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Effects of Nicotinic Acid Deficiency on the Levels of Various Metabolites in the Serum of Quail

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Body weight gain in the niacin deficient group of quail was markedly lowered as compared to that of the control group, but heart, kidney and liver weight were slightly reduced relative to the body weight. Nicotinic acid deficiency resulted in the significant increase of serum glucose level but the serum cholesterol, albumin and total protein levels were not affected to any extent. Glutamic oxaloacetate transaminase and glutamic pyruvate transaminase activities were significantly enhanced but alkaline phosphatase and lactic dehydrogenase activities were not influenced. Tryptophan and tyrosine levels were remarkably reduced and a similar observation was also made with aspartic acid, glutamic acid and alanine plus serine. However, the levels of basic amino acids such as arginine, histidine and lysine plus branched chain amino acids such as isoleucine, leucine and valine were not affected.

KEY WORDS: Nicotinic acid, Free amino acids, Enzymes, Metabolites

Nicotinic acid (pyridine-3-carboxilic acid) and its amide (nicotinamide) are usually converted to their coenzyme forms *in vivo*. These coenzyme forms such as NAD and NADP serve as hydrogen carriers in the biological oxidation-reduction (Friedrich, 1988). Briggs *et al.* (1943) reported that chick fed nicotinic acid deficient diet showed poor growth and a marked reduction of nicotinic acid and NAD content of breast muscle and perosis. In a similar experiment conducted by Garcia-Bunuel *et al.* (1962) the levels of liver nicotinamide coenzymes and activities of α-glycerophosphate dehydrogenase and isocitrate dehydrogenase were significantly lowered but glutamic dehydrogenase activitiy virtually unchanged.

Recently Park (1985) reported that nicotinic acid deficiency caused a dramatic reduction of NAD and NADPH in the pectoral muscle but not in the liver and brain. The activities of metabolically related enzymes such as lactic dehydrogenase, glutamic dehydrogenase, glyceraldehyde-3-phosphate dehydrogenase, malic enzyme and tryptophan pyrrolase in the liver or pectoral muscle were not affected. Interestingly enough, activities of these enzymes that did not correspond with nicotinamide coenzyme under this nutritional stress is not clearly established yet. Very little information is available regarding the effects of nicotinic acid deficiency on the metabolism of physiological metabolites such as glucose, cholesterol, proteins, and free amino acids in the serum of animals.

Therefore the current study is designed to investigate alterations of glucose, cholesterol, albumin, total protein, enzymes, and free amino acid metabolism observed under the nicotinic acid deficiency.

A part of this work was presented at the meeting of the Zoological Society of Korea held at Kang Won University in October 27, 1990.

Materials and Methods

Chemicals

All chemicals used were of analytical grade and

purchased from Sigma Chemical Co., St. Louis, Mo., USA.

Treatment of Animals

Quail weighing approximately 13.6 g were divided into three groups of 20 and were fed their respective diets *ad libitum* except for the pair-fed group which was pair-fed to the nicotinic acid free group. All animals had free access to water. The nicotinic acid free diet (U.S. Biochemical Corporation, Cleveland, OH, USA) consisted of (grams per kilogram): 180, vitamin-free casein: 587.7, glucose: 100, gelatin: 0.3, L-cysteine: 50, soybean oil: 10, calcium phosphate: 50, salt mix and 22 vitamin mix. At the end of the experiment the animals were sacrificed by decapitation and the blood was collected to obtain the serum and the pectoral muscle tissue was rapidly removed and stored at −70°C.

Determination of Metabolites and Enzymes

The determination of glucose level was essentially based on the oxidation of glucose by glucose oxidase and of o-dianisidine by peroxidase (Falis, 1963). The concentration of cholesterol was determined by Abell *et al.* (1952), and protein by Lowry *et al.* (1951).

Alkaline phosphatase activity was assayed by Salomen *et al.* (1964), glutamic oxaloacetate transaminase and glutamic pyruvate transaminase activities by Amador and Wacker (1962) and lactic dehydrogenase activity by Fritz (1967).

