

«Research Note»

## Fructosyl-Valine Orally Administrated to Chickens is Absorbed from Gastrointestinal Tract

Natsuki Takahashi and Kazumi Kita

Laboratory of Animal Nutrition, Faculty of Agriculture, Iwate University, Morioka, Iwate 020-8550, Japan

Amadori products are non-enzymatically formed by binding carbonyl groups and amino groups. Glycated amino acids generated by reacting amino acid and glucose are also in a group of Amadori products of which the transport and metabolism have been investigated mainly in mammals but not in avians. In the present study, therefore, we examined whether dietary fructosyl-valine, which is one of the glycated amino acids, orally administrated to chickens can be incorporated into blood or not. Fructosyl-valine was orally administrated to the chicken and blood samples were collected at 0, 20, 40, 60, 120 and 180 min after administration. Plasma concentration of fructosyl-valine was measured by using LC/MS. The plasma concentration of fructosyl-valine was increased by passing time from 0 to 180 min after administration, and no change was observed in the control group. Conclusively, it was clarified that fructosyl-valine orally administrated to the chicken could be absorbed from gastrointestinal tract and incorporated into blood.

**Key words:** Amadori product, fructosyl-valine, gastrointestinal tract, glycation, oral administration, plasma concentration

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### Introduction

The amino-carbonyl reaction is a non-enzymatic reaction between carbonyl and amino groups, which results in forming Schiff base, and then forms Amadori product through Amadori rearrangement (Hodge, 1955). This reaction can be easily taken place not only in the body but also in foods (Meitinger *et al.*, 2014). Amadori products generated from glucose and amino acids might contribute to animal's health because it has been reported that this compounds have several biological effects such as antioxidant (Ide *et al.*, 1999), antihyperglycemia (Ha *et al.*, 2011), and so on. Compared to mammalians, avian species have the unique characteristics such as high blood glucose concentration and high body temperature (Hazelwood and Lorenz, 1959), which would easily lead to generate Amadori products. In our previous study, advanced glycation end products (AGEs), which are generated from Amadori products, were more accumulated in spleen, liver and kidney of chickens than other tissues (Kita, 2014). These results suggest that Amadori products like AGEs could be incorporated into several tissues. It has been reported that glycated amino acids could

be absorbed from gastrointestinal tract of rats (Sgarbieri *et al.*, 1973; Hultsch *et al.*, 2006). Although the radioactivity of Amadori products and their metabolites were measured after administrating radioactive Amadori products into rats, the actual amounts of Amadori products in tissues and plasma have not been directly measured yet. Therefore, the aim of the present study is to measure plasma concentration of fructosyl-valine in young chickens orally administrated with fructosyl-valine.

### Materials and Methods

#### *Preparation of Fructosyl-valine*

Fructosyl-valine (N- $\alpha$ -(1-deoxyfructosyl)valine) was prepared by modifying the previous method (Wang *et al.*, 2008). Briefly, anhydrous glucose (20 mmol), malonic acid (5 mmol) and L-valine (20 mmol) were refluxed in anhydrous methanol (30 mL) under nitrogen gas for 6 h. After filtration to remove the unreacted glucose and L-valine, the filtrate was concentrated, and the almost same volume of anhydrous acetone was added, which resulted in precipitating fructosyl-valine. Precipitated fructosyl-valine was dissolved in distilled water and then precipitated with anhydrous acetone in order to eliminate unreacted valine remained with fructosyl-valine. Then, the product was vacuum-dried and stored under nitrogen atmosphere until used. The identification and determination of its purity were performed using LC/MS.

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Correspondence: K. Kita. Laboratory of Animal Nutrition, Faculty of Agriculture, Iwate University, Morioka, Iwate 020-8550, Japan.

