無菌及び通常ニワトリヒナの肝臓及び腸管蛋白質合成に及ぼす重炭酸アンモニウム添加の影響

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Effect of Supplementing with Ammonium Bicarbonate on Protein Synthesis of Liver and Gastrointestinal Tract in Germ-Free and Conventional Chicks

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The present study was conducted to investigate whether or not ammonia was responsible for the increased intestinal protein synthesis in the chicken harbouring the conventional gut microflora. In the presence or absence of the gut microflora, the chicks were allowed free access to a practical diet, to which ammonium bicarbonate was added at 4.2 g/kg diet, for 10 days from 7 to 17 days of age, and on the last day rate of liver and intestinal protein synthesis was measured by injecting radioactive phenylalanine through a wing vein. The results indicated that almost no significant effect of ammonia supplementation on protein synthesis of the tissues except for the liver was observed in both germ-free and conventional chicks. Thus, at the dietary level tested, which was considered to reflect the amount of ammonia involved in the entero-hepatic recycling, i. e. released in the gut and absorbed by the host bird, biological significance of the role of ammonia in enhanced protein synthesis of the liver and gut by the association with normal microflora was not substantiated.

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Key words: gut microflora, ammonia, liver protein synthesis, intestinal protein synthesis, chick

Introduction

The presence of the gut microflora increased protein synthesis in the lower gut of chickens (MURAMATSU *et al.*, 1987). The enhanced protein synthesis in the lower gut was more remarkable on a practical diet than on a purified diet (MURAMATSU *et al.*, 1988), suggesting that certain metabolites released by bacterial actions in the gut lumen may be responsible for this. MURAMATSU (1990) has pointed out that volatile fatty acids, ammonia or endotoxin would probably be the most likely compounds that cause increased protein synthesis in the lower gut of the chicken.

Recently, however, it was found that in germ-free (GF) and conventional (CV) chickens acetic acid tested as a representative of volatile fatty acids failed to enhance protein synthesis of the chicken hind gut when added to a diet (MURAMATSU *et al.*, 1993). Because the effects of supplementing a diet with acetate on protein synthesis and protein: DNA ratios in the gut were opposite to those expected by the presence of the gut microflora, volatile fatty acids would little, if any, contribute to the elevated lower gut protein synthesis of conventional birds. The present study was conducted,

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therefore, to clarify whether or not ammonia is responsible for the increased gut protein synthesis since not only ammonia is a major metabolite produced by the gut microflora in the gastrointestinal tract of the chicken, but also it could be a metabolite likely to cause elevated intestinal protein synthesis (MURAMATSU, 1990).

Materials and Methods

The method for producing GF birds was described elsewhere (YOKOTA *et al.*, 1984). Throughout the entire experimental period the birds were cared for under Guidelines for Animal Experimentation, laid down by the Committee of Experimental Animal Care, Nagoya University. After hatching, the GF and CV chicks were maintained on a practical diet which was fortified with vitamins to compensate possible losses of vitamins due to irradiation sterilization (COATES *et al.*, 1963). At 7 days of age, they were distributed into two experimental groups of 8 birds in each environment so that the average body weight of the experimental groups was as uniform as possible. The GF and CV chicks were reared individually in metabolism cages placed in a thermostatically-controlled room, and were allowed free access to experimental diets and water for the following 10 days. The composition of the experimental diets is given in Table 1. As a source of ammonia, ammonium bicarbonate was chosen because bicarbonate can be metabolized to give CO_2 so that the effect of anion could be minimized. The level of ammonia supplementation was determined three times as high as the estimated amount of entero-hepatic recyling originating from uric acid and urea as reported by EMMANUEL and HOWARD (1978). The factor of three was arbitrarily set to take into account of extra release of ammonia from deamination of amino acids by bacterial actions in the gut of CV birds. Ambient temperature was kept at 28±1℃, and light was provided continuously for 24 hours a day. Body weight and feed intake were recorded on alternate days for the 10-day experimental period.

In the morning of the last day, at 17 days of age, the birds were injected intravenously with redioactive [^{3}H] phenylalanine through a wing vein, and four birds in each environment were sacrificed at 2 and 10 min after the injection. The measurement of protein synthesis in liver, duodenum, jejuno-ileum and cecum was done as

Table 1. Composition of experimental diets

Diet	Control	NH_3
Chick mash[1]	941.7	937.5
Cellulose	34.3	34.3
Vitamin A+E in corn oil	16.0	16.0
Vitamin mixture[2]	8.0	8.0
Ammonium bicarbonate	—	4.2
Calculated value:		
CP (%)	18.00	18.47
ME (kcal/kg)	2837	2825

[1] Chick 15 (Marubeni Shiryo Co. Ltd., Tokyo).

[2] MURAMATSU *et al.* (1987).

described previously (MURAMATSU *at al.*, 1987). Protein and RNA contents in the tissues were analyzed according to the methods of LOWRY *et al.* (1951), and MUNRO and FLECK (1969), respectively.

