《Research Note》

β -Alanine Enhances Brain and Muscle Carnosine Levels in Broiler Chicks

Shozo Tomonaga¹, Mitsuhiro Matsumoto² and Mitsuhiro Furuse¹

¹Laboratory of Regulation in Metabolism and Behavior, Graduate School of Bioresource and Bioenvironmental Sciences, Kyushu University, Higashi-ku, Fukuoka-shi, Japan

²Livestock and Grassland Research Division, NARO Kyushu Okinawa Agricultural Research Center, Koshi-shi, Kumamoto-ken, Japan

Carnosine and its methylated derivative anserine are dipeptides present in high levels in chicken muscles. They are antioxidants and putative neurotransmitters. If administration of β -alanine, one of the constituents of carnosine, could increase levels of these dipeptides in the brain and muscles, it may improve brain function and increase commercial values of the chicken meat. In the present study, we investigated whether orally administered β -alanine could increase these dipeptide levels in the brain, *Musculus pectoralis superficialis*, and plasma in broiler chicks. Broilers (2 days old) were given oral doses of β -alanine (0.176, 0.88, 4.4 and 22 mmol/kg) twice a day for 5 days. Carnosine levels were dose-dependently increased in the brain, *Musculus pectoralis superficialis*, and plasma while no influence of anserine was detected. These results suggest that supplemental β -alanine could be effective in increasing carnosine levels in the brain and muscle of broiler chicks.

Key words: β -alanine, brain, broiler, carnosine, muscle

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Introduction

Carnosine (β -alanyl-L-histidine) and its derivative anserine (β -alanyl-1-methyl-L-histidine) are high in the breast muscle (*Musculus pectoralis superficialis*, MPS) of chickens (Nishimura *et al.*, 1988; Aristoy and Toldra, 2004). They are known as antioxidants (Kohen *et al.*, 1988) and putative neurotransmitters (Tomonaga *et al.*, 2004; Tomonaga *et al.*, 2005a). Carnosine could enhance meat stability in storage by its antioxidant activity (Djenane *et al.*, 2004). Not only that, carnosine seems to contribute to the nutritional quality of meat because dietary carnosine has a hypoglycemic effect (Yamano *et al.*, 2001), hypotensive action (Niiijima *et al.*, 2002), antidepressant-like activity (Tomonaga *et al.*, 2008), and anti-aging effects (Gallant *et al.*, 2000). Carnosine is synthesized from β -alanine (β -Ala) and histidine (His) by carnosine synthase (Drozak *et al.*, 2010), and anserine is mainly formed from carnosine by a methylation reaction (Boldyrev and Severin, 1990), while Horinishi *et al.* (1978) reported that a purified enzyme from chickens could methylate His to generate anserine by direct synthesis. On the other hand, carnosinase, which hydrolyzed carnosine or an-

serine to its constituent amino acids, was confirmed to be present in rats and humans. The rate of carnosine hydrolysis is 3–4-fold higher than that of anserine (Pegova *et al.*, 2000).

The influence of constitutive amino acids of these dipeptides, especially β -Ala, has been studied. Intraperitoneal injections of β -Ala caused increases in the carnosine concentration in the olfactory bulb in the brain and in the gastrocnemius muscle of mice. Cessation of β -Ala treatment for 5 days caused an increase of anserine concentration in the gastrocnemius muscle in mice (Margolis *et al.*, 1985). Dietary β -Ala plus His supplementation increased carnosine concentration of *type IIA* and *IIB* muscle fibers in horses (Dunnett and Harris, 1999). In humans, supplementation of β -Ala could increase carnosine levels of the soleus and gastrocnemius muscles (Derave *et al.*, 2007).

