«Research Note»

Incorporation of ^{15}N -Urea in Chicks

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Under certain circumstances, effectiveness of non-protein nitrogen has been studied. OKUMURA *et al.*¹⁾ reported that growth of conventional chicks given a basal diet containing adequate amounts of all the essential but none of the non-essential amino acids is improved by the urea supplements, and concluded that the gut microorganisms are responsible for the growth-promoting effect of urea. OKUMURA *et al.*²⁾ studied the extent of the effectiveness of dietary urea and reported that dietary supplemented urea is beneficial for the chicks up to 5.15% under the condition where dietary non-essential amino acids are limiting.

Use of ^{15}N -labelled compounds as a means of studying their incorporation into body tissue of the chick is limited, although studies with other species are more extensive. It has been shown that rats³⁾, pigs^{4,5)} and human⁶⁾ effectively utilised non-protein nitrogen administered as ^{15}N -labelled compounds, when protein intake from normal sources was restricted.

The purpose of this study was to know the direct evidence of incorporating dietary urea into body proteins and the metabolism of dietary urea. Chicks were fed an essential amino acid mixture with or without ^{15}N -labelled urea and showed the evidence of the ^{15}N -nitrogen enrichment of the protein fraction of blood plasma, muscle, liver and kidney.

Materials and Methods

Day old single comb White Leghorn male chicks were housed in electrically heated brooders. They received a commercial chick mash for 8 days and were then selected and distributed into 3 experimental groups so that the body weight was as uniform as possible. At this stage they were housed in individual metabolism cages²⁾ so that feed consumption could be measured and droppings could be collected quantitatively. The experiment was done using 5 chicks to each dietary treatment. The basal diet was designed to supply the essential amino acids in amounts considered adequate for the growing chick as reported by LEE and BLAIR⁷⁾. Growth studies using these diets have been reported by OKUMURA *et al.*^{1,2)} It contained (g/kg): essential amino acid mixture²⁾ 101.8, corn oil 150, sucrose 100, cellulose 30, mineral mixture⁸⁾ 56.3, vitamin mixture²⁾ 2, choline chloride 1.5, antacid mixture 10 and corn starch 548.4. The antacid mixture contained equal amounts of NaHCO_3 and $\text{Al}(\text{OH})_3$. Where necessary, 1.03 and 2.06% of urea were added at the expense of the corn starch.

Feeding experiments were carried out for 8 days. For last 4 days, normal urea in the urea containing diets was replaced with 3% atom excess ^{15}N -urea and droppings were collected into 200 ml 0.05 M sulfuric acid in deep, stainless-steel traps beneath the metabolism

cages. Blood was taken by heart puncture. Chicks were then killed by a blow on the head and tissue was removed immediately. Upper and lower gut contents were collected as described by OKUMURA *et al.*²⁾ The entire liver and kidney were removed, washed free of residual blood in physiological saline solution (0.9 g NaCl per 100 ml), weighed and frozen. The entire pectoral muscles were taken and treated in the same manner as the liver and kidney.

The samples of liver, kidney and muscle were thawed and homogenized in physiological saline. Samples were treated with 2% trichloroacetic acid to precipitate the protein. The samples were then subjected to Kjeldahl digestion to isolate the nitrogen as ammonia, which was then converted to N₂ for mass spectral analysis for ¹⁵N⁹⁾.

Results and Discussion

Table 1 gives values for body weight gain, feed consumption, feed efficiency, N retention and N utilisation. These results are in agreement with the earlier reports^{1,2)}.

Table 2 gives ¹⁵N concentrations in diets, droppings, gut contents and tissue trichloro-

Table 1. Effect of dietary urea on body weight gain, feed consumption, feed efficiency, N retention and N utilisation

Dietary urea	Body wt gain	Feed consumption	Feed efficiency	N retention ²	N utilisation ³
(%)	(g/bird/8 days)		(%)		
0	16.0±2.9 ^{1a}	64.4±4.3 ^a	24.2±2.8 ^a	7.3±0.2 ^a	47.2±1.6 ^a
1.03	22.2±1.7 ^a	68.6±4.4 ^a	32.9±2.4 ^a	9.5±0.7 ^b	47.1±3.4 ^a
2.06	25.4±3.2 ^a	79.0±6.3 ^a	31.8±1.5 ^a	12.5±1.2 ^b	49.6±5.0 ^a

1. Mean±S.E. (n=5) Values without a common letter are significantly different (a, b P<0.05).

2. N retention=(mg N intake-mg N excreted)/g feed consumption

3. N utilisation=(mg N intake-mg N excreted)×100/mg N intake

Table 2. ¹⁵N concentration in diets, droppings, gut contents and tissue trichloroacetic acid precipitates

