice versa* and these two classes of fatty acids are competitive with each other in metabolic processes. The appropriate ratio of n-6:n-3 PUFA in diets is deemed to be essential for increasing antioxidant and anti-stress capacity and maintaining health status. To date, however, a reasonable range of n-6:n-3 PUFA ratios has not been clearly established; moreover, there are very few publications focusing on the ratios impacting the antioxidation functions of vital organs such as liver (Wang et al., 2009).

The Yangzhou goose is a major breed in China, and was approved as the first national goose breed by the National Examination and Approval Committee of Domestic Animal and Poultry Breeds in 2006. Research on Yangzhou goose has mainly been concentrated on growth performance, nutritional requirements, gastrointestinal tract morphology, etc. (Shi et al., 2007; Wang et al., 2008, 2010; Liu et al., 2010). The effects of n-6:n-3 PUFA ratios on liver oxidation susceptibility in this breed have not been reported up to now. The hypothesis of the present study was that dietary n-6:n-3 PUFA ratios are associated with liver antioxidant function in geese. Thus, the Yangzhou goose was taken as the experimental bird in this study aimed at assessing the effects of n-6:n-3 PUFA ratios on liver oxidation resistance by feeding diets with different n-6:n-3 PUFA ratios, and also at providing some references for the scientific utilization of oil feeds.

MATERIAL AND METHODS

Experimental birds and feeding

The feeding experiment was conducted from November 2009 to March 2010, at the experimental farm of Yangzhou University (Yangzhou, Jiangsu Province, P.R. China). All animal handing protocols were approved by the Yangzhou University Animal Care and Use Committee. A total of 160 healthy Yangzhou geese (80 male and 80 female) with a similar weight

(0.407±0.023 kg), aged 21 days, were randomly divided into 4 groups. Each group contained 4 replications, 10 birds each in a block (5 male and 5 female). Each group of birds was randomly allocated to one of four diets (n-6:n-3 PUFA ratios were 12:1, 9:1, 6:1, and 3:1, respectively) shown in Table 1. A maize-peanut meal basal diet was designed referring to NRC (1994) and other studies on this breed. Palmitic and oleic acids were purchased from Zibo Wanyou Chemical Co. Shandong Province (China), and the purity of palmitic and oleic acids were 99.9 and 99.8%, respectively. The birds were fed with 1/3 original diet + 2/3 experimental diet for 2 days, 1/2 original diet + 1/2 experimental diet for 2 days, 2/3 original diet + 1/3 experimental diet for 3 days; and then were fed with the whole experimental diet to start the feeding experiment. No vaccinations were given throughout the experimental period, and 3 birds in total died (mortality rate: 1.86%).

Table 1 Composition and nutritive level[1] of experimental diet (% unless otherwise stated)

Composition	n-6:n-3 PUFA ratios			
	A (12:1)	B (9:1)	C (6:1)	D (3:1)
Maize	66.20	66.20	66.20	66.20
Soyabean meal	17.30	17.30	17.30	17.30
Lucerne powder	10.70	10.70	10.70	10.70
Peanut oil	1.30	1.28	1.16	1.06
Sunflower seed oil	0.16	0.15	0.16	0.08
Linseed oil	0.10	0.13	0.19	0.32
Palmitic acid	0.37	0.37	0.38	0.40
Oleic acid	0.07	0.07	0.11	0.14
Calcium hydrogen phosphate	1.20	1.20	1.20	1.20
Stone powder[2]	0.60	0.60	0.60	0.60
L-Lysine hydrochloride	0.35	0.35	0.35	0.35
DL-Methionine	0.15	0.15	0.15	0.15
Sodium chloride	0.50	0.50	0.50	0.50
Premix[3]	1.00	1.00	1.00	1.00
Metabolizable energy, ME, MJ/kg[4]	11.70	11.70	11.70	11.70
Crude protein	14.04	14.41	14.35	14.22
n-6 PUFA[5]	0.60	0.60	0.56	0.50
n-3 PUFA[5]	0.05	0.07	0.09	0.16
n-6:n-3 PUFA ratio	12.00:1	8.57:1	6.22:1	3.12:1