We have speculated that if administration of β -Ala, one of the constituents of carnosine, could increase levels of these dipeptides in the brain and muscles, it may improve brain function and increase commercial values (enhancement of storage stability and nutritional quality) of the chicken meat. To clarify this speculation, we first revealed that oral administration of β -Ala (22 mmol/kg twice a day, from 2 days old for 5 days) increased both dipeptide concentrations in the brain but failed to increase the sum of the dipeptide concentrations in the MPS of layer-type chicks (Tomonaga *et al.*, 2005b). Next, we revealed that dietary β -Ala (0.5, 1, and 2% in commercial diet, from 4 weeks old for 4 weeks) enhanced the carnosine and anserine levels in the brain, but

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Correspondence: S. Tomonaga, Laboratory of Regulation in Metabolism and Behavior, Graduate School of Bioresource and Bioenvironmental Sciences, Kyushu University, Higashi-ku, Fukuoka-shi, 812–8581, Japan.
(E-mail: shozo@brs.kyushu-u.ac.jp)

not muscles (MPS, *Musculus pectoralis profundus* and *Musculus biceps femoris*), in broilers (Tomonaga *et al.*, 2006). From these results, we speculated that the methods used in these previous studies may not be appropriate for increasing the dipeptides in the chicken muscles because of the strain difference, inadequate treatment period and method for injection. In the present study, therefore, we evaluated a different method, oral administration of β -Ala (0.176, 0.88, 4.4 and 22 mmol/kg twice a day, from 2 days old for 5 days) in broiler chicks. Among the free amino acids, in addition to the constituent amino acids, we focused on taurine. Administration of β -Ala induces tissue taurine depletion because β -Ala competes with taurine for uptake mechanisms (Hu *et al.*, 2000; Tomonaga *et al.*, 2005b, 2006). Furthermore, tissue taurine depletion evokes physiological dysfunctions such as growth retardation (Sturman, 1993; Hu *et al.*, 2000). Therefore, we assumed that it is important to investigate tissue taurine depletion for the evaluation of the effect of supplemental β -Ala.

Materials and Methods

One-day-old male broiler chicks (Chanky) were purchased from a local hatchery (Kyushu Hatchery, Fukuoka, Japan). Food (Toyohashi Feed Mills Co., Ltd., Toyohashi, Japan) and water were freely accessible. The chicks were reared as a group until they were 1 day old. From 2 to 7 days old, birds were individually reared. The birds were distributed into experimental groups based on their body weight so that the average body weight of each group was as uniform as possible. Experimental procedures followed the guidance for Animal Experiments in the Faculty of Agriculture and in the Graduate Course of Kyushu University and the Law (No. 105) and Notification (No. 6) of the Government. β -Ala was purchased from Wako (Osaka, Japan). The birds (2 days old) were orally given distilled water (10 ml/kg, control) or β -Ala (0.176, 0.88, 4.4 or 22 mmol/10 ml/kg) twice a day for 5 days. The number of birds used in each group was 7 or 8. At 7 days old, all birds were killed following the anesthesia with isoflurane (Mylan Inc., Tokyo, Japan) and blood, whole brain tissue and MPS were collected. Blood taken from the jugular vein was collected into heparinized tubes. The blood was centrifuged at 10,000 g for 10 min at 4°C and the plasma was removed and stored at -80°C until assaying.

Whole brain and MPS tissues were weighed and stored at -80°C prior to amino acid analysis. For MPS weight, the right side was weighed; from this, total MPS weight was extrapolated. The method of sampling for the amino acid analysis has been described elsewhere (Tomonaga *et al.*, 2005) with some modifications. The tissue (brain or MPS) was homogenized in 0.2 mol/L ice-cold perchloric acid and the homogenate was cooled on ice for 30 min for deproteinization. The homogenate was centrifuged at 20,000 x g for 15 min at 0°C. Next, the pH of the supernatant was adjusted to approximately 3.0 by adding 1 mol/L sodium acetate. The sample was filtered through a 0.2- μ m filter (Millipore, Bedford, USA). The plasma was diluted with sulfosalicylic acid solution (20 mg/ml) and shaken for 15 min. Then the homo-

genate was centrifuged at 2,000 x g for 15 min at 4°C. Next, the sample was filtered through a 0.2- μ m filter (Millipore, Bedford, USA). Then, 40 μ l of each filtrate was applied to the Amino Acid Analyzer L-8800 (Hitachi High-Tech-nologies Co. Ltd., Hitachi, Japan). A standard L-amino acid solution was prepared by diluting a commercially available L-amino acid solution (Type AN II and Type B, Wako, Osaka, Japan) with water. Carnosine, anserine, their constituent amino acids (β -Ala, histidine, and 1-methyl-histidine) and taurine were analyzed.

