The Journal of Veterinary Medical Science

FULL PAPER

Physiology

Supplementation with rice bran hydrolysates reduces oxidative stress and improves lipid profiles in adult dogs

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ABSTRACT. Oxidative stress is defined as an imbalance between reactive oxygen species (ROS) production and antioxidant defense mechanisms of the body. An overproduction of ROS leads to lipid and protein oxidation, injuring the cells both in normal and pathological conditions. Rice bran protein hydrolysates (RBH) has potent antioxidant, anti-inflammatory, anti-angiotensin converting enzyme (ACE) and hypolipidemic effects. Little is known, however, about the effects of RBH in dogs. The present study evaluated the antioxidative, anti-ACE and metabolic effects of RBH in adult dogs. Eighteen adult dogs were divided into 2 groups: control (n=7) and RBH supplemented groups (n=11), received a diet with the same nutritional compositions. The RBH supplemented group was fed with RBH 500 mg/kg body weight (BW) mixed with food for 30 days. BW, blood glucose, lipid profiles, liver enzymes, electrocardiography (ECG), plasma ACE activity, oxidative stress and antioxidant biomarkers were determined on day 0 and day 30 of supplementation periods. Results showed that RBH decreased oxidative stress and increased antioxidant biomarkers by significantly reducing plasma malondialdehyde (MDA) and protein carbonyl, enhanced blood glutathione (GSH) and improved the GSH redox ratio. Moreover, decreased LDL-C and increased HDL-C levels were found after RBH supplementation whereas BW, blood glucose, liver enzymes, plasma ACE activity, plasma catalase (CAT) and superoxide dismutase (SOD) activity and cardiac function were not significantly changed. These results suggest that RBH may help to lower the risk of oxidative stress and dyslipidemia in adult dogs.

KEYWORDS: antioxidant, dog, dyslipidemia, oxidative stress, rice bran hydrolysates

J. Vet. Med. Sci.
85(7): 727–734, 2023
doi: 10.1292/jvms.22-0513

Received: 21 November 2022
Accepted: 3 May 2023
Advanced Epub:
23 May 2023

Oxidative stress is defined as the imbalance between the production and neutralization of reactive oxygen species (ROS) [1]. It can occur from a reduction of antioxidants, an excess of ROS, or both [25]. An increase in ROS level leads to lipid peroxidation, protein oxidation and damages the DNA strands leading to cellular dysfunction and death [16, 26]. Oxidative stress plays a major role in the pathogenesis of numerous disease processes affecting human and companion animals, including neoplasia, heart disease, cancer, diabetes mellitus (DM), as well as trauma and burns [16, 25]. In normal conditions, age is the greatest influence on free radical generation [42]. A previous study proposed that decreased levels of antioxidants and an increased amount of ROS contributed to oxidative damage to lipids and protein in the brains of older dogs and those changes are apparently increased with age in dogs [33]. In addition, some investigators showed that ROS generation increased with aging [22, 36]. These findings indicated the importance of oxidative stress both in pathological and normal conditions. Treatment for oxidative stress was aimed to suppress the damaging effect of ROS and oxidative stress which included decreased ROS generation, augmenting endogenous antioxidants, or scavenging existing ROS [25], and it has been proven that antioxidative substances supplementation may have a positive effect [17].

Rice bran is a by-product of the milling process which is derived from milling of the brown rice to white rice. Each year, over 63 million tons of rice bran are produced and most of it is utilized in animal feeding [20]. In animal farm feeding, up to 40% of dietary intake of poultry, pigs and cows is comprised of rice bran and fat [34] whereas in companion animals, rice bran is an ingredient commonly used in pet foods [10]. Rice bran is an excellent source of essential amino acids and contains several bioactive molecules especially phytochemicals which have medical and nutritional properties in the management or prevention of chronic diseases such as type-2 DM, obesity and cardiovascular disease [12, 34]. This indicates that rice bran was primarily used as an animal feed and it is

