

Nutrient Interactions and Toxicity

Adult Cockatiels (*Nymphicus hollandicus*) Metabolically Adapt to High Protein Diets^{1,2}

Elizabeth A. Koutsos, Jeanne Smith,* Leslie W. Woods[†] and Kirk C. Klasing³

Department of Animal Science, *Veterinary Medicine Resource Services and [†]California Animal Health and Food Safety Laboratory, University of California, Davis, CA

ABSTRACT To determine the ability of cockatiels (*Nymphicus hollandicus*), a granivorous avian species, to adapt metabolically to high dietary protein levels, adult males ($n = 26$) were fed isocaloric diets containing 11, 20, 35 or 70% crude protein (CP) for 11 mo. Throughout the trial, body weight and breast muscle weight were maintained by 11, 20 or 70% CP. The 35% CP diet resulted in significantly greater body weight ($P < 0.05$) and whole-body lipid content ($P < 0.05$) compared with the 11% CP diet. The 20% CP diet resulted in greater breast muscle mass compared with 70% CP ($P < 0.05$). Activity of the amino acid catabolic enzymes alanine aminotransferase, aspartate aminotransferase and arginase as well as the gluconeogenic enzyme phosphoenolpyruvate carboxykinase were significantly increased with 70% CP ($P < 0.05$). Serum essential amino acids, urea and uric acid were also increased with 70% CP ($P < 0.05$), but the magnitude of their increase was similar to that found in omnivorous chickens fed a similar diet. There was no evidence of visceral gout, articular gout or renal pathology; however liver lesion severity, and specifically liver lipogranuloma severity, was significantly increased above 11% CP ($P < 0.05$). We conclude that cockatiels are able to up-regulate enzymes for amino acid catabolism as well as mechanisms for nitrogen excretion in response to high dietary protein levels, and that high dietary protein levels are not associated with kidney dysfunction in this avian species. J. Nutr. 131: 2014–2020, 2001.

KEY WORDS: • Psittacine • protein toxicity • adaptation • maintenance • amino acid catabolism • cockatiels

The dietary preferences, gastrointestinal morphology and metabolic capabilities of animals have been intimately intertwined during evolution, and the degree of dietary specialization is extremely variable across the animal kingdom. Omnivorous, or generalist species, consume a variety of plant and animal foods that frequently change in relative proportion. These species possess the digestive and metabolic plasticity to adapt to a wide variation in dietary macronutrient proportions (1,2). For example, omnivorous species such as chickens, Japanese quail, rats, pigs and humans are capable of up- or down-regulating enzymes for amino acid catabolism and are able to utilize diets with either a very low or very high protein content (3–7). In contrast, nonomnivorous species often specialize on a narrow range of food items of very uniform nutritional content. For example, faunivorous (animal matter-eaters) species such as barn owls, vultures, alligators, trout and cats select high protein:low carbohydrate food items (protein specialists) and have little capability to regulate their amino acid and carbohydrate metabolizing pathways (8–12). Granivorous (grain-eaters), frugivorous (fruit-eaters) and nectarivorous (nectar-eaters) species select low protein:high car-

bohydrate foods (carbohydrate specialists). In contrast to faunivores, these species have low requirements for dietary amino acids and appear to be able to conserve amino acids by tight regulation of amino acid catabolism (13). Analogous to the poor metabolic adaptations by faunivores, it might be expected that carbohydrate specialists would have a poor capacity to adapt to high protein diets. However, the metabolic plasticity of carbohydrate selectors has received very little attention. For this reason, we tested the capacity of cockatiels, an avian granivore, to adapt to high protein diets. In their native areas of Australia, wild cockatiels select seeds with 8.8–14% crude protein (CP)⁴ and consume little or no animal matter (14). Furthermore, the popular literature is rife with anecdotes of protein intolerance by this species.

The purpose of this experiment was to determine the effect of high dietary protein on the health of adult cockatiels at maintenance, as well as to examine the ability of these birds to adapt metabolically to high protein diets. To assess the possibility of protein toxicity, we examined the maintenance of body weight and composition, deposition of uric acid precipitates in organs and joints, and the development of pathology in kidney and liver. Metabolic adaptation was assessed by

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³ To whom correspondence should be addressed.

E-mail: kcklasing@ucdavis.edu.