All data were statistically analyzed by regression analysis for the dose of β -Ala treatment and amino acid or dipeptide level. Statistical analysis was conducted using the commercially available StatView software package (SAS 1998).

Results

At the beginning of the experiment, the number of birds used in each group was 7 (control, 0.176, and 22 mmol β -Ala groups) or 8 (0.88 and 4.4 mmol β -Ala groups). However, two birds (one in the 4.4 mmol β -Ala group and one in the 22 mmol β -Ala group) died during the experimental periods. Furthermore, two samples (one in the control group and one in the 0.88 mmol/kg β -Ala group) were lost during sampling. Therefore, for growth and various tissue parameters, the numbers of birds in each group were: control group, 7; 0.176 mmol β -Ala group, 7; 0.88 mmol β -Ala group, 8; 4.4 mmol β -Ala group, 7; and 22 mmol β -Ala group, 6. For analysis of amino acid and dipeptide levels, the numbers of birds in each group were: control group, 6; 0.176 mmol β -Ala group, 7; 0.88 mmol β -Ala group, 7; 4.4 mmol β -Ala group, 7; and 22 mmol β -Ala group, 6. In the analysis of brain carnosine, some sample levels were below the detection limit and they were not included in the analysis. Therefore, the numbers of birds in each group were: control group, 4; 0.176 mmol β -Ala group, 5; 0.88 mmol β -Ala group, 7; 4.4 mmol β -Ala group, 7; and 22 mmol β -Ala group, 5.

Table 1 represents the effect of β -Ala on growth and various tissue weights in broilers. There were no dose-dependent effects on all parameters.

Tables 2 and 3 indicate the effect of β -Ala on carnosine, anserine, their constituent amino acids and taurine levels in the brain, MPS and plasma of broilers. We presented only the data in which significant correlations were detected in Table 3. In the brain, MPS and plasma, there were significant positive correlations between the dose of injected β -Ala and carnosine or β -Ala levels. On the other hand, there were significant negative correlations between the dose of injected β -Ala and taurine levels in the brain, MPS and plasma.

Discussion

β -Ala dose-dependently increased carnosine levels in the brain and MPS of broiler-type chicks. These results were consistent with our previous study using the same treatment in layer-type chicks (Tomonaga *et al.*, 2005b). On the other hand, the effect was somehow different in anserine metabolism: brain and MPS anserine levels were not influenced (brain level was increased and MPS level was decreased in

Table 1. Effect of β -alanine on growth and various tissue weights in broiler chicks

	β -Alanine (mmol/kg)				
	0	0.176	0.88	4.4	22
Initial Body weight (g)	52 $\pm$ 1	52 $\pm$ 1	51 $\pm$ 2	52 $\pm$ 1	53 $\pm$ 1
Feed consumption (g/5 days)	109 $\pm$ 8	111 $\pm$ 4	105 $\pm$ 4	110 $\pm$ 3	107 $\pm$ 4
Body weight gain (g/5 days)	108 $\pm$ 7	111 $\pm$ 4	110 $\pm$ 5	111 $\pm$ 3	105 $\pm$ 2
Feed efficiency (Body weight gain/feed consumption)	1.01 $\pm$ 0.02	1.00 $\pm$ 0.02	1.00 $\pm$ 0.03	1.00 $\pm$ 0.01	1.02 $\pm$ 0.03
MPS weight (g)	3.69 $\pm$ 0.37	3.86 $\pm$ 0.31	3.11 $\pm$ 0.21	3.62 $\pm$ 0.25	3.21 $\pm$ 0.29
Whole brain weight (g)	1.29 $\pm$ 0.02	1.25 $\pm$ 0.02	1.25 $\pm$ 0.04	1.26 $\pm$ 0.04	1.27 $\pm$ 0.02

β -Alanine was orally administered twice a day for five days. MPS indicates *Musculus pectoralis superficialis*. For MPS weight, the right side was weighed and extrapolated to the whole weight. Values are means $\pm$ standard error of the mean.