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gaining importance commercially in human diets due to the beneficial nutritive and biological effect. Rice bran hydrolysates (RBH) which is prepared from defatted rice bran and possesses antioxidant, anti-inflammatory and angiotensin converting enzyme inhibiting (ACEi) activity [37, 38]. Previous studies have reported the beneficial effects of RBH in hypertension, insulin resistance and metabolic syndrome in rats by moderating hypertension and mitigating cardiovascular risk factors through vasodilatory, antioxidant activities, an ACEi property, ameliorating dyslipidemia, insulin resistance, anti-inflammation, reduction of oxidative stress, cardiac autonomic dysfunction, cardiovascular remodeling and arterial stiffness [3–5, 37, 38]. Little is known, however, about the potential health benefits of RBH in companion animals. A previous study in dogs found that inclusion of stabilized rice bran or defatted rice bran had no adverse effect in diet nutrient digestibility, fecal characteristics or changes in inflammatory immune mediators [39]. Furthermore, a study using a canine fecal inoculum showed that with a 24 hr fermentation, rice bran resulted in greater concentrations of propionate, butyrate and lactate when compared with rice bran combined with one of probiotics or probiotic alone [30]. This result indicates that rice bran may be an economic alternative to prebiotic supplementation of pet foods [30]. Considering the beneficial effect of RBH in rats and positive results of rice bran supplementation in dog diets, the health benefits of RBH supplementation in dogs is interesting. It is therefore, in the present study the effects of RBH on metabolic parameters, cardiac function, ACE activity, oxidative stress and antioxidant parameters were determined in adult dogs.

MATERIALS AND METHODS

Animals

Eighteen adult dogs of mixed breed, 9 males and 9 females aged 4.9 ± 0.76 years with an average body weight of 19.17 ± 1.28 kg, were enrolled in this study. Before the recruitment, physical examinations, biochemical profiles and electrocardiography were performed to confirm good health status of all dogs and owner consent was obtained for each dog. All dogs were housed at the Experimental Dog Building, Faculty of Veterinary Medicine, Khon Kaen University, where the dogs were individually housed in 150 cm ×150 cm ×150 cm identical cages with environmentally enriched facilities. Dogs were allowed to play and socialize twice daily with other dogs and people for at least 1 hr/time. Ethical approval for this study was obtained from the Institutional Animal Care and Use Committee of Khon Kaen University (IACUC-KKU-24/62) and was in accordance with the Guidelines for Care and Use of Animals for Scientific Purposes of the National Research Council of Thailand.

Experimental design

For 5 days before experimental initiation, all dogs were given a commercial diet with free access to drinking water. The nutrition composition of the pretrial food was crude protein 6.0% (min.), crude fat 2.5% (min.), crude fiber 1.5% (max.) and moisture 86.0% (max.). This period was used to standardize the oxidative and antioxidant status of the dogs. All dogs were then divided into 2 groups: control group (7 dogs, 4 males and 3 females) and RBH supplemented group (11 dogs, 5 males and 6 females). Both groups received a standard diet with the same nutritional compositions, excepting dogs in the RBH supplemented group who received a standard diet added with an RBH of 500 mg/kg BW. During the experimental periods (30 days), body weights (BW) were recorded weekly and all dogs were fed to maintain protocol entry body weight. Food was fed individually *ad libitum* with a controlled amount based on recorded weight and historical food intake. Water was available *ad libitum* throughout the study period.

For evaluation of the effect of RBH supplementation; BW, modified body mass index (MBMI), waist to truncal length ratio (WTLR), blood glucose, lipid profiles, liver enzymes, electrocardiography (ECG), angiotensin converting enzyme (ACE) activity, oxidative stress and antioxidant markers were determined at the beginning (Day 0) and at the end of the trial period (Day 30).

RBH supplementation

RBH was obtained from the faculty of Agro-Industry, Chiang Mai University (Chiang Mai, Thailand). RBH preparation is described in a previous study [38]. The composition of RBH was: protein content: 27.47% (wet basis), fat: 3.45%, and moisture: 1.44%. The polyphenolic compounds in RBH consisted of gallic acid 0.392 g/kg, eriodictyol 0.0083 g/kg, apigenin 0.1042 g/kg, isoquercetin 1.412 g/kg, kaempferol 0.0334 g/kg, quercetin 1.053 g/kg, rutin 0.8893 g/kg, catechin 0.6453 g/kg and tannic acid 0.6686 g/kg (Central Laboratory, Bangkok, Thailand).

During the 30 days of the supplement, all dogs in this RBH supplemented group were given 500 mg/kg of RBH mixed with a meal per day. The doses of RBH were chosen based on the results of preliminary observations and previous studies [37, 38]. BW was measured every week and the RBH supplement dose was adjusted. Each time of supplementation, RBH was mixed with a small amount of food and given to the dogs before feeding the remainder of the meal to assure that all dogs received RBH at a dose 500 mg/kg/day.

