Equixanthin™

Natural Astaxanthin from *Paracoccus carotinifaciens*

Grafton New Zealand Ltd.

Contact us

webmaster@grafton-nz.com

What is Equixanthin™ ?

- Equixanthin™ is produced by a natural (non-GMO) soil microbe using highly controlled cGMP fermentation and DSP facilities.

- Equixanthin™ is a new, all natural source of Astaxanthin in the n on-esterified form with 3S,3'S isomer.

- It is differentiated from other sources by being a natural blend of a umber of antioxidant xanthophylls.

- In addition to Astaxanthin, Equixanthin™ also contains Adonirubin and Adonixanthin, xanthophylls with 2-3 times the antioxidant power of Astaxanthin.

- Equixanthin™ is stable and safe. Passed safety study for feed additives. < 3% loss after 1 month at room temperature(25℃).

- Equixanthin™ improves many health conditions in horses: primarily azoturia/tying-up, susceptibility to ulcers, skin diseases, fertility issues in stallions and mares and throat infections in sale yearlings.

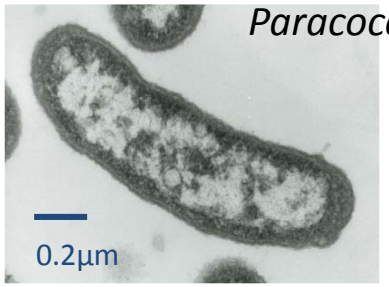
Astaxanthin Boosts Performance without Burning-out

Astaxanthin, one of carotenoid family is nature's most powerful antioxidant. In racehorses,astaxanthin has been shown to reduce oxidative stress and maximize muscle performance resulting in the followings;

- **BOOSTS** power output and muscle endurance.
- **LOWERS** lactic acid, fatigue and muscle soreness.
- **REDUCES** muscle damage and inflammation.
- **IMPROVES** blood flow and antioxidant status.
- **PROMOTES** muscle fat metabolism during exercise.
- **MAKES** the coat shiny.

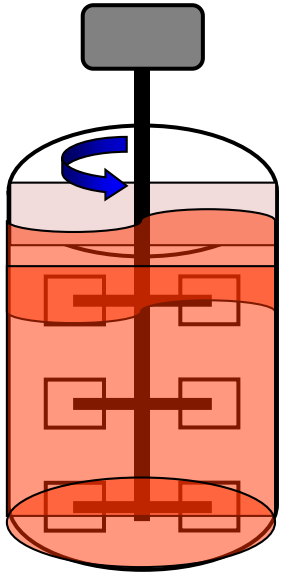
None of the other antioxidants simultaneously exhibits the efficacy described above at lower dosage and in safety.

Equixanthin™ Production Overview



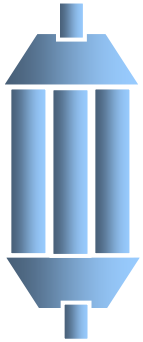
Paracoccus carotinifaciens (natural soil microbe)

