

Effects of astaxanthin supplementation in healthy and obese dogs

Tae Murai¹
Koh Kawasumi¹
Kumi Tominaga²
Yuki Okada¹
Motoo Kobayashi¹
Toshiro Arai¹

¹Laboratory of Veterinary Biochemistry, School of Veterinary Medicine, Nippon Veterinary and Life Science University, Musashino, Tokyo 180-8602, Japan; ²Research and Development Division, AstaReal Co. Ltd., Minato-ku, Tokyo 105-0011, Japan

Background: Since astaxanthin (ASX) has potent anti-oxidative effects with inhibitory action of lipid peroxidation and singlet oxygen quenching activity, it is widely used as a functional food for keeping good health in human. Obesity is a risk factor for various metabolic disorders. It is characterized by low-grade chronic inflammation based on oxidative stress by excessively produced ROS. From the point of preventive medicine, natural compounds have been proposed as potential therapeutic agents in the prevention of metabolic disorder in companion animals. The purpose of this study is to evaluate the effects of ASX supplementation in healthy and obese dogs.

Materials and methods: Ten healthy beagle dogs and 5 clinically obese dogs were used in this study. The healthy beagle dogs were randomly divided into 2 groups as follows: control and test groups. The test group dogs received ASX supplementation mixed with the food for 6 weeks. Five clinically obese dogs received ASX supplementation for 8 weeks. Metabolites, hormones and enzymes were measured before and after ASX supplementation.

Results: In the healthy dog groups, after 6 weeks, plasma triglyceride (TG) and malondialdehyde concentrations and lactate dehydrogenase (LDH) values significantly decreased in the test group. There was no significant difference in the control group. In clinically obese dogs, plasma TG concentration decreased after 8 weeks of ASX supplementation. Plasma alanine aminotransferase and LDH values clearly decreased in all 5 dogs and 4 dogs out of 5 dogs, respectively.

Conclusion: ASX supplementation (0.3 mg/kg body weight/day) for 6 weeks in healthy dogs and 8 weeks in obese dogs induced the elevation of antioxidant function and of liver function by ameliorating lipid metabolism.

Keywords: astaxanthin, obese dogs, lipid metabolism, anti-oxidative activity, liver function

Introduction

The incidence of obesity and its associated diseases has been increased in dogs and cats as well as in human.^{1,2} Since obesity causes physical inactivity and oxidative stress related diseases that are induced by obesity-based metabolic syndrome, body weight (BW) reduction is required for obese animals. However, the satisfactory result of weight reduction is limited in the animals with pathological obesity induced by accumulated visceral fat, causing slight systemic inflammation.³ Fat accumulation and oxidative stress impair the function of mitochondria via morphological alteration, increased membrane peroxidation, decreased ATP level, increased ROS production, defective mitochondrial β -oxidation and increased mitochondrial permeabilization.⁴ Increase in circulating nonesterified fatty acids (NEFAs) due to excessive accumulated

Correspondence: Koh Kawasumi
Laboratory of Veterinary Biochemistry,
School of Veterinary Medicine, Nippon
Veterinary and Life Science University,
1-7-1 Kyonancho, Musashino,
Tokyo 180-8602, Japan
Tel +81 422 31 4151
Fax +81 422 31 7841
Email kawasumi224@nvl.ac.jp

visceral fat is confirmed in obese animals. Fat accumulation triggers free radical production and insults additional inflammation.⁵ Excess amount of ROS is also produced via accelerated β -oxidation of fatty acids. Such overproduced ROS is attributed to one of the pathogens for obesity and its associated diseases.⁶ Consequently, some antioxidants appear to be effective to ameliorate obesity conditions in animals.⁷⁻⁹ Experimental studies of mice administered obesity-inducing diet combined with ASX showed anti-diabetic and anti-obesity effects by improved insulin (INS) sensitivities and liver function.^{10,11} The study showed the suppression of fat tissue weight gain by ASX in a dose-dependent manner.¹¹

