Effects of Dietary RNA and Adenine on Feed Intake and Kidney Weight and Function in Adult Cockerels

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ABSTRACT : This study was conducted with adult cockerels to determine whether dietary RNA affects feed intake and renal weight and function, and if the responses are similar to dietary adenine. Chickens were *ad libitum* fed a RNA diet (100.0 g/kg) or an adenine diet (9.1 g/kg) for 14 d and catheterized in right jugular vein, hepatic portal vein and both urethers, and saline together with para-amino hippuric acid and sodium thiosulfate was continuously infused into them to evaluate renal functions. Dietary RNA reduced feed intake and body weight, and dietary adenine increased kidney weight expressed as a proportion of body weight ($p < 0.05$). Feed intake and body weight on the adenine diet and kidney weight on the RNA diet showed similar though non significant tendencies. No calculi were detected in the kidney in chickens fed either the RNA or adenine diets. Plasma inorganic phosphate (IP), Ca and 1,25 $(OH)_2$ vitamin D_3 concentrations were increased by dietary RNA and adenine, although the increases of IP and Ca in adenine-fed chickens were not significant. Uric acid and urea concentrations in the blood plasma were unaffected by dietary RNA or adenine. Both dietary RNA and adenine increased renal blood flow rates 3.5-3.7 fold, renal plasma flow rates 3.4-3.7 fold and glomerular filtration rates (GFR) 2.9-3.0 fold ($p < 0.01$). Clearance of urea, IP and Ca were also enhanced by dietary RNA, but not by dietary adenine. However, neither RNA nor adenine affected uric acid clearance. Only IP clearance was significantly augmented at the glomerular level by dietary RNA ($p < 0.05$). Glomerular filtration of uric acid, urea, IP and Ca and reabsorption of urea, IP and Ca at the renal tubule were increased by dietary RNA and adenine ($p < 0.05$), whereas tubular secretion of uric acid was decreased by both dietary treatments. It is concluded that dietary adenine is effective in changing renal function and P and Ca metabolism in chickens.
(**Key words**: Chicken, Renal Function, RNA and Adenine Diet, Feed Intake)

INTRODUCTION

Single-cell protein consisting of a more or less processed cell mass of bacteria, yeasts or algae grown on a variety of substrates, is considered a potentially valuable source of protein for the nutrition of animals and humans, because of its well balanced amino acid composition. It is also characterized by a high (< 15%) content of nucleic acids. According to our previous studies, some yeast proteins or RNA of their main component fed to chicks depressed feed intake and body weight gain (Karasawa and Kubota, 1986, 1990, Kubota and Karasawa, 1994), and dietary RNA also resulted in enlarged kidneys and induced the deposition of renal calculi mainly composed of calcium phosphate (Kubota and Karasawa, 1994).

This study was conducted with adult cockerels to clarify whether dietary RNA has adverse effects on feed intake and kidney size as in chicks, and on renal function, and if the responses are similar to those of adenine, a major constituent of RNA.

MATERIALS AND METHODS

The experimental birds were 14-month-old Single Comb White Leghorn adult cockerels, and four birds were allotted to each dietary group with a mean initial body weight of 2.3 kg. They were housed individually in metabolism cages in a room with controlled lighting (14L: 10D) and allowed free access to the control, RNA or adenine diets and water for 14 d. The experimental diets all contained 150 g protein (/kg) (table 1): The control diet was a purified diet containing egg albumen (P type, Kewpie Egg Co., Tokyo) as the sole source of protein, and RNA and adenine diets contained 100.0 g RNA (/kg) and 9.0 g adenine (/kg) at the expense of egg albumen,

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respectively. The adenine content of the RNA diet was almost equal to that of the adenine diet. Details of the control diet were given in a previous paper (Karasawa et al., 1973).

Table 1. Composition of experimental diets (%)

Ingredients	Control diet	10% RNA diet	0.9% Adenine diet
Egg albumen	18.90	5.04	15.20
Cornstarch	67.12	69.46	69.91
Cellulose powder	4.00	4.00	4.00
Corn oil	2.00	2.00	2.00
Mineral mixture[1]	6.49	6.49	6.49
Vitamin mixture[1]	1.22	1.22	1.22
Choline chloride	0.27	0.27	0.27
RNA[2]		11.52	
Adenine[3]			0.91
P content	0.7	1.4	0.7
Ca content	1.2	1.2	1.2
Protein content	15	15	15

[1] Nesheim et al. J. Nutr. 78:89 (1962).
[2] [T.G], purity 86.84%, Kohjin Co., Ltd., Tokyo, Japan.
[3] minimum 99%, Sigma Chemical Co., Mo. U.S.A.

