EFFECTS OF INSTANT PUMPKIN SOUP: IN VIVO ANALYSIS ON WISTAR RAT'S GROWTH, SERUM Β-CAROTENE, ANTIOXIDANT ENZYME

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ABSTRACT

This study aims to determine the effect of instant pumpkin soup consumption on the rats' growth, β-carotene levels, Antioxidant Enzyme, Malondialdehyde, and lipid profile. 30 male Wistar rats, 8-10 weeks, were divided equally into six groups. Control-negative (C-) rats were maintained on the AIN-93 diet. Control positive (C+) rats were given AIN-93+beef fat. Instant pumpkin soup groups (IPS 20, 40, 60, and 80) were given AIN-93+beef fat+instant pumpkin soup (20, 40, 60, and 80 g/kg of standard diet, respectively). Blood serum was collected and subjected to lipid profile, MDA (TBARS method), antioxidant analysis (DPPH), endogenous antioxidant enzyme, and β-carotene analysis. Data were analyzed using ANOVA followed by post hoc LSD. All rats in each group were shown a significant growth pattern. The antioxidant activity of IPS treatment groups showed insignificant (p>0.05) improvement in a dose-dependent manner. Insignificant improvements in antioxidant enzymes were also found in IPS Treatment groups. There is a significant (p<0.05) reduction of MDA in the treatment groups, with IPS 80 as the most potent dose.

Keywords: pumpkin, instant soup, growth, β-carotene, lipid profile antioxidant, oxidative stress

"تأثيرات حساء اليقطين الفوري: تحليل في الجسم الحي على نمو جرذان ويستار، ومستوى البيتا كاروتين في المصل، وإنزيمات مضادات الأكسدة"

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المستخلص

يحتوي اليقطين على الفينولات والفلافونويدات والفيتامينات المعروفة بفوائدها الصحية .قد يؤدي ابتكار صنع حساء اليقطين الفوري (IPS)إلى تحسين قبول اليقطين .تهدف هذه الدراسة إلى تحديد تأثير استهلاك حساء اليقطين الفوري على نمو الفئران ومستويات بيتا كاروتين وإنزيم مضاد للأكسدة ومالونديالدهيد وملف الدهون .تم تقسيم 30فئران ويستار ذكر، تتراوح أعمارهم بين 8و 10 أسابيع، بالتساوي إلى ست مجموعات .تم الحفاظ على الفئران السلبية الضابطة (–C) على نظام AIN–93 الغذائي .تم إعطاء الفئران الإيجابية الضابطة + AIN–93 (+ C)دهن البقر .تم إعطاء مجموعات حساء اليقطين الفوري IPS 20) و 40 و 60 و + AIN–93 (80 دهن البقر +حساء اليقطين الفوري 20) و 40 و 60 و 80 جم / كجم من النظام الغذائي القياسي، على التوالي .(تم جمع مصل الدم وإخضاعه لتحليل الدهون، و) MDA طريقة (TBARS، وتحليل مضادات الأكسدة (DPPH)، وإنزيم مضاد الأكسدة الداخلي، وتحليل بيتا كاروتين .تم تحليل البيانات باستخدام تحليل التباين متبوعًا بتحليل LSD بعد التجربة .أظهرت جميع الفئران في كل مجموعة نمط نمو ملحوظ .أظهر نشاط مضادات الأكسدة في مجموعات علاج IPS تحسنًا ضئيلًا (p>0.05)بطريقة تعتمد على الجرعة .كما تم العثور على تحسنات طفيفة في إنزيمات مضادات الأكسدة في مجموعات علاج .IPSهناك انخفاض كبير (p<0.05) في MDA في مجموعات العلاج، مع PS 80 اكأقوى جرعة.