Haematococcus pluvialis, known as an important source of natural astaxanthin (ASX), is a freshwater microalga belonging to the family *Chlamydomonadaceae*. When the alga experiences environmental stress conditions, ASX is created and acts like a force field that protects the nuclear DNA and lipids against UV-induced oxidation.¹² ASX (3,3'-dihydroxy- β,β' -carotene-4,4'-dione) is a nontoxic and organic fat-soluble xanthophyll carotenoid. In comparison to other phytochemicals, ASX has previously been reported to possess a significantly greater antioxidant function,^{13,14} with its antioxidant activities quantified as 10-fold greater than other carotenoids, such as β -carotene, and 100-fold greater than α -tocopherol (vitamin E).¹⁵ ASX accumulates in the liver, especially in the microsomal and mitochondrial fractions of the liver tissue.^{16,17} This substance has been shown to prevent oxidative damage to the liver, improve metabolic profiles, and reduce hepatic inflammation.¹⁸⁻²⁰ From the above, excessive fat accumulation and oxidative stress and liver function are closely related.

In this study, we measured plasma metabolites and hormone concentrations and enzyme activities involved in energy metabolism in healthy and obese dogs with ASX supplementation for several weeks. The purpose of this study was to evaluate the effect of ASX supplementation in obese and healthy dogs.

Materials and methods

Animals

Ten healthy beagle dogs and 5 clinically obese dogs were used in this study. Their body condition score (BCS) was evaluated by the 5-point scale system (1, very thin; 2, underweight; 3, ideal; 4, overweight and 5, obese). BCS of 10 healthy dogs was 3. The average age of them was 2 years (1–3 years), and the average BW was 10.4 kg (9.7–11.1 kg). They were randomly divided into 2 groups: control group (dog no 1–5) and test group (dog no 6–10). As preparation of the study, they were given commercial diet (Nippon Pet Food Co., Ltd., Tokyo, Japan) for 2 months. The nutrient composition of the food is crude protein 21.7%, crude fat 10.1%, crude ash 6.3%, crude fiber 2.7%, linoleic acid 1.72%, moisture 8.7% and nitrogen-free extract 50.5%. All the dogs were kept under controlled conditions and professionally supervised at Narita Animal Science Laboratory Co., Ltd. (Narita, Japan) prior to and during the study period. Ethical approval for this study was obtained from Narita Animal Science Laboratory Co., Ltd. Research Animal Ethical Committee (17-C042).

Five clinically obese dogs were recruited from local primary veterinary practice as shown in Table 1. The selected 5 dogs met the following 3 conditions: 1) BW must be more than 20% of its ideal weight; 2) symptoms of chronic disease are properly managed and 3) owner is reliable, and good compliance can be received with the complete consent. Case numbers 1, 2 and 3 have been treated for their chronic diseases by certain therapeutic agents for years, and the clinical signs have been well controlled for long time. Case numbers 4 and 5 have taken no medications.

ASX supplementation

ASX that is *H. pluvialis* biomass (AstaReal® AW1011; Asta-Real Inc., Moses Lake, WA, USA) was used in this study. In the healthy beagle dogs, 5 test group dogs were given 1 dose of

Table 1 Profiles of clinically overweight and obese dogs

Case Number	1		2		3		4		5	
	0W	8W	0W	8W	0W	8W	0W	8W	0W	8W
Breed	Miniature Dachshund		Mongrel		Miniature Schnauzer		Kishu dog		Labrador retriever	
Age (years)	10		14		11		8		3	
Sex	NM		NM		NM		NF		NM	
Clinical complication	Hypothyroidism		Cushing syndrome		Hypothyroidism, Arthritis		Arthritis		None	
BW (Kg)	10.0	11.3	15.4	15.6	7.3	7.2	25.5	25.5	59.5	59.6
Ideal weight (Kg)	7		11		6		15		35	
BCS	5	5	4	4	4	4	5	5	5	5

Abbreviations: BCS, body condition score; BW, body weight; NF, neutered female; NM, neutered male; W, weeks.

0.3 mg/kg ASX with a meal per day. BW and BCS were measured every week, and the ASX supplement dose was adjusted. For clinical cases, 0.3 mg /kg /day of ASX was given in a single dose or in divided doses with the food on a daily basis.

