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Effects of Dietary Protein Restriction on Circulating Concentrations of Growth Hormone in Growing Domestic Fowl (*Gallus domesticus*) (41282)

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Abstract. Two- to three-week-old broiler chicks were fed isocaloric purified diets with varying levels of protein (soy protein supplemented with glycine and methionine). The growth rates of the birds were monitored throughout the period of 14 days on the purified diets, while the circulating concentrations of growth hormone (GH) were determined at the end of this period. As expected, decreases in the dietary protein level were associated with concomitant reductions in growth rate. While this decrease in growth rate was observed with chicks fed 12 and 18% protein (compared with 24%), circulating concentrations of GH were observed to be only elevated with severe protein restriction (10% and lower). There was also evidence for some progressive increase in plasma concentrations of GH as dietary protein levels were decreased.

It is well established that the plasma concentration of growth hormone (GH) can be affected by diet, circulating concentrations of nutrients (including glucose, free fatty acids, and arginine) and insulin. While these effects are found in all species investigated, there are considerable differences between species in terms of both the magnitude of the effect as well as to whether GH release if stimulated or inhibited. For instance, fasting is reported to elevate plasma concentrations of GH in man (1), pigs (2), and rabbits (3), but had little effect in ruminants (3–5) and even depressed GH secretion in rats (6, 7). A similar situation seems to exist in chronic malnutrition, with GH levels elevated in protein malnutrition in man (8) but reduced in rats (9). Insulin administration, and the resultant hypoglycemia, frequently affects plasma concentrations of GH in a manner similar to that induced by fasting. In primates, insulin is a potent stimulus for GH release (1, 10), as it is in ruminants (2). It is less effective in pigs (2) and depresses circulating concentrations of GH in rats and rabbits (e.g., 3, 6, 11).

The domestic fowl offers considerable advantages to the study of the interactions of nutrition, growth, and GH. These include its fast growth rate and the availability of strains with different growth characteristics. Furthermore, its nutritional requirements have been subjected to intense investigation. Moreover, circulating concentrations of GH in the chicken, as measured by radioimmunoassay (12), have been demonstrated to be high during the period of rapid growth (13). This high GH concentration in young chickens can be further elevated by starvation for periods of 24 or 48 hr (14) or chronic reduction in food intake (15). However, the plasma GH concentration is reduced by insulin-induced hypoglycemia (14), as well as by glucagon and epinephrine-induced hyperglycemia (14, 16).

Materials and Methods. The experimental diets are shown in Table I. All the birds used in these studies were broiler "meat type" heavy chickens. In study 1, the broiler birds were obtained from Cobb (Concord, Mass.) while studies 2 and 3 employed a Cornish New Hampshire cross (these breeds having very similar characteristics). At 1 day old, the birds were sexed and the sexes were reared separately in battery cages. The birds were subject to a 16L:8D photoperiod with food (a commercial diet) and water available *ad libitum*. The 14-day-old (study 1), 21-day-old (study 2), and 17- and 20-day-old male and 20-day-old female (study 3) birds were weighed and assigned to groups such that the mean weight per group was uniform. Groups of 10 chicks were held in separate cages and fed various purified diets for 2

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TABLE I. BASAL DIET[a]

Assay soy protein[b]	3.01
DL-methionine	0.048
Glycine	0.06
Alfalfa leaf meal	1.00
Fiber, non-nutritional[c]	2.00
Corn oil, refined	3.00
Mineral mixture[d]	5.40
Vitamin mixture[e]	0.20
Choline Cl (70%)	0.30
Starch	20.00
Glucose monohydrate	64.982
Total	100.00

[a] The basal diet contained, by calculation, 3% protein (N × 6.25). Other diets contained 6, 12, 18, and 24% protein. For each 3% (experiment 1) protein increase to the basal 3% protein content, 3.188% isolated soy protein, 0.052% DL-methionine, and 0.064% glycine were included at the expense of glucose monohydrate. The values given are percentages.

[b] Purina Assay Protein RP-100.

[c] Solka Floc, Brown Co., New York.