الكلمات المفتاحية :اليقطين، الحساء الفوري، النمو، بيتا كاروتين، مضادات الأكسدة في الملف الدهني، الإجهاد التأكسدي

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INTRODUCTION

Pumpkin (*Cucurbita moschata*) is a widely cultivated functional vegetable with a distinctive flavor and scent. This nutrient-rich vegetable contains phenolates, flavonoids, β-carotene, vitamin C, α-tocopherol, carbohydrates, and amino acids, which received considerable attention for its health benefits (25). The reddish-yellow color of the pumpkin depends on the presence of carotenoids. Fresh pumpkin contains 234.21 to 404.98 μg/g of carotenoids. The major carotenoid of this species is β-carotene, accounting for 60% of the carotenoid content, and is also considered a potent antioxidant(7). Pumpkin has been widely explored in the food industry for commercial production, such as snacks, desserts, cereals, pudding, and instant soup (1,30). Instant pumpkin soup is a dry food product processed with a thickening agent (37). The drying process may use a cabinet dryer with attention to the thickness of the pulp to provide a standardized characteristic of the instant soup (29). Thickening agents also play an important role in the production of instant pumpkin soup (10). Corn starch is a common thickening agent for instant soup (21). Recent studies found that porang (*Amorphophallus muelleri*), a native Indonesian tuber plant, is a promising source of hydrophilic polysaccharides called glucomannan (13, 34). This glucomannan contains abundant hydroxyl and carbonyl groups that may improve viscoelasticity (22, 35), rheology, and microstructure of the product (12, 36), strengthening its potential as a thickening agent. The health benefits of pumpkin are widely reported. Strong antioxidant characteristics from β-carotene in pumpkin bakery products were confirmed to improve rats' lipid profile *in vivo* (8). Pumpkin also contains vitamin C, Vitamin E, flavonoid, and phenol that act synergically as a potent exogenous antioxidant. Several minerals, such as Cu, Fe, Zn, and Se, found in pumpkins also support their health benefits as a cofactor for antioxidant enzymes (17). Conceptually, the human body can produce endogenous antioxidants. Some types of antioxidants produced by the body are superoxide dismutase (SOD), catalase and glutathione peroxidase enzymes(4,33). These three enzymes play a role in suppressing oxidation reactions in the body with ROS, such as O_2- and H_2O_2. The SOD enzyme converts superoxide radicals into hydrogen peroxide and oxygen molecules. The catalase and glutathione peroxidase enzymes convert hydrogen peroxide into H_2O(33). Antioxidants found in the body and those found outside the body can cooperate to preserve or restore redox equilibrium. Interacting ROS and antioxidants are regarded as functionally linked redox-active chemicals; they are essential parts of an organism's redox processes (26). This study aimed to explore the health-promoting effects of instant pumpkin soup, consisting of Wistar Rat's Growth, Serum β-carotene, Antioxidant Enzyme, Malondialdehyde, and Lipid Profile.

MATERIALS AND METHODS

This study used 30 male Wistar rats, eight to ten weeks old, 150-200 g of body weight, pumpkins (*Cucurbita moschata* var. Bokor) obtained from the local market, chicken broth, skim milk, AIN 93 as a standard feed for rats, SOD Kit (ABBKINE, China), Catalase Kit (ELABSCIENCE, China), and Glutation Peroxidase Kit (ELABSCIENCE, China), Cholesterol Kit (DiaSys, Germany), Triglycerides Kit (DiaSys, Germany), HDL Kit (DiaSys, Germany), DPPH (Sigma Aldrich, USA), ethanol, and petroleum ether.

Preparation of Instant Pumpkin Soup

Pumpkins, (*Cucurbita moschata* var. Bokor) were steamed at 90°C for 10 minutes to soften the flesh. The steamed pumpkins were then blended for several minutes until mashed thoroughly. The pumpkin pulp was added with 6% skimmed milk, 50% chicken broth, 0.5% Porang flour, and some seasonings. The pulp was dried using a cabinet dryer for 6 hours at 50 - 60°C. The dried pulp is then crushed using a blender and sifted using a 60-mesh sieve. Instant pumpkin soup was analyzed for its proximate content and fibers.