Blood sampling

Fasting blood samples were collected before initiation of the study, after 6 weeks in healthy dogs, and after 8 weeks in clinically obese dogs, respectively. Collected blood was dispensed in a heparinized tube and centrifuged at $400 \times g$ for 10 minutes at 4°C to collect plasma. Plasma was stored at -80°C until use.

Metabolite, hormone and enzyme analyses

Glucose (GLU), total cholesterol (TC), triglyceride (TG), total protein (TP), blood urea nitrogen (BUN), creatinine (CRE) concentrations and alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) activities were measured using an auto-analyzer (JCA-BM2250; JEOL, Tokyo, Japan) at FUJIFILM Monolith Co., Ltd (Tokyo, Japan). Plasma NEFA concentration was measured using the NEFA-C test kit (Wako Pure Chemical Industries, Ltd., Osaka, Japan). Plasma

malondialdehyde (MDA) concentration was measured using the NWLSS™ Malondialdehyde assay kit (Northwest Life Science Specialties, LLC, Vancouver, Canada). Plasma INS, adiponectin (ADN) and TNF α were measured by the Rat Insulin ELISA kit (AKRIN-010T; Shibayagi Co., Gumma, Japan), mouse/rat adiponectin ELISA kit (Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan) and TNF α Dog ELISA kit (LS-F1347-1; Life Span Bioscience, Inc, Seattle, WA, USA), respectively.

Statistical analysis

All values were calculated using Microsoft Excel. The data were expressed as mean \pm standard error (SE). Statistical analysis was performed using the 2-tailed, paired *t*-test. Statistical significance was designated as $P < 0.05$, and a high level of significance was designated as $P < 0.01$.

Results

Comparisons of biomarker levels in healthy dogs in the control group and the test group are shown in Table 2. The values of plasma TG and MDA concentrations and LDH significantly decreased in the test group dogs ($P < 0.05$, $P < 0.01$), and there was no significant difference in the control group. After 6 weeks, TGs significantly decreased ($P < 0.05$) in the test group, MDA and LDH also significantly decreased in

Table 2 Comparison of biomarkers level of healthy dogs with and without astaxanthin supplementation

Parameter	without ASX (n=5)		with ASX (n=5)	
	0 week	6 weeks	0 week	6 weeks
Body weight (kg)	10.5 \pm 0.2	11.3 \pm 0.3	10.3 \pm 0.2	11.1 \pm 0.3
Body condition score	3	3	3	3
Glucose (mg/dL)	87.4 \pm 1.8	79.8 \pm 2.1	90.6 \pm 2.5	87.4 \pm 2.3
Triglyceride (mg/dL)	30.6 \pm 1.3	38.0 \pm 6.6	37.2 \pm 1.3	23.6 \pm 4.1 *
Total cholesterol (mg/dL)	144.8 \pm 20.0	131.6 \pm 8.9	113.8 \pm 6.5	133.6 \pm 9.1
NEFA (mEq/L)	0.7 \pm 0.1	0.7 \pm 0.1	0.8 \pm 0.0	1.0 \pm 0.1
Total protein (g/dL)	7.1 \pm 0.1	7.0 \pm 0.1	6.8 \pm 0.1	6.6 \pm 0.1
Blood urea nitrogen (mg/dL)	13.4 \pm 0.7	12.0 \pm 0.7	12.4 \pm 0.7	10.2 \pm 0.6
Creatinine (mg/dL)	0.7 \pm 0.0	0.7 \pm 0.0	0.7 \pm 0.0	0.7 \pm 0.1
Malondialdehyde ($\mu\text{mol/L}$)	1.5 \pm 0.2	1.5 \pm 0.1	1.5 \pm 0.2	0.9 \pm 0.0 ^{***}
Insulin (ng/mL)	0.2 \pm 0.0	0.3 \pm 0.1	0.3 \pm 0.1	0.3 \pm 0.1
Adiponectin ($\mu\text{g/mL}$)	18.4 \pm 4.0	27.5 \pm 3.3	27.0 \pm 6.3	30.5 \pm 4.3
TNF- α (pg/mL)	0.1 \pm 0.0	0.1 \pm 0.0	0.1 \pm 0.0	0.1 \pm 0.0
AST (IU/L)	32.2 \pm 1.8	35.2 \pm 2.4	37.2 \pm 1.3	33.6 \pm 1.5
ALT (IU/L)	28.5 \pm 4.1	30.2 \pm 5.2	31.6 \pm 1.2	36.4 \pm 2.9
ALP (IU/L)	184.0 \pm 46.3	166.6 \pm 36.0	152.4 \pm 8.7	152.6 \pm 8.7
LDH (IU/L)	83.4 \pm 5.7	113.6 \pm 12.6	89.4 \pm 6.3	57.4 \pm 2.4 ^{***}