At about noon on day 14 after start of feeding of experimental diets, each of 12 chickens used in this experiment was catheterized in right jugular vein, hepatic portal vein and both ureters on a separate day according to our previous report (Karasawa et al., 1973), and there was a 12-day-lag to the greatest extent in an experimental time. In order to evaluate the renal functions of chickens fed with RNA and adenine diets, saline (9 g NaCl/L) together with para-amino hippuric acid (200 mg/100 ml) and sodium thiosulfate (200 mg/100 ml) was continuously infused into the chickens through the hepatic portal catheter at the rate of 0.4 ml per min per kg body weight for 60 min. Whole urine excreted naturally through the ureter catheter was collected at 10 min intervals after 30 min from commencement of infusion and about 5 ml of blood was withdrawn through the jugular catheter at 35, 45 and 55 min after start of infusion. Glomerular filtration rate was assumed as clearance of sodium thiosulfate, and renal plasma flow as clearance of para-amino hippuric acid. Glomerular filtration, tubular secretion and reabsorption of uric acid, urea, IP and Ca were calculated as follows; The glomerular filtration was obtained by multiplying GFR with plasma concentration of each substance, the reabsorption by subtracting the urinary excretion of each substance from the value of the glomerular filtration, and the secretion by subtracting the glomerular filtration from the urinary excretion, because the substance is not secreted but reabsorbed when the clearance is smaller than the GFR and it is not reabsorbed but secreted when their relation is opposite.

Immediately following completion of infusion birds were killed by exsanguination and the kidneys were excised and weighed. A portion of fresh kidney was fixed with ethanol : chloroform : acetic acid (6:3:1) and stained with hematoxylin and eosin for pathological examination. A portion of the fixed kidney was examined for calcium salts by the method of Dahl (1952).

Hematocrit value of fresh blood was determined by the capillary method using an ultracentrifuge at 10,000xg for 5 min. Urea-N, uric acid, inorganic phosphorus and calcium in blood and urine were determined by an autoanalyzer (Hitachi 7450, Hitachi Co., Tokyo) using Merck auto UN (Kanto Chemical Co., Tokyo), uric acid-HRII, inorganic phosphorus-HRII and calcium-HR (Wako Junyaku Co., Osaka), respectively. Para-amino hippuric acid was determined by the colorimetric method and sodium thiosulfate by the titration method (Kanai, 1993). Plasma $1,25(OH)_2$ vitamin D_3 concentration was measured by the radioreceptor assay method using bovine mammary receptor (Watanabe et al., 1994).

Data were statistically analyzed by Student's t test (Snedecor and Cochran, 1967).

RESULTS

Dietary RNA significantly reduced feed intake and body weight ($p < 0.05$) (table 2). A similar tendency was also observed in chickens fed on an adenine diet although the effect was not significant. Kidney weight expressed as a proportion of body weight was significantly increased by dietary adenine ($p < 0.05$) and also it tended to increase in chickens fed a RNA diet although not significant. No calculi, however, were histochemically observed in the kidney either in chickens fed RNA and adenine.

Mean concentrations of uric acid, urea, inorganic phosphorus (IP), calcium (Ca), and $1,25\ (OH)_2$ vitamin D_3 in the blood plasma taken at 35, 45 and 55 min after start of infusion with sodium thiosulfate and para-amino hippuric acid solution are presented in table 2. Because steady states of these concentrations were attained within 35 min, the mean concentrations during the period 35-55 min were used for the calculation of parameters of renal function. Plasma IP, Ca and $1,25\ (OH)_2$ vitamin D_3 concentrations were increased by dietary RNA and adenine ($p < 0.05$), although the increases of IP and Ca

in adenine-fed chickens were not significant. Urea concentrations in the blood plasma were not affected by dietary RNA or adenine. Plasma uric acid concentration was increased on RNA diet ($p < 0.05$).