Data were treated statistically by a two-way analysis of variance, and significance of differences between means was assessed by a protected LSD method (SNEDECOR and COCHRAN, 1980) using the General Linear Model procedure of Statistical Analysis System (1985). The significance of differences between diet means within the same environment was not tested unless significant interaction between environment and diet was detected.

Results and Discussion

The values for body weight gain, feed intake, and feed efficiency are given in Table 2. No significant main effects of diet, and environment, and the interaction were found in body weight gain and feed efficiency. Feed intake was significantly higher in GF birds than in CV counterparts, but it was not affected by ammonia supplementation.

Table 3 gives the values for protein content and protein synthesis in the tissues. Except for the liver, tissue protein content was significantly higher in the CV birds

Table 2. Effect of supplementing with ammonium bicarbonate on body weight, feed intake, and feed efficiency in germ-free (GF) and conventional (CV) chicks

Env[1]	Diet	Body wt gain	Feed intake (g/10 days)	Feed efficiency (%)
GF	Control	100	181	55.0
	NH_3[2]	103	197	57.7
	GF mean	101	189	56.4
CV	Control	89	180	49.8
	NH_3	95	170	55.8
	CV mean	92	175[+]	52.8
SEM (12 df) for				
	GF vs. CV means	3.1	4.3	2.4
	Any two means	4.5	6.1	3.5

Source	df	Analysis of variance Mean square		
Env (E)	1	320 ns	784*	50.4 ns
Diet (D)	1	72 ns	36 ns	74.0 ns
E×D	1	6 ns	676 ns	12.6 ns
Residual	12	79	147	47.9

[1] Environment.

[2] Ammonium bicarbonate was added at 0.42%.

The number of birds used was 4 per treatment.

Significance level: ns, not significant; *, $P<0.05$.

[+] Significantly different from the GF mean at $P<0.05$

Table 3. Effect of supplementing with ammonium bicarbonate on protein contents and protein synthesis of liver and gastrointestinal tract in germ-free (GF) and conventional (CV) chicks

Env[1]	Diet	Protein contents				Protein synthesis			
		Liver	Duodenum J+I[2] (mg/100 g body wt)		Cecum	Liver	Duodenum J+I[2] (mg/100 g body wt per day)		Cecum
GF	Control	741	206	350	43.4	456	140	203	23.4
	NH_3	718	204	342	49.5	619#	136	173	26.3
	GF mean	730	205	346	46.5	538	138	188	24.9
CV	Control	692	275	446	55.0	544	236	298	39.1
	NH_3	723	342	442	54.9	519	255	279	31.3
	CV mean	708	309^{++}	444^{++}	55.0^{+}	532	246^{++}	289^{++}	35.3^{+}
SEM (12df) for									
	GF vs. CV mean	27	13	14	2	31	7	15	3
	Individual mean	38	18	20	4	44	10	22	4

Source	df	Analysis of variance (Mean square)							
Env (E)	1	1,936 ns	43,264**	38,416**	289	144 ns	46,225**	40,401**	429*
Diet (D)	1	49 ns	4,224 ns	144 ns	36 ns	3,944 ns	196 ns	2,500 ns	25 ns
E×D	1	3,114 ns	4,346 ns	16 ns	38 ns	50,444*	558 ns	22 ns	114 ns
Residual	12	5,808	1,334	1,575	48	7,583	417	1,892	59

[1] Environmet.
[2] Jejunum+Ileum.
[3] Ammonium bicarbonate was added at 0.42%.
The number of birds used was 4 per treatment.
Significance level: ns, not significant; **, $P<0.01$; *, $P<0.05$.
Significantly different from the control within the same environment at $P<0.05$.
$^{++}$, $^{+}$ Significantly different from the GF mean at $P<0.01$ ($^{++}$) and $P<0.05$ ($^{+}$).

than in the GF counterparts with no significant effect of either the dietary treatment, i.e. ammonia supplementation or the interaction between the diet and environment. Higher protein content of the gastrointestinal tract in the presence of the gut microflora is in good agreement with our previous findings (Muramatsu *et al.*, 1987, 1988, 1993). Liver protein synthesis of the GF birds was significantly elevated by supplementing with ammonia, whereas this was not so in the CV controls. It might imply that ammonia, which is released from amino acid deamination in the gut lumen by the bacterial actions and subsequently absorbed by the host birds (Coates, 1980), could modulate liver protein synthesis to a certain extent (Muramatsu *et al.*, 1983, 1990). The findings of Topping and Visek (1977) that ammonia increased DNA and RNA synthesis, and probably protein synthesis in intestinal cells *in vitro* are in line with the above speculation. The reason for little effect in the CV state might be that there is already enough ammonia present in the gut (Salter and Coates, 1971) with respect to the supplementary level, which would correspond to the amount of enterohepatic circulation of ammonia, and therefore the effect of further supply from the diet as the ammonium salt might be masked. If this explanation were correct at all, the liver protein synthesis in the CV chick on average should have been higher than that

in the GF bird as found previously (MURAMATSU *at al.*, 1983, 1987). However, this was not the case in the present study. At this stage, therefore, it is premature to draw any biological significance for the role of ammonia in the liver protein synthesis.