⁴ Abbreviations used: ALT, alanine aminotransferase; AST, aspartate aminotransferase; CP, crude protein; GK, glucokinase; PEPCK, phosphoenolpyruvate carboxykinase; PK, pyruvate kinase; TEAA, total essential amino acids; TNEAA, total nonessential amino acids.

changes in amino acid and carbohydrate metabolizing enzymes in the liver and kidney as well as serum metabolite concentrations.

MATERIALS AND METHODS

Animals and diets. Adult male cockatiels ($n = 26$; UC Davis cockatiel colony), 2–3 y of age, with average body weight of 93 g, were housed individually in $0.3 \times 0.3 \times 0.6 \text{ m}^3$ wire cages at 24°C under a 12-h light: 12-h dark lighting schedule. Cockatiels were randomly assigned to one of four dietary treatments, which were isocaloric and contained either 11% CP ($n = 6$), 20% CP ($n = 7$), 35% CP ($n = 6$) or 70% CP ($n = 7$) (Table 1). Diets were formulated for identical acid-base balance, calculated as $\text{meq}(\text{Na} + \text{K} + \text{Ca} - \text{P} - \text{Cl})$. Diets were mixed, ~50% water was added and diets were then pelleted through a commercial sausage grinder (Hollymatic model # GMG 180A, Countryside, IL) with a 5-mm diameter die. Pelleted diets were crumbled by hand to ~12–15 mm length, and then dried overnight at 55°C . All diets were stored at 4°C before use. Birds were acclimated to the 11% CP diet for 1 mo. After this acclimation period, birds assigned to the higher protein diets were switched to their assigned experimental diets over a period of several weeks to prevent acute protein toxicity. Birds assigned to 20, 35 or 70% CP were initially switched to 20% CP. One week later, birds assigned to

35 or 70% CP were switched to 35% CP, and so on, until all birds were being fed their assigned experimental diet (d 1 of the experiment). Cockatiels consumed the experimental diets ad libitum for 11 mo and had free access to deionized water. After 10 mo of consuming the experimental diets, water intake was measured using 100-mL water bottles, graduated in 1-mL increments, with a 1.5-cm drinking surface (BioServe Frenchtown, NJ). The University of California at Davis Animal Care and Use Committee approved all animal protocols.

Tissue sampling and processing. All birds were weighed at the onset of the trial and monthly thereafter. Feed was not withdrawn before the time of weighing, bleeding or necropsy. Blood (1 mL) was taken from the jugular at the onset and termination of the experiment and twice in between, on d 34 and 178. Freshly drawn blood was used to make a blood smear and drawn into a hematocrit tube. The remaining blood was allowed to clot for 3 h and serum was frozen until analysis. After 11 mo of consuming the experimental diets, all birds were killed by isoflurane (Merial Animal Health, Iselin, NJ) anesthesia followed by isoflurane overdose. Kidney, liver, hock joint and pericardium were immediately dissected from the birds. One kidney and one liver lobe were flash-frozen between two aluminum plates in liquid N and stored at -60°C before enzymatic analysis. All other tissues were fixed in 10% buffered formalin for histopathologic analysis. The breast was removed, weighed and returned to the bird of origin. Birds were then weighed and freeze-dried at a shelf temperature of 30°C (Virtis Freeze Drier, Model # 50 SRC, Gardiner, NY) for 24 h for moisture determination. Subsequently, all birds were placed in Soxhlet units for lipid extraction using a modified AOAC procedure (15). Birds were extracted for 7 d with petroleum ether, followed by 3 d with acetone. Birds were then dried overnight at 55°C , weighed, and lipid content was calculated by difference.

Analysis of blood samples. Hematocrit tubes were centrifuged in a microcapillary centrifuge (International Equipment, Needham, MA), and hematocrit values were determined using a microcapillary reader (International Equipment). Blood smears were stained with hematoxylin-eosin and examined microscopically for total leukocyte number, as well as monocyte, lymphocyte, basophil, heterophil and eosinophil number. Serum samples were analyzed by standard methods for clinical chemistry parameters (Clinical Chemistry Laboratory, UC Davis, Veterinary Medical Teaching Hospital) including cholesterol, creatine kinase, lactate dehydrogenase, uric acid, urea N, calcium, albumin, globulin, glucose and total protein. In addition, serum samples were analyzed for plasma amino acid concentration by HPLC as described by Bidlingmeyer et al. (16).

