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¹⁵N Transfer from ¹⁵N-Urea and ¹⁵N-Diammonium Citrate into Tissue Proteins in Normal Cats Fed with an Optimal Protein Diet

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(Received 24 December 1984/Accepted 26 July 1985)

ABSTRACT. To investigate the utilization of NPN in cats, the ¹⁵N contents in the proteins of the blood serum, liver and muscle, and the ¹⁵N retention rate into the body were determined after the oral administration of ¹⁵N-urea and ¹⁵N-diammonium citrate to normal cats. The incorporation of ¹⁵N into these proteins was observed in all cats. The ¹⁵N retention rate into body was higher in cats given ¹⁵N-DAC than that in cats given ¹⁵N-urea.—**KEY WORDS:** cat, ¹⁵N-NPN.

Jpn. J. Vet. Sci. 47(6): 995–998, 1985

It is well known that the oral, administered non-protein nitrogen (NPN) can be used as a source of nitrogen for the protein synthesis in non-ruminants, since the ¹⁵N from ¹⁵N-NPN can be incorporated into various tissue proteins in rat [1, 5, 6, 11, 13, 17, 18, 21], man [7, 8, 15, 16, 20, 22] and pig [2–4, 9, 12, 14]. It is also suggested that urea is a less effective form than other ammonium salts such as the sources of NPN compounds [6, 11, 17]. But, little is known about the utilization of NPN in cats. For this purpose, the ¹⁵N incorporation into tissue proteins and the ¹⁵N retention rate into the body were determined after the administration of ¹⁵N-urea and ¹⁵N-diammonium citrate (DAC) to normal cats fed an optimal protein diet.

Two male and two female cats, 3.75 ± 0.35 Kg (mean $\pm$ SD) body weight (BW) and 1.35 ± 0.34 years old in age, were used. They were individually placed in a metal metabolism cage. For 7 days, all cats were fed an optimal protein diet, containing 26.64% crude protein (CP) and metabolisable energy (ME) 3.304 Kcal/g (dry-matter basis). The diet was given twice a day at a rate of 22.0 g/Kg of BW/day/cat. Then, one male and one

female cats (Cats 1 and 2) were given 0.1000 g ¹⁵N-urea (50.3 atom % ¹⁵N)/Kg of BW with the diet. The remaining two cats (Cats 3 and 4) were given 0.1883 g ¹⁵N-DAC (51.8 atom % ¹⁵N)/Kg of BW with the diet.

The urine and feces were collected every day in a total of 6 days after the administration of ¹⁵N-urea and ¹⁵N-DAC. On the 6th day following the administration of ¹⁵N labelled substances, the blood was drawn and the liver and muscle (*M. gluteus*) were removed after bleeding.

The liver and muscle were washed thoroughly and homogenized in the cold (4°C), saline solution (0.85 g NaCl/100 ml). These homogenates and the blood serum were treated with one fourth of their volume of 50% (W/V) trichloroacetic acid (TCA) solution, and centrifuged at 1,600 g for 15 minutes. The precipitates were washed three times with 5% TCA solution. The TCA precipitates were hydrolyzed, distilled and titrated for the determination of nitrogen content by the Kjeldahl method. The ¹⁵N content was determined by a modified method of the Rittenberg's method using an apparatus of ¹⁵N analyzer (Jasco NIA-1 ¹⁵N Analyzer). The determina-

Table 1. ^{15}N content in three fractions of blood serum, liver and muscle in cats administered ^{15}N -urea and ^{15}N -DAC^{a)}