Fermentation

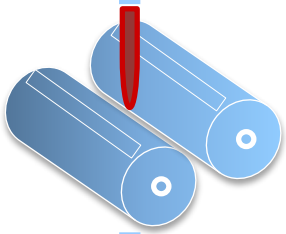


Pasteurization

Concentration

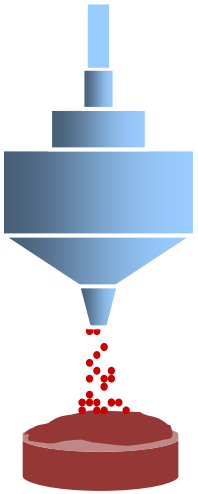


Drying

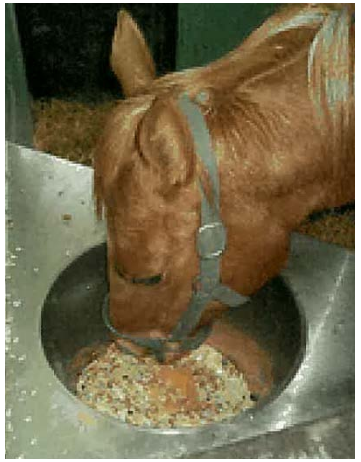


Grinding

Mixing



Test assay

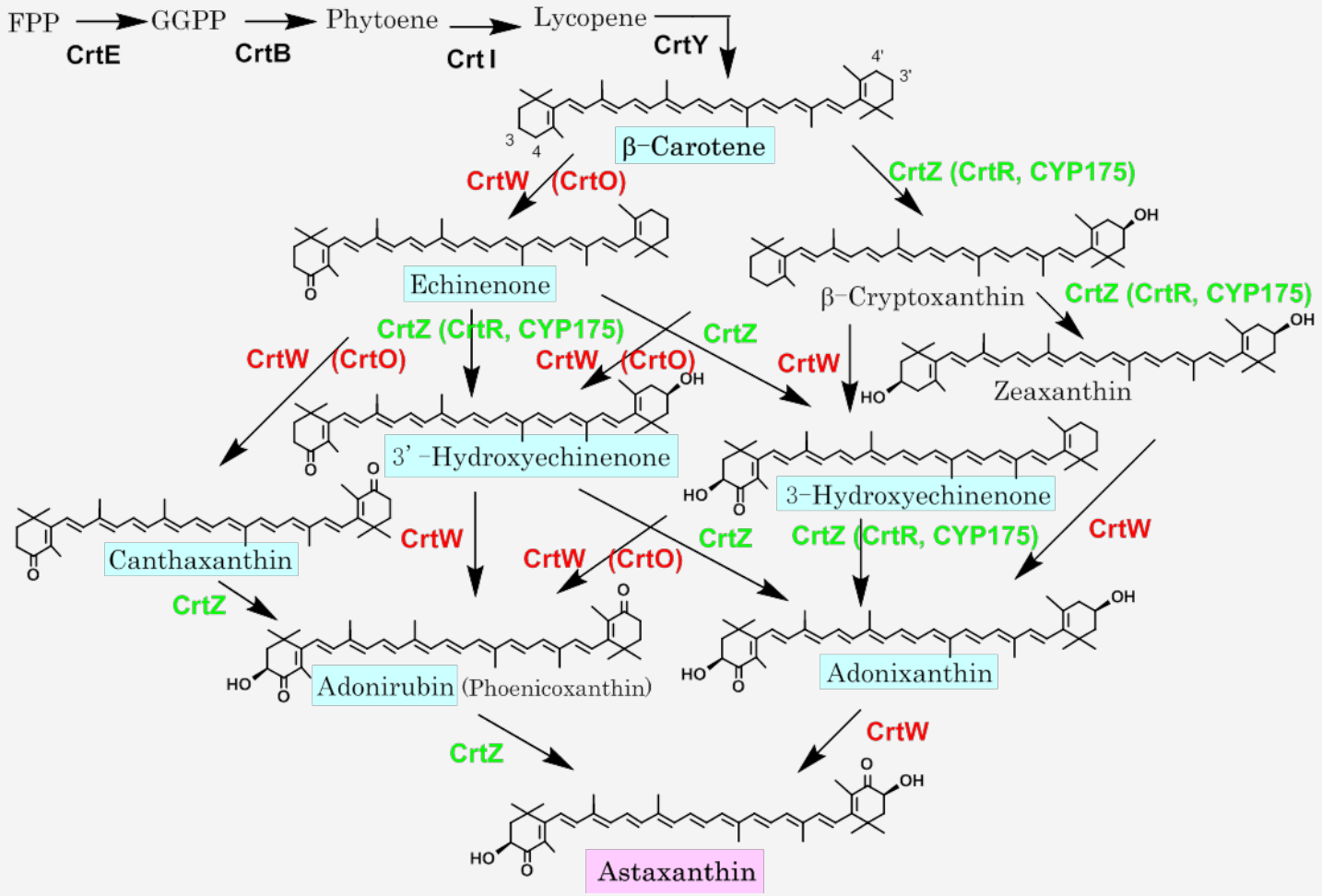


Equixanthin™

Special mention: Ethoxyquin free, Non-feed & food additive, Non-GMO, RAC free, Dioxins free

Biosynthetic Pathway of Carotenoids

Paracoccus carotinifaciens (natural soil microbe)



Composition of Equixanthin™

ITEM			TYPICAL CONTENT
Carotenoids			
	Astaxanthin	g/kg	21.7
	Adonirubin	g/kg	5.3
	Adonixanthin	g/kg	2.9
	Other Carotenoids	g/kg	4.1
	Total Carotenoids	g/kg	34.0
Main Components			
	Crude Protein	g/kg	596
	Crude Fat	g/kg	37
	Crude Ash	g/kg	43
	Carbohydrate	g/kg	285

ITEM			TYPICAL CONTENT
Inorganic Component, etc.			
	Na	g/kg	5.4
	P	g/kg	6.7
	Fe	g/kg	1.9
	Ca	g/kg	3.4
	K	g/kg	2.5
	Mg	g/kg	2.7
	Lead	ppm	ND (QL: 0.1ppm)
	Arsenic	ppm	ND (QL: 0.1ppm)
	Mercury	ppm	ND (QL: 0.1ppm)
	Salmonella	25g	Negative

ND: Not detected, QL: Quantification Limit

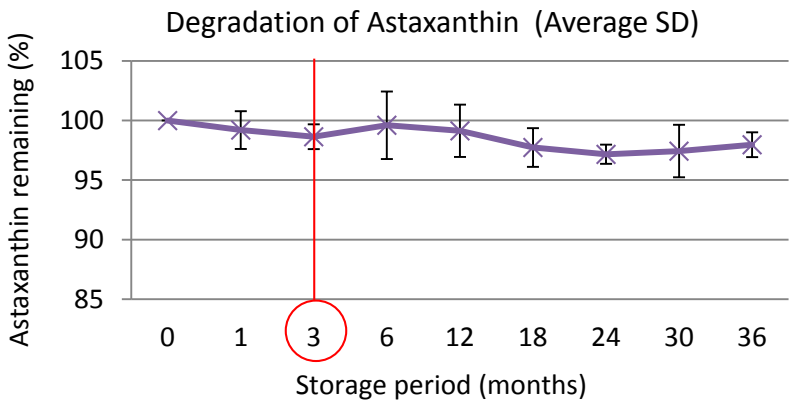
Safety Study of Equixanthin™

- Bacterial Reverse Mutation Test (Ames test)
  - ☞ Equixanthin™ is considered to be non-mutagenic.
- Chromosome aberration Test (Human lymphocytes *in vitro*)
  - ☞ Equixanthin™ is considered to be non-mutagenic.
- Micronucleus Test (Rats)
  - ☞ Equixanthin™ dose not induce micronuclei in bone marrow cells.
- Acute Oral Toxicity Test (Rats)
  - ☞ The acute oral median lethal dose (LD_{50}) of Equixanthin™ is higher than 5000 mg/kg.
- 90-Day Toxicity Study (Rats)
  - ☞ No observed adverse effect level (NOAEL) of Equixanthin™ is higher than 3000 mg/kg/day.
- Acute Inhalation Toxicity study (Rats)
  - ☞ The acute inhalation median lethal concentration (4hr LC_{50}) of Equixanthin™ is greater than 4.92 mg/L.