Postmortem examination and histopathology. Birds were examined for visual evidence of visceral, articular or renal gout. Any other gross abnormalities were also noted. Kidney, liver and pericardium were fixed in 10% buffered neutral formalin and processed for histopathology. Livers sections were graded from one to five with least severely affected livers (rare, single vacuolated cell-small granulomas) receiving a grade of one and most severely affected livers (numerous, large lipogranulomas) receiving a grade of five. Kidney sections were graded similarly on the basis of the frequency and size of inflammatory or degenerative foci, with grade one denoting the least severely affected and grade five the most severely affected.

Tissue enzyme activities. Enzymatic activity in liver and kidney samples was analyzed as described previously by Myers and Klasing (11). Briefly, tissue samples were prepared for enzyme analysis by placing them on dry ice and breaking them into pieces no larger than 6–7 mm across in a -20°C cold room. Tissue samples were weighed into test tubes and 9 parts of ice-cold 0.14 mol/L KCl were added for alanine aminotransferase (ALT; EC 2.6.1.2), aspartate aminotransferase (AST; EC 2.6.1.1) and pyruvate kinase (PK; EC 2.7.1.40). For phosphoenolpyruvate carboxykinase (PEPCK; EC 4.1.1.31) assays, 9 parts of ice-cold deionized water were added. Samples were homogenized on ice with a Polytron (Brinkmann Instruments, Westbury, NY) twice at half-maximum power for 15 s. Homogenates were centrifuged for 30 min at $14,000 \times g$ in a 5°C cold room in an Eppendorf centrifuge (Brinkmann). For glucokinase (GK; EC 2.7.1.1) assay, 1 part liver was added to 5 parts 0.15 mol/L KCl, 0.005 mol/L sodium EDTA and 5 mmol/L MgCl_2 , pH 7.0, then homogenized with a Teflon pestle twice for 15 s. The homogenate was centrifuged

TABLE 1

Composition of diets fed to adult male cockatiels at maintenance for assessment of metabolic adaptation to changing dietary crude protein level¹

Ingredient	Diet			
	11% CP	20% CP	35% CP	70% CP
	g/kg diet			
Isolated soy protein ²	120	220	370	780
Cornstarch	729	642	511	152
Cellulose ³	75.5	65.3	50.1	0.0
Soybean oil	40	40	40	40
Vitamin Mix ⁴	2.5	2.5	2.5	2.5
Mineral Mix ⁴	2.5	2.5	2.5	2.5
$\text{MgSO}_4 \cdot 7 \text{H}_2\text{O}$	5.5	5.5	5.5	5.5
Dicalcium phosphate	13.3	11.3	8.0	0.15
CaCO_3	3.6	4.0	4.6	15.0
NaCl	4.6	4.6	4.6	3.4
Na_2CO_3	1.3	0.9	0.4	0.0
KHCO_3	7.7	7.4	6.8	3.2
KCl	0.0	0.0	0.0	1.5
Choline Chloride	2.0	2.0	2.0	2.0
D-L-Met	2.0	0.0	0.0	0.0
	Calculated composition			
ME, kJ/kg	14.57	14.57	14.57	14.57
Acid-base balance, meq ⁴	0.253	0.254	0.253	0.256

¹ Abbreviations: CP, crude protein; ME, metabolizable energy.

² ARDEX R Isolated Soy Protein, ADM, Decatur, IL.

³ Celufil, United States Biochemical, Cleveland, OH.

⁴ Vitamin mix supplied (per kg diet): 15 mg thiamin HCl, 15 mg riboflavin, 20 mg Ca-pantothenate, 50 mg nicotinic acid, 7.8 mg pyridoxine HCl, 6 mg folacin, 0.6 mg biotin, 0.02 mg vitamin B-12, 18 mg retinol palmitate, 0.31 mg cholecalciferol, 20 mg *dl*- α -tocopherol acetate, 15 mg menadione, 50 mg ascorbate, 100 mg ethoxyquin in cornstarch. Mineral mix supplied (per kg diet): 1.0 g KCl, 350 mg $\text{MnSO}_4 \cdot \text{H}_2\text{O}$, 120 mg $\text{ZnSO}_4 \cdot 5 \text{H}_2\text{O}$, 500 mg $\text{FeSO}_4 \cdot 7 \text{H}_2\text{O}$, 30 mg $\text{Cu SO}_4 \cdot 5 \text{H}_2\text{O}$, 0.2 mg Na_2SeO_3 , 2 mg KIO_4 , 1.7 mg CoCl_2 , 123 mg $\text{Mg SO}_4 \cdot 7 \text{H}_2\text{O}$, 8.3 mg $\text{Na}_2\text{MoO}_4 \cdot 2 \text{H}_2\text{O}$, in cornstarch.