Table 2. Effects of dietary RNA and adenine on feed intake, body weight and kidney weight, and plasma uric acid, urea, inorganic phosphorus, calcium and $1,25(OH)_2$ vitamin D_3 concentrations in chickens

	Diet		
	Control	RNA	Adenine
Feed intake (g/kgBW/d)	49.87 ± 2.28	29.90 ± 7.48*	39.51 ± 9.01
Body weight (kg)	2.37 ± 0.11	2.06 ± 0.07*	2.22 ± 0.08
Kidney (g/kgBW)	2.20 ± 0.11	2.58 ± 0.28	2.61 ± 0.14*
Renal calculus	ND	ND	ND
Plasma (mg/100 ml)			
Uric acid	4.14 ± 0.30	6.99 ± 0.90*	3.32 ± 0.27
Urea-N	0.86 ± 0.25	0.62 ± 0.15	0.72 ± 0.20
Inorganic phosphorus	5.80 ± 0.19	8.81 ± 0.50*	7.11 ± 0.63
Calcium	18.08 ± 0.73	23.7 ± 1.68*	19.88 ± 0.58
$1,25(OH)_2$ vitamin D_3	16.88 ± 1.23	30.7 ± 5.32*	26.30 ± 2.84*

Values are means ± SEM of 4 chickens. Blood was taken at 55 min after start of infusion.
ND: not detected. * $p < 0.05$.

Table 3 shows effects of dietary RNA and adenine on parameters of renal function in adult cockerels. Both RNA and adenine in the diet increased renal blood flow rates 3.5-3.7 fold, plasma blood flow rates 3.4-3.7 fold and glomerular filtration rates (GFR) 2.9-3.0 fold ($p < 0.01$). Clearance of urea, IP and Ca were also enhanced by dietary RNA ($p < 0.05$), but not significantly by dietary adenine. However, neither dietary RNA nor adenine affected uric acid clearance. Dietary RNA significantly augmented only IP clearance of the above substances at the glomerular level ($p < 0.05$), but adenine affected none of them.

Table 3. Effects of dietary RNA and adenine on parameters of renal function

	Diet		
	Control	RNA	Adenine
RBF (ml/min/kg BW)	5.91 ± 1.13	22.01 ± 6.23*	20.82 ± 4.95*
RPF (ml/min/kg BW)	3.39 ± 0.54	11.47 ± 3.05*	12.59 ± 2.77*
GFR (ml/min/kg BW)	2.39 ± 0.33	6.94 ± 0.66*	7.07 ± 1.58*
Clearance (ml/min/kg BW)			
Uric acid	7.45 ± 2.39	6.85 ± 0.93	6.56 ± 0.98
Urea	1.63 ± 0.48	3.21 ± 0.28*	2.13 ± 0.51
Inorganic phosphorus	0.23 ± 0.06	0.67 ± 0.11*	0.40 ± 0.13
Calcium	0.04 ± 0.01	0.10 ± 0.03*	0.05 ± 0.01
Clearance/GFR			
Uric acid	2.92 ± 0.74	0.99 ± 0.10*	1.17 ± 0.40
Urea	0.66 ± 0.17	0.49 ± 0.07	0.33 ± 0.10
Inorganic phosphorus	0.10 ± 0.03	0.10 ± 0.02	0.06 ± 0.02
Calcium	0.01 ± 0.00	0.01 ± 0.01	0.01 ± 0.00

Values are means ± SEM of 4 chickens.
* $p < 0.05$.
RBF: renal blood flow rate.
RPF: renal plasma flow rate.
GFR: glomerular filtration rate.

Table 4 presents effects of dietary RNA and adenine on glomerular filtration, tubular secretion and reabsorption of uric acid, urea, IP and Ca in the kidney of adult cockerels. Glomerular filtration of uric acid, urea, IP and Ca, and reabsorption of urea, IP and Ca at the renal tubule, were increased by dietary RNA and adenine. The tubular secretion of uric acid was decreased substantially but not significantly by both dietary treatments.