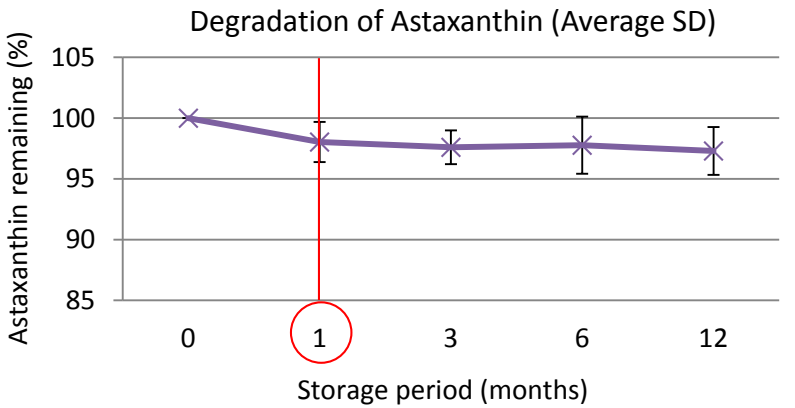
Special mention: All studies were conducted in compliance with GLP.

Preservation Stability of Equixanthin™

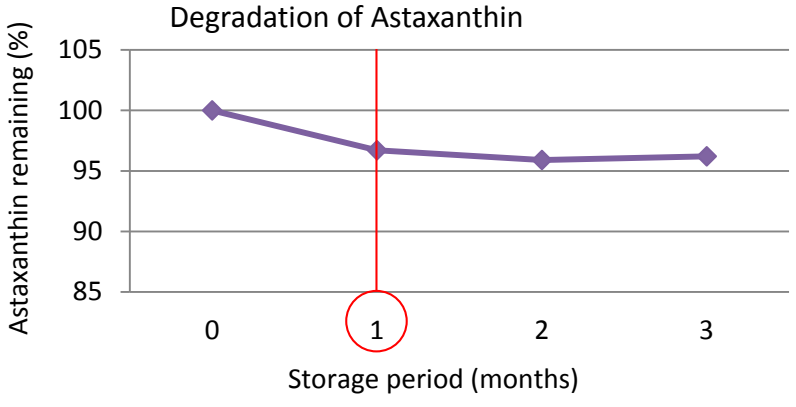
Conditions: **25℃**, 20-45% R.H.
Packaging: Usual seal in aluminum bag



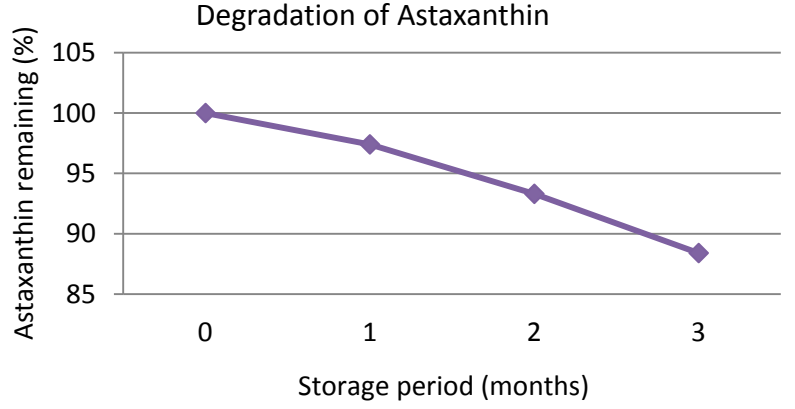
Conditions: **40℃**, 75% R.H.
Packaging: Usual seal in aluminum bag