⁴ Acid-base balance (meq/kg diet) calculated as $\text{meq}(\text{Na} + \text{K} + \text{Ca} - \text{P} - \text{Cl})$.

(Sorvall RC 100, DuPont, Wilmington, DE) for 1 h at $105,000 \times g$ at 4°C , and the supernatant was assayed for GK activity as previously described (17). The assays for ALT and AST were according to procedures described by Segal and Matsuzawa (18); arginase (EC 3.5.3.1) was according to Tamir and Ratner (19) and PEPCK and FBP were according to Opie and Newsholme (20). Enzyme activity was measured in a multicell thermostatically controlled spectrophotometer (Shimadzu, Kyoto, Japan) and was expressed as substrate consumed per minute per milligram of protein. Protein was determined by Coomassie dye binding using a protein assay kit (# 5656; Sigma, St. Louis, MO).

Statistical analysis. All data were analyzed by a general linear model (SAS Institute, Cary, NC). Data collected at only one time point (water consumption, enzyme activities and histopathology) were analyzed by one-way ANOVA for the effect of dietary treatment. Data collected at multiple time points throughout the study were analyzed for main effects due to diet and time and for the interactions of diet and time by two-way ANOVA for repeated measures. Birds were nested within diets, and the model accounted for the random variation among birds. The Pdiff procedure of SAS was used to determine whether mean values were significantly different at $P < 0.05$, with Bonferonni adjustment for α critical for data analyzed by repeated measures (21). Regression analysis of dietary protein level on liver enzyme activities and on serum urea and uric acid concentrations on d 34, 178 and 330 was accomplished using JMP (SAS Institute).

RESULTS

Body weight and composition. Change in body weight (calculated as body weight at each time point – initial body weight) was not significantly different for birds eating 11 or 20% CP (Fig. 1). There was no consistent change in body weight for birds consuming 35% CP until d 252, at which point 35% CP resulted in a consistent positive change in body weight compared with birds fed all other diets ($P < 0.05$). Birds consuming 70% CP maintained body weight; however, on d 330, body weight of birds eating 70% CP was significantly reduced compared with those eating 20 or 35% CP ($P < 0.05$). Body composition was also affected by dietary CP level (Table 2). Dry matter content was lower in birds fed 35% CP compared with those fed all other diets ($P < 0.05$), whereas breast muscle mass was greater in birds fed 20% CP compared with those fed 70% CP ($P < 0.05$). Finally, lipid content, on a wet weight (Table 2) or dry matter basis (data not shown), was significantly greater in birds fed 35% CP compared with all other dietary treatments ($P < 0.05$).

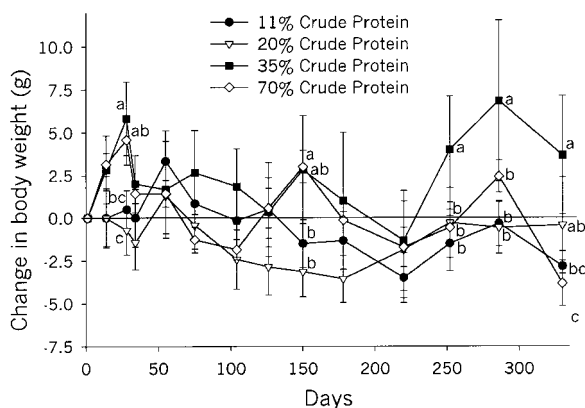


FIGURE 1 Effect of dietary crude protein level on body weight change in adult male cockatiels. Birds were fed diets varying from 11 to 70% crude protein for 11 mo. Values are means \pm SEM, $n = 5-7$ birds. Means at a time with different superscripts differ, $P < 0.05$.