[1] values are expressed on a DM basis; [2] a special feed material to supply calcium in China; [3] provided per kg of premix; IU: vit. A 9.000; vit. D 1 400; vit. E 15; mg: vit. K 1.5; thiamin 2.2; riboflavin 3.8; vit. B_{12} 12; pantothenic acid 12; nicotinic 75; choline 1 400; folic acid 0.5; Mg 600; Fe 96; Mn 66; Se 0.1; and Zn 60; [4]metabolizable energy are calculated values; [5] the content of n-6 (n-3) PUFA presented are the total, including linoleic acid (linolenic acid) and other kinds of n-6 (n-3) fatty acids such as arachidonic acid (AA, 20:4 n-6), etc

The N content of feed was determined by the Kjeldahl process (method number 948·13; AOAC, 1990). Fatty acids were analysed by gas chromatography (SHIMADZU GC-14B gas chromatograph, Shimadzu Corp. Kyoto, Japan) with

methyl esterification by 10% sulphuric acid in methanol (H_2SO_4-CH_3OH). The gas chromatography (GC) analyses were performed using a fused silica capillary column (DB-FFAP), 30 m × 0.25 mm inner diameter (ID) × 0.25 μm film thickness (J&W Scientific, Agilent Technologies), a split/splitless injector, an automatic sampler (model AOC-17), and flame ionization detection in a SHIMADZU GC-14B gas chromatograph (Shimadzu Corp. Kyoto, Japan). The initial temperature programme was: 130°C with a 1 min hold; ramp: 4°C /min to 178°C, 1°C /min to 225°C, and then 40°C /min to 245°C, with a 13 min hold. The carrier gas was H_2, with a linear velocity of 60 cm/s; a constant pressure of 102.4 kPa was used. Fatty acid analysis was performed by autoinjection of 1 μl of each sample at a split ratio of 7.5:1. The FID temperature was 250°C, with air and nitrogen make-up gas flow rates of 450 and 10 ml/min.

Sampling design and sample handling

Eight birds of each group were selected randomly, slaughtered quickly after a 24-h fast, on d 42, d 56 and d 70. Isolated livers were placed in an ice tray and index measurements were conducted as soon as possible. Liver tissues were mixed with 0.85% (w/v) saline solution at a proportion of 1:10. The homogenates were centrifuged at 9 000 *g* for 10 min, and the supernatant fluid was collected for index testing.

Index determination and test methods

Assay kits for total superoxide dismutase (T-SOD) activity, glutathione peroxidase (GSH-Px) activity, catalase (CAT) activity, malondialdehyde (MDA), hydrogen peroxide (H_2O_2), nitric oxide (NO), and tissue protein content (Prot.) were purchased from Jiancheng Com. Nanjing (China).

Tissue Prot. - the homogenate was centrifuged at 12.000 *g* for 10 min at 4°C, and the supernatant was diluted to make a 1% solution with normal saline. A Coomassie brilliant blue protein kit was used to determine protein content.

T-SOD - A colorimetric method was used to determine T-SOD activity. One unit of SOD was defined as the enzyme activity causing 50% inhibition in the nitroblue tetrazolium (NBT) reduction rate maintained at 37°C. SOD activity was expressed as U/mg tissue protein. The determination was conducted according to the manufacturer's instructions.

GSH-Px - 5,5-double thio-nitrobenzoic acid (DTNB colour method) was used to examine GSH-Px activity. One unit of GSHPx activity was defined as the amount of enzyme required to decrease the glutathione (GSH) concentration of 1 μmol/l, per min per mg of tissue protein at 37°C. The activity of GSH-Px was

expressed as U/mg tissue protein. The determination was performed according to the kit instructions.

CAT - A catalase ELISA kit was used in which the colour change was measured spectrophotometrically at a wavelength of 450 ± 2 nm. The concentration of CAT in the samples was then determined by comparing the optical density (O.D.) of the samples to the standard curve. The determination was performed according to the kit instructions.