Experimental Protoco

The experimental protocol to assess the rat's lipid profile, growth, and serum β-carotene is shown in Figure (1). Male Wistar rats (n=30) were kept in a stainless-steel cage at the Animal Testing Laboratory, Universitas

Muhammadiyah Semarang. Rats were divided into six equal groups. Rats with abnormal behavior, weakness sign, and food rejection are considered excluded.

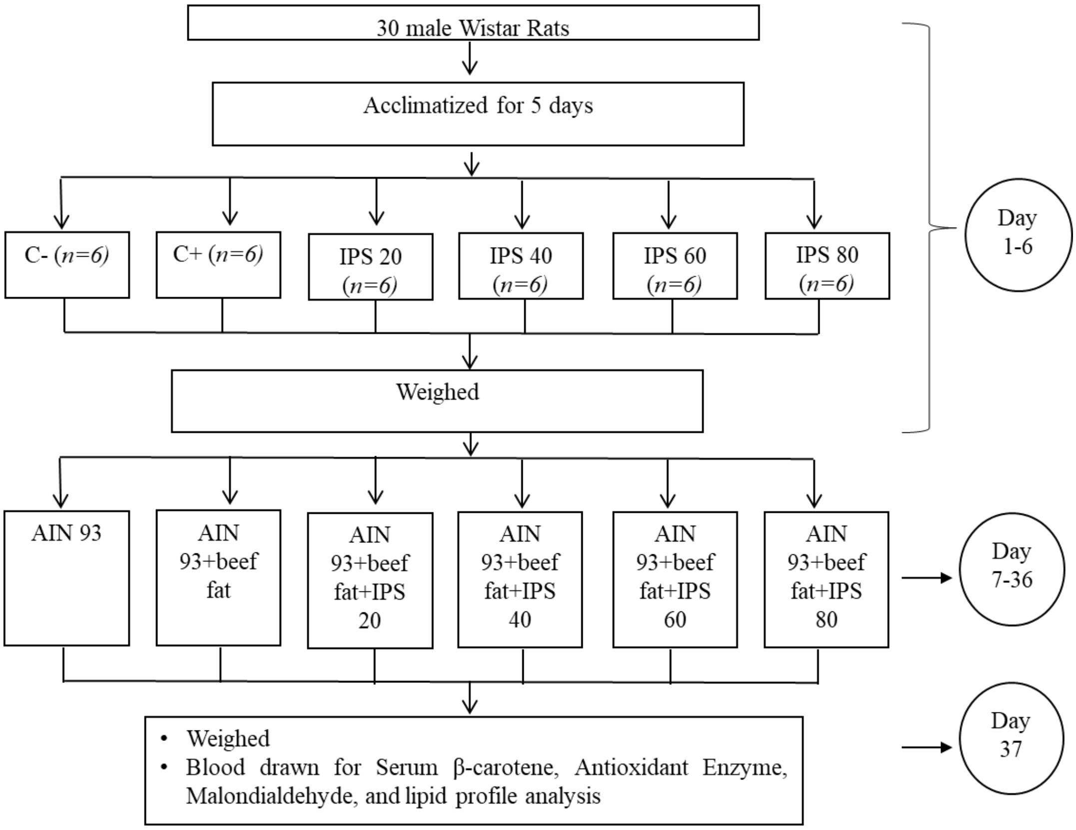

Figure 1. Study Protocol

Rats were fed according to Table 1. The control negative (C-) group received a standard diet of AIN-93. Control positive (C+) were given AIN-93 enriched with beef fat. Instant pumpkin soup groups (IPS 20, 40, 60, and 80) were maintained on a diet consisting of AIN-93 + beef fat + different doses of instant pumpkin soup (20, 40, 60, and 80 g/kg of standard diet, respectively). All rats were weighed pre- and post-treatments. The rats were acclimatized for five days prior to treatment. The protocol and the study's experimental design were approved by the Ethics Committee Research.