Notes: Data are presented as the mean \pm SE. Statistical significance is indicated by asterisks. *Significantly different ($p < 0.05$) from the value at 0 week in the test group with ASX (paired *t*-test). **Significantly different ($p < 0.01$) from the value at 0 week in the test group with ASX (paired *t*-test). ***Significantly different ($p < 0.01$) from the value at 6 weeks of the control group without ASX (paired *t*-test).

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ASX, astaxanthin; LDH, lactate dehydrogenase; NEFA, non-esterified fatty acid; SE, standard error. SE, standard error.

the test group ($P<0.01$) (Figure 1). Moreover, upon comparing control and test groups in 6 weeks, the values of MDA and LDH significantly decreased ($P<0.01$) in the test group ($P<0.01$) (Figure 1). In healthy dogs, with or without ASX supplementation, plasma GLU, TC, NEFA, TP, BUN, CRE, INS, ADN, AST and ALP values showed no significant change before and after 6 weeks of trail (Table 2).

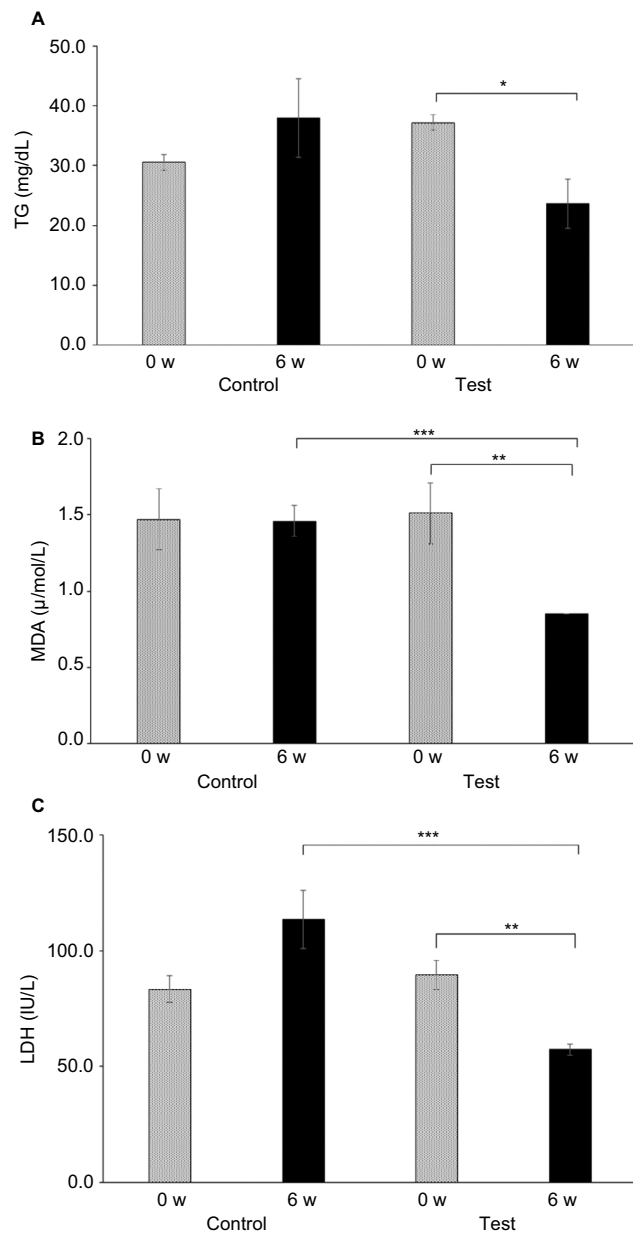


Figure 1 Comparison of TG, MDA and LDH levels in healthy beagle dogs with or without ASX. **(A)** TG (mg/dL), **(B)** MDA ($\mu\text{mol/L}$) and **(C)** LDH (IU/L).

