Diurnal Change in the Rates of N^ε-methylhistidine Excretion in Laying Hens

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Abstract N^ε-methylhistidine (N^ε-MH) excretions were measured in laying and non-laying hens to show the role of the skeletal muscle as a source of amino acids for egg protein synthesis. Four 10-month old laying hens and 3 117-day old non-laying hens were housed in individual cages and fed the experimental diet containing 15% protein. The diet was made mainly of corn and purified soybean protein. After 7 days of pre-feeding of the experimental diet, whole excreta samples were collected to measure N^ε-MH every 4 hours in the daytime starting at 7 a.m. The excreta samples were also collected in the evenings (7 p.m.-7 a.m.) of the seventh and eighth days. In the laying hen, the peak of N^ε-MH excretion was observed between 4-8 hours after oviposition, and the rate of N^ε-MH excretion at the peak was about 2 times the rate before oviposition. However, in the non-laying hen, there was no diurnal variation of the rate of N^ε-MH excretion, and the rates were much lower than those of the laying hen. The results clearly show that there is a diurnal variation of the skeletal muscle protein breakdown in the laying hen and that skeletal muscle protein breakdown is accelerated when egg protein is intensively synthesized.

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Key words : laying hen, N^ε-methylhistidine, protein breakdown

The skeletal muscle is thought to be a source providing amino acids for egg protein synthesis especially when egg protein is synthesized at a high rate⁹⁾ while there is no paper reporting on the rate of muscle protein breakdown in the laying hen. If dietary supply of amino acids does not meet the requirement for egg protein synthesis, skeletal muscles should function as suppliers of amino acids. It is often observed that the hen lays an egg more than 3 days after fasting. However, there is little investigation to show the contributions of protein

breakdown in tissues to supply amino acids for egg protein synthesis because there is no suitable method¹⁴⁾.

The purpose of the present investigation is to provide evidence showing that the skeletal muscle functions as a pool for amino acids for egg protein synthesis. The following is the speculation of the present investigation ; since temporal shortage of amino acids supply occurs when egg protein synthesis is very intensive, muscle protein is broken down to meet the need of egg protein synthesis.

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Excretion of N^{τ} -methylhistidine is widely used as an index of muscle protein breakdown^{4,15,16}, and we have been using this index to investigate the muscle protein metabolism in chickens⁶. Therefore, we conducted two experiments to test the above mentioned speculation using laying hens and non-laying hens; intensive synthesis of egg white protein should be followed by an increase in the excretion of N^{τ} -MH.

Materials and Methods

Four 10-month old Single Comb White Leghorn hens weighing 1.52 ± 0.2 (S.D.) kg and having 91 ± 3 (S.D.) % egg production rate on the average were used in the first experiment. Average food intake was 78 g/day. The birds laid at around 8 a.m. of the day before excreta collection. They were housed in individual cages and fed an experimental diet (CP 15%, Table 1) made mainly of ground corn and purified soybean protein (Fujipro-R, Fujiseiyu Co. Ltd., Osaka) and water *ad libitum* during an 8-day experimental period. The experiment was conducted in a temperature-controlled environment ($24 \pm 1^{\circ}\text{C}$) with a 14-hour light (6:00 a.m.-8:00 p.m.) and a 10-hour dark cycle. On day 8 of the experiment, excreta samples were collected 3 times every 4 hours starting at 7:00 a.m. Excreta samples during the night (7:00 p.m.-7:00 a.m.) were collected twice on day 7 and 8. The time of oviposition on day 8 was recorded, and the body weight of the birds were measured after the excreta collection. Feed intake was measured, and the hourly mean value was calculated to estimate the contribution of the feed to N^{τ} -MH excretion. The second experiment was conducted in the same manner as the first experiment using 3 non-laying hens (117 days old). However, since a hen lost body weight during the experiment, N^{τ} -MH analysis was performed using the excreta samples of the remaining two hens. N^{τ} -MH content in the excreta and the feed were measured by the

Table 1. Composition of experimental diet

Ingredients	Amount
	g/kg
Ground corn	835
Purified soybean protein	82
Dehydrated alfalfa meal	43
DL-Methionine	1.2
L-lysine HCl	2.6
Vitamin mix ¹⁾	2.7
Mineral mix ²⁾	33.5
Analytical composition	
Crude protein (nitrogen $\times 6.25$)	138
Crude fat	14
Crude fibre	21
Metabolizable energy (kcal/kg)	3130
N^{τ} -Methylhistidine (mmol/kg)	0.04

¹⁾ Contained (g/kg): retinol palmitate 1,750,000 IU, cholecalciferol 200,000 IU, dl- α -tocopherol acetate 2.5, menadione 0.25, thiamine HCl 1.0, riboflavin 2.5, pyridoxine 0.5, cyanocobalamin 0.01, nicotinic acid 10, calcium pantothenate 4, folic acid 0.2, biotin 0.025 and choline chloride 300.