(E-mail: kitak@iwate-u.ac.jp)

### Animals and Experimental Procedures

Newly hatched single-comb White Leghorn male chicks were obtained from a local hatchery (Koiwai Farm Co., Ltd, Shizukuishi, Iwate, Japan). Chicks were fed a commercial chick mash diet (crude protein: 207 g/kg, metabolizable energy: 12.1 MJ/kg; Toyohashi Feed Mills Co., Ltd, Toyohashi, Aichi, Japan) from hatching until 8 d of age in an electrically heated brooder. At this age, 40 birds were selected and used for the experiment. After fasting for 12 h, fructosyl-valine dissolved in distilled water (300  $\mu$ mol/kg body weight) was orally administrated to chickens using a stomach tube. Control chickens were administrated the equivalent volume of distilled water. At 20, 40, 60, 120 and 180 min (60 and 180 min in control) after administration, blood samples were taken from heart after light anesthesia with diethylether. The samples at 0 min after administration were obtained from the chickens not administrated anything. Blood samples were centrifuged for 20 min at 5,000 $\times$ g, 4 $^{\circ}$ C to separate plasma. Plasma samples were stored at -20 $^{\circ}$ C until analyzed. Animal care was in compliance with applicable guidelines from the Iwate University Animal Care and Use Committee.

### Deproteinization and Delipidation of Plasma Samples

The method to remove protein and lipid was described previously (Kita *et al.*, 2013). Before deproteinization and delipidation, 62.5  $\mu$ L of 1/3 mM N-methyl-valine was added into 500  $\mu$ L of plasma as internal standard. Then, the plasma samples were mixed with 2 mL of acetonitrile for deproteinization. After deproteinization, lipid was removed by mixing with chloroform and methanol (2:1, v/v). For purifying fructosyl-valine, cation-exchange resin was used.

### Measurement of Plasma Fructosyl-valine using LC/MS

Separation and detection of fructosyl-valine and N-methyl-valine was performed using LC/MS with an electrospray positive ionization (ESI) (LCMS-8040, Shimadzu Corporation, Kyoto, Japan). The mobile phase was ultrapure water. The flow rate was 400  $\mu$ L/min. The sample injection volume was 3  $\mu$ L. Reverse phase HPLC column (100 $\times$ 2 mm I.D., Gemini 3  $\mu$ m C18 110  $\text{Å}$ , Phenomenex, Torrance, CA, USA) was used. Fructosyl-valine and N-methyl-valine were measured in positive ion mode using Q3 selected ion monitoring (Q3 SIM). The values for m/z of positive ion of fructosyl-valine and N-methyl-valine were 280.29 and 132.17, respectively. The concentration of fructosyl-valine was corrected with the recovery rate of N-methyl-valine.

The ratio of detected fructosyl-valine in chicken plasma to the amount of fructosyl-valine orally administrated to young chickens was calculated. Calculation was performed using the ratio of 3.5–6.5 mL/100 g body weight (Whittow, 2000) as the volume of chicken plasma.

### Statistical Analysis

All data are presented as mean  $\pm$  SE. Statistical analysis of data was performed by one-way ANOVA and Tukey's HSD test for multiple comparisons ( $P < 0.05$ ) using the General Linear Model Procedures of SAS (SAS version 9.3, SAS Institute, 2011).

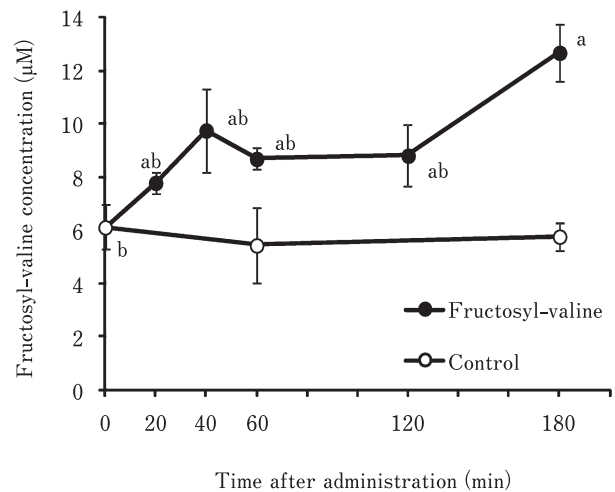


Fig. 1. **Time course change in plasma fructosyl-valine concentration in young chickens orally administrated with fructosyl-valine.** Fructosyl-valine dissolved in distilled water (300  $\mu$ mol/kg) was orally administrated to the crop of young chickens. Control chickens were administrated distilled water alone. At 20, 40, 60, 120 and 180 min (60 and 180 min in control) after administration, blood samples were taken from heart and plasma fructosyl-valine concentration was measured by using LC/MS. <sup>a,b</sup> Means with different superscript letters are significantly different ( $P < 0.05$ ). Values were means  $\pm$  SE.  $n = 5$ .