NO - NO was assayed using the Griess method described by Saha et al. (2004). A standard curve of NO was determined by adding PBS with different concentrations of sodium nitrite solution to 40 to 160 μl of Griess reagent, the solution was mixed and after 20 min the O.D. of the solution was measured spectrophotometrically at a wavelength of 550±2 nm. Samples of 40 μl were taken and the NO content (μmol/ml) in the sample was calculated according to the O.D. of the samples and the NO standard curve.

MDA, H_2O_2 - MDA was detected by the thiobarbituric acid (TBA) method; H_2O_2 was examined by coloration method; the determinations were performed according to the kit instructions.

Statistical analysis

Experimental data were processed using Microsoft Excel 2003 (Microsoft Corp. Redmond, WA), and the results are presented as means. Statistical analysis was carried out by ANOVA with the posthoc multiple comparison test of Tukey using SPSS software (SPSS Inc., Chicago, IL). P-values of <0.05 were considered statistically significant.

RESULTS

Effects of n-6:n-3 PUFA ratios on T-SOD, GSH-Px, and CAT in liver

The results presented in Table 2 showed that with increasing age, the level of liver T-SOD in each group declined to a minimum on d 70, and the differences between ages were significant ($P<0.05$). Comparison of treatments showed that in general, T-SOD increased as n-6:n-3 PUFA ratios decreased, and there were significant differences between groups ($P<0.05$). In the 3:1 group it was significantly higher than the remaining groups ($P<0.05$), in the 6:1 group it was significantly higher than groups 9:1 and 12:1 ($P<0.05$), however, the difference was not significant between groups 9:1 and 12:1 ($P>0.05$).

GSH-Px levels had a tendency to decline with age (Table 2). In group 12:1 in particular, a significant difference was found between ages ($P<0.05$),

Table 2. Effects of n-6:n-3 PUFA ratios on liver total superoxide dismutase (T-SOD), glutathione peroxidase (GSH-Px), and catalase (CAT) in liver

Enzymes	Age (d)	A (12:1)	B (9:1)	C (6:1)	D (3:1)	Mean	F-value	P-value
		$53.51^{a,C}$	$56.80^{a,C}$	$67.94^{a,B}$	$74.08^{a,A}$	63.24	60.681	0.000
	42	n=7	n=8	n=7	n=8	n=30		
		$41.03^{b,C}$	$44.09^{b,C}$	$60.05^{b,B}$	$66.00^{b,A}$	53.07	91.810	0.000
	56	n=8	n=7	n=8	n=8	n=31		
T-SOD		$40.08^{b,C}$	$39.03^{c,C}$	$53.83^{c,B}$	$66.00^{b,A}$	49.73	156.998	0.000
U/mg	70	n=8	n=8	N=8	n=8	n=32		
	Mean	44.50	46.60	60.20	68.69			
		n=23	n=23	n=23	n=24			
	F-value	45.584	80.335	22.519	12.753			
	P-value	0.000	0.000	0.000	0.000			
		$20.34^{a,B}$	$18.23^{a,B}$	$27.09^{a,A}$	$29.01^{a,A}$	23.66	87.477	0.000
	42	n=7	n=8	n=7	n=8	n=30		
		$16.16^{b,B}$	$15.97^{b,B}$	$23.00^{ab,A}$	$24.99^{b,A}$	20.16	89.499	0.000
	56	n=8	n=7	n=8	n=8	n=31		
GSH-Px		$14.10^{c,C}$	$15.04^{b,C}$	$20.00^{b,B}$	$24.01^{b,A}$	18.29	90.837	0.000
U/mg	70	n=8	n=8	n=8	n=8	n=32		
	Mean	16.72	16.38	22.97	26.00			
		n=23	n=23	n=23	n=24			
	F-value	90.935	18.818	10.674	17.832			
	P-value	0.000	0.000	0.001	0.000			
		$5.67^{a,B}$	$5.91^{a,B}$	$6.06^{a,B}$	$6.93^{a,A}$	6.16	10.676	0.000
	42	n=7	n=8	n=7	n=8	n=30		
		$5.43^{a,A}$	$5.90^{b,A}$	$5.90^{b,A}$	$6.11^{b,A}$	5.83	2.055	0.130
	56	n=8	n=7	n=8	n=8	n=31		
		$4.54^{b,B}$	$4.45^{b,B}$	$4.76^{b,B}$	$5.86^{b,A}$	4.91	0.217	0.000
CAT	70	n=8	n=8	n=8	n=8	n=32		
U/mg	Mean	5.19	5.34	5.58	6.30			
		n=23	n=23	n=23	n=24			
	F-value	11.521	19.025	16.872	10.618			
	P-value	0.000	0.000	0.000	0.001			