Determination of Free Amino Acids by HPLC

The reverse-phase HPLC with fluorometric detection (Waters model 510, Mitford, MA) was employed to measure free amino acids. The deproteinization involved the filtration of the serum sample through the membrane filter (0.45 μm) followed by centricon treatment. The detection system was a 420-AC fluorometer equipped with a monochromator at the exitation side and a filter at the emission side. The mobil phase, consisting of buffer A (50 μM Na_2HPO_4 plus 50 μM sodium acetate, pH 7.0-tetrahydrofolate in 945: 55 ratio) and buffer B (methanol-CH_3CN-H_2O in 45 : 10 : 45 ratio), was pumped at a flow rate of 2 ml/min (4,000 psi) at 45°C.

Statistical Analysis

Analysis of variance was conducted according to Snedecor (1956) and treatment differences were subjected to the Student-Newman-Keuls multiple-range test as outlined by Kirk (1968).

Results and Discussion

The growth patterns of young quail in response to various dietary treatments are illustrated in Figure 1. Quail fed with the nicotinic acid deficient diet grew only slightly. A very similar growth pattern was observed with the pair-fed control. In contrast, quail fed with diets supplemented with nicotinic acid (100 mg/3 kg diet) grew in a steady rate and after 28 days their body weights were almost twice heavier than the other two groups. These results indicate that nicotinic acid is an essential nutrient for the normal growth of the animal (Friedrich, 1988).

Mean body weight gains of the control group were significantly ($p < 0.01$) higher than those of the other two groups (Table 1). Similarly, the

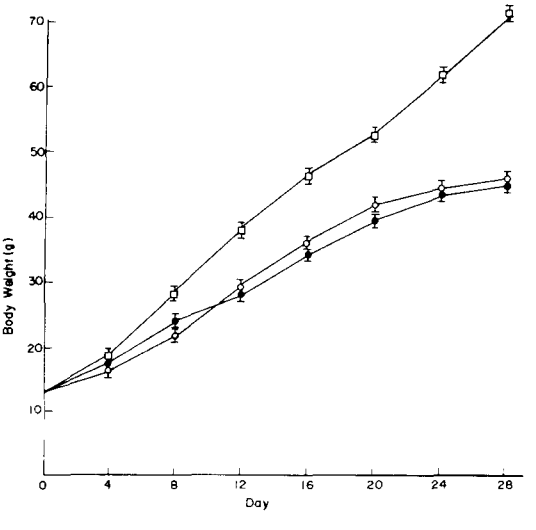

Fig. 1. Growth pattern of quail fed with various diets. control: −□−□−, NFD: −○−○−, Pair-fed: −●−●− (NFD: Nicotinic acid deficiency).

Table 1. Effects of nicotinic acid deficiency on body weight(g) and organ weight(g) in quail.

Treatment	Initial body weight	Final body weight	Weight gain	Brain weight	Heart weight	Kidney weight	Liver weight
Control	13.6 ± 0.4^{a}	72.0 ± 5.0^{A}	58.4 ± 3.8^{A}	0.62 ± 0.05^{a}	0.58 ± 0.03^{a}	1.00 ± 0.1^{a}	2.58 ± 0.3^{a}
Nicotinic acid deficient	13.6 ± 0.4^{a}	44.5 ± 3.2^{B}	30.9 ± 2.5^{B}	0.58 ± 0.02^{a}	0.44 ± 0.02^{b}	0.83 ± 0.08^{b}	1.76 ± 0.2^{b}
Pair-fed	13.6 ± 0.4^{a}	43.2 ± 3.0^{B}	29.6 ± 2.3^{B}	0.57 ± 0.02^{a}	0.45 ± 0.02^{b}	0.73 ± 0.1^{b}	1.54 ± 0.2^{b}

All values are expressed in terms of means ± S.D. of 4 experiments.
Means not sharing a common superscript letter within a column are significantly different. $^{ab}P < 0.05$. $^{AB}P < 0.01$.

Table 2. Effects of nicotinic acid deficiency on metabolites and proteins in the serum of quail.