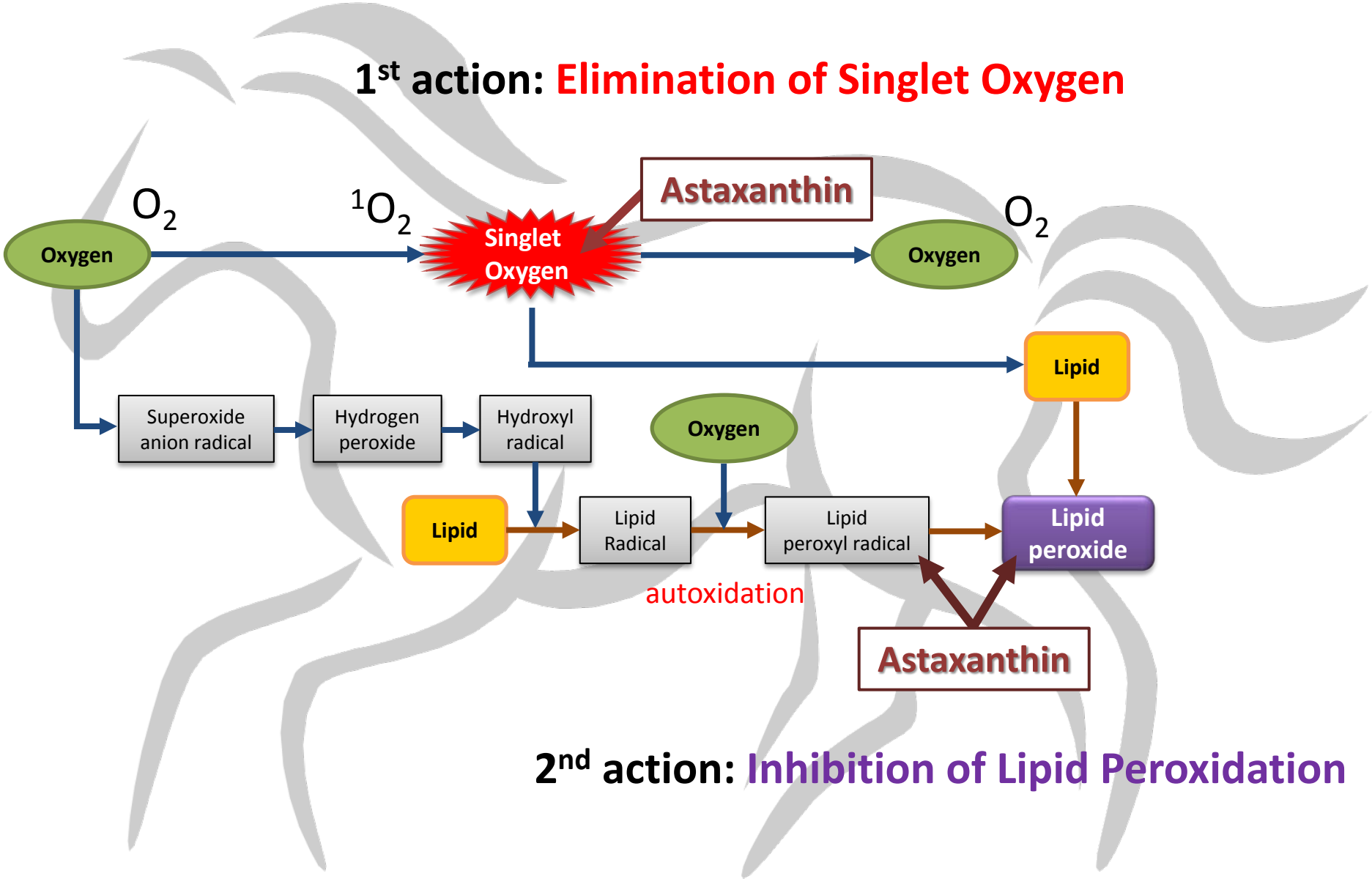
Condition: **60℃**
Packaging: Usual seal in aluminum bag



Conditions: **25℃**, 20-45% R.H.
Packaging: **Open** aluminum bag in the dark



Antioxidant Action of Astaxanthin



Utility in Clinical Practice by Astaxanthin

1. Antioxidant action

- muscle fatigue resistance
- prevention of tying-up syndrome
- improvement of conception rate
- adjustment of estrous cycle & heat-stress relief
- enhancement of immune system
- anti-inflammatory & anti-photoaging effect
- prevention of metabolic syndrome, etc.

2. Unknown action

- improvement of intestinal flora ?

Do not drop the body weight !

High-fat diet)

Abnormal intestinal flora

Astaxanthin effect

High-fat diet + **0.02 % Astaxanthin**

It's extremely important for health maintenance.

Normal intestinal flora

【Authority】Effects of Astaxanthin on Intestinal Micro flora in Mice Fed a High-fat Diet. *Anti-aging medicine.* **10**(4), 77-91, 2013

Improvement of Tying-up Syndrome (1)

All data was provided by Fumio Sato※, DVM, PhD.
※ Japan Racing Association Hidaka Training and Research Center,　E-mail: fumio_sato@jra.go.jp

1. Methods

(1) Horses

63 thoroughbreds,　age: 20.6±1.1 months,　body weight (BW): 452.1±23.8 kg

(2) Groups

- administered group: 29 horses (♂19, ♀10)
- non-administered group: 29 horses (♂16, ♀13)

(3) Administration

- Dose: 37.5 mg astaxanthin※ and 1500 mg L-carnitine
- Number of doses a day: Twice a day (morning and afternoon)
- Total daily dose: 75 mg astaxanthin※ and 3000 mg L-carnitine
- Administration period: 8 weeks