Notes: Statistical significance is indicated by asterisks. *Significantly different ($P<0.05$) from the value at 0 week in the test group with ASX (paired *t*-test). **Significantly different ($P<0.01$) from the value at 0 week in the test group with ASX (paired *t*-test). ***Significantly different ($P<0.01$) from the value at 6 weeks of the control group without ASX (paired *t*-test).

Abbreviations: ASX, astaxanthin; LDH, lactate dehydrogenase; MDA, malondialdehyde; TG, triglyceride; W, weeks.

The results of obese dogs are shown in Table 3. TG values clearly decreased after 8 weeks of ASX supplementation, and ALT and LDH values also remarkably decreased in all 5 dogs and in 4 out of the 5 dogs, respectively. Plasma GLU, TC, NEFA, TP, BUN, CRE, INS, ADN, AST and ALP values showed no major change in clinically obese dogs on ASX supplementation after 8 weeks.

Although statistical comparison is impossible, simple comparison of the TG, ALT, MDA and LDH values of the healthy dog groups with individual obese dogs is done (Figure 2). The change before and after ASX supplementation was more pronounced in obese dogs. In each obese dog, ALT values were always higher than those of healthy dogs regardless of ASX supplementation.

BW and BCS showed no changes after ASX supplementation.

Discussion

Obesity is characterized by low-grade chronic inflammation.²¹ This continuous inflammation due to obesity induces severe metabolic disorders such as hypertension, vascular disorders, diabetes mellitus and others.²² Increased circulating NEFAs from accumulated visceral fat cause inflammation and INS resistance by directly activating plasma membrane receptors, such as toll-like receptor 4,²³ followed by elevation of inflammatory reaction via NF- κ B.^{24,25} On the other hand, excessive amount of NEFA enhances overproduction of ROS in the process of β -oxidation of fatty acids in the mitochondria of various tissues. Overproduction of ROS induces oxidative stress. Consequently, systemic inflammatory components were confirmed in obesity.^{26,27} Adipose tissue, a population of adipocytes, not only acts as an energy reservoir but also has physiological activities such as angiogenesis and wound healing, and adipocytes produce and secrete adipokines involved in energy metabolism.²⁸ Accumulated visceral fat in obese animals induces high concentrations of plasma NEFA, circulating C-reactive protein (CRP) and MDA.^{29,30} Excessive amount of visceral fat is suspected as a contributing factor to various metabolic disorders in obese animals. From the above mentioned findings, antioxidant substances are considered to decrease oxidative stress and to ameliorate metabolic disorders caused due to obesity.

Obese animals encountered in veterinary practice are individually unique in their background. In general, dietary supplements and functional foods are often recommended for animals that require the health care intervention from veterinary professionals, such as aging, chronic diseases and

Table 3 Changes in biomarkers level of clinically overweight and obese dogs with astaxanthin supplementation

