Nutrient Interactions and Toxicity Research Communication

Excess Dietary Methionine Markedly Increases the Vitamin B-6 Requirement of Young Chicks

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ABSTRACT A soy-protein isolate diet that contained essentially no bioavailable vitamin B-6 was used to establish the quantitative effect of excess dietary methionine on the vitamin B-6 requirement of young chicks. When made adequate in vitamin B-6, chicks fed the basal diet required 2 g/kg supplemental L-methionine to achieve maximal growth, and 10 g/kg additional L-methionine (total = 12 g/kg) was found to be a tolerable excess level that would not depress voluntary food intake or growth rate. When chicks were fed seven graded doses of supplemental pyridoxine (PN) in diets that contained either adequate (2 g/kg) or excess (12 g/kg) methionine, the vitamin B-6 requirement for maximal growth was found to increase (P < 0.01) from 0.73 to 1.05 mg/kg, a 44% increase, when 10 g/kg excess methionine was present in the diet. Indeed, this level of excess dietary methionine depressed (P < 0.01) growth at all PN dose levels ≤1 mg/kg, but not at PN doses of 1.2 or 1.4 mg/kg. Because dietary intakes of both vitamin B-6 and methionine can affect plasma homocysteine levels, dietary methionine (and protein) intake should be considered important factors in setting safe and adequate requirement levels for vitamin B-6. J. Nutr. 130: 3055–3058, 2000.

KEY WORDS: • vitamin B-6 • methionine • protein • chicks • requirements

Excess intake of protein exacerbates vitamin B-6 deficiency (Bai et al. 1991, Bender 1985, Canham et al. 1969, Driskell 1984, Leklem 1991, Morgan et al. 1946). The chick studies of Daghir and Shah (1973) and Gries and Scott (1972), together with the rat study of Okada et al. (1998), also provided qualitative evidence that excess protein increases the dietary requirement for vitamin B-6. Our recent chick work (Scherer and Baker 2000) demonstrated that doubling the protein level from 200 to 400 g/kg, using methionine (Met)-fortified soyprotein isolate, increased the vitamin B-6 requirement for maximal growth by 45%. We questioned whether this effect was due to protein (or excess amino acids) per se, or whether there might be a single amino acid, e.g., Met, that might be causing most of the effect.

Vitamin B-6 [as pyridoxal phosphate (PLP)], is intimately involved in sulfur amino acid (SAA) metabolism. In the transsulfuration pathway, homocysteine (+ serine) conversion to cystathionine, and cystathionine conversion to cysteine, α-ketobutyrate and ammonia require PLP. Of the homocysteine produced from Met catabolism in mammals, an estimated 50% is remethylated to Met, and roughly half of the homocysteine remethylation that occurs uses 5-methyltetrahydrofolate as a methyl donor (Finkelman 1990). The biosynthesis of serine, with its subsequent conversion to glycine, generates a methyl group, and this PLP-requiring reaction is an important contributor to the folate pool for use in remethylating homocysteine to Met (Martinez et al. 2000). Thus, in the overall process of transsulfuration, there are three key PLP-requiring reactions. In addition, several S-adenosylmethionine–requiring reactions also require PLP as a cofactor, e.g., the conversion of ornithine to putrescine, putrescine to spermidine and spermidine to spermine. Moreover, one of the pathways in cysteine catabolism involves transamination, which is a PLP-dependent reaction.

Because vitamin B-6 status can affect the level of both homocysteine (Leklem 1991, Martinez et al. 2000, Rassin et al. 1977, Ubbink et al. 1996, Wilcken and Wilcken 1998) and cystathionine (Andersson et al. 1990, Leklem 1990, Linkswiler 1981) in blood and urine, we attempted herein to use the chick as a model for purposes of determining whether excess dietary Met per se might increase the dietary need for vitamin B-6. In a quantitative study involving both vitamin B-6 and Met, the chick is a very useful animal model in that transsulfuration in avian species is similar to that in mammals (Emmert et al. 1996). Moreover, chicks, unlike rats, do not practice coprophagy, a factor that could confound interactive results of a vitamin B-6 dosing study.