Table 1 The composition of Standard and IPS-enriched feed

Materials	Treatments					
	C-	C+	IPS 20	IPS 40	IPS 60	IPS 80
Skimmed milk (g)	377	377	372.70	368.39	364.35	359.77
Corn starch (g)	620.70	440.70	199.30	173.32	148.88	121.37
Instant pumpkin soup (g)	-	-	20	40	60	80
Corn oil (g)	42.29	42.29	41.28	40.25	39.31	38.23
Sucrosa (g)	100	100	100	100	100	100
CMC (g)	50	50	50	50	50	50
Vitamin mix AIN 93 (g)	10	10	10	10	10	10
Mineral mix AIN 93 (g)	35	35	35	35	35	35
L-cystine (g)	1.80	1.80	1.80	1.80	1.80	1.80
Choline bitartrate (g)	2.50	2.50	2.50	2.50	2.50	2.50
Beef fat (g)	-	180	180	180	180	180

The control negative (C-) group received a standard diet of AIN-93. Control positive (C+) were given AIN-93 enriched with beef fat. Instant pumpkin soup groups (IPS 20, 40, 60, and 80) were given AIN-93 + beef fat + different doses of IPS (20, 40, 60, and 80 g/kg of standard diet, respectively).

The blood samples were taken after 30 days of treatment from plexus retro-orbital for approximately 4 ml. The blood was centrifuged for 10 minutes at 4000 rpm to separate the platelet and the serum. The collected serum was subjected to lipid profile, MDA (TBARS method), antioxidant activity (DPPH), endogenous antioxidant enzyme and β-carotene analysis. Endogenous antioxidant enzymes consist of SOD (ABBKINE method), Catalase (ELABSCIENCE method), and Glutation Peroxidase (ELABSCIENCE method). Lipid profile analysis includes cholesterol and HDL (CHOD-PAP method) and triglycerides (glycerol-3-phospate oxidase; GPO method).

Antioxidant Activity

A total of 180 µL of plasma was pipetted into a 2 ml microtube, then added with 1620 µL of 2,2-diphenyl-1-pycrilhydrazyl reagent (DPPH Sigma Aldrich, USA) 0.004 percent w/v. The control used was a DPPH reagent of as much as 1620 µL. The samples and controls were then stored in a dark room for 60 minutes and then centrifuged for 10 minutes at a speed of 800 µg. The supernatant was pipetted into a microplate as much as 100 µL, then measured using a microplate reader spectrophotometer at a wavelength of 517 nm, and the analysis was carried out in duplicate. The absorbance of each sample was obtained and its antioxidant activity was calculated using the % RSA formula

$$\textbf{\% Antioxidant activity} = \frac{Absorbance - Sample\ absorbance}{Absorbansi\ kontrol} \textbf{ x 100\%}$$

Statistical Analysis

The normality of the data was tested using the Shapiro-Wilk test. The significance between Pre- and post-test weight differences was analyzed using a paired t-test. The determination of weight, antioxidant activity, MDA, SOD, catalase, and lipid profile differences between groups was analyzed using ANOVA followed by LSD. The differences in the statistical result were considered significant for $p<0.05$.

RESULTS AND DISCUSSION

Proximate, Fiber, and β-carotene Analysis of Instant Pumpkin Soup

This study evaluated the Proximate, fiber, and β-carotene content of the IPS formula enriched with 0.5% of Porang flour. As shown in Table (2), the IPS formula contains higher protein, fat, carbohydrate, and fiber content than the raw ingredients. Raw pumpkin has 11.77% of carbohydrates, 2.02 % of protein, and 0.064 % of fat (25). Adding some ingredients, such as skimmed milk, beef fat, and porang flour, increase the macro mineral content in this IPS formula significantly.