TABLE 2

Dry matter (DM) content, lipid content, breast muscle mass and daily water intake in adult male cockatiels fed different levels of dietary crude protein (CP) for 11 mo¹

Diet	Dry matter	Lipid	Breast muscle	Water intake
				<i>g/kg wet weight</i>
11% CP	607 \pm 9.0 ^a	90 \pm 10.5 ^b	235 \pm 7.8 ^{ab}	17.8 \pm 37.2 ^a
20% CP	602 \pm 8.0 ^a	97 \pm 13.8 ^b	255 \pm 6.3 ^a	9.2 \pm 6.6 ^b
35% CP	562 \pm 22.2 ^b	156 \pm 32.4 ^a	234 \pm 13.4 ^{ab}	10.1 \pm 13.2 ^b
70% CP	633 \pm 6.4 ^a	45 \pm 10.0 ^b	217 \pm 5.8 ^b	17.2 \pm 24.8 ^a
P-value	0.006	0.004	0.01	0.0001

¹ Values are means \pm SEM, $n = 5-7$ birds. Means within columns with different superscripts differ, $P < 0.05$.

Water intake, behavior and general health. Water intake (Table 2) was significantly greater in birds fed 70 or 11% CP compared with those fed 20 or 35% CP ($P < 0.05$). The birds were frequently observed for indices of general health, including posture, feather positioning and integrity, mobility, feed wastage and consistency of droppings. No diet-related changes in these variables were noted. There was no mortality with the exception of one bird fed 11% CP, which died on d 315 of unknown causes. Necropsy revealed no signs of inflammation, renal dysfunction or other pathology in this bird.

Enzyme activity. The effect of dietary protein level on the activity of liver and kidney enzymes is shown in Table 3. In the liver, glucokinase activity was significantly decreased at dietary protein concentrations $>11\%$ CP ($P < 0.05$). PEPCK activity was not affected in the liver, but was significantly greater in the kidney of birds fed 70% CP compared with those fed 11% CP ($P < 0.05$). Liver and kidney ALT and AST activities generally increased with increasing level of dietary CP, and the difference was significant ($P < 0.05$) between 70% CP and the lower levels. Arginase activity was significantly greater in birds fed 70% CP compared with those fed 11% CP in the liver, and compared with those fed all dietary protein levels in the kidney ($P < 0.05$). In addition, kidney arginase activity was greater in birds fed 35% CP compared with those fed 11 or 20% CP ($P < 0.05$).

Blood chemistry. Serum uric acid concentrations increased linearly with dietary protein levels ($P < 0.001$; $r^2 = 0.60$). Uric acid was significantly greater in birds fed 70% CP ($P < 0.05$) compared with 11, 20 or 35% CP (Fig. 2A). In addition, serum urea concentrations increased linearly with dietary protein levels ($P < 0.0002$; $r^2 = 0.68$). Urea was significantly increased in birds fed 70% CP ($P < 0.05$) compared with all other dietary treatments (Fig. 2B).

Dietary protein concentration affected serum amino acid concentration in several instances. Serum aspartate, cysteine, leucine, methionine and total essential amino acids (TEAA) were significantly increased in birds fed 70% CP compared with all other diets ($P < 0.05$) (data not shown). In addition, serum valine was significantly increased in birds fed 70 and 35% CP compared with 11 and 20% CP; serum methionine was significantly increased with 35% CP compared with 11 and 20% CP; TEAA were significantly increased with 35% CP compared with 11% CP ($P < 0.05$). In contrast, serum phenylalanine was significantly decreased with 70% CP compared with all other dietary treatments ($P < 0.05$). Total nonessential amino acids (TNEAA) were not affected by dietary CP level. Mean serum amino acid values ($\mu\text{mol/L}$) \pm SEM for birds fed 11% CP were as follows: Asp = 48 ± 6 ; Ser = 177 ± 41 ;

TABLE 3

Effect of feeding different crude protein (CP) levels for 11 mo on hepatic and renal enzyme activities of adult male cockatiels at maintenance¹