It is well documented that an elevation in the circulating level of homocysteine represents an independent risk factor for cardiovascular disease in humans (Wilcken and Wilcken 1998). Thus, if excess Met ingestion caused by high protein diets were to exacerbate vitamin B-6 deficiency, also a factor that causes homocysteinemia (Martinez et al. 2000, Miller et al. 1994, Selhub et al. 1998, Smolin and Benevenga 1984), high protein or high Met diets might appropriately be added to the growing list of factors that contribute to cardiovascular disease.

MATERIALS AND METHODS

General procedures. All procedures were approved by the University of Illinois Committee on Laboratory Animal Care.

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2 Abbreviations used: PLP, pyridoxal phosphate; PN, pyridoxine; SAA, sulfur amino acids.
Two bioassays were conducted with male chicks from the cross of New Hampshire males and Columbian females (University of Illinois Poultry Farm, Urbana, IL). Chicks were housed in thermostatically controlled battery pens equipped with raised wire floors in an environmentally controlled laboratory room with 24-h continuous fluorescent lighting. All equipment, including batteries, feeders, water trays and feed mixing equipment, was of stainless steel construction. Water and experimental diets were freely available, and diets were formulated to meet or exceed NRC (1994) requirements for all essential nutrients with the exception of vitamin B-6. Chicks were fed a conventional 24% crude protein diet during the first 7 d posthatching. On the morning of d 8 posthatching, after 16 h without either feed or water, the chicks were wingbanded, weighed and then assigned to battery pens in a manner that ensured minimal variation in initial body weight among pens. The two experiments involved four pens of four chicks for each diet during a 12-d experimental feeding period of 8–20 d posthatching.

**Basal diet.** The basal soy-protein isolate diet (Table 1) was developed and characterized over several years for purposes of studying utilization of several nutrients (Baker et al. 1999, Emmert and Baker 1995 and 1997, Patel and Baker 1996). The soy-protein isolate product used was a functional alcohol-extracted soy product (Ardex AF, ADM, Decatur, IL). Chemical analysis of this product yielded the following results: 824 g/kg crude protein (macro-Kjeldahl), 49 g/kg lipid (chloroform-methanol extraction), 89 g/kg H2O, 21.8 mg/kg B-12, 40 mg/kg ZnCO3, 0.1 g; FeSO4.7H2O, 0.415 g; MnSO4.7H2O, 3.5 g; ZnSO4.7H2O, 1 mg; CoSO4.7H2O, 20 mg; MgSO4.7H2O, 20 mg; NaHCO3, 5.0 g; KI, 40 mg; CaCO3, 20 g; K2HPO4, 9 g; NaCl, 8.8 g; MgSO4.7H2O, 3.5 g; ZnO, 0.1 g; FeSO4.7H2O, 0.415 g; MnSO4.7H2O, 0.65 g; CuSO4.5H2O, 20 mg; H3BO3, 9 mg; Na2MoO4.2H2O, 9 mg; KI, 40 mg; Na2SeO3, 0.215 mg; Na2SO3, 0.415 g; MnSO4.7H2O, 1 mg; Na2SeO3, 0.215 mg. Chemical analysis of this product yielded the following results: 824 g/kg crude protein (macro-Kjeldahl), 49 g/kg lipid (chloroform-methanol extraction), 89 g/kg H2O, 21.8 mg/kg B-12, 40 mg/kg ZnCO3, 0.1 g; FeSO4.7H2O, 0.415 g; MnSO4.7H2O, 3.5 g; ZnSO4.7H2O, 1 mg; CoSO4.7H2O, 20 mg; MgSO4.7H2O, 20 mg; NaHCO3, 5.0 g; KI, 40 mg; CaCO3, 20 g; K2HPO4, 9 g; NaCl, 8.8 g; MgSO4.7H2O, 3.5 g; ZnO, 0.1 g; FeSO4.7H2O, 0.415 g; MnSO4.7H2O, 0.65 g; CuSO4.5H2O, 20 mg; H3BO3, 9 mg; Na2MoO4.2H2O, 9 mg; KI, 40 mg; Na2SeO3, 0.215 mg; Na2SO3, 0.415 g; MnSO4.7H2O, 1 mg; Na2SeO3, 0.215 mg.