## Results

The time course change in plasma fructosyl-valine concentration in chickens orally administrated with fructosyl-valine is shown in Fig. 1. Plasma fructosyl-valine concentration in chickens administrated distilled water alone did not significantly change between before and after administration. On the other hand, when chickens were orally administrated with fructosyl-valine, plasma fructosyl-valine concentration gradually increased after administration, and significant change was observed at 180 min after administration compared to 0 min. These results suggested that fructosyl-valine could be absorbed from gastrointestinal tract and incorporated into blood. The ratio of detected fructosyl-valine in chicken plasma to the amount of fructosyl-valine orally administrated to young chickens is shown in Table 1. The ratio detected fructosyl-valine in chicken plasma was approximate 0.1% at 180 min after oral administration.

## Discussion

Previously, two types of glycated tryptophan compounds in the plasma of chickens were successfully detected and quantified by using LC/MS (Makino *et al.*, 2015). In the present study, as shown in Fig. 1, fructosyl-valine, one of glycated amino acids, was also successfully detected and measured in the chicken plasma.

In young chicken, we revealed that the concentrations of glucose and amino acids reached a peak at 20 min after oral

**Table 1. The ratio (%) of fructosyl-valine detected in chicken plasma to the amount of fructosyl-valine orally administrated to young chickens**

	Time after administration (min)				
	20	40	60	120	180
Ratio (%)	0.02–0.037	0.043–0.079	0.03–0.056	0.032–0.059	0.077–0.143

Calculation was performed using the ratio of 3.5–6.5 mL / 100 g body weight (Whittow, 2000) as volume of plasma volume.

administration with the mixture of glucose and amino acids (Ito and Kita, 2013). But, in the case of fructosyl-valine, the concentration in plasma fructosyl-valine was consecutively elevated until 180 min after oral administration. Similar results were observed in rats orally administrated with  $^{14}\text{C}$ -labeled leucine or  $^{14}\text{C}$ -labeled fructosyl-leucine (Sgarbieri *et al.*, 1973). They measured time course change in the radioactivity of leucine and fructosyl-leucine in the serum and revealed that the radioactivity of  $^{14}\text{C}$ -fructosyl-leucine slowly increased by 8.5 h and then decreased gradually, which differed from that of  $^{14}\text{C}$ -leucine.

As shown in Table 1, only a few fructosyl-valine was detected in chicken plasma, which is very low compared to the amount of administration. In mammals, some Amadori products generated from glucose and amino acids are poorly absorbed because absorption of these compounds might be performed from the large intestine by passive diffusion (Erbersdobler and Faist, 2001; Sgarbieri *et al.*, 1973). In the present study, fructosyl-valine, one of the Amadori products, was also slowly absorbed into blood, which suggests this mechanism might be applied to birds.

It is reliable that plasma fructosyl-valine concentration is regulated by reversible reactions, generation and decomposition of fructosyl-valine. There are some reports as to the specific enzyme acting on glycated proteins, and this enzyme is called fructosamine 3-kinase (FN3K) (Delplanque *et al.*, 2004; Veiga-Da-Chunha *et al.*, 2006). FN3K phosphorylates the third carbon on the sugar moiety of fructosamines and leads to deglycation. Birds also have this enzyme (Delplanque *et al.*, 2004), which suggests that plasma concentration of fructosyl-valine in chickens may be decreased and regulated by this enzyme. There are some reports indicating the excretion of Amadori products into urine. A large part of radiolabeled Amadori product intravenously administrated to rats was excreted into urine (Hultsch *et al.*, 2006). This phenomenon also suggests that the plasma concentration of fructosyl-valine can be regulated by urinary excretion of this compound.

In this study, it was revealed that fructosyl-valine can be absorbed from gastrointestinal tract in chickens. As stated above, plasma fructosyl-valine concentration is changed slowly compared to glucose and amino acids, and the change in plasma fructosyl-valine would be regulated by generation, degradation and excretion of this compound. These points should be studied in the future.

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