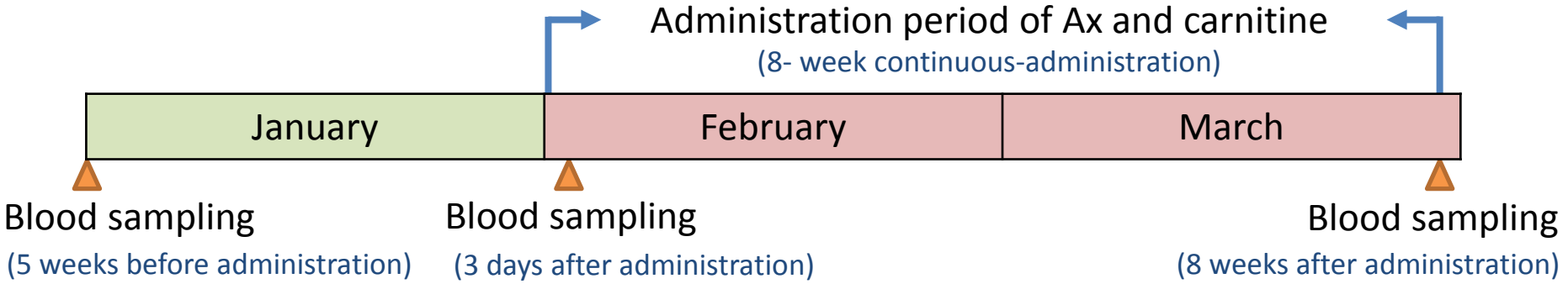
)

Improvement of Tying-up Syndrome (2)

All data was provided by Fumio Sato※, DVM, PhD.
※ Japan Racing Association Hidaka Training and Research Center,　E-mail: fumio_sato@jra.go.jp

1. Methods

(4) Administration period, Blood sampling and Training menus



High-intensity training in Jan.
- Canter (9.0 m/s)
  1600 m flat course × 1
- Gallop (10 m/s)
  1000 m slope course × 1

High-intensity training from Feb. to Mar.
- Canter (9.0 m/s)
  1600 m flat course × 1
- Gallop (10 – 12.5 m/s)
  1000 m slope course × 2

(5) Clinical study of onset rate of Tying-up syndrome

Stiffness,　Muscle Pain,　Reluctance to move

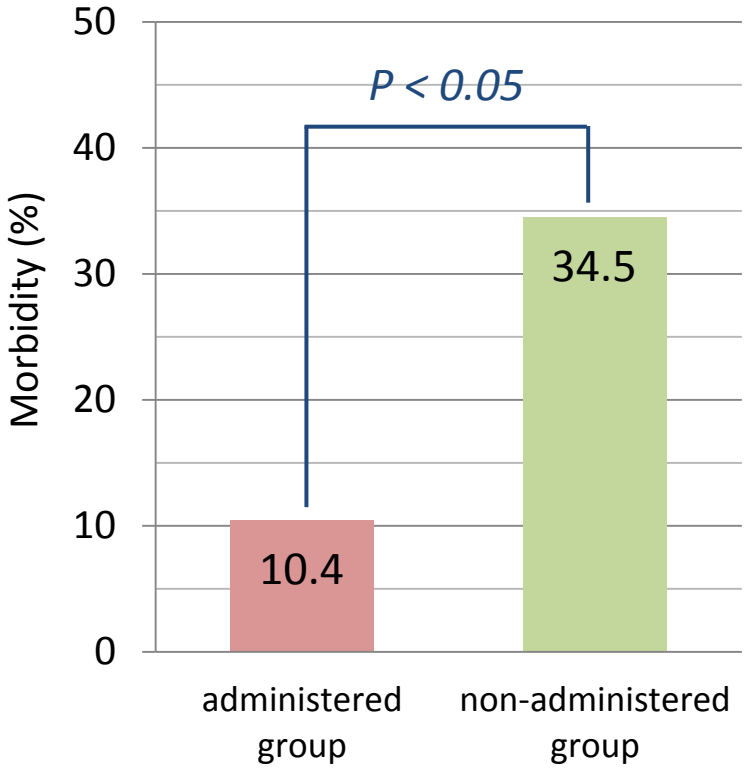
Improvement of Tying-up Syndrome (3)

All data was provided by Fumio Sato※, DVM, PhD.

※ Japan Racing Association Hidaka Training and Research Center,　E-mail: fumio_sato@jra.go.jp

2. Results

(1) Tying-up Syndrome onset



Recurrence rate in non-administered group

50% (5/10 heads)

Group	Horse No.	Gender	Date of onset	Resting days
A	1	♂	1-Mar	0
A	2	♂	30-Mar	0
A	3	♂	23-Feb	2
B	1	♂	8-Mar	0
B	2	♂	22-Feb	5
			8-Mar	0
			18-Mar	1
B	3	♀	25-Mar	9
B	4	♀	8-Mar	0
B	5	♀	1-Mar	2
			8-Mar	0
			22-Mar	4
			30-Mar	0
B	6	♀	29-Mar	0
B	7	♀	1-Mar	0
			31-Mar	0
B	8	♀	12-Mar	2
B	9	♀	22-Feb	0
			23-Mar	0
			25-Mar	0
B	10	♀	22-Feb	0
			18-Mar	0
			23-Mar	0
			25-Mar	0
			29-Mar	0