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Previous work from our laboratory had established that 2 g/kg of supplemental DL-Met would meet the minimal level of SAA required for maximal growth of chicks fed the basal diet containing a superequivalent level (5 mg/kg) of pyridoxine (PN) (Marovich et al. 1982, Baker 1994). Dietary additions of PN·HCl and DL-Met were made at the expense of cornstarch. Because milligram quantities of PN·HCl were involved in the construction of individual diets, PN additions were accomplished by first dissolving PN·HCl in deionized water (1 g PN/L), after which appropriate quantities of this solution were pipetted, premixed, and then added to the individual diets.

**Experiment 1.** Graded levels of excess supplemental DL-Met were added to the basal diet made superequivalent in vitamin B-6 (5 mg/kg DL-Met) to determine a level of excess Met that could be tolerated without causing a growth depression. Previous results from our laboratory had shown that 10 g/kg of excess DL-Met added to conventional corn-soybean meal diet would not depress either weight gain or food efficiency (Hale and Baker 1993). Thus, 10, 20 or 30 g/kg of added DL-Met were tested in the soy-protein isolate semipurified diet to establish whether a 10 g/kg DL-Met supplement would similarly be a tolerable excess in this diet.

**Experiment 2.** A 2 × 7 factorial arrangement of treatments was used in this bioassay, involving two levels of supplemental DL-Met added to the Met-adequate basal diet (none, i.e., adequate, and 10 g/kg, i.e., excess) and seven graded doses of PN ranging from 0.20 to 1.4 mg/kg. Our previous work with PN additions to the basal diet shown in Table 1 indicated that these dosage levels of PN would cover both the linear and plateau portions of the growth-response curve. The objective of the bioassay was to determine whether excess Met might depress growth at deficient but not at adequate levels of vitamin B-6, and also to define dietary requirements for vitamin B-6 under conditions of adequate and excess dietary Met.

**Statistical analyses.** Both experiments were completely randomized designs. After ANOVA of pen means data, orthogonal single df comparisons were made to evaluate treatment differences (Steel and Torrie 1980). Linear and quadratic responses to excess Met were evaluated in Experiment 1, and Met and PN (linear and quadratic) main effects and their interaction were determined in Experiment 2. The weight gain data in Experiment 2 were also fitted to a one-slope broken-line model (Robbins et al. 1979, Robbins 1986) in which gain was regressed on dietary PN level for chicks fed either an adequate or an excess level of Met.

**RESULTS AND DISCUSSION**

The results of Experiment 1 (Table 2) clearly established that 10 g/kg of supplemental DL-Met represented a tolerable

| TABLE 2 |

<table>
<thead>
<tr>
<th>Diet</th>
<th>Weight gain2</th>
<th>Food intake2</th>
<th>Gain:food2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal diet (B)3</td>
<td>161</td>
<td>187</td>
<td>860</td>
</tr>
<tr>
<td>B + 10 g/kg DL-Met</td>
<td>163</td>
<td>185</td>
<td>880</td>
</tr>
<tr>
<td>B + 20 g/kg DL-Met</td>
<td>67</td>
<td>112</td>
<td>599</td>
</tr>
<tr>
<td>B + 30 g/kg DL-Met</td>
<td>13</td>
<td>70</td>
<td>187</td>
</tr>
<tr>
<td>SEM</td>
<td>5</td>
<td>13</td>
<td>30</td>
</tr>
</tbody>
</table>

1 Data are mean values of four pens of four male chicks during a 12-d experimental feeding period; average initial body weight was 99 g.
2 Quadratic (P < 0.01) decrease.
3 The basal diet (Table 1) contained adequate levels of supplemental DL-Met (2 g/kg) and pyridoxine (5 mg/kg) as established previously (Emmert and Baker 1995, Scherer and Baker 2000).