[d] Composition (in percentage of total diet): $Ca_3(PO_4)_2$, 0.85; KH_2PO_4, 1.05; NaCl, 0.80; $CaCO_3$, 1.90; Fe-gluconate, 0.052; $MgSO_4$, 0.25; $MnSO_4 \cdot H_2O$, 0.02; KI, 0.001; $CuSO_4$, 0.00128; $ZnCO_3$, 0.01; and $Na_2MoO_4 \cdot 2H_2O$, 0.001.

[e] In milligrams per kilogram of total diet: thiamin–HCl, 25; riboflavin, 16; Ca-pantothenate, 20; pyridoxine–HCl, 6; biotin, 0.6; folic acid, 4; 2 methyl-1,4-naphthoquinone, 5; cobalamine, 0.02; ascorbic acid, 250; niacin, 150; retinyl acetate (100 IU/mg), 100; cholecalciferol (250 IU/mg), 2.4; DL-α-tocopheryl acetate (0.5 IU/mg), 20. Some of the vitamins used in this study were donated by Merck, Sharp and Dohme, Rahway, N.J., and Hoffman–LaRoche, Nutley, N.J.

weeks. At that time the birds were reweighed and blood samples taken following decapitation. After centrifugation, plasmas were stored at −20° prior to determining GH concentration. All plasmas from a single experiment were assayed together to eliminate interassay variation. Determinations were made in duplicate and at two concentrations by the method of Harvey and Scanes (12). Data from each experiment were analyzed by ANOVA.

Results. Data from the three studies is summarized in Table II, with cumulative results from all studies shown in Table III. In all studies growth, as indicated by body weight, was markedly affected by the dietary protein concentration, with the greatest differences between feeding regimens being observed in experiment 1. In experiment 1, the effect of a wide spectrum of dietary protein concentration on plasma concentrations of GH was investigated. In both sexes, reduction of the dietary protein concentration from 12 to 6% was accompanied by elevated plasma levels of GH (the increase in males being 34 ng/ml or 54% and in females 49 ng/ml or 163%) (see Table II). In males there was a further increase in circulatory concentrations of GH when the protein concentration of the feed was reduced to 3%. It was therefore decided to investigate the effects of protein concentrations between 12 and 6% and 6 and 3% in more detail (in experiments 2 and 3, respectively). Again, in experiment 2 plasma levels of GH were increased as the feed protein concentration was reduced, this being apparent at 10% protein in males and 7.5% in females. However, further reduction of protein concentration in diet did not have any additional effect on circulating concentrations of GH. In experiment 3, low-protein diets again elevated circulating GH concentrations. In this case, however, there is evidence that graded reduction of protein concentration in the diets (between 6.2 and 3.3%) was accompanied by a further increase in GH levels. This was particularly apparent in the male birds.

Further analysis of the results of the three studies was performed in an attempt to show whether the effect of dietary protein level is a graded response (Table III). It would appear that circulating GH levels are not elevated until the dietary protein concentration is below 10%. A further increase in GH levels is seen in birds on a diet with protein below 5% (Tables II and III).

Discussion. It is apparent that, in the growing chicken, low levels of protein in the diet both reduce growth rate and increase circulating levels of GH. This is similar to the situation in humans where protein malnutrition is accompanied by elevated plasma GH levels (8) but differs from that in the rat where circulating GH concentrations are depressed (9). The mechanism by which reduction of dietary protein concentration affects the plasma level of GH is not clear. Reduction in circulatory concentration of all or specific amino acids may affect either the clearance or secretion

TABLE II. Plasma Growth Hormone (GH) Concentration ± SEM in Young (4-Week-Old) Chickens on Diets with Different Protein Levels