$^{A-C}$ values with different capital letter superscripts in the same row means significant difference ($P<0.05$); $^{a-c}$ values with different small letter superscripts in the same line means significant difference ($P<0.05$)

but no significant differences were detected between d 56 and d 70 in the three other groups ($P>0.05$). Among treatments, GSH-Px increased as the n-6:n-3 PUFA ratios decreased. On d 42 and d 56, GSH-Px was higher in the 3:1 and 6:1 groups, and significantly higher than in groups 9:1 and 12:1 ($P<0.05$), however, the differences between groups 3:1 and 6:1 ($P>0.05$), 9:1, and 12:1 ($P>0.05$) were not significant; on d 70, the 3:1 group was significantly higher than the three other groups ($P<0.05$).

Overall, the levels of liver CAT also declined with increasing age (Table 2). CAT on d 42 in each group was significantly higher than on d 70

(P<0.05). Comparison among treatments showed that on d 42 and d 70, CAT in 3:1 was significantly higher than in the three other groups (P<0.05), while differences among the other three groups were not significant (P>0.05); at d 56, there was no difference in CAT among treatments (P>0.05).

Effects of n-6:n-3 PUFA ratios on MDA, H_2O_2, and NO in liver

Table 3 showed that MDA increased with age and was distinctly higher on d 7. In the 12:1 group, significant differences existed in MDA between ages (P<0.05), while the differences were not significant between d 56 and d 70 (P>0.05) in groups 9:1 and 6:1. MDA levels decreased as the n-6:n-3 PUFA ratios decreased

Table 3. Effects of n-6:n-3 PUFA ratios on liver malondialdehyde (MDA), hydrogen peroxide (H_2O_2), and nitric oxide (NO) in liver