Table 2. Proximate and Fiber Analysis of Instant Pumpkin Soup

Composition	Amount (%)
Water	6.99±0.08
Protein	7.94±0.47
Fat	5.08±0.88
Carbohydrate	74.23±0.92
Ash	5.75±0.40
Fiber	11.38±0.74

The IPS formula in this study also contains 12,04 mg/100 g of β-caroten, three times higher than the raw form and 11.38% of fiber. The fiber content is about 8,21% higher than raw pumpkins with the same variety (25). A previous study found that porang flour may have 53.46% of soluble dietary fiber. Glucomannan is the most water-soluble dietary fiber in porang flour 34). This formula's improvement of dietary fiber is expected to be due to adding 0.5% of porang flour.

Table 3. Rat's body weight Pre- and Post- Treatment

Treatment	Body weight (g)		Growth (g)
	Before	After	
Negative control	189.25±10.24^{a}	235.48±25.80^{b}	46.23±19.81^{a}
Positive control	180.00±6.67^{a}	239.08±22.10^{b}	59.08±17.05^{a}
IPS 20	159.00±19.86^{a}	220.54±22.47^{b}	61.54±11.17^{a}
IPS 40	164.00±10.12^{a}	229.94±22.94^{b}	65.94±14.88^{ab}
IPS 60	163.80±13.74^{a}	237.54±13.04^{b}	73.74±12.34^{ab}
IPS 80	165.00±9.03^{a}	248.90±24.75^{b}	83.90±18.78^{b}

The control negative (C-) group received a standard diet of AIN-93. Control positive (C+) were given AIN-93 enriched with beef fat. Instant pumpkin soup groups (IPS 20, 40, 60, and 80) were given AIN-93 + beef fat + different doses of IPS (20, 40, 60, and 80 g/kg of standard diet, respectively). Pre- and post-test differences were analyzed using a t-test. Weight differences between groups were analyzed using ANOVA followed by LSD. Alphabetical superscripts showed a significance level.

The Effect of IPS on Rat's Body Weight

Growth in this study was assessed by body weight improvement. This study demonstrated that all rats in the control and IPS groups showed significant weight increase. The IPS groups presented a growth-increasing trend in a dose-dependent manner. The highest improvement was presented in the IPS 80 group. Post-hoc analysis found that the IPS 80 group significantly differed from the negative and positive control groups and the IPS 20 group (Table 3). As mentioned, the IPS formula contains higher macro-mineral content than raw pumpkins. These properties may support the growth of the rats. As a main ingredient of IPS, the pumpkin also contains several nutrients such as polysaccharides, β-carotene, and several vitamins and minerals. Prior studies found that polysaccharides in pumpkin may stimulate the growth of intestinal bacteria, such as *Bifidobacterium bifidium, Bifidobacterium longum, and Lactobacillus brevis*, thus related to the improvement of individual growth (14). All the nutrients inside the pumpkin, including β-carotene, polysaccharides, vitamins, and minerals, synergistically act as potent antioxidants that may reduce oxidative stress (9,14), therefore promoting the beneficial effect of growth and development, reproduction, cell differentiation, immunity, cardioprotective effect, and anti-carcinogenesis (2,14,20).