Treatment	Glucose (mg/ml)	Cholesterol (mg/ml)	Albumin (mg/ml)	Total protein (mg/ml)
Control	3.17 ± 0.31^{a}	2.33 ± 0.2^{a}	13 ± 1.9^{a}	29 ± 3^{a}
Nicotinic acid deficient	4.37 ± 0.40^{b}	2.44 ± 0.2^{a}	14 ± 2.3^{a}	32 ± 4^{a}
Pair-fed	2.98 ± 0.28^{a}	2.40 ± 0.2^{a}	15 ± 2.5^{a}	30 ± 3^{a}

All values are expressed in terms of means ± S.D. of 4 experiments.
Means not sharing a common superscript letter within a column are significantly different. ($P < 0.05$).

average heart, kidney and liver weights of the control group were significantly ($p < 0.05$) higher than those of the other two groups. However, there were no significant differences in body weight gain, brain weight, heart weight, kidney weight, and liver weight between nicotinic acid deficient group and pair-fed group. As expected, brain weights were not affected by nicotinic acid deficiency.

The effects of nicotinic acid deficiency on levels of some metabolites and proteins are presented in Table 2. Nicotinic acid deficiency state gave rise to a marked increase in glucose level as compared to two other groups. It can be speculated that either the reduced metabolism of glucose or the accelerated breakdown of glycogen under the physiological stress could be responsible for the elevated levels of glucose (Tanese *et al.*, 1983). This kind of hyperglycemic phenomenon was also well demonstrated in animals administered 6-aminonicotinamide which is a potent antimetabolite of nicotinic acid (Park *et al.*, 1990).

The level of cholesterol was not affected by nicotinic acid deficiency. Similarly, no significant difference was observed regarding the percentage of cholesterol, total lipid and phospholipid in the brain of rat fed on nicotinic acid deficient diet (Nakashima and Suzue, 1982). Likewise, the average content of albumin and total protein among the three treatment groups were not significantly different ($p < 0.05$). In regard to the protein concentration a very similar observation was made with liver and pectoral muscle tissues among the three treatments (Park, 1985).

The specific activity levels of serum enzymes in response to three different groups are summarized in Table 3. The specific activity of serum glutamic oxaloacetate transaminase and glutamic pyruvate transaminase of the nicotinic acid deficient group was approximately twofold higher ($p < 0.05$) than that in the control group. However, there were no significant differences ($p > 0.05$) in enzyme activities between the nicotinic acid deficient group and pair-fed group. The results of these studies would

Table 3. Effects of nicotinic acid deficiency on serum enzyme activities of quail (μmol/min/mg protein).

Treatment	Alkaline phosphatase	GOT	GPT	Lactic dehydrogenase
Control	30 ± 3.2^a	7.5 ± 1.0^a	6.6 ± 1.0^a	9.6 ± 1.2^a
Nicotinic acid deficient	29 ± 3.0^a	12.7 ± 2.0^b	10.7 ± 1.5^b	9.0 ± 0.9^a
Pair-fed	32 ± 3.5^a	14.3 ± 2.7^b	11.0 ± 2.0^b	10.4 ± 1.5^a

All values are expressed in terms of means ± S.D. of 4 experiments.
Means not sharing a common superscript letter within a column are significantly different. ($P < 0.05$).
GOT: glutamic oxaloacetic transaminase, GPT: glutamic pyruvate transaminase.

indicate that transaminase activities were not specifically affected by nicotinic acid status alone but rather was affected by general nutritional stress. Under these circumstances it can be hypothesized that the enhanced activities of these transaminases might result in channelling of some key intermediates into citric acid cycle and consequently either lead to the elevated gluconeogenesis or replenish the necessary energy source such as ATP (Voet and Voet, 1990). The specific activities of serum alkaline phosphatase and lactic dehydrogenase were not influenced by nicotinic acid status or pair-feeding treatment. In the case of lactic dehydrogenase activity a very similar observation was also made with liver and pectoral muscle tissues which were not influenced by nicotinic acid deficiency (Park, 1985). Similarly, in thermostability studies of enzymes, *in vivo* the non-allosteric enzyme such as lactic dehydrogenase was much less readily inactivated by the heat treatment as compared to the allosteric enzyme such as glyceraldehyde-3-phosphate dehydrogenase (Park, unpublished data). This effect may arise from subtle differences in the native conformation of these dehydrogenases (Marangos and Constantinides, 1974). Overall these results would imply that the changes in enzyme activities can not be directly ascribed to the effect of nicotinic acid deficiency since similar alterations also occurred in both the nicotinic acid deficient group and the pair-fed group.