A: administered group
B: non-administered group

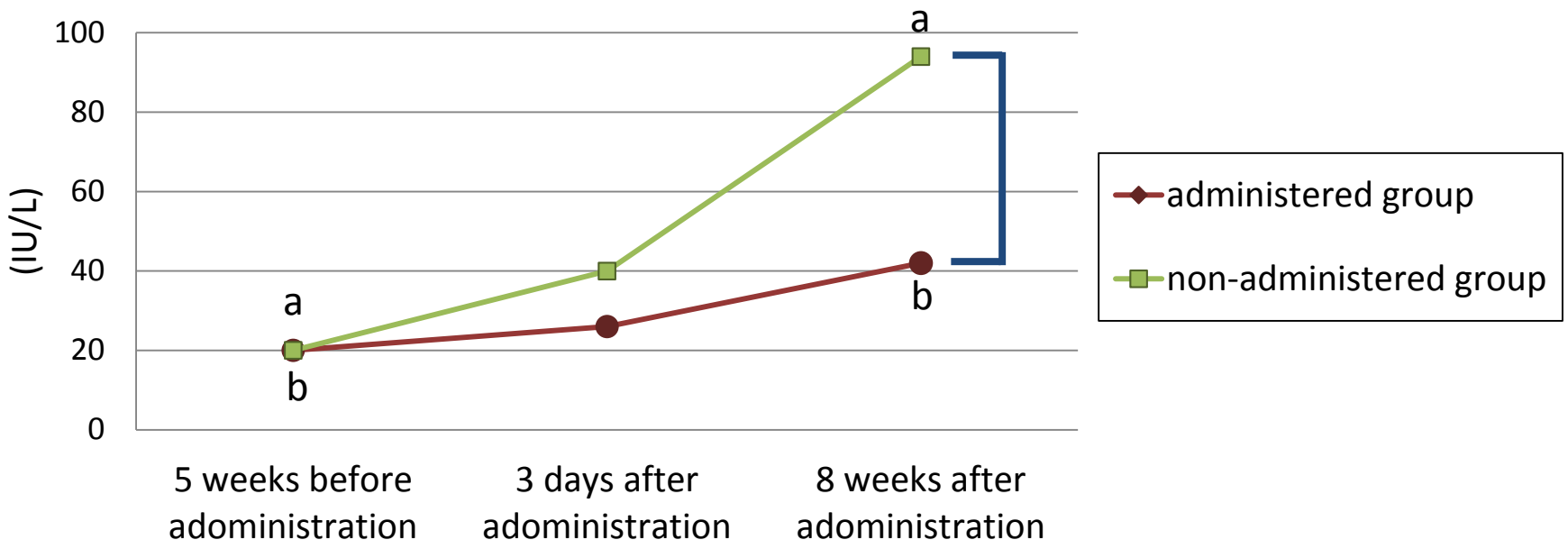
Improvement of Tying-up Syndrome (4)

All data was provided by Fumio Sato※, DVM, PhD.
※ Japan Racing Association Hidaka Training and Research Center,　E-mail: fumio_sato@jra.go.jp

2. Results

(2) Creatine Kinase (CK) ☞

Creatine kinase is one of the marker for rhabdomyolysis (severe muscle breakdown).



a and b: matching symbols within each group, $P < 0.001$
Bar: between administered group and non-administered group, $p < 0.05$

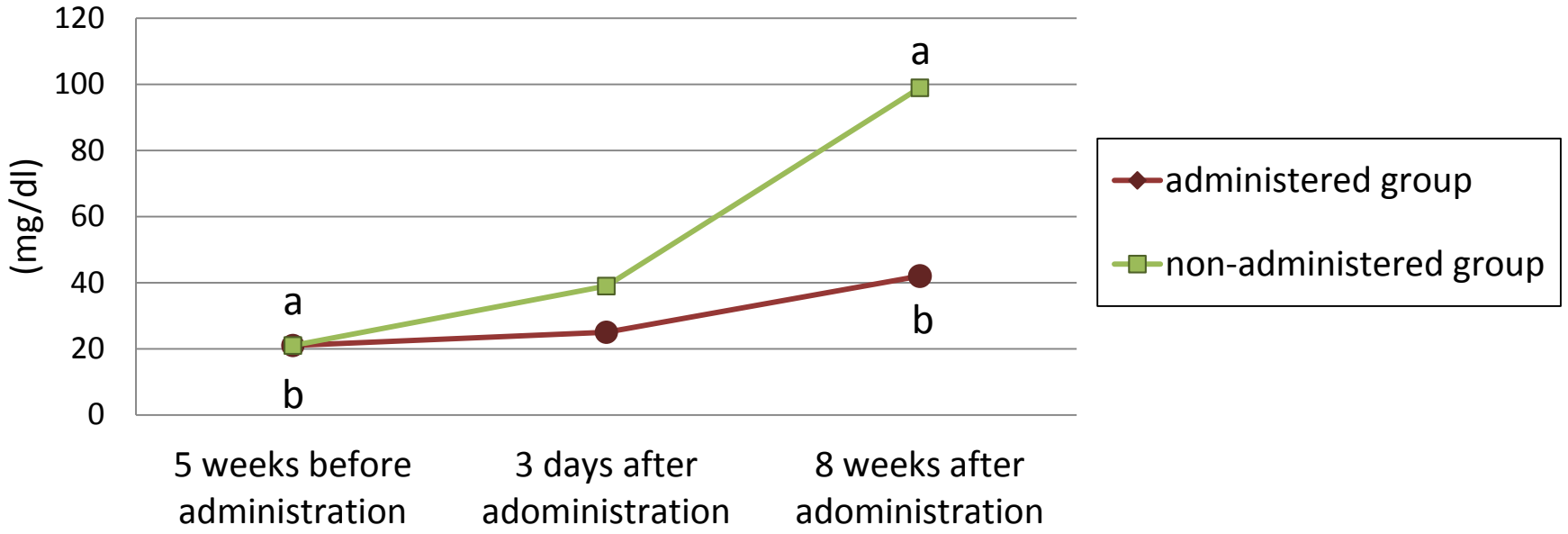
Improvement of Tying-up Syndrome (5)