Sample	¹⁵ N concentration (atom % excess)		
	1.03% urea diet	2.06% urea diet	
Diets	0.711	1.161	
Droppings (from day 4 to day 8)	0.994±0.020 ¹	1.552±0.032	P<0.001
Upper gut contents	0.187±0.049	0.246±0.037	N S
Lower gut contents	0.164±0.023	0.252±0.041	N S
Trichloroacetic acid precipitates			
Pectoral muscle	0.019±0.000	0.029±0.005	N S
Plasma	0.056±0.058 [#]	0.163±0.020 ^{##}	N S
Liver	0.095±0.007	0.149±0.016	P<0.05
Kidney	0.046±0.006	0.077±0.009	P<0.05

1. Mean±S.E. (n=5)

The corresponding control group samples were used as a measure of the natural abundance of ¹⁵N.

[#] Three missing values.

^{##} Two missing values.

acetic acid precipitates. The 1.03 and 2.06% urea diets contained 0.711 and 1.161 atom % of ^{15}N in the total N in excess of the natural abundance of ^{15}N , respectively. These concentrations of ^{15}N account for averages of 94.2 and 100.4% of the calculated administered dosage of ^{15}N . ^{15}N content of upper and lower gut contents both on 1.03 and 2.06% urea diets were accounting for a small fraction of ^{15}N compared to those of the droppings (feces plus urine). Namely, large parts of ^{15}N in the droppings were from the urine. This suggests that dietary urea was readily absorbed from the intestine as reported by VISEK¹⁰⁾.

The ^{15}N concentration in trichloroacetic acid precipitates of blood plasma and liver was highest followed by kidney and pectoral muscle. There is a small but definite amount of the administered urea N incorporated into the pectoral muscle protein.

Although the present experiment prove that there is an incorporation of N originating from urea into protein in chicks, the incorporation may reflect not only net incorporation but an exchange of amino nitrogen in the body¹¹⁾. ^{15}N incorporation into tissue trichloroacetic acid precipitates were higher in 2.06% urea diet group than 1.03% urea diet group and significant differences were found in liver and kidney between two groups. Thus the present experiment indicate that definite amount of the administered urea incorporated into the body protein. Supposing that about 50% of the body weight of the chick is the muscle weight by calculating the total creatinine content of the muscle and carcass in our unpublished data, the amounts of ^{15}N incorporated into the whole muscle protein are expected to be 4.5 and 3.0% of the administered ^{15}N in 1.03 and 2.06% urea diets, respectively.

Summary

Experiments were conducted to study the fate of ^{15}N supplied as ^{15}N -labelled urea in terms of excretion and incorporation into body tissue protein.

The ^{15}N of dietary ^{15}N -labelled urea was incorporated into the trichloroacetic acid insoluble fractions of blood plasma, liver, kidney and pectoral muscle. The result indicates that dietary urea is used in protein synthesis by the chick.

This work was supported in part by a grant-in-aid for Scientific Research from the Ministry of Education.

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ニワトリヒナによる ^{15}N 尿素の体内へのとり込み

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通常ヒナは飼料中に可欠アミノ酸が不足していると、飼料に添加された尿素を利用できることが知られている。そこで本実験では ^{15}N で標識した尿素を用い、尿素 N のヒナの体蛋白質へのとり込みを調べた。

白色レグホン種雄 8 日齢のヒナを 8 日間代謝ケージで飼育した。実験飼料は尿素添加レベルを 0, 1.03, 2.06 % とし、後半の 4 日間は添加尿素の ^{15}N が 3 atom % excess となるように調製したものを給与した。糞尿混合排泄物は実験期間の後半 4 日間採集した。実験終了時に血液を採集し、ヒナを屠殺した。その後直ちに深胸筋、肝臓、腎臓を別出し、 ^{15}N の蛋白質へのとり込みを

測定した。また上部および下部腸管内容物中、糞尿混合排泄物中の ^{15}N についても測定した。

ヒナの増体量、飼料摂取量、飼料効率、 N 蓄積率、 N 利用率は前報^{1,2)} と同様な結果を示した。各臓器における蛋白質中の ^{15}N 濃度 (atom % excess) は血漿、肝臓で最も高く、次いで腎臓、深胸筋の順となった。またこれらの臓器の蛋白質中の ^{15}N 濃度は尿素添加レベルが 2.06% の方が 1.03% より高い値を示した。以上の結果より、ヒナの体蛋白質合成に尿素由来の N が利用されることが明らかとなった。

(家禽会誌, 16, 45~48, 1979)