²⁾ Contained (g/kg): CaCO_3 210, CaHPO_4 660, NaCl 113, $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$ 6.6, $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ 4.0, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ 5.0, $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ 0.233, NaIO_3 0.016 and H_2SeO_3 0.006.

method of Hayashi *et al.*⁷⁾, and the rate of muscle protein breakdown was calculated as reported previously⁶. However, the calculation was performed assuming that the content of the skeletal muscle was 40% of the body weight and 80% of the excreted N^{τ} -MH was derived from the skeletal muscle. N^{τ} -MH concentration in the muscle was $0.743 \mu\text{mol/g}$. The results are expressed as mean $\pm$ S.D., and the significance of differences between the means was assessed by the Duncan's multiple range test.

Results and Discussion

Diurnal changes in the rate of N^{τ} -MH excretion and the rate of muscle protein breakdown in the laying hen are shown in Table 2 and Fig. 1, respectively. All the birds laid at around 8:00 a.m. except one bird which laid at

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Table 2. Diurnal changes in the rate of N¹⁵-methylhistidine (N¹⁵-MH) excretion in the laying hen

Time of measurement	Body weight	Total skeletal muscle N ¹⁵ -MH content	Total excreted N ¹⁵ -MH	N ¹⁵ -MH derived from feed	N ¹⁵ -MH derived from skeletal muscle
hours after oviposition	kg	μmol	$\mu\text{mol}/\text{hour}$	$\mu\text{mol}/\text{hour}$	$\mu\text{mol}/\text{hour}$
-12-0 hr	1.52 $\pm$ 0.20 ¹⁾	452 $\pm$ 59	1.25 $\pm$ 0.21 ^a	0.13 $\pm$ 0.02	0.90 $\pm$ 0.16 ^a
0-4 hr	"	"	1.42 $\pm$ 0.33 ^a	"	1.03 $\pm$ 0.26 ^a
4-8 hr	"	"	2.09 $\pm$ 0.23 ^b	"	1.57 $\pm$ 0.18 ^b
8-12 hr	"	"	1.94 $\pm$ 0.25 ^b	"	1.45 $\pm$ 0.20 ^b
12-24 hr	"	"	1.30 $\pm$ 0.20 ^a	"	0.94 $\pm$ 0.18 ^a

¹⁾ Values are means $\pm$ S.D. of 4 observations, and means bearing different superscripts are significantly different ($P<0.05$).

Fig. 1. Diurnal change in the rate of muscle protein breakdown in the laying hen. Width of the pole shows the time of collecting excreta samples. Values are means with their standard deviation represented by vertical bars. *Values differ from that before laying: $P<0.01$.

around 1:00 p.m. Therefore, the datum between 7:00 and 11:00 a.m. of this bird was combined with the data taken during nighttime of the other birds to illustrate the changes after oviposition. N¹⁵-MH excretion increased significantly after oviposition and peaked between 4-8 hours after oviposition and then decreased to the level before oviposition.

Although the physiological role of the skeletal muscle in amino acid metabolism is still obscure, many researchers suggest that the skeletal muscle plays important roles¹³⁾. Since the skeletal muscle is the biggest organ,

changes in the amino acid metabolism in the muscle is necessarily followed by changes in the whole body amino acid metabolism. If an animal faces food shortage, the muscle provides amino acids and energy to maintain body functions. Thus, it is natural to think that the skeletal muscle has an active role in protein metabolism in animals. Egg white protein secretion and synthesis are highest when the ovum passes through the magnum as reported by kumagai and Ishibashi¹⁰⁾ and Hiramoto *et al.*⁸⁾, respectively. The ovum reaches the magnum about 15-20 minutes after ovulation and stays there for about 3 hours. The amount of protein contained in an egg white is about 4 g, and this is 37% of the protein provided by the diet per day. However, in the *ad libitum* feeding state, amino acids are absorbed continuously, even 10 hours after the last meal⁹⁾. Therefore, in the laying hen, dietary supply of amino acids does not seem to be enough to meet the temporal needs of the egg white protein synthesis, and thus other amino acids sources should be considered.

To show the active role of the muscle in synthesizing egg white protein, we examined the diurnal variation of the rate of muscle protein breakdown in the laying hen using N¹⁵-MH method. Although the rate of N¹⁵-MH excretion is a good index of skeletal muscle protein breakdown in many species of animals including chickens¹²⁾, caution is needed because

Table 3. Diurnal changes in the rates of N¹⁵-methylhistidine (N¹⁵-MH) excretion and muscle protein breakdown (Kd) in the non-laying hen