Diet	Liver					Kidney				
	GK ²	PEPCK	ALT	AST	ARG	PEPCK	ALT	AST	ARG	
	<i>μmol substrate/(mg protein · min)</i>									
11% CP	62.3 ± 3.0 ^a	33.2 ± 5.0	55.4 ± 2.6 ^b	211.8 ± 11.3 ^c	0.052 ± 0.01 ^b	13.3 ± 1.7 ^b	33.3 ± 3.5 ^b	160.7 ± 9.9 ^{ab}	0.710 ± 0.07 ^c	
20% CP	51.4 ± 1.4 ^b	35.8 ± 4.3	59.0 ± 1.8 ^b	247.7 ± 12.6 ^{cb}	0.070 ± 0.01 ^{ab}	17.2 ± 2.5 ^{ab}	33.6 ± 3.4 ^b	151.1 ± 11.1 ^b	1.140 ± 0.08 ^c	
35% CP	46.1 ± 1.8 ^b	36.5 ± 5.2	65.7 ± 6.0 ^b	278.7 ± 9.9 ^b	0.058 ± 0.01 ^{ab}	17.3 ± 1.8 ^{ab}	41.3 ± 4.1 ^{ab}	166.3 ± 12.1 ^{ab}	2.230 ± 0.28 ^b	
70% CP	47.4 ± 1.2 ^b	29.4 ± 6.0	98.9 ± 4.1 ^a	349.7 ± 17.3 ^a	0.086 ± 0.01 ^a	20.9 ± 2.8 ^a	49.5 ± 3.2 ^a	187.3 ± 10.2 ^a	2.800 ± 0.16 ^a	
ANOVA										
<i>P</i> -value	0.0001	0.76	0.0001	0.0001	0.09	0.17	0.008	0.13	0.0001	
Regression										
<i>P</i> -value	0.0026	0.437	0.0001	0.0001	0.04	0.038	0.0006	0.025	0.0001	
<i>r</i> ²	0.32	0.025	0.77	0.71	0.16	0.17	0.39	0.19	0.74	

¹ Values are means ± SEM, *n* = 5–7 birds. Means within columns with different superscripts differ, *P* < 0.05.

² Abbreviations: ALT, alanine aminotransferase; ARG, arginase; AST, aspartate aminotransferase; GK: glucokinase; PEPCK: phosphoenolpyruvate carboxykinase.

Glu = 303 ± 21; Gln = 218 ± 24; Pro = 374 ± 58; Gly = 435 ± 30; Ala = 577 ± 43; Tyr = 236 ± 6; Cys = 58 ± 10; Thr = 374 ± 22; Val = 235 ± 12; Met = 75 ± 8; Ile = 192 ± 25; Leu = 373 ± 31; Phe = 144 ± 6; Lys = 464 ± 31; His = 77 ± 17; Arg = 373 ± 17; Trp = 59 ± 5; TEAA = 2366 ± 64; TNEAA = 2417 ± 103.

There were no significant differences in any other blood variables due to dietary treatment. Mean values ± SEM for each were as follows: ammonia (μmol/L) = 89 ± 6; cholesterol (mmol/L) = 5.74 ± 0.09; lactate dehydrogenase (IU/L) = 275 ± 17.4; calcium (mmol/L) = 1.40 ± 0.04; albumin (g/L) = 12 ± 0.2; globulin (g/L) = 9.5 ± 0.1; glucose (mmol/L) = 18.98 ± 0.26; total protein (g/L) = 21 ± 0.3; hematocrit (%) = 41.1 ± 1.0; heterophil (%) = 62.3 ± 0.39; lymphocyte (%) = 36.2 ± 0.45; monocyte (%) = 0.75 ± 0.11; eosinophil (%) = 0.25 ± 0.05; and basophil (%) = 1.7 ± 0.14.

Postmortem and histopathology. No evidence of visceral, articular or renal gout was found in any of the birds at necropsy. One bird fed 70% CP showed breast muscle atrophy and dark intestinal contents with excess gas formation. No other gross lesions were found in any of the treatment groups. There was no evidence of substantive renal pathology due to dietary treatment (Table 4). Sixteen of 25 birds had lesions in the kidneys; these were characterized by infrequent foci of mild interstitial mononuclear inflammatory cell infiltrates, and 12 of these birds also had focal or infrequent multifocal mild tubular dilatation with occasional tubular luminal mineral concretions. Renal changes were considered background changes and not related to dietary protein levels because four of the five birds fed low protein diets had lesions in the kidneys. In general, lesions in the kidneys of most birds were mild when present and likely not clinically important (Fig. 3A).