Body dimensions and morphometric measurements

Tape measurements were used to accessed body dimensions. Truncal length (TL, cm) was measured from the front of the chest at the shoulder level to the point of buttock and waist circumference (WC, cm) was measured over the dorsal spine of the 4th lumbar vertebrae using a tape wrapped around the dog abdomen in a standing position [40]. TL and WC were used for the modified body mass index (MBMI) and the waist-to-truncal length ratio (WTLR) calculation was as previously described [40]. These parameters were useful for evaluation of abdominal obesity and general body composition in dogs.

Electrocardiographic recording

ECG was performed by a single-channel automatic electrocardiograph (Cardisuny C100 FUKUDA M.E., Nagareyama, Japan). In

brief, all dogs were held in the standard position, right lateral recumbency, on a blanket and the electrodes were attached directly to the dog's skin. The electrocardiography was performed using standard limb leads [41].

Blood sampling

Blood was collected from each dog before and after 30 days of RBH supplementation. Before the blood samples were collected, dogs were fasted for 12 hr and then 3 mL of blood was sampled from the cephalic or saphenous veins. Blood samples were stored in 3 tubes, one without anticoagulant, the second with heparin and the third with potassium-EDTA. Serum samples were obtained by blood centrifugation at 5,000 × *g* for 15 min at 25°C and plasma samples were separated by blood centrifugation at 3,500 × *g* for 15 min at 4°C. The samples were stored at −20°C until analysis.

Fasting blood glucose, lipid profiles and liver enzymes

Fasting blood glucose (FBG) was determined in blood samples collected from each dog using a glucometer (ACCU-CHEK®, Roche Diagnostics GmbH, Mannheim, Germany). Serum concentrations of lipid profiles (cholesterol, triglyceride (TG), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C)) were measured by using enzymatic and colorimetric methods (Roche Diagnostics, Bangkok, Thailand). The liver function was evaluated by measuring liver enzymes, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) concentrations in plasma using a kinetic rate method [6, 15, 21].

Plasma angiotensin converting enzyme activity

Plasma angiotensin converting enzyme (ACE) activity was measured by using the *o*-phthalaldehyde (OPA)-chromogenic reaction as previously described with slight modifications [3, 9]. In brief, 25 µL of plasma sample were mixed with 15 mM Hip-His-Leu solution in sodium borate buffer and incubated at 37°C for 30 min. The color reactions were formed by OPA reagent added and the background absorbance were assessed from plasma samples diluted in 150 µL buffer. After 20 min incubation at room temperature, the absorbance was determined with a spectrophotometer (Ultrospec 6300 *pro*. Biochrom Ltd., Cambridge, UK) at 390 nm. The results were assessed according to a standard curve of ACE solutions (15–120 mU/mL).

Plasma oxidative stress and antioxidant markers

Plasma malondialdehyde and protein carbonyl: Malondialdehyde (MDA) concentration as a marker of lipid peroxidation, was quantified by measuring thiobarbituric acid (TBA) reactive substance and protein carbonyl level as a marker of protein oxidation, were determined in the plasma following a previously described method [29].

Blood glutathione: The reduced glutathione (GSH) and the oxidized forms, glutathione disulfide (GSSG), indicator of the cellular redox state were measured in the whole blood following the method described previously [29]. In brief, 100 µL of whole blood was immediately reacted with 33 mM 1-methyl-2 vinyl-pyridinum trifate (M2VP) or distilled water and subsequently protein was precipitated by adding 5% cold metaphosphoric acid (MPA). After centrifugation, the supernatant was used in the enzymatic coupling assay for GSH by reading optical density at 412 nm using a spectrophotometer (Biochrom Ltd., Cambridge, UK). The redox ratio was calculated as GSH/GSSG.

Plasma catalase activity: Catalase (CAT) activity was determined in plasma by Goth's colorimetric method as previously described [13] with slight modifications. In brief, a 20 µL sample of plasma was incubated in H_2O_2 substrate for 1 min., then the enzymatic reaction was stopped by adding ammonium molybdate. The formation of a yellow complex by ammonium molybdate and H_2O_2 was measured at the intensity at 405 nm and the results were presented as kU/L.

Plasma superoxide dismutase (SOD) activity was determined with the corresponding detection kits (Sigma-Aldrich, Merck KGaA, Darmstadt, Germany) according to the manufacturer's protocols by colorimetric analysis using a spectrophotometer.

Statistical analysis

All data were expressed as the means ± standard error of the mean (SEM). Two-way repeated measures analysis of variance (ANOVA) followed by Duncan's *post hoc* test were used to determine the differences in investigated parameters between control and RBH supplemented groups. Values were considered to have a significant at P value <0.05.