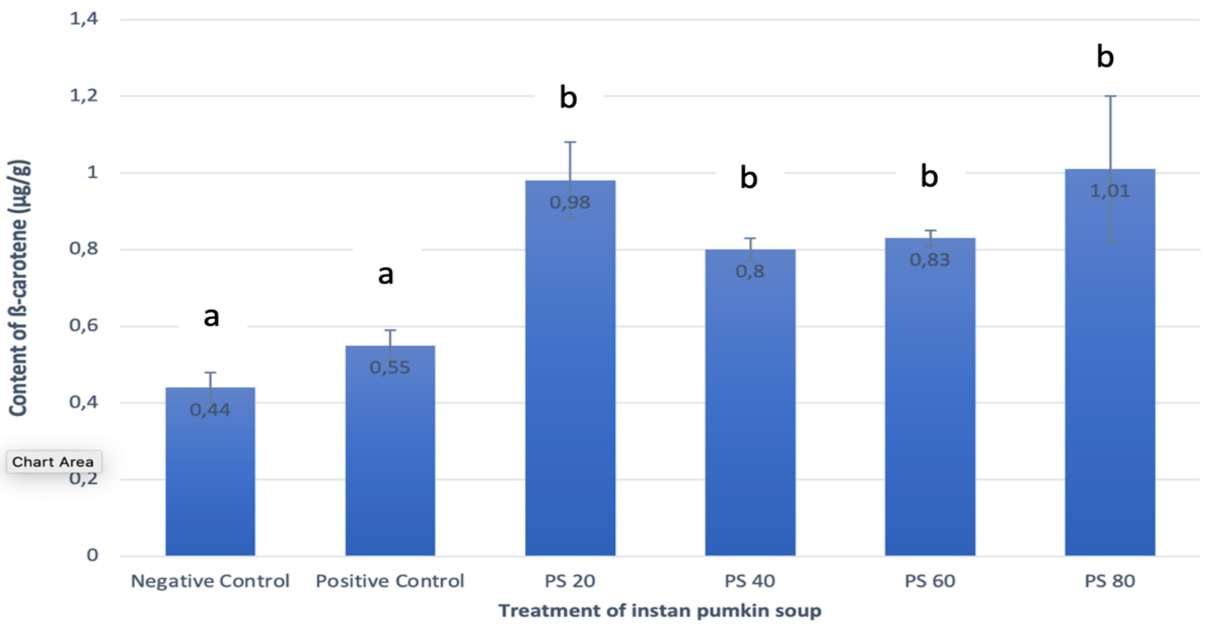

Figure 2. The effect of treatments on the Serum β-carotene (different alphabet showed significant difference)

The Effect of IPS on Serum β-carotene

Serum β-carotene was analyzed 30 days after treatments. The study showed that treatments increase Serum β-carotene in all groups. The improvement of Serum β-carotene in C+ group is higher than C-but not significant. The presence of Serum β-carotene may result from the presence of corn oil as one of the standard feed's ingredients (38). Serum β-carotene of the IPS groups was significantly higher (p<0.05) than the control groups. The highest Serum β-carotene was detected in the IPS 80 group, emphasizing the abundant β-carotene content in the pumpkin (25). β-Carotene plays a vital role as an antioxidant by scavenging Reactive oxygen species (ROS) in the form of single molecular oxygen and peroxyl radicals. There are three mechanisms exist for carotenoids to scavenge free radicals: electron transfer (oxidation, reduction: CAR + ROO → CAR^{+} + ROO^{-}), hydrogen abstraction (CAR + ROO → CAR + ROOH), and addition (CAR + ROO → ROOCAR) (23). β-Carotene serves as a powerful chain-breaking antioxidant (28,31); β-carotene consumption has a linear correlation with serum β-carotene in the blood (3).