In contrast, there was a significant increase in liver pathology (*P* < 0.05) for birds consuming 20, 35 or 70% CP compared with 11% CP. Specifically, the severity of lipogranuloma lesions, characterized by sinusoidal and periportal clusters of macrophages with cytoplasmic lipid vacuoles, increased with increasing dietary protein level (*P* < 0.05) (Table 4; Fig. 3 B-D). Single vacuolated cells were intimately associated with the sinusoidal and perisinusoidal space and were likely Ito cells (vitamin A–storing cells, fat storing cells, lipocytes, stellate

cells). Only one bird had hepatocellular micro- and macrovesicular lipidosis.

DISCUSSION

The requirement for dietary protein for cockatiels at maintenance has not been determined experimentally. Therefore, assumptions were made, on the basis of related data, to choose a level of dietary protein that would serve as a low protein control diet [see (22–24)]. Therefore, an 11% CP diet was chosen as the control. A 20% CP diet was included in this experiment because this level is often supplied in commercial diets used for breeding, growth and maintenance. The high dietary protein levels of 35 and 70% CP were chosen to ensure that if there was a limit to up-regulation of enzyme activity or nitrogen excretion in cockatiels, a protein toxicity would be reached.

Excess levels of dietary amino acids cause decreased food intake, muscle deposition and body weight in chickens and rats (25). In the present experiment, body weight and breast muscle size were maintained by 11, 20 and 70% CP, whereas 35% CP caused a significant increase in body weight, accompanied by an increase in whole-body lipid content. These data indicate that levels of CP well above expected maintenance requirements did not cause overt protein toxicity. An increase in fat deposition at high levels of dietary protein (35%) was surprising; lipid accretion may be due to increased energy consumption or to a shift in metabolic priorities. However, the fact that breast muscle weight of these birds was similar to that of the 11 and 20% CP treatment groups argues in favor of increased energy intake and not a change in the partitioning of dietary energy toward fat storage at the expense of other tissue types.

Water intake was significantly greater in birds fed 70 and 11% CP compared with 20 or 35% CP. Additional water for the excretion of nitrogen end products may be responsible for increased water consumption by birds eating 70% CP. In the case of the increase by cockatiels eating 11% CP, an explanation is less clear. However, in growing broiler chickens, levels of dietary protein below NRC recommendations (17% CP) resulted in a significant increase in water intake (26). A