Table 4. Effects of dietary RNA and adenine on glomerular filtration, tubular secretion and reabsorption of uric acid, urea, inorganic phosphorus(IP) and calcium(Ca) in the kidney of chickens

Substance	Dietary treatment	Glomerular filtration	Tubular secretion	Reabsorption	Urinary excretion
		mg/10 min/kg BW			
Uric acid	Control	0.96 ± 0.12	1.79 ± 0.70	0	2.76 ± 0.73
	RNA	4.96 ± 0.90*	0.35 ± 0.35	0.34 ± 0.22	4.97 ± 1.07
	Adenine	2.35 ± 0.61*	0.64 ± 0.50	0.77 ± 0.44	2.21 ± 0.58
Urea-N	Control	0.20 ± 0.03	0	0.08 ± 0.04	0.13 ± 0.03
	RNA	0.62 ± 0.16*	0	0.29 ± 0.04*	0.32 ± 0.12
	Adenine	0.49 ± 0.10*	0	0.35 ± 0.09*	0.16 ± 0.05
IP	Control	1.17 ± 0.36	0	1.07 ± 0.33	0.13 ± 0.03
	RNA	6.31 ± 1.11*	0	5.70 ± 1.04*	0.61 ± 0.33*
	Adenine	5.43 ± 1.98	0	5.10 ± 1.85	0.33 ± 0.13
Ca	Control	4.82 ± 0.80	0	4.76 ± 0.79	0.07 ± 0.02
	RNA	16.23 ± 0.52*	0	16.02 ± 0.53*	0.21 ± 0.08
	Adenine	15.04 ± 3.22*	0	14.95 ± 3.22*	0.09 ± 0.02

Values are means ± SEM of 4 adult cockerels.
* Significantly different from control ($p < 0.05$).

DISCUSSION

The present results show 10% dietary RNA to decrease feed intake and enlarge the kidneys in adult chickens, which is in agreement with an earlier findings with young chicks (Kubota and Karasawa, 1994). The 0.9 % adenine diet caused similar though non significant tendencies in feed intake and body weight and a significant increase in kidney weight. Therefore, the adverse effects of the RNA diet on chicks might be derived from adenine in dietary RNA.

The RBF and RPF obtained from adult cockerels in the present experiment were low compared with those determined previously with young chicks and laying hens (Glahn et al., 1988; Roberts, 1992; Wideman et al., 1985), whereas GFR was essentially similar. These findings suggest that RBF and RPF are affected by age and/or sex but that GFR is not.

In the present experiment 1,25 $(OH)_2$ vitamin D_3 concentration in the blood increased similarly in chickens fed both RNA and adenine as compared with control birds, although the RNA diet contained twice as much P as the control and adenine diets. Therefore, the increase in 1,25 $(OH)_2$ vitamin D_3 concentration in the blood of RNA-fed chickens might be due to dietary adenine rather than P levels. 1,25 $(OH)_2$ vitamin D_3 has been reported to have a stimulatory effect on bone resorption (Kim, 1993; Raiz et al., 1972), and in the present experiment, deitary RNA increased IP and Ca concentrations in the blood and dietary adenine showed a similar though less marked effect. Consequently, the increases in IP and Ca concentrations in the blood of chickens fed RNA and adenine may result from a release of IP and Ca from bone through the effect of 1,25 $(OH)_2$ vitamin D_3 on bone resorption.

The present experiment indicated that dietary RNA and adenine similarly increased RBF, RPF and GFR in adult cockerels. It is reported that the administration of 1 α OH vitamin D_3 to humans causes a rise in GFR (Eke and Winterborn, 1983) and that this metabolite is transformed to 1,25 $(OH)_2$ vitamin D_3 in the rat liver (Fukushima et al., 1975). The injection of angiotensin II into the kidney portal system in chickens also induces increases in GFR and RPF by inhibiting the tubular Na^+ and water reabsorption (Cuypers et al., 1993). Since 1,25 $(OH)_2$ vitamin D_3 concentration was augmented in the

blood of chickens fed both RNA and adenine, the increases in RPF, RBF and GFR observed in the present experiment may be caused through increased 1,25 $(OH)_2$ vitamin D_3 and angiotensin II.

When P was intravenously infused into chickens, blood P concentration increased but GFR tended to decrease (Wideman et al., 1980). Therefore, it is unlikely that the high contents of P in the RNA diet is involved in an increase in GFR in the present study.