All data was provided by Fumio Sato※, DVM, PhD.
※ Japan Racing Association Hidaka Training and Research Center,　E-mail: fumio_sato@jra.go.jp

2. Results

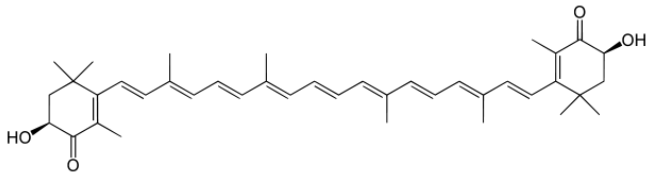
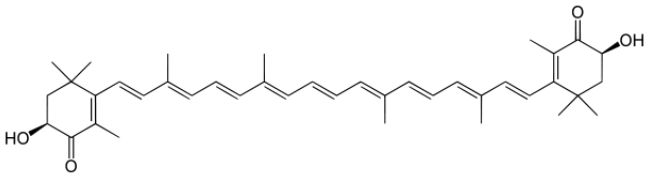
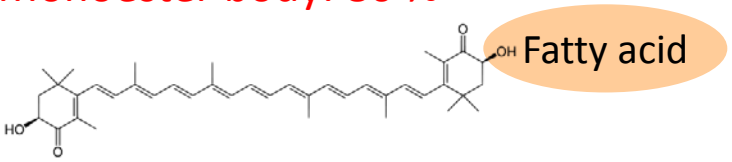
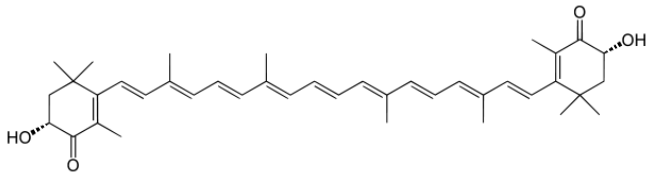
(3) Lactate Dehydrogenase Isoenzyme-5 (LDH-5) ☞

LDH-5 is the marker for skeletal muscle injury



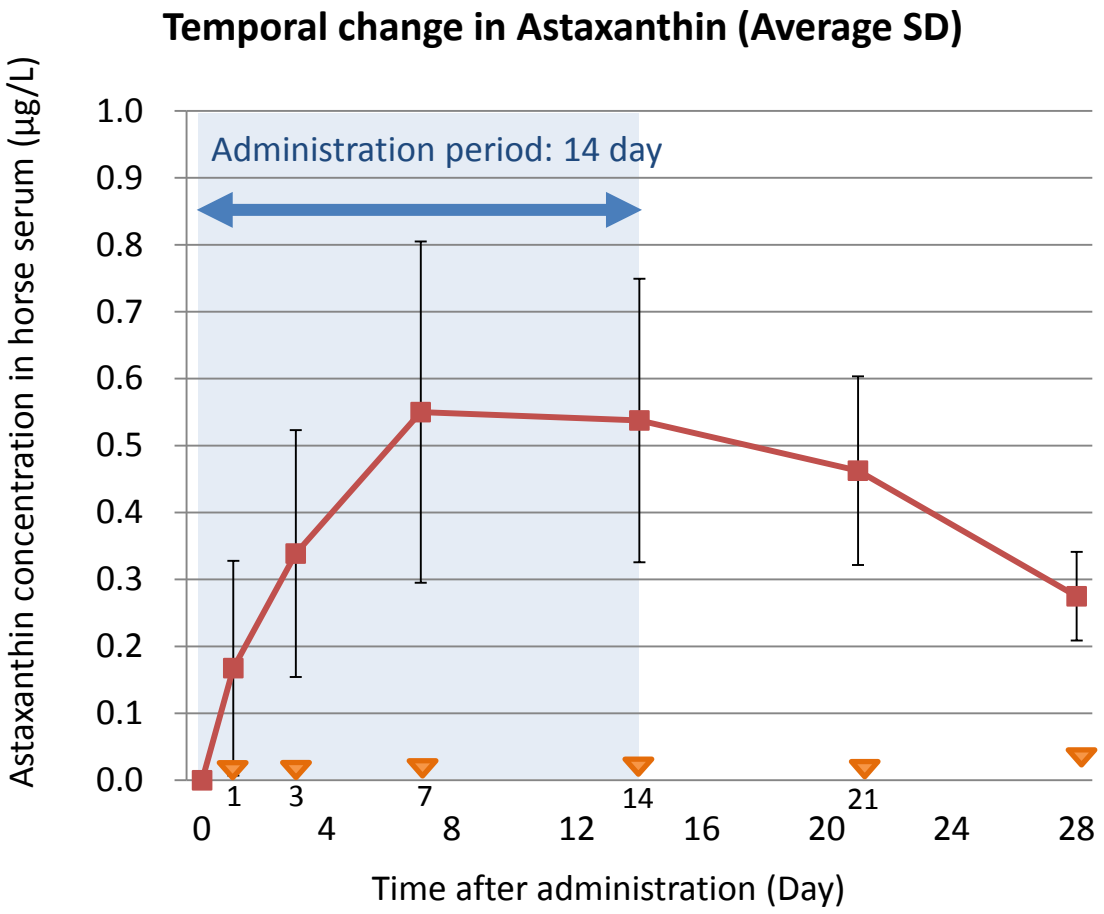
a and b: matching symbols within each group, $P < 0.001$