RESULTS

Effect of RBH supplementation on body weight, body dimensions and metabolic variables

Comparisons of metabolic profiles between baseline values (Day 0) and after 30 days of RBH supplementation (Day 30) in healthy dogs are shown in Table 1. There were no significant differences in BW, MBMI, WTLR, FBG and liver enzymes between before and after 30 days of supplement. In the control group, all metabolic parameters showed no significant differences between baseline values and after 30 days of trial (Table 1). The values of serum HDL-C concentrations significantly increased and LDL-C concentrations significantly decreased after 30 days of RBH supplementation, and when compared with the control group (P<0.05; Table 1). Serum cholesterol and TG levels showed no significant changes before and after 30 days of supplement. The values of FBG, lipid profiles and liver enzymes both in baseline and after RBH supplementation remained within normal ranges (Table 1).

Table 1. Changes in body weight and metabolic variables in adult dogs with and without rice bran hydrolysates supplementation

Variables	Control		RBH supplemented	
	Day 0	Day 30	Day 0	Day 30
Body weight (kg)	19.71 ± 2.63	20.43 ± 2.51	18.36 ± 1.42	17.73 ± 1.32
MBMI (kg/m^2)	96.81 ± 8.93	95.70 ± 4.69	96.07 ± 6.17	93.15 ± 6.62
WTLR	1.16 ± 0.06	1.09 ± 0.06	1.21 ± 0.07	1.11 ± 0.07
Lipid profiles				
Cholesterol (mg/dL)	159.43 ± 6.83	155.29 ± 14.73	151.36 ± 18.43	134.36 ± 15.84
Triglycerides (mg/dL)	42.14 ± 2.74	40.29 ± 3.15	45.80 ± 6.93	41.70 ± 3.72
HDL-C (mg/dL)	54.69 ± 4.42	49.33 ± 3.51	58.40 ± 7.72	69.00 ± 8.85*,**
LDL-C (mg/dL)	84.83 ± 10.82	78.89 ± 7.20	80.00 ± 12.90	53.70 ± 8.07*,**
FBG (mg/dL)	81.00 ± 1.43	82.57 ± 1.84	82.50 ± 2.23	83.58 ± 2.17
Liver enzymes				
AST (U/L)	20.00 ± 1.40	22.57 ± 1.46	19.27 ± 2.49	16.45 ± 1.12
ALT (U/L)	25.57 ± 2.74	30.86 ± 2.70	23.45 ± 7.43	23.36 ± 5.24
ALP (U/L)	22.29 ± 5.31	27.14 ± 4.49	21.45 ± 7.71	25.73 ± 6.92

Results are expressed as mean ± SEM. *P<0.05 vs. Day 0 of the RBH supplemented group, **P<0.05 vs. Day 30 of the control group. RBH, rice bran hydrolysates; MBMI, modified body mass index; WTLR, waist to truncal length ratio; HDL-C, high density lipoprotein-cholesterol; LDL-C, low density lipoprotein-cholesterol; FBG, fasting blood glucose; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; Day 0, baseline value; Day 30, after 30 days of trial.

Table 2. Change in electrocardiography parameters in adult dogs with and without rice bran hydrolysates supplementation

Parameters	Control				RBH supplemented			
	Day 0		Day 30		Day 0		Day 30	
	Amplitude (mV)	Duration (sec)	Amplitude (mV)	Duration (sec)	Amplitude (mV)	Duration (sec)	Amplitude (mV)	Duration (sec)
P wave	0.19 ± 0.02	0.04 ± 0.00	0.22 ± 0.04	0.04 ± 0.00	0.18 ± 0.01	0.04 ± 0.00	0.17 ± 0.02	0.04 ± 0.00
P-R interval	-	0.11 ± 0.01	-	0.11 ± 0.01	-	0.12 ± 0.01	-	0.12 ± 0.01
Q wave	0.41 ± 0.05	0.01 ± 0.00	0.36 ± 0.12	0.01 ± 0.00	0.48 ± 0.07	0.02 ± 0.00	0.50 ± 0.10	0.02 ± 0.00
R wave	1.40 ± 0.14	0.03 ± 0.00	1.36 ± 0.18	0.02 ± 0.00	1.43 ± 0.20	0.02 ± 0.00	1.47 ± 0.12	0.02 ± 0.00
S wave	0.11 ± 0.02	0.01 ± 0.00	0.20 ± 0.06	0.03 ± 0.01	0.13 ± 0.04	0.02 ± 0.00	0.13 ± 0.03	0.02 ± 0.00
QRS complex	-	0.05 ± 0.00	-	0.05 ± 0.00	-	0.06 ± 0.00	-	0.07 ± 0.00
S-T segment	-	0.09 ± 0.01	-	0.08 ± 0.01	-	0.10 ± 0.01	-	0.11 ± 0.00
T wave	0.29 ± 0.03	0.05 ± 0.01	0.25 ± 0.03	0.05 ± 0.01	0.21 ± 0.06	0.05 ± 0.01	0.20 ± 0.06	0.05 ± 0.01