Protein (%)	Males		Females	
	Body weight (g)	Plasma GH (ng/ml)	Body weight (g)	Plasma GH (ng/ml)
Experiment 1				
3	125 ± 7	154 ± 10	146 ± 5	55 ± 7
6	177 ± 8***	97 ± 17**	193 ± 5**	79 ± 16
12	314 ± 10***	63 ± 8*	368 ± 15***	30 ± 6***
18	434 ± 13***	54 ± 6	437 ± 8***	33 ± 3
24	450 ± 11	46 ± 4	468 ± 10*	27 ± 3
Experiment 2				
5	397 ± 11[a]	228 ± 40[a]	230 ± 4[a]	290 ± 60[a]
6	339 ± 12[b,*]	172 ± 26[a,b]	257 ± 10[a]	344 ± 68[a]
7.5	324 ± 14[a,b]	208 ± 42[a]	310 ± 7[b,***]	322 ± 101[a]
10	424 ± 9[c,***]	174 ± 31[a,b]	336 ± 7[c,*]	128 ± 25[b,*]
13	424 ± 16[c]	80 ± 18[b]	346 ± 8[c]	135 ± 44[b]
Experiment 3				
3.3	185 ± 4[a]	173 ± 16[a,b]	230 ± 8[a]	153 ± 21[a,b]
3.7	190 ± 5[a]	194 ± 17[a]	228 ± 6[a]	186 ± 13[a]
4.6	201 ± 6[a,b]	176 ± 17[a,b]	241 ± 9[a]	106 ± 14[b,*]
5.1	216 ± 9[b,c]	143 ± 14[b]	251 ± 13[a,b]	121 ± 18[a,b]
5.6	236 ± 7[c,d]	150 ± 21[a,b]	253 ± 11[a,b]	109 ± 35[b]
6.2	257 ± 13[d]	106 ± 12[c]	272 ± 8[b]	118 ± 31[a,b]
23	516 ± 15[e,***]	44 ± 8[e,**]	516 ± 15[e,***]	39 ± 21[e,*]

Note. N = 10 throughout experiments 1–3 (except N = 20 in males in experiment 3). Initial body weights were: Experiment 1, 137 ± 3 (N = 50) for males and 139 ± 2 (N = 50) for females; Experiment 2, 285 ± 3(50) for males and 235 ± 2(50) for females; Experiment 3, 321 ± 3(140) for males and 263 ± 3(70) for females. *P < 0.05; * *P < 0.01; * * *P < 0.001 compared with next lowest protein level.

[a,b,c,d,e] Data with different subscript letter differ, $p < 0.05$.

of GH. The latter effect may be due to influence of amino acids at a hypothalamic or anterior pituitary gland level. Indeed it is possible that reductions in dietary tyrosine and/or tryptophan lead to decreases in hypothalamic neurotransmitters (dopamine and norepinephrine and serotonin, respectively) and hence on the release of hypothalamic–hypophysiotropic factors and GH.

In the present studies, the chicks consumed less of the isocaloric diets with lower protein levels. It may be therefore possible that GH release may be influenced by the reduction in food intake (and hence via cir-

TABLE III. Cumulative Data on the Effect of Protein Levels on Circulating Growth Hormone Concentration

Protein concentration (%)	Plasma growth hormone concentration			
	ng/ml		%[a]	
	Male	Female	Male	Female
0–5	183 ± 9(77)	158 ± 13(49)	378 ± 19(77)	293 ± 26(49)
5–10	145 ± 9(97)*	174 ± 22(70)	267 ± 15(97)***	249 ± 25(70)
10–15	71 ± 9(19)***	80 ± 15(19)**	108 ± 11(20)***	100 ± 19(19)**
15–20	54 ± 6(10)	33 ± 3(10)	99 ± 11(10)	110 ± 11(10)
20–25	45 ± 5(30)	33 ± 10(20)	92 ± 6(30)	95 ± 27(20)

Note. Mean ± SEM. N is in parentheses. *P < 0.05; * *P < 0.01; * * *P < 0.001 compared with next lowest protein level.

[a] Plasma GH concentration expressed as a percentage of mean GH concentration in groups in each experiment receiving greater than 10% protein. (This transformation was made to overcome differences between individual experiments.)

culating metabolites and gastrointestinal/pancreatic hormones) or by the depressed appetite indirectly affecting the hypothalamic control of GH. Some support for this comes from the observations that fasting stimulates, while insulin and glucagon depress, GH release in young chickens (14). Moreover, feeding growing chicks on a diet deficient in essential fatty acid, is accompanied by increases in circulating concentration of GH compared with those on a replete diet (15). The identity of the metabolite/hormone affecting GH secretion remains to be elucidated. It does not appear, however, that glucose or insulin act alone (14). The increases in GH concentration in the circulation with reduced dietary protein (e.g., Table III) may have an important metabolic role. Perhaps the elevated plasma GH concentrations prestimulate tissues in anticipation for the period of so called "catch up" growth which would occur once dietary protein levels were elevated.

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