The effects of nicotinic acid deficiency on levels of free amino acids in the serum of quail are presented in Table 4. Tryptophan and tyrosine levels were remarkably reduced under nicotinic acid deficiency and a similar observation was also made with aspartic acid, glutamic acid and alanine plus serine. Tryptophan is an unusual amino acid in which it has been purported to possess unique roles in metabolic regulation (Sidransky, 1985). Sidransky *et al.* (1984) also reported that tryptophan produces hormone-like properties in promoting protein synthesis in rat liver by altering the permeability of the nuclear envelope and facilitating translocation of mRNA from the nucleus to

Table 4. Effects of nicotinic acid deficiency on levels of free amino acids in the serum of quail (nmol/ml).

Amino acids	Control	Nicotinic acid deficient	Pair-fed
Aspartic acid	102	46	84
Glutamic acid	264	193	302
Arginine	270	272	383
Lysine	423	417	845
Histidine	59	46	78
Alanine	885	406	661
Glycine	887	645	1582
Serine	2006	1264	2060
Threonine	229	326	397
Methionine	68	62	81
Isoleucine	130	129	160
Leucine	190	181	263
Valine	388	329	557
Phenylalanine	158	154	182
Tryptophan	42	22	62
Tyrosine	303	156	429

the cytoplasm. Recently, the activity of porcine muscle ribosomes was intimately associated with enhanced concentrations of tryptophan (Lin *et al.*, 1988). It is very likely that tryptophan has the potential to regulate muscle protein synthesis in a manner beyond serving simply as a component of protein. In our present studies the lower level of trytophan may be responsible for the dramatic reduction of total body weight gain as a result of the reduced rate of protein synthesis in the nicotinic acid deficient group. Furthermore, our recent studies showed that the various tissues of quail under the niacin deficiency had the differential turnover rates of proteins. The high turnover rate of proteins was primarily attributed to the combined effects of reduced synthesis rate and enhanced degradation rate of proteins (Park *et al.*, 1991). The metabolic significance of the lower levels of aspartic acid, glutamic acid, serine and alanine under nicotinic acid deficiency can not be fully assessed at this point. In the pair-fed group the levels of arginine, lysine, glycine, leucine and valine were higher than those in the control group and nicotinic acid deficient group. However, there were no significant differences in concentrations of these amino acids between the latter two groups. These results would indicate that the increase in free amino acid levels in pair-fed group relative to the other two groups arose from the metabolic disturbances caused by the restricted feeding pattern. The biochemical mechanism for the reduction of tyrosine is not clear yet. Further studies should be carried out to elucidate the physiologic role of tyrosine in this ragard.

In conclusion it may be stated that nicotinic acid deficiency resulted in the marked increase of glucose level whereas cholesterol, albumin and total protein levels were not affected. Tryptophan level, in particular, was markedly reduced and tyrosine, aspartic acid, glutamic acid, alanine serine levels also followed the same trend.

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Nicotinic acid 결핍이 메추리 혈청의 여러 대사물질 수준에 미치는 효과

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Nicotinic acid 결핍시 메추리는 심각한 체중의 감소를 보였으며, 심장 및 간의 무게도 약간 감소하였다. 포도당의 농도는 현저하게 증가하였으나 콜레스테롤, 알부민 그리고 총 단백질 양의 변화는 없었다. Glutamic oxaloacetate transminase와 glutamic pyruvate transaminase의 활성은 증가하였으나 alkaline phosphatase와 LDH의 활성은 변화가 없었다. 혈청속의 아미노산 중 tryptophan, tryosine, aspartic acid, glutamic acid 등의 농도는 감소하였으나, arginine, histidine, lysine 등은 변화가 없었다.