Tissue	Fraction	^{15}N -urea				^{15}N -DAC			
		Cat 1 (M) ^{b)}		Cat 2 (F) ^{c)}		Cat 3 (M)		Cat 4 (F)	
		A ^{d)}	B ^{e)}	A	B	A	B	A	B
Blood serum	Whole	0.044	100	0.060	100	0.050	100	0.041	100
	Protein ^{f)}	0.041	74.3	0.087	72.1	0.054	90.3	0.039	74.1
	NPN ^{g)}	—	25.7	—	27.9	—	9.7	—	25.9
Liver	Whole	0.039	100	0.047	100	0.046	100	0.031	100
	Protein	0.046	78.4	0.066	81.9	0.064	77.2	0.062	75.7
	NPN	—	21.6	—	18.1	—	22.8	—	24.3
Muscle (M. gluteus)	Whole	0.035	100	0.024	100	0.037	100	0.034	100
	Protein	0.044	94.0	0.035	60.1	0.047	96.9	0.039	79.7
	NPN	—	6.0	—	39.9	—	3.1	—	20.3

a) Diammonium citrate.

b) Male.

c) Female.

d) ^{15}N content (atom % excess ^{15}N): Trichloroacetic acid (TCA) precipitates of the liver and muscle in a cat unsupplemented ^{15}N -urea and ^{15}N -DAC were used to measure the natural abundance of ^{15}N , which contained an average of 0.368 atom % ^{15}N .e) ^{15}N incorporation rate calculated as 100% in whole tissue (%).

f) TCA precipitated-fraction in tissue.

g) Non-protein-nitrogen fraction in tissue.

tions of the nitrogen and ^{15}N contents in the urine and feces and in the whole materials of the blood serum, liver and muscle were also done by the same methods.

The incorporations of ^{15}N from ^{15}N -urea and ^{15}N -DAC into tissue proteins were observed in all cats since the ^{15}N contents in the TCA precipitates of the blood serum, liver and muscle were respectively higher than the natural abundance of ^{15}N in one cat given only the diet without ^{15}N labelled substances (Table 1). It was suggested, in our previous studies [2–4], that the nitrogen of urea was a possible source of the nitrogen needed for protein synthesis only when the urea was converted into ammonia nitrogen by the action of intestinal-bacterial urease, but the oral DAC was autolyzed to ammonia nitrogen and absorbed as a source of the nitrogen in the intestine without this action. Therefore, it was assumed that the ^{15}N of ^{15}N - NH_4^+ from the ^{15}N -urea and ^{15}N -DAC orally administered to cats was incorporated into the TCA

precipitates of the liver, blood serum and muscle, perhaps including the nucleoprotein in the liver TCA-precipitates. Kornberg and Davies found the significant excess of ^{15}N in the liver, muscle, kidney and heart of one cat when ^{15}N -urea was injected subcutaneously [10].

The ^{15}N incorporation rate in the TCA-precipitated fraction was higher than that in the NPN fraction in each tissue of all cats given ^{15}N -urea and ^{15}N -DAC (Table 1). This indicated that the ^{15}N of both ^{15}N -urea and ^{15}N -DAC was mainly used as a source of nitrogen for protein synthesis. The higher ^{15}N contents were observed in the TCA precipitates of the livers in most cats (Table 1). An similar result was reported by Kornberg and Davies [10]. They found also that the ^{15}N distribution in the liver after the ^{15}N -urea was injected to cat was higher in the protein fraction than that in the amide in the NPN fraction. The possibility of the utilization of ammonia for protein synthesis was explained

Table 2. Excretion and retention rates of ^{15}N administered as ^{15}N -urea and ^{15}N -DAC to cats

	^{15}N -urea		^{15}N -DAC	
	Cat 1 (M)	Cat 2 (F)	Cat 3 (M)	Cat 4 (F)
^{15}N excretion rate (%) ^{a)}				
Urine 0–1st day	71.60	75.48	60.03	45.30
1st–2nd	8.27	12.31	5.06	19.72
2nd–3rd	1.69	1.49	4.68	3.01
3rd–4th	2.10	1.33	1.84	3.07
4th–5th	1.25	1.13	2.94	— ^{b)}
5th–6th	0.70	0.53	1.42	8.21
Urine 0–6th	87.62	92.27	79.96	79.32
Feces 0–1st day	0.82	—	1.11	1.50
1st–2nd	0.79	1.50	1.64	2.18
2nd–3rd	0.49	—	0.32	1.48
3rd–4th	0.32	0.48	0.35	0.79
4th–5th	0.79	—	0.69	0.42
5th–6th	0.48	0.63	1.05	0.68
Feces 0–6th	3.69	2.62	5.16	7.05
Total ^{15}N excretion rate (%)	91.31	94.89	85.12	86.37
Total ^{15}N retention rate (%)	8.69	5.11	14.88	13.63