Case Number	1		2		3		4		5	
	0W	8W	0W	8W	0W	8W	0W	8W	0W	8W
Breed	Miniature Dachshund		Mongrel		Miniature Schnauzer		Kishu dog		Labrador retriever	
Age (years)	10		14		11		8		3	
Sex	NM		NM		NM		NF		NM	
Clinical complication	Hypothyroidism		Cushing syndrome		Hypothyroidism, Arthritis		Arthritis		None	
Body weight (kg)	10	11.3	15.4	15.6	7.3	7.2	25.5	25.5	59.5	59.6
Body condition score	5	5	4	4	4	4	5	5	5	5
Glucose (mg/dL)	87	87	102	87	91	92	74	83	93	104
Triglyceride (mg/dL)	89	57	270	73	450	99	21	18	117	41
Total cholesterol (mg/dL)	162	148	360	297	178	188	224	230	173	179
NEFA (mEq/L)	0.54	0.54	0.58	0.63	1.15	0.39	0.58	0.60	0.46	0.54
Total protein (g/dL)	8.2	8.1	5.8	5.7	7.6	6.8	7.4	7.3	6.6	6.6
Blood urea nitrogen (mg/dL)	23	24	16	21	14	11	16	15	14	11
Creatinine (mg/dL)	0.6	0.6	0.4	0.4	0.4	0.3	0.7	0.7	0.9	0.8
Malondialdehyde (μ mol/L)	1.63	2.74	3.85	1.95	10.97	3.06	1.73	1.89	3.06	2.42
Insulin (ng/mL)	3.6	5.5	3.5	1.0	4.7	3.2	4.3	3.8	0.5	0.7
Adiponectin (μ g/mL)	10.8	13.9	2.0	1.5	NT	0.14	4.8	3.4	9.7	13.3
AST (IU/L)	31	39	25	24	211	32	21	18	52	38
ALT (IU/L)	250	99	270	215	123	74	72	61	135	97
ALP (IU/L)	1460	352	4714	4215	348	404	2122	2130	118	126
LDH (IU/L)	68	173	74	64	7417	177	73	34	187	76

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ASX, astaxanthin; LDH, lactate dehydrogenase; NEFA, non-esterified fatty acid; NF, neutered female; NM, neutered male; NT, not tested; W, weeks.

critical obesity as well. Considering these circumstances, obese dogs participated in this experiment were various age, sex, and species.

In this study, ASX supplementation showed clear antioxidative effects in both healthy and obese dogs. In the healthy dogs, after ASX supplementation for 6 weeks, plasma TG and oxidative stress biomarker MDA concentrations decreased significantly. ASX revealed antioxidant activity in healthy dogs. In the same way, plasma TG, MDA, ALT and LDH values decreased, especially, TG and ALT values remarkably decreased. Antioxidant effects of ASX supplementation in obese dogs are more apparent than those in the healthy dogs. Since those positive effects were observed in all obese dogs with different underlying diseases, it is considered that ASX supplementation could be effective on the antioxidant activity and also improve the hepatic function.

ASX prevents diseases in heart and kidneys³¹ and liver²⁰ from oxidative stress, in addition ASX can decrease plasma MDA concentrations and improve the pathological signs of animal diabetic nephropathy.³² In this study, ASX supplementation indicated to be effective on elevation of antioxidant function and on amelioration of metabolic functions in liver. Long-term intake of ASX inhibits the

elevations in BW and adipose tissue weight caused by a high-fat diet in mice tested for 60 days.¹¹ Chronic ASX administration significantly improve increased body weight, hyperglycemia, hyperinsulinemia and increased plasma levels of TNF α and IL-6 observed in the study of obese model mice.³³ However, ASX supplementation does not influence BW reduction in obese dogs in this study. In the mice experiments, ASX was given in relatively high dose such as 6, 12 and 30 mg/kg for 60 days. The dose of ASX used in this experiment was set up based on the standard recommended dose to human. Considering the lifespan of the mouse, the duration of 60 days for the mouse corresponds to 1 year or more in the dog. We speculate that longer administration period in obese dogs of more than 2 months could bring the farther positive effect. ASX improved oxidative stress biomarkers by suppressing lipid peroxidation and stimulating the liver function. However, weight reduction effect could not be achieved only by supplementation of ASX to obese dogs.

Moreover, safety of ASX supplementation is advocated by EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) in previous report.³⁴ Considering that ASX has wide safety range,³⁵ continuous supplementation of ASX seems to be effective to prevent the prevalence