Results are expressed as mean ± SEM. *P<0.05 vs. Day 0 of the RBH supplemented group, **P<0.05 vs. Day 30 of the control group. RBH, rice bran hydrolysates; Day 0, baseline value; Day 30, after 30 days of trial.

Effect of RBH supplementation on electrocardiographic parameters

ECG was used to evaluate cardiac function in the dogs. After RBH supplementation, no significant changes in all parameters of ECG occurred when compared with their own baseline and the control group. All values of ECG both in control and RBH supplemented groups remained within normal physiological ranges as shown in Table 2.

Effect of RBH supplementation on plasma ACE activity

ACE plays an important role in the formation of angiotensin II (Ang II) and regulation of blood pressure. As presented in Fig. 1, after 30 days of RBH administration there was a tendency to be a decrease in plasma ACE activity. No significant differences were observed when compared to the baseline values of ACE activity (Day 0). In addition, there was no difference in plasma ACE activity between control and RBH supplemented groups in 30 days of the trial (Fig. 1).

Effect of RBH supplementation on oxidative stress and antioxidant markers

As shown in Fig. 2A and 2B, there were significant decreases in plasma MDA and protein carbonyl after 30 days of RBH supplementation when compared to their own baseline and the control group, indicating that RBH alleviated lipid peroxidation and protein oxidation in healthy dogs (P<0.05). Moreover, RBH supplementation significantly increased the levels of blood GSH and the redox ratio of GSH/GSSG when compared to their own baseline values and the control group (P<0.05; Fig. 3C and 3D). No significant changes were observed in the activity of the enzymatic antioxidant CAT and SOD activities in plasma both in control and RBH supplemented groups after 30 days of the experiment (Fig. 3A and 3B). In control dogs, all these parameters showed no significant differences between baseline and after 30 days of trial (Table 1).

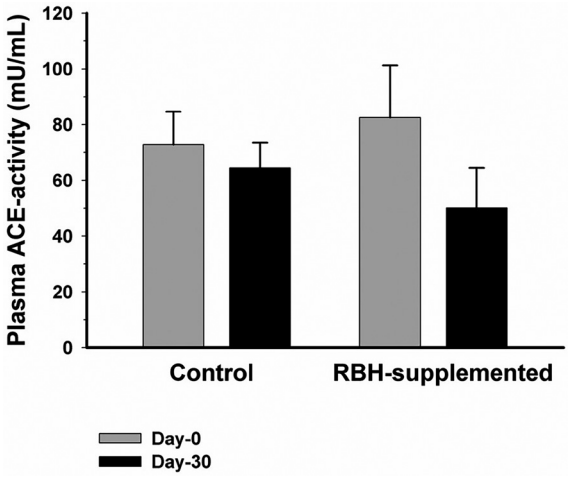

Fig. 1. Effect of rice bran hydrolysates on plasma angiotensin converting enzyme activity in adult dogs. Values are expressed as mean ± SEM. RBH, rice bran hydrolysates; ACE, angiotensin converting enzyme.