a) ^{15}N excretion rate was calculated as follows:

$$\frac{[\text{Total excreted-N content (mg N/day)} \times ^{15}\text{N content (atom \% excess } ^{15}\text{N})]}{\div \text{administered } ^{15}\text{N content from } ^{15}\text{N-urea or } ^{15}\text{N-DAC (mg } ^{15}\text{N})} \times 100(\%).$$

b) No excretion.

as followed; the ^{15}N from the ^{15}N -ammonia was firstly incorporated into the nitrogen of glutamic acid by the action of glutamate dehydrogenase system in the liver, and the ^{15}N from glutamic acid was secondly distributed into the nitrogen of other amino acids [5, 16]. It was also shown that the oral, administered NPN to non-ruminants was mainly used as a source of nitrogen for the synthesis of non-essential amino acids, in rat [5, 7], pig [3, 4] and man [8], but this utilization of NPN was not elucidated in cats.

On the other hand, it was reported that urea was a less effective form than ammonium salts as a source of ammonia nitrogen for protein synthesis, since the excretion rate of ^{15}N into the urine and feces after the administration of ^{15}N -urea and various ^{15}N -ammonium salts to animals was the highest when ^{15}N -urea used, but the ^{15}N retention rate into

the body was conversely the lowest in case of using ^{15}N -urea [6, 11, 17]. The similar results were obtained in this study; i.e., total excretion rate of ^{15}N into the urine and feces during 6 days was higher in cats given ^{15}N -urea than that in cats given ^{15}N -DAC, and the ^{15}N retention rate into the body was lower when 3qtNurea was used in cats as compared to when ^{15}N -DAC was used (Table 2). The oral, administered ^{15}N -urea to non-ruminants was rapidly absorbed as the ^{15}N -urea from the upper small intestine and was entered into the blood flow [9, 13, 14]. Then, the ^{15}N -urea in blood was mostly excreted into the urine within a short time after the administration of ^{15}N -urea [9, 12–14] and a part of ^{15}N -urea in blood was re-excreted into the gastrointestinal tract and was hydrolyzed to $^{15}\text{N-NH}_4^+$ by the action of bacterial urease [2, 3]. The oral ^{15}N -DAC was, however, entirely autolyzed

to $^{15}\text{N-NH}_4^+$ in the intestine and absorbed into the body in the hen [19]. Therefore, it seemed that the efficiency of the ^{15}N released from $^{15}\text{N-NH}_4^+$, which can be used for the protein synthesis, was greater in cats administered $^{15}\text{N-DAC}$ than that in cats administered $^{15}\text{N-urea}$.

ACKNOWLEDGEMENT. This work was supported by a Grant-in-Aid for Scientific Research (No. 59760234) from the Ministry of Education, Science and Culture of Japan.

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要 約

ネコにおける経口投与 ^{15}N 標識尿素および ^{15}N 標識クエン酸 2 アンモニウム (DAC) 由来 ^{15}N の組織タンパク質への取り込みと体内保留率について (短報): 出口栄三郎 (鹿児島大学農学部附属家畜病院) —— ^{15}N 標識尿素および ^{15}N 標識 DAC を適タンパク質飼料 (CP 26.64%, ME 3.304 Kcal/g) 給与正常ネコ各 2 頭 (♂ 1, ♀ 1) にそれぞれ経口投与した。血清、肝臓および中殿筋の組織タンパク質分画への ^{15}N 標識尿素および ^{15}N 標識 DAC 由来 ^{15}N の取り込みは全頭のネコで認められ、その ^{15}N 含有率は非タンパク態窒素分画のそれよりも高かった。 ^{15}N の体内保留率は ^{15}N 標識 DAC 投与ネコが ^{15}N 標識尿素投与ネコに比較して高かった。