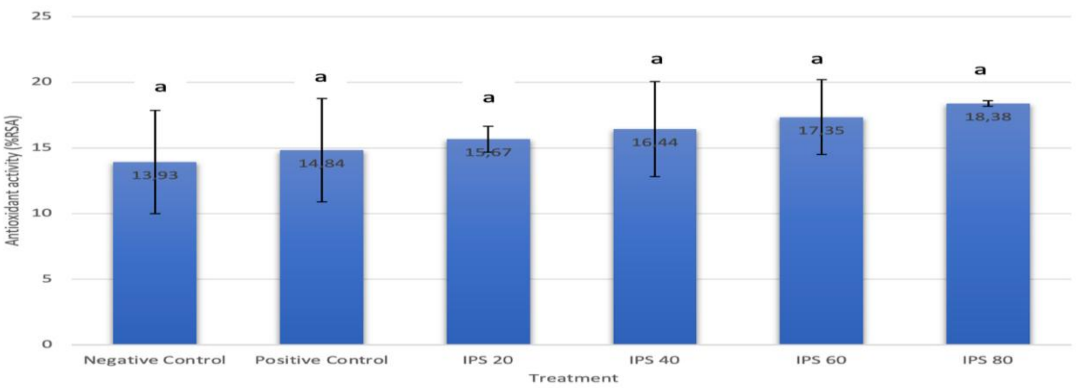

Figure 3. The relationship between instant pumpkin soup consumption anda antioxidant activity of rat blood serum. Same letter indicates no significant difference (p>0.05)

The Effect of IPS on Antioxidant Activity

Figure (3) shows the antioxidant activity in the rat's serum. The average antioxidant activity of the rats ranged between 13.93 -18.38% RSA. The data showed that antioxidant activity in the treatment groups was increased in a dose-dependent trend even though the result was not statistically significant (p>0.05). It has been demonstrated that consuming a high-fat diet leads to abnormalities in the metabolism of fatty acids and damages the heart's energy metabolism, primarily through the uncoupling of oxidative phosphorylation and the maladaptation of free fatty acid oxidation, while also lowering the expression of peroxisome-proliferator-activated receptor alpha (PPARα). Furthermore, a high-fat diet causes endothelial cells to produce more reactive oxygen species (ROS) in a toll-like receptor 4 (TLR4)-dependent manner, linked to decreased adiponectin expression in white adipose tissue (24). This study found that the positive control group also showed higher antioxidant activity compared to negative control group. This result is thought to be due to the high endogenous antioxidant capacity of the rats that combat the free radicals. According to the theory, antioxidants from both the body (endogenous) and the environment (exogenous) work synergistically to preserve redox equilibrium. Many of the antioxidants in whole plant meals, such as polyphenols and flavonoids, function as immunomodulators, antioxidant-related-element stimulants, and free radical scavengers. Certain antioxidants stabilize and neutralize dangerous free radicals by donating electrons. Other antioxidants combat the chemicals that create free radicals before they initiate the processes that lead to oxidative damage (5,16). The high antioxidant content in IPS was influenced by pumpkin as the main ingredient of IPS. It is interesting to explore whether increasing the amount of instant pumpkin soup added to the feed may improve the antioxidant activity of the rats. Moreover, the duration of treatment may be the factor that may improve the result.

Table 4. Endogenous Antioxidant Enxyme of rats with instant pumpkin soup treatment

Endogenous Antioxidants	Treatment					
	Negative Control	Positive Control	IPS 20	IPS 40	IPS 60	IPS 80
SOD (Unit/ml)	40,49±8,65^{a}	31,46±5,15^{a}	56,99±9,99^{b}	60,95±5,95^{b}	53,45±8,28^{b}	58,90±9,87^{b}
Catalase (Unit/ml)	58,47±13,81^{a}	49,80±13,63^{a}	63,78±7,97^{a}	61,85±13,63^{a}	78,55±6,83^{b}	82,73±8,19^{b}
Gluthation Peroxidase (Unit/ml)	28,76±2,29^{a}	29,36±0,79^{a}	29,28±0,58^{a}	30,61±1,10^{a}	32,05±3,93^{a}	31,65±2,60^{a}

The control negative (C-) group received a standard diet of AIN-93. Control positive (C+) were given AIN-93 enriched with beef fat. Instant pumpkin soup groups (IPS 20, 40, 60, and 80) were given AIN-93 + beef fat + different doses of IPS (20, 40, 60, and 80 g/kg of standard diet, respectively). Lipid profile parameter differences between groups were analyzed using ANOVA followed by LSD. Alphabetical superscripts showed a significance level; a: significant compared to control group; b: significant compared to treatment group

Table 4 shows three endogenous antioxidant enzyme levels, Superoxide Dismutase (SOD), Glutathione Peroxidase (GPx), and Catalase (CAT).