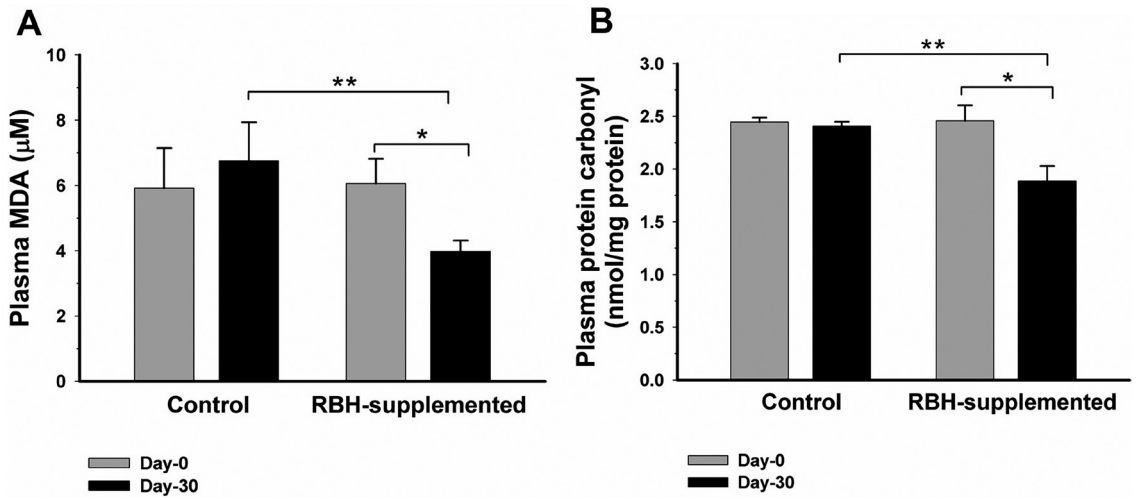

Fig. 2. Effect of rice bran hydrolysates on oxidative stress markers in adult dogs. Plasma malondialdehyde activity (**A**), Plasma protein carbonyl levels (**B**). Values are expressed as mean ± SEM. **P*<0.05 vs. Day 0 of the RBH supplemented group, ***P*<0.05 vs. Day 30 of the control group. RBH, rice bran hydrolysates; MDA, malondialdehyde.

DISCUSSION

The purpose of this study was to evaluate the health benefits of RBH in controlling oxidative stress and to provide information on the values of lipid profiles, blood glucose, liver enzymes, ACE activity and cardiac function after supplementation with RBH for 30 days in adult dogs. The main findings of this study are dietary supplementation with RBH appeared to decrease oxidative stress and increase antioxidant biomarkers. In addition, decreased LDL-C and increased HDL-C levels were found after RBH supplementation whereas serum TG and cholesterol, plasma ACE activity, blood glucose, liver enzymes, body weight and cardiac function were not significantly different between baseline and after 30 days of RBH supplementation.

It's widely believed that free radicals are constantly generated *in vivo* and are significant contributors to the development of chronic degenerative disorders such as neurodegenerative disease [14, 32], cancer [24] and cardiovascular disease [35]. Studies in humans and rats indicated that consuming food products high in antioxidants or antioxidant supplements are related with decreased risk of cancer [2], coronary heart disease [35], metabolic syndrome [37, 38], hypertension [3, 18] and diabetes mellitus [5] by decreasing oxidative stress, reducing inflammation and increasing antioxidant biomarkers. Previous studies demonstrated that rice bran and RBH possess antioxidant and free radical scavenging activities in obese, hypertensive and metabolic syndrome rats [3, 19, 37]. In this study, after RBH supplementation for 30 days, plasma MDA and protein carbonyl concentrations which are oxidative stress biomarkers, decreased significantly. In addition, blood GSH was also significantly increased after RBH supplementation in adult dogs. Glutathione