Time of measurement	Body weight	Total skeletal muscle N ¹⁵ -MH content	Total excreted N ¹⁵ -MH	N ¹⁵ -MH derived from feed	N ¹⁵ -MH derived from skeletal muscle	Kd
hours after oviposition	kg	μ mol	μ mol/hour	μ mol/hour	μ mol/hour	%/day
-12-0 hr	1.49 $\pm$ 0.1 ¹⁾	443 $\pm$ 30	0.54 $\pm$ 0.06	0.11 $\pm$ 0.01	0.34 $\pm$ 0.03	1.9 $\pm$ 0.1
0-4 hr	"	"	0.73 $\pm$ 0.11	"	0.49 $\pm$ 0.03	2.7 $\pm$ 0.2
4-8 hr	"	"	0.75 $\pm$ 0.22	"	0.51 $\pm$ 0.16	2.7 $\pm$ 0.6
8-12 hr	"	"	0.64 $\pm$ 0.17	"	0.42 $\pm$ 0.12	2.3 $\pm$ 0.5
12-24 hr	"	"	0.56 $\pm$ 0.28	"	0.44 $\pm$ 0.09	2.4 $\pm$ 0.3

¹⁾ Values are means $\pm$ S.D. of 2 observations, and means bearing different superscripts are significantly different ($P < 0.05$).

balenine, a peptide containing N¹⁵-MH, is accumulated in the muscle in some strain of chickens and thus followed by the underestimation of the rate of muscle protein breakdown⁵.

The diurnal variation of N¹⁵-MH excretion in the laying hen was quite significant while there was no diurnal variation in the non-laying hen as shown in Table 3. As it was suspected that the diurnal variation of the rate of muscle protein breakdown might be common in young hens, we used a young non-laying hen as the control in the present experiment. The time course of the changes in N¹⁵-MH excretion in the laying hen was almost consistent with that of the secretion of egg white protein¹⁰. Although the peak of N¹⁵-MH excretion was observed a few hours after oviposition, this delay of N¹⁵-MH excretion is thought to be the time needed to transport and store N¹⁵-MII in the cloaca before excretion. The rate of breakdown before oviposition was 4.8%/day and it was 8.4%/day at the time when N¹⁵-MH excretion peaked. Thus, the absolute rates of muscle protein breakdown are calculated to be 1.2 g/hour and 2.1 g/hour, respectively. The amount of amino acids supplied from the diet is calculated to be about 0.5 g/hr. Thus, the absolute rate observed at the peak (2.1 g/hour) is thought to be large enough to meet the temporal requirement for egg white protein synthesis. Furthermore, the average rate of

whole day muscle protein breakdown calculated as reported previously⁶) was much higher in the laying hen (5.6%/day) than in the non-laying hen (2.3%/day) though older animals usually have lower breakdown rates⁶.

Although we have little information concerning diurnal variation of muscle protein breakdown, it is reported that the diurnal variation of the rate of muscle protein synthesis is small in human³) and rat²). However, in the laying hen, diurnal changes in both the rates of synthesis and breakdown exist as reported by Hiramoto *et al.*⁸) and shown in the present experiment. Since egg white protein content is about 4 g and this is synthesized within about 3 hours while dietary supply of amino acids is fairly constant as mentioned previously, a temporal amino acid shortage occurs. It is well known that the major inflow of amino acids into the metabolic pool is via the diet or the breakdown of tissue proteins, mainly the skeletal muscle proteins, and, therefore, if protein intake is below the requirement, alterations in whole body protein amino acid metabolism occur mainly via the changes in rates of body protein synthesis and breakdown¹⁷). Corticosteroids are known to be strong accelerators of muscle protein breakdown¹¹), and maximum blood concentration of corticosterone had been observed 44 minutes before oviposition¹). The diurnal variation of

muscle protein breakdown in the laying hen could be associated with the changes in the level of this hormone. In conclusion, it is indicated in the present investigation that the skeletal muscle functions as a pool supplying amino acids for egg protein synthesis.

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産卵鶏における N^{ϵ} -メチルヒスチジン排泄の日内変動

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筋肉蛋白質が卵蛋白質合成のためのアミノ酸のプールとして機能していることを示す目的で産卵鶏と非産卵鶏における N^{ϵ} -メチルヒスチジン (N^{ϵ} -MH) 排泄の日内変動を調べた。4羽の産卵中の白レグ (10ヵ月齢) および3羽の産卵開始前の白レグ (117日齢) を試験飼料で7日間予備飼育した後, 36時間にわたって4時間間隔で排泄物を採取した。ただし, 夜間 (19:00-7:00) は12時間間隔として2回採取した。その結果, 産卵鶏においては, 産卵後4-8時間に明らかな N^{ϵ} -MH 排泄のピークが認められ, このときの値は産卵前 (夜間) の約2倍であった。一方, 非産卵鶏においては N^{ϵ} -MH の排泄は少なく, さらに, 日内変動は認められなかった。

以上の結果は卵蛋白質が合成されるときには筋肉蛋白質の分解が促進され卵蛋白質合成に必要なアミノ酸が供給されることを示唆している。

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