1 Like rats, mice and pigs, avian species use the D-isomer of Met almost as efficiently as the L-isomer (Baker 1994).
excess in that weight gain, voluntary food intake and gain:food ratio were not reduced (P > 0.10) from this level of excess Met. Excess Met doses of 20 or 30 g/kg, however, depressed all measures of response. This bioassay established that chicks fed the basal diet containing adequate Met and surfeit PN could tolerate a 10 g/kg Met excess dose without exhibiting a growth depression. The 10 g/kg level of excess Met was therefore used in Experiment 2 to evaluate the effects of excess Met on responses to graded increments of PN.

Weight gain data for Experiment 2 are shown in Figure 1 in which the observed pen means data points for PN doses at each level of Met were fitted to one-slope broken lines. The breakpoint estimate of the PN requirement for maximal growth occurred at 0.73 ± 0.04 mg/kg for chicks fed diets with adequate Met and at 1.05 ± 0.04 mg/kg for chicks fed diets with 10 g/kg excess Met. The difference was significant (P < 0.01) and represented a 44% increase in the vitamin B-6 requirement of chicks fed excess Met. The interaction of PN level × Met level was significant (P < 0.01), and this is explained by the finding that weight gain was depressed by excess Met at all PN dose levels ≤1.0 mg/kg, but the growth depression did not occur at higher PN dose levels of 1.2 and 1.4 mg/kg. Food intake and gain:food ratio data are not shown, but responses in these criteria generally followed the same pattern as gain responses.

One could question whether lower doses of excess Met (or cystine) would similarly depress growth of vitamin B-6–deficient chicks, and also whether such doses would increase the dietary requirement for vitamin B-6. We have not addressed the latter question, but we have unpublished results showing that as little as 3.3 g/kg of excess Met will depress the growth rate of vitamin B-6–deficient chicks. A level of 10 g/kg of excess dietary L-cystine, however, had no effect on the performance of vitamin B-6–deficient chicks.

It appears that the excess Met contained in excess protein may explain a good portion of the excess protein—exacerbating effect on vitamin B-6 utilization. On the basis of the recent work of Martinez et al. (2000), the need for PLP in catalyzing the serine hydroxymethyltransferase and γ-cystathionase reactions may be more important than the need for PLP in the cystathionine β-synthase reaction. Indeed, Sato et al. (1996) showed that vitamin B-6 deficiency in rats increases the proportion of hepatic γ-cystathionase in apoenzyme form, and also increases the catabolism of the enzyme. We did not measure plasma homocysteine in our chicks, but deficiencies of vitamin B-6 are known to elevate plasma homocysteine (Martinez et al. 2000, Miller et al. 1994, Selhub et al. 1993, Smolin and Benevenga 1984). Miller et al. (1994) also found that Met loading was additive with vitamin B-6 deficiency in causing elevations of plasma homocysteine in rats.

That a level of Met that does not depress growth (when dietary vitamin B-6 is superadequate) increases the vitamin B-6 requirement of chicks fed excess Met by 44%, approximately the same as that caused by a doubling of the protein (Scherer and Baker 2000), implies that Met is the primary component of excess protein that is causing this effect. Most amino acids require PLP in their catabolism, but Met catabolism requires PLP in several steps. Also, Met is well established as being among the most toxic of all amino acids when fed at excess levels in a diet (Edmonds and Baker 1987, Edmonds et al. 1987).

LITERATURE CITED


Robbins, K. R. (1986) A method, SAS program, and example for fitting the broken line to growth data, University of Tennessee Research Report 86–09. University of Tennessee Agricultural Experimental Station, Knoxville, TN.