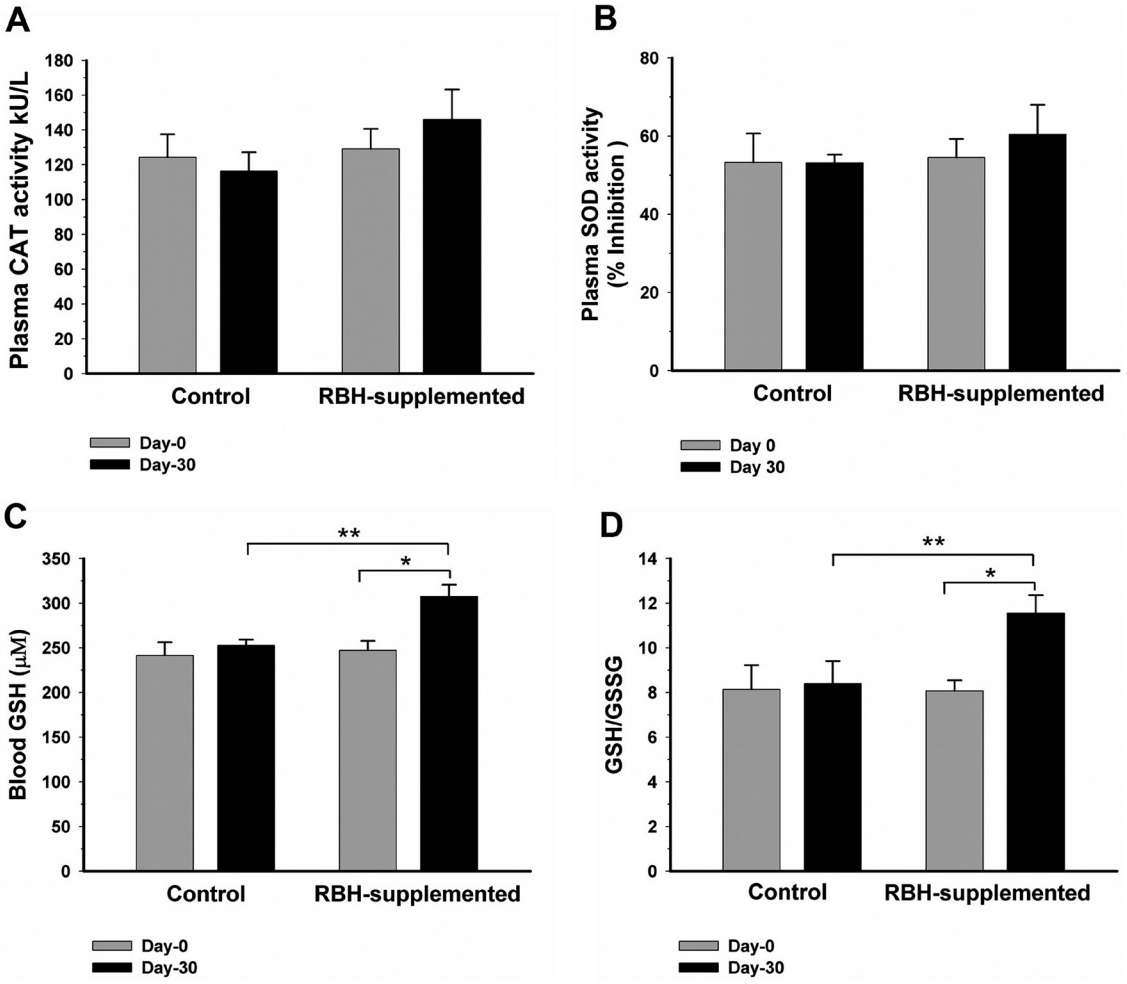

Fig. 3. Effect of rice bran hydrolysates on antioxidant markers in adult dogs. Plasma catalase activity (**A**), Plasma superoxide dismutase activity (**B**), Blood glutathione levels (**C**), Reduced glutathione/oxidized glutathione ratio (**D**). Values are expressed as mean ± SEM. **P*<0.05 vs. Day 0 of the RBH supplemented group, ***P*<0.05 vs. Day 30 of the control group. RBH, rice bran hydrolysates; CAT, catalase; SOD, superoxide dismutase; GSH, glutathione; GSSG, oxidized glutathione.

is the substrate used by the glutathione peroxidase enzyme which detoxifies hydrogen peroxide and exists in two forms: reduced glutathione (GSH) and the oxidized form (GSSG) [25]. Reduced glutathione is oxidized to GSSG during oxidative stress. Therefore, measurement of the ratio of the GSH to the GSSG content can be used to assess oxidative damage via depletion of reduced glutathione in dogs and cats [7]. This is in line with results of the current study, which showed that blood GSH and the GSH/GSSG ratio were significantly increased whereas oxidative stress biomarkers were significantly decreased after RBH supplementation. These results support the potential beneficial effects of RBH as antioxidants in adult dogs. The RBH supplementation did not influence the activity of enzymatic antioxidant CAT and SOD in adult dogs in this study. CAT is an enzymatic antioxidant that works in conjunction with SOD by catalyzing the breakdown of H_2O_2 and restoration to water. In a normal condition, CAT is of no great importance to most cell types, but in the presence of oxidative stress especially in acute or severe oxidative stress, this antioxidant enzyme is the most adaptive and plays an important role in cell defense against oxidative damage [42]. This may account for the results of no statistically significant differences in CAT activity found in this study. Furthermore, a previous study has found that CAT activity in heart tissue of control rats was not significantly changed after RBH treatment for 6 weeks [38]. In addition, another previous study showed that CAT activity was unchanged in vitamin A supplemented rats [11]. These findings coincided with the current study's results. The most abundant antioxidant enzyme and the first line of defense against ROS in animals is SOD [25, 42]. In this study, plasma SOD activity was unchanged after supplementation with RBH for 30 days. A previous study in rats demonstrated that supplementation with vitamin A at 8,000 IU/kg could increase liver SOD activity whereas that was unchanged after 4,000 IU/kg vitamin A intake [8]. Moreover, they also found that SOD activities in erythrocytes were not different when comparing vitamin A supplementary groups and the vitamin A-deficient group whereas liver SOD activity was increased in the vitamin A supplemented group which resulted from the differences of oxidative stress in tissues [8]. These results indicated that an appropriate dosage of antioxidant and degree of oxidative stress affected these different tissues differently to SOD activity. These findings may explain the results of no statistically significant differences in SOD activity in this current study.