Table 2. Effect of β -alanine on amino acid and dipeptide levels in broiler chicks

Tissue	Contents	β -Alanine (mmol/kg)				
		0	0.176	0.88	4.4	22
Brain	Carnosine	27 $\pm$ 5	27 $\pm$ 7	39 $\pm$ 5	35 $\pm$ 4	49 $\pm$ 12
	Anserine	298 $\pm$ 21	304 $\pm$ 32	328 $\pm$ 28	378 $\pm$ 28	370 $\pm$ 45
	β -Alanine	28 $\pm$ 2	30 $\pm$ 2	29 $\pm$ 2	32 $\pm$ 2	51 $\pm$ 8
	Histidine	70 $\pm$ 4	65 $\pm$ 7	74 $\pm$ 5	82 $\pm$ 6	79 $\pm$ 10
	1-Methylhistidine	27 $\pm$ 5	22 $\pm$ 5	20 $\pm$ 5	25 $\pm$ 4	18 $\pm$ 3
	Taurine	5180 $\pm$ 294	5111 $\pm$ 358	5092 $\pm$ 288	4664 $\pm$ 338	3929 $\pm$ 261
MPS	Carnosine	6877 $\pm$ 856	6379 $\pm$ 1258	6116 $\pm$ 878	9162 $\pm$ 1453	11396 $\pm$ 501
	Anserine	7380 $\pm$ 585	7699 $\pm$ 644	6882 $\pm$ 532	7611 $\pm$ 635	7236 $\pm$ 699
	β -Alanine	83 $\pm$ 10	113 $\pm$ 44	109 $\pm$ 17	109 $\pm$ 16	264 $\pm$ 54
	Histidine	258 $\pm$ 19	244 $\pm$ 17	256 $\pm$ 17	312 $\pm$ 27	292 $\pm$ 29
	1-Methylhistidine	105 $\pm$ 16	100 $\pm$ 15	86 $\pm$ 14	100 $\pm$ 17	69 $\pm$ 11
	Taurine	2721 $\pm$ 121	2787 $\pm$ 199	2655 $\pm$ 164	2484 $\pm$ 224	1627 $\pm$ 101
Plasma	Carnosine	7 $\pm$ 1	6 $\pm$ 1	7 $\pm$ 1	11 $\pm$ 3	13 $\pm$ 1
	Anserine	14 $\pm$ 2	12 $\pm$ 1	15 $\pm$ 5	16 $\pm$ 4	18 $\pm$ 2
	β -Alanine	11 $\pm$ 2	16 $\pm$ 5	11 $\pm$ 1	16 $\pm$ 3	56 $\pm$ 5
	Histidine	98 $\pm$ 13	87 $\pm$ 6	102 $\pm$ 10	112 $\pm$ 8	122 $\pm$ 21
	1-Methylhistidine	41 $\pm$ 6	33 $\pm$ 4	25 $\pm$ 3	32 $\pm$ 4	30 $\pm$ 7
	Taurine	354 $\pm$ 29	332 $\pm$ 29	325 $\pm$ 26	269 $\pm$ 25	199 $\pm$ 19

β -Alanine was orally administered twice a day for five days. MPS indicates *Musculus pectoralis superficialis*. Values are means $\pm$ standard error of the mean. Brain and MPS levels are in nmol/g wet tissue. Plasma level is in μ M.