In contrast to the liver, protein synthesis in the gastrointestinal tract including duodenum, jejuno-ileum and cecum was not affected by ammonia supplementation, but significantly increased by the presence of the gut microflora. Because increased protein synthesis by the presence of the gut microflora was more evident in the gut, especially in the lower gut, than in the liver (MURAMATSU *et al.*, 1987, 1993), the effect of ammonia supplementation should have been exerted clearly in the lower gut if ammonia would be responsible for increased gut protein synthesis. However, obviously this is not true, at least, at the supplementary level used in the present study.

In the present study, the supplementary level of ammonia was set to mimic the amount of entero-hepatic recycling *in vivo*, mainly originating from uric acid and urea secreted into the gut (EMMANUEL and HOWARD, 1978). However, most dietary ammonia supplemented as ammonium bicarbonate could have already been absorbed before reaching the lower gut where the bacterial activity is supposed to be highest (FULLER, 1984). Because of this, the possibility that ammonia is involved in the enhanced intestinal protein synthesis in the CV state cannot be entirely ruled out. To deliver

Table 4. Effect of supplementing with ammonium bicarbonate on protein synthesis per unit RNA of liver and gastrointestinal tract in germ-free (GF) and conventional (CV) chicks

Env[1]	Diet	protein synthesis per unit RNA			
		Liver	Duodenum	J+I[2]	Cecum
		(mg synthesis/mg RNA per day)			
GF	Control	37.7	20.6	19.5	18.6
	NH_3[3]	26.8	22.2	17.2	19.3
	GF mean	32.3	21.4	18.4	19.0
CV	Control	29.1	24.8	16.2	17.2
	NH_3[3]	27.8	22.7	16.9	14.8
	CV mean	28.5	23.8	16.6	16.0
SEM	(12 df) for				
	GF vs CV means	2.4	1.1	0.8	1.2
	Individual means	3.3	1.5	1.1	1.8
		Analysis of variance			
Source	df	Mean square			
Env (E)	1	57.8 ns	23.0 ns	13.0 ns	36.0 ns
Diet (D)	1	148.8 ns	0.3 ns	2.6 ns	2.9 ns
E×D	1	92.2 ns	12.7 ns	9.0 ns	8.4 ns
Residual	12	44.7	9.3	4.6	12.3

[1] Environment.
[2] Jejunum+Ileum.
[3] Ammonium bicarbonate was added at 0.42%.
The number of birds used was 4 per treatment.
Significance level: ns, not significant.

efficiently dietary ammonium salt to the lower gut, pH-sensitive coating or other means of protection from absorption may be necessary. Only when this is attained, the role of ammonia in modulated gut protein synthesis may be understood more thoroughly.

The values for protein synthesis per unit RNA are given in Table 4. No significant main effects of diet and environment, and the interaction were detected in any of the tissues measured, suggesting that the observed changes in tissue protein synthesis, and as a result, protein contents would be brought about by the changes in RNA contents *per se*. As protein synthesis per unit RNA may reflect a minimal translation rate (WATERLOW *et al.*, 1978), the effect of metabolites released by bacterial actions on tissue protein synthesis would occur at either the transcription step or at the stabilization of majority of mRNA, both of which may be unlikely to change the minimal translation rate, but could enrich mRNA concentrations in general. In experiment *in vitro*, ammonia was considered to increase DNA and RNA synthesis in the intestine (TOPPING and VISEK, 1977), and thereby could affect cell life span (VISEK, 1972). This might offer the possible way of ammonia involvement, although being ambiguous from the present results, in modified protein synthesis in the liver and intestine with the association of normal gut bacteria.

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無菌及び通常ニワトリヒナの肝臓及び腸管蛋白質合成におよぼす重炭酸アンモニウム添加の影響

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通常の腸内細菌を保有するニワトリでみられる腸組織の蛋白質合成増加に対して，アンモニアがその原因となつているかどうかを確かめるために本試験を行った。腸内細菌の有無の状態で，ニワトリヒナに 10 日間，7 日齢から 17 日齢まで重炭酸アンモニウムを 4.2g/kg 添加した飼料を自由摂取させて飼育し，最終日に翼下静脈より放射性フェニルアラニンを注射後，肝臓ならびに腸組織の蛋白質合成速度を測定した。その結果，無菌及び通常の両環境においても肝臓を除き組織蛋白質合成にはアンモニア添加の効果はほとんどみられなかった。以上の結果より，本実験では腸内で放出され，後に宿主ニワトリによって吸収されるような腸肝循環に関与する量に相当すると考えられるレベルでは，通常の腸内細菌の存在によって生じるアンモニアが肝臓や腸組織蛋白質合成増加に果たす役割の生物学的意義を明らかにすることはできなかった。 （家禽会誌，***30***: *263-269, 1993*）

キーワード：腸内細菌，アンモニア，肝臓蛋白質合成，腸管蛋白質合成，ニワトリ