ACE plays a major role in the regulation of blood pressure and formation of Ang II by proteolysis of Ang I to the active form, Ang II. Previous studies demonstrated that RBH poses an ACE-inhibiting effect both *in vitro* [23] and in an *in vivo* model such as the hypertensive and metabolic syndrome [3, 18, 37]. In this study, plasma ACE activity was not significantly changed after RBH supplementation for 30 days consistent with previous results in which normal control rats received RBH for 6 weeks [3, 37, 38]. Furthermore, RBH is reported to have a hypoglycemic effect and an anti-dyslipidemic activity [5, 37]. In the present study, there were significantly decreased LDL-C in serum and significantly increased HDL-C in serum after RBH supplementation for 30 days whereas in other serum lipid profiles (cholesterol and TG) there were no differences. LDL is directly associated with development of cardiovascular disease, whereas HDL has an inverse relationship [28]. Therefore, these results show the beneficial effect of RBH on serum lipid profiles in adult dogs. Previous studies have shown that RBH mitigates dyslipidemia by decreasing cholesterol, TG and LDL-C together with increased HDL-C levels in serum after RBH administration for 6 weeks [5, 37] and the mechanism of this effect was associated with the suppression of lipogenic genes (Srebf1 and Fasn) expression [5]. In this current study, it was observed that serum cholesterol level trended to decrease but was not significantly different after 30 days of RBH supplementation. This may be due to the differences of supplementation time periods. Some studies, however, found that the levels of serum total cholesterol and TG were decreased after 4 weeks of rice bran administration at a dose of 2,205 mg/kg/day [27]. Taken together, investigations on longer supplementation periods and a higher dosage of RBH may be needed for further exploration.

Moreover, this study found that the levels of blood glucose were within normal ranges, and the values were not different between baseline and after 30 days supplementation. The plausible mechanism may be due to the counter regulatory control of blood glucose level of the body by two hormone, insulin and glucagon. Previous studies showed that RBH possessed a hypoglycemic effect in the metabolic syndrome and diabetic rats under the conditions of insulin resistance whereas administration of this substance in normal control rats has not found this effect [5, 31, 37]. Consistent with previous studies, this study was performed on healthy adult dogs in which the counter regulatory system of blood glucose is properly functioning, thereby, the blood glucose level remains unchanged. In addition, this study evaluated the effect of RBH supplementation on body weight, liver enzymes and cardiac function. All of these parameters were not significantly different between baseline and after 30 days of RBH supplementation. These results indicated that RBH (500 mg/kg/day) is safe in a 30-day supplementation as there was no sign of toxicity nor abnormalities in various clinical variables in adult dogs and thereby supports the use of RBH in lowering the risk of oxidative stress and dyslipidemia.

This study has some limitations that should be taken into consideration since a direct measurement of the level of ROS in the blood samples was not been evaluated in this study. We found some interesting information, however, that RBH supplementation reduces oxidative stress by decreasing oxidative stress biomarkers and increasing the GSH antioxidant defense system when compared to their own baseline and the control group.

In conclusion, the findings of this study show that oxidative stress can be found in adult dogs and RBH supplementation can improve antioxidant status and alleviated oxidative stress by modulating oxidative stress biomarkers and enhancing the GSH antioxidant defense system. Moreover, the beneficial effect of RBH on serum lipid profiles is evident in adult dogs. Further studies with long-term, dose-dependent effects of RBH supplementation and a large number of dogs are necessary to clarify the usefulness of RBH as an antioxidative substance in the veterinary field. In addition, considering the health benefits of RBH, supplementation with RBH could be beneficial for many pathological conditions in dogs, such as cardiovascular diseases, diabetes mellitus, dyslipidemia, and also in aging dogs.

CONFLICT OF INTERESTS. The authors declare no conflict of interest.

ACKNOWLEDGMENTS. This work was supported by grant from the Research Fund, Faculty of Veterinary Medicine, Khon Kaen University, 2019. We would like to acknowledge Prof. James Arthur Will, for editing the manuscript via Publication Clinic KKU, Thailand.

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