Substances	Days	A (12:1)	B (9:1)	C (6:1)	D (3:1)	Mean	F-value	P-value
		$2.46^{c,A}$	$2.59^{b,A}$	$1.90^{b,B}$	$2.10^{b,B}$	2.27	24.644	0.000
	42	n=7	n=8	n=7	n=8	n=30		
		$4.03^{b,A}$	$3.90^{a,A}$	$2.30^{a,B}$	$2.10^{b,B}$	3.06	204.506	0.000
	56	n=8	n=7	n=8	n=8	n=31		
MDA		$4.31^{a,A}$	$4.12^{a,A}$	$2.34^{a,B}$	$2.35^{a,B}$	3.29	222.119	0.000
nmol/mg	70	n=8	n=8	n=8	n=8	n=32		
	Mean	3.65	3.55	2.23	2.18			
		n=23	n=23	n=23	n=24			
	F-value	173.961	100.052	10.886	5.390			
	P-value	0.000	0.000	0.001	0.013			
		$1.21^{c,A}$	$1.24^{c,A}$	$0.86^{b,B}$	$0.91^{b,B}$	1.06	20.446	0.000
	42	n=7	n=8	n=7	n=8	n=30		
		$1.72^{b,A}$	$1.64^{b,A}$	$1.51^{a,A,B}$	$1.32^{a,B}$	1.55	8.337	0.000
	56	n=8	n=7	n=8	n=8	n=31		
H_2O_2		$2.24^{a,A}$	$1.96^{a,B}$	$1.43^{a,C}$	$1.36^{a,C}$	1.76	44.283	0.000
μmol/mg	70	n=8	n=8	n=8	n=8	n=32		
	Mean	1.75	1.63	1.28	1.20			
		n=23	n=23:1	n=23	n=24			
	F-value	55.745	42.765	57.719	32.477			
	P-value	0.000	0.000	0.000	0.000			
		$7.82^{b,A}$	$7.33^{a,A}$	$6.60^{a,B}$	$6.30^{a,B}$	7.00	27.049	0.000
	42	n=7	n=8	n=7	n=8	n=30		
		$8.91^{a,A}$	$7.50^{a,B}$	$6.90^{a,B}$	$6.49^{a,B}$	7.45	42.586	0.000
	56	n=8	n=7	n=8	n=8	n=31		
NO		$9.40^{a,A}$	$7.90^{a,B}$	$7.01^{a,C}$	$6.55^{a,C}$	7.71	81.921	0.000
μmol/mg	70	n=8	n=8	n=8	n=8	n=32		
	Mean	8.75	7.66	6.85	6.45			
		n=23	n=23	n=23	n=24			
	F-value	19.409	2.325	1.488	1.720			
	P-value	0.000	0.122	0.249	0.203			

$^{A-C}$ values with different capital letter superscripts in the same row means significant difference (P<0.05); $^{a-c}$ values with different small letter superscripts in the same line means significant difference (P<0.05)

in all analysed ages and were significantly lower in groups 3:1 and 6:1 than the other two groups ($P<0.05$), but the differences between 3:1 and 6:1 ($P>0.05$), and between 9:1 and 12:1 ($P>0.05$) were not significant.

The results of H_2O_2 shown in Table 3 indicated that H_2O_2 levels increased when the geese aged, and significant differences were detected between sampling ages ($P<0.05$). It was further observed that H_2O_2 decreased as the n-6:n-3 PUFA ratios decreased and, moreover, 3:1 and 6:1 were significantly lower than in the other two groups at d 42 ($P<0.05$); in group 3:1 it was significantly lower than in groups 9:1 and 12:1 on d 56 ($P<0.05$); and when the geese were aged 70 days, in groups 3:1 and 6:1 H_2O_2 was significantly lower than in 9:1 ($P<0.05$), and in 9:1, it was significantly lower than 12:1 ($P<0.05$).

The NO level increased with age in each group (Table 3), with the rise in group 12:1 reaching significance ($P<0.05$), while in the other three groups the changes were not significant among ages ($P>0.05$). With the decrease of n-6:n-3 PUFA ratios, NO had a tendency to decrease in general. On d 42, in groups 3:1 and 6:1 it was significantly lower than in the other two groups ($P<0.05$); on d 56 and 70, in group 12:1 NO was significantly higher than the other three groups ($P<0.05$).

DISCUSSION

Effects of n-6:n-3 PUFA ratios on T-SOD, GSH-Px, CAT in liver. The body always produces free radicals but, at the same time, has a free radical scavenging system, which protects cytosolic organelles from the damaging effects of the hydroperoxides formed by normal aerobic metabolism (Bertram and Hass, 2008). If the balance is destroyed, however, excessive free radicals might attack cell membranes as well as nucleic acids, proteins, and other biological macromolecules, leading eventually to damage of cell function. SOD is one of the most important enzymes that act as cellular antioxidants. It is present in the cytoplasm and mitochondria in order to maintain a low concentration of superoxide anions, which are the major oxygen radical. GSH-Px is another important peroxidase present in the body, and plays a key role in protecting the cell membrane. GSH-Px reduces the concentrations of H_2O_2 formed during respiration, and specifically catalyzes the glutathione reduction of lipid peroxides (Johnson et al., 2003; Lu, 2006). CAT is a kind of removal reagent, and can promote catalysis of H_2O_2 to H_2O and O_2, and consequently protects cells from H_2O_2 toxicities (Desagher et al., 1996). Therefore, SOD, GSH-Px, and CAT activity are important indicators reflecting the body's antioxidant capacity (Levin, 1988; Sun and Liu, 2009).