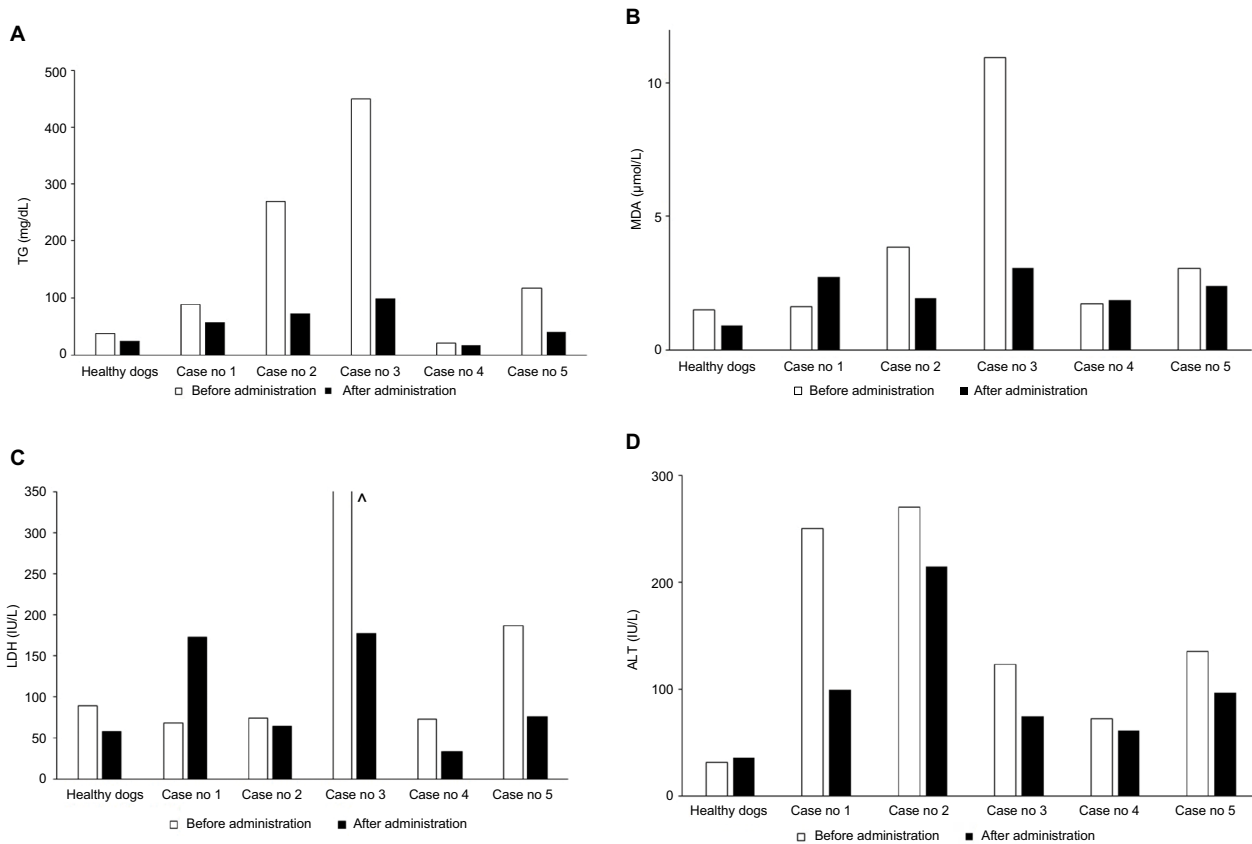


Figure 2 Comparison of TG, MDA, ALT and LDH levels in healthy and obese dogs before and after ASX supplementation.

Notes: (A) TG (mg/dL), (B) MDA (µmol/L), (C) LDH (IU/L) and (D) ALT (IU/L). [^]The value exceeded the display range.

Abbreviations: ALT, alanine aminotransferase; ASX, astaxanthin; LDH, lactate dehydrogenase; MDA, malondialdehyde; TG, triglyceride; W, weeks.

of visceral fat-type obesity. Further studies with different doses of ASX supplementation are necessary to clarify the usefulness of ASX as an antioxidant supplement in a large number of dogs with different severities of obesity.

Conclusion

ASX supplementation (0.3 mg/kg BW/day) in food for 6 weeks in healthy dogs and for 8 weeks in obese dogs effectively activated antioxidant function and liver function followed by improved lipid metabolism.

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Disclosure

Kumi Tominaga is a researcher working in AstaReal Co.Ltd. The authors report no other conflicts of interest in this work.

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