Table 3. Regression analysis of the effect of β -alanine on tissue carnosine, β -alanine or taurine level in broiler chicks

Tissue	Contents	Regression equation	R ²	P value
Brain	Carnosine	Y=31.7(SE 3.5)+0.8(SE 0.4)X	0.16	<0.05
	β -Alanine	Y=28.7(SE 1.9)+1(SE 0.2) X	0.457	<0.0001
	Taurine	Y=5092(SE 160)–55(SE 17) X	0.258	<0.005
MPS	Carnosine	Y=6763(SE 572)+224(SE 59)X	0.313	<0.001
	β -Alanine	Y=94.4(SE 16.5)+7.5(SE 1.7)X	0.383	<0.0001
	Taurine	Y=2732(SE 88)–51(SE 9) X	0.494	<0.0001
Plasma	Carnosine	Y=7(SE 0.9)+0.3(SE 0.1)X	0.229	<0.005
	β -Alanine	Y=11.1(SE 1.8)+2(SE 0.2)X	0.781	<0.0001
	Taurine	Y=329(SE 14)–6(SE 1) X	0.381	<0.001

β -Alanine was orally administered twice a day for five days. Y indicates level in each tissue (μ mol/g wet tissue) or in plasma (μ M). X indicates dose of administered β -alanine. MPS indicates *Musculus pectoralis superficialis*.

the layer-type chicks). Regulation of carnosine and anserine metabolisms may be somehow different between layer-type and broiler-type chicks. Indeed, brain and MPS levels of these dipeptides and their constituents in the control group differed from those in the control group of layer-type chicks of the same age in the previous study, for example, β -Ala level of the MPS in layer-type chicks is approximately 2-fold higher than that in broiler-type chicks (Tomonaga *et al.*, 2005b). Clarification of the difference in these dipeptide metabolisms between broiler- and layer-type chicks may be useful for breeding because it may help to select chickens that contain dipeptides in high levels. On the other hand, the effectiveness in increasing dipeptide levels may be higher in younger chicks because we previously confirmed that dietary β -Ala (0.5, 1 and 2% in commercial diet, from 4 weeks old for 4 weeks) enhances brain, but not muscle (MPS, *Musculus pectoralis profundus*, and *Musculus biceps femoris*), dipeptide levels in broilers (Tomonaga *et al.*, 2006). To improve present method, it can be speculated that subsequent cessation for several days may be effective in increasing both dipeptide levels because intraperitoneal injections of β -Ala increased carnosine concentrations in the olfactory bulb in the brain and in gastrocnemius muscle and subsequent cessation of β -Ala treatment for 5 days caused an increase of anserine concentration in the gastrocnemius muscle in mice (Margolis *et al.*, 1985). On the other hand, as shown in studies in horses (Dunnett and Harris, 1999), supplementation with β -Ala plus His may be more effective than supplementation with β -Ala alone. Further studies are necessary.

β -Ala levels of brain, MPS and plasma were dose-dependently increased while their taurine levels were decreased in a dose-dependent manner. Taurine depletion was commonly observed following β -Ala treatment because β -Ala competes with taurine for uptake (Hu *et al.*, 2000; Tomonaga *et al.*, 2005b, 2006). The supplemental β -Ala in the present study successfully competed with taurine. It is not clear why not only tissue but also plasma taurine level decreased. We hypothesized that temporarily increased taurine in the plasma might accelerate some physiological mechanisms of taurine excretion and as a result, plasma taurine decreased too much. Further study should be done to clarify this hypothesis. As pointed out in the introduction, tissue taurine depletion could induce physiological dysfunctions such as growth retardation (Sturman, 1993; Hu *et al.*, 2000). In the present study, no significant negative effects were observed in growth parameters such as feed consumption, body weight gain, feed efficiency, MPS weight and whole brain weight (Table 1). On the other hand, as shown in the results section, two chicks died in higher β -Ala groups. Therefore, further study should be done to clarify whether present method influences the health in chickens.

Thus, supplemental β -Ala given to broiler chicks seems effective in increasing the carnosine in their MPS and brain. This may improve brain function and/or increase the commercial value of meat. To effectively increase the dipeptides in chickens, further nutritional studies are needed. In addition, other methods, such as stimulation of carnosine syn-

thesis and/or suppression of its degradation by a method of molecular biology, may be effective. In particular, carnosine synthase was recently identified in chickens (Drozak *et al.*, 2010) and this may make molecular studies easier to perform. Further studies are needed to determine how to increase dipeptides in broilers.

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