Dietary RNA and adenine caused the kidneys to enlarge, as observed in an earlier study with young chicks (Kubota and Karasawa, 1994), although in the present study there was no deposition of inorganic calculi in the kidney. Almost equal increases in RPF, RBF and GFR between chickens fed RNA and adenine suggest that the enlargement of the kidney is due mainly to accelerated renal blood flow resulting from the increased 1,25 $(OH)_2$ vitamin D_3 arising from the high dietary adenine levels in both diets. The increased 1,25 $(OH)_2$ vitamin D_3 also causes a disarrangement of P and Ca metabolism in the body.

The present experiment demonstrated that dietary adenine is effective in changing renal function and altering P and Ca metabolism in chickens.

REFERENCES

Cuypers, Y., P. Mcconaghey and P. Steels. 1993. The direct renal tubular effect of angiotensin II in chickens. Renal Physiol. Biochem. 16:325-332.

Dahl, L. K. 1952. A simple and sensitive histochemical method for calcium. Proc. Soc. Exp. Biol. Med. 80:474-479.

Eke, F. U. and M. H. Winterborn. 1983. Effect of low dose 1 α-hydroxycholecalciferol on glomerular filtration rate in moderate renal failure. Arch./Dis. Child. 58:810-813.

Fukushima, M., Y. Suzuki, Y. Toira, I. Matsunaga, K. Ochi and H. Nagano. 1975. Metabolism of 1 α-hydroxy vitamin D_3 to 1 α, 25-dihydroxyvitamin D_3 in perfused rat liver. Biochem. Biophys. Res. Commun. 66:632-638.

Glahn, R. P., R. F. Wideman and B. S. Cowen. 1988. Effect of dietary acidification and alkalinization on urolith formation and renal function in single comb white leghorn laying hens. Poultry Sci. 67:1694-1701.

Kanai, M. 1993. Clinical test method (30th Ed.), Kinbara Press, Tokyo. (in Japanese).

Karasawa, Y. and T. Kubota. 1986. Utilization of dietary yeasts by chickens. J. Fac. Agric. Shinshu Univ. 23:79-86.

Karasawa, Y. and T. Kubota. 1990. Effect of RNA on growth and food intake of young chicks. Jpn. Poult. Sci. 27:165-172.

Karasawa, Y., I. Tasaki, H. Yokota and F. Shibata. 1973. Effect of infused glutamine on uric acid synthesis in chickens fed high and low protein diets. J. Nutr. 103:526-529.

Kim, J. 1993. The action and mechanisms of vitamin D (Vitamin D, edited by Ogata E., T. Suda and Y. Ogura), Kodan-sha Scientific, Tokyo. (in Japanese).

Kubota, T. and Y. Karasawa. 1994. Adverse effects of low concentrations of dietary RNA addition on the growth, food intake and kidney weight of young chickens. Br. Poult. Sci. 35:585-588.

Raisz, L. G., C. L. Trummel, M. F. Holic and H. F. Deluca. 1972. 1,25-Dihydroxycholecalciferol: A potent stimulator of bone resorption in tissue culture. Science. 175:768-769.

Roberts, J. R. 1992. Renal function and plasma arginine vasotocin during an acute salt load in feral chickens. J. Comp. Physiol. B 162:54-58.

Snedecor, G. W. and W. G. Cochran. 1967. Statistical Method, Iowa State University Press, Ames. IA.

Watanabe, Y., T. Kubota, E. Suzumura, T. Suzuki, M. Yonezawa, T. Isigami, M. Ichikawa and Y. Seino. 1994. 1, 25-dihydroxyvitamin D radioreceptor assay using bovine mammary gland receptor and non-high performance liquid chromatographic purification. Clin. Chim. Acta. 225:187-194.

Wideman, R. F., N. B. Clark and E. J. Braun. 1980. Effects of phosphate loading and parathyroid hormone on starling renal phosphate excretion. Am. J. Physiol. 239:F233-F243.

Wideman, R. F., J. A. Closser, W. B. Roush and B. S. Cowen. 1985. Urolithiasis in pullets and laying hens: Role of dietary calcium and phosphorus. Poultry Sci. 64:2300-2307.