The Effect of IPS on Endogenous Antioxidant Enzyme

The SOD enzyme activity was lowest (31.46 Units/ml) in the positive control group. In comparison, the highest activity was observed in the group receiving 40 g/kg instant pumpkin soup in standard feed (60.95 Units/ml). Statistical analysis revealed a significant difference ($p<0.05$) in SOD enzyme activity among the various dietary treatments. Interestingly, the negative control group exhibited a higher SOD enzyme activity compared to the positive control group, but this difference was not statistically significant. This observation suggests that dietary beef fat supplementation does not exert a substantial influence on SOD enzyme activity. Of the catalase activity, the positive control group showed the lowest activity (49.80 Units/ml), and the IPS 80 group showed the highest (82,73 Units/ml). Significant differences were observed on IPS 20 and IPS 40 groups. Thus, statistical analysis showed that Instant pumpkin soup affects the serum catalase of rats significantly ($p < 0.05$). There is no significant difference between negative and positive control, indicating that beef fat does not affect catalase activity. The treatment group, which included the 40, 60, and 80 groups, showed an increasing trend of serum catalase in a dose-dependent manner. The average serum catalase activity of IPS 60 and IPS 80 significantly differed from that of the IPS 20 and IPS 40 groups. The lowest glutathione peroxidase enzyme activity was in the negative control group (28.76 Units/ml), while the highest was in the 60 g/kg standard feed instant pumpkin soup group (32.05 Units/ml). The average value was almost the same in all groups of rats given control treatment (negative and positive) and instant pumpkin soup. The value of the positive control group was slightly different from the negative control and was not significantly different. This shows that the provision of beef fat does not affect the activity of the glutathione peroxidase enzyme. There are two types of endogenous antioxidants: enzymes and non-enzymatically endogenous antioxidants. The majority of endogenous antioxidants are enzymes, including glutathione reductase (GR), glutathione peroxidase (GPx), catalase (CAT), and superoxide dismutase (SOD). Conversely, the body produces non-enzymatic endogenous antioxidants such as glutathione and lipoic acid as a result of metabolism(11). The enzyme plays a crucial role in converting superoxide radicals (O_2-) into hydrogen peroxide (H_2O_2) and oxygen (O_2). Hydrogen peroxide (H_2O_2), a reactive oxidant, is subsequently neutralized by catalase and glutathione peroxidase enzymes into water (H_2O). This coordinated action of these three antioxidant enzymes effectively controls oxidative stress (18,33). The SOD enzyme activity data for the instant pumpkin soup groups showed a significant difference compared to the control groups (negative and positive). This finding strongly indicates that instant pumpkin soup supplementation has a pronounced effect on SOD enzyme activity. The observed elevation in SOD enzyme activity suggests an enhanced antioxidant status in the subjects, potentially leading to an improved ability to counteract pro-oxidant species. The glutathione peroxidase enzyme is pivotal in neutralizing oxidative species generated within the body. This enzyme effectively converts hydrogen peroxide (H_2O_2) into water (H_2O) and oxygen (O_2), thereby eliminating the oxidative potential of these reactive molecules. The end products of glutathione peroxidase-catalyzed reactions are non-oxidative compounds.(19). The statistical analysis showed that all treatment groups had glutathione peroxidase activity in rat serum that was not significantly different ($p>0.05$). However, the provision of feed containing instant pumpkin soup has a higher tendency than the control groups (both negative and positive group). If rats are maintained with instant pumpkin soup for a longer period, they may have significantly higher glutathione peroxidase enzyme activity.

Conclusions

All rats experienced significant growth during maintenance. Enriching IPS may improve serum β-carotene and lipid profiles in several ways. Although insignificant, IPS treatment may improve antioxidant activity and endogenous antioxidant levels, thus lowering MDA levels.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

DECLARATION OF FUND

The authors declare that they have not received a fund.

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