Research has shown that n-3 PUFA not only scavenges free radicals directly, but also improves the activity of antioxidant enzymes such as SOD, GSH-Px, thus effectively eliminates free radicals and lipid peroxides, improves the body's

antioxidant and anti-aging capability (Liu, 2009). The current work showed that the activities of liver T-SOD, GSH-Px, and CAT decreased with age, and indicated that the ability to eliminate oxygen radicals, antioxidant capacity, and anti-aging properties tended to decline gradually with age. The activities of liver T-SOD, GSH-Px, and CAT increased as the n-6:n-3 PUFA ratios decreased. Among four groups, the 3:1 and 6:1 groups were significantly higher than the other two groups, and the 3:1 group was significantly higher than 6:1, confirming previous studies in which n-3 PUFA could increase antioxidant capacity. n-3 PUFA may improve the antioxidant enzyme activities by increasing the expression of their genes, and, in our opinion, this should be investigated promptly.

Effects of n-6:n-3 PUFA ratios on MDA, H_2O_2, and NO in the liver. The unsaturated fatty acids in lipids are easily damaged by oxidative free radicals and produce end-products of lipid peroxidation, specifically, MDA (Mayne, 2003; Cortinas et al., 2004). MDA causes cell toxicity, cross-linking proteins within and between molecules, leading to cell damage, releasing a large number of inflammatory mediators. MDA has a significant inhibitory effect on mitochondrial respiratory function, pyruvate dehydrogenase, α-ketoglutarate dehydrogenase, etc. (Long et al., 2005). The toxicity of lipid hydroperoxides to animals has best been illustrated by the lethal phenotype of glutathione peroxidase 4 (GPX4) knockout mice. These animals did not survive past embryonic day 8, indicating that the lipid hydroperoxidase was absolutely essential for life (Muller et al., 2007). Despite being a simple molecule, NO is a fundamental component in the fields of neuroscience, physiology, and immunology (Culotta and Koshland, 1992; Lander et al., 1993; Macphail et al., 2003). NO and O_2^{-1} play key roles in toxic oxidation, can inactivate enzymes, inhibit respiratory chain enzymes, destroy mitochondrial structure, and cause oxidative damage (Forsythe et al., 2002). Thus, MDA, H_2O_2, and NO levels can reflect the body's lipid peroxidation and indirectly reflect the degree of cell membrane damage (Erden-Inal et al., 2001).

Studies have shown that n-3 series PUFA-rich oils have functions of removing lipid peroxides and protecting cells from the effects of oxygen free radical damage. The present work showed that MDA, H_2O_2, and NO increased with age. As is known, cells decrease oxidation resistance gradually when the body ages. In addition, MDA, H_2O_2, and NO levels in the liver tended to decrease as n-6:n-3 PUFA ratios decreased. This might be attributed to the increase of antioxidant enzyme activities by n-3 PUFA, as described above. Among the treatments, the 3:1 and 6:1 group was significantly lower than the two other groups. This result demonstrated that low n-6:n-3 PUFA ratios in diets could increase the ability to remove lipid peroxides of liver cells, and thus improve the antioxidant and anti-aging capacity.

CONCLUSIONS

Based on the results of this study, it can be concluded that the antioxidant function of the liver was influenced by dietary n-6:n-3 PUFA ratios. Diets containing low n-6:n-3 PUFA ratios (3:1 or 6:1) could improve the activities of enzymes such as total superoxidase dismutase, glutatione peroxide, and catalase and decrease the level of substances such as hydrogen peroxide, malondialdehyde and nitric oxide in the liver. This is relevant to improving the antioxidant capability of Yangzhou goslings aged from 42 to 70 days. Future studies should be undertaken to clarify the range of this ratio.

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