Type of Natural Astaxanthin

	Stereoisomer	Ester binding
Equizanthin™ (*Paracoccus carotinifaciens*)	 3S,3S' - compound	Free body: 100 %
Green alga (*Haematococcus pluvialis*)	 3S,3S' - compound	Monoester body: 80 %  Fatty acid Diester body: 12 % Free body: 8 %
Yeast (*Phaffia rhodozyma*)	 3R,3R' - compound	Free body: 100 %

Astaxanthin is present in the horse body as free body.

Administration of Equixanthin™



Test methods

1. Horses
   - 8 thoroughbreds (breeding mares)
   - age: 3～14 years
   - body weight: 651±35 kg
2. Dose
   - 50 mg astaxanthin※ × 2 times /day
   - total daily dose: Ax 100 mg / head

   ※ Equixanthin™ (Grafton NZ Ltd.)
3. Administration Period
   - 14 day continuous-administration
4. Blood Sampling Point
   - 1, 3, 7, 14, 21, 28 day
5. Analysis of Astaxanthin in serum
   - HPLC
   - Silica gel, Acetone: Hexane (2:8)

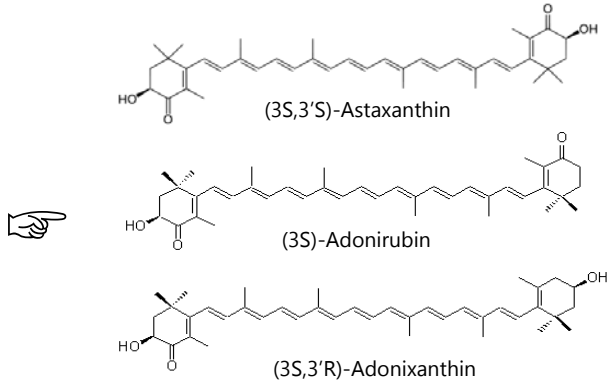
Blood concentration of astaxanthin achieves a stable state from day 7 after the start of the administration.

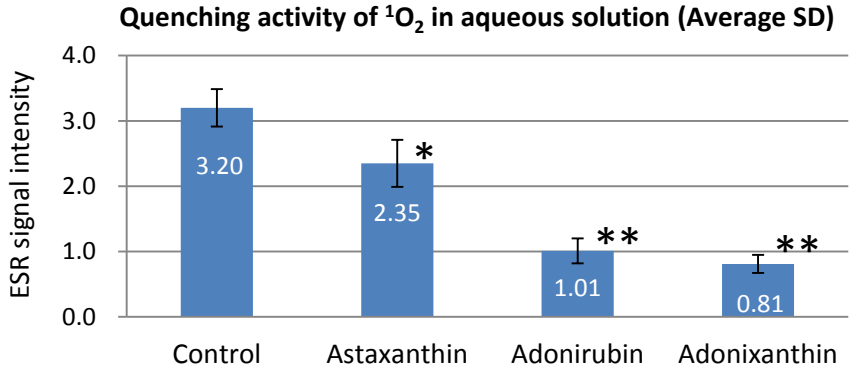
Anti-oxidative Activity of Adonirubin and Adonixanthin

(1) Elimination of Singlet Oxygen ($^1O_2$)

Adonirubin and Adonixanthin react more efficiently with $^1O_2$ than astaxanthin.

Carotenoid	Relative activity
Astaxanthin	1
Adonirubin	2.3
Adonixanthin	2.9





Quenching activity of $^1O_2$ produced by a hematoporphyrin-UVA system in aqueous solution by carotenoids. The reaction mixtures consisted of 62.5 µM hematoporphyrin and 50 mM TMPD dissolved in 50 mM phosphate buffer, and 625 µM astaxanthin, adonirubin, or adonixanthin dissolved in acetone at 22℃ in a total volume of 0.2 mL of 50 mM phosphate buffer (pH 7.5). Results of ESR spin-trapping experiments are expressed as the mean ± SD of three repeated experiments. Statistical analysis was performed by analysis of variance (ANOVA) followed by Post-hoc test with a 1 % or 5 % significance of difference. There were significant differences (* $P < 0.05$, ** $P < 0.01$) in the $^1O_2$ quenching activities of carotenoid treatment groups compared with the control group.

(2) Inhibition of Lipid Peroxidation

Adonirubin and Adonixanthin show slightly weaker activity than astaxanthin.

【Authority】Anti-oxidative, anti-tumor-promoting, and anti-carcinogenic activities of adonirubin and adonixanthin. *J. Oleo Sci.* **62**(3), 181-186, 2013

Carotenoid Composition of *Haematococcus pluvialis*

Carotenoids	Typical Composition Ratio (%)
Monoesters of Astaxanthin	70
Diesters of Astaxanthin	10
Free Astaxanthin	5
Lutein	5
Zeaxanthin	3
Canthaxanthin	3
Violaxanthin	1
Neoxanthin	1
β-Carotene	1
Others	1
Total	100