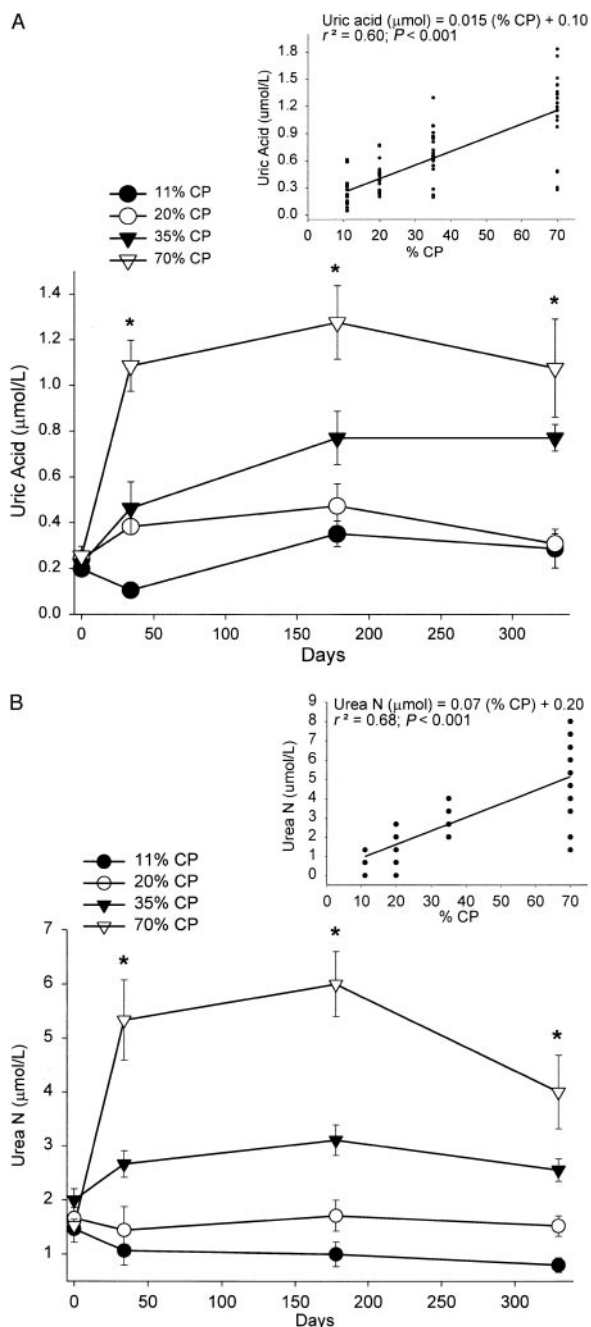


FIGURE 2 Effect of dietary crude protein (CP) level on serum uric acid (panel A) and urea N (panel B) concentrations in adult male cockatiels. Birds were fed diets varying from 11 to 70% CP for 11 mo. Serum uric acid and urea N concentrations were measured on d 0, 34, 178 and 348. Values are means \pm SEM, $n = 5-7$ birds. Inset: Regression of dietary protein level and uric acid or urea levels on d 34, 178, and 348. *Different from all other dietary treatments, $P < 0.05$.

mechanism for this change in water intake has yet to be proposed.

Liver and kidney enzymes involved in amino acid catabolism generally increased with increasing protein level, which has been demonstrated in the omnivorous chicken and quail (4-7,27). These data suggest that cockatiels are able to up-regulate enzymes for amino acid catabolism in a manner similar to that of omnivores. Cockatiels were also able to adapt to low dietary glucose levels as indicated by maintenance of serum glucose and altered activity of kidney PEPCK and liver

glucokinase. Because cockatiels have evolved consuming low protein, high carbohydrate diets, the ability to catabolize high levels of dietary protein and to use amino acids for glucose synthesis could indicate that these activities are retained for other purposes. Increases in the rate of amino acid catabolism and gluconeogenesis are also important in acute starvation and the catabolic response to infection or trauma (28), and it may be that these are the primary reasons for the retention of metabolic plasticity.

Kidney arginase and serum urea concentrations increased with increasing dietary protein. These data are in contrast to work done in growing broiler chickens, which demonstrated that a twofold increase in protein level above dietary requirements did not increase blood urea levels (4). However, in the present experiment, assuming a maintenance CP requirement of $\sim 11\%$, a sixfold increase in dietary protein was tested. This substantial increase may account for differences in urea N data. Traditionally, blood urea is considered to have little clinical importance in avian species; however, it has been suggested that this variable may be useful to detect early renal failure (29). In the case of these cockatiels, high concentrations of blood urea N were not associated with renal pathology, but were correlated with dietary protein concentration.

We observed a marked increase in serum levels of uric acid at 70% CP, indicating that increased uric acid synthesis accompanied increased amino acid catabolism. However, there were no significant differences in uric acid levels among the 11, 20 and 35% CP groups. In breeding parakeets, levels of CP from 13 to 25% did not affect plasma uric acid concentration (24), but higher concentrations of dietary protein were not tested. In chickens, uric acid synthesis increases with increasing dietary CP (30), and plasma uric acid concentrations of chickens fed a similarly high CP diet (31) are elevated to the same extent that we observed in cockatiels. In both chickens and cockatiels fed very high protein diets, uric acid synthesis appears to keep pace with amino acid catabolism because blood ammonia levels do not rise.

In avian clinical medicine, uric acid concentration is considered to be indicative of renal function (29). Further, many avian species presenting with high plasma uric acid levels are diagnosed with gout and renal dysfunction. The cause of these pathologies is often attributed to protein toxicity (32). However, renal sections in the present experiment showed no evidence of pathology at either the gross or the histologic level, which suggests that high uric acid concentrations may be indicative of dietary protein concentration and not renal damage. In chickens, gout seems to be associated primarily

TABLE 4

Effect of feeding different crude protein (CP) levels for 11 mo on liver and kidney lesion severity in adult male cockatiels at maintenance^{1,2}

Diet	Kidney lesion severity	Liver lesion severity	Liver lipogranuloma severity
11% CP	1.8 \pm 0.58	1.0 \pm 0.00 ^b	0.8 \pm 0.20 ^b
20% CP	1.0 \pm 0.44	2.6 \pm 0.30 ^a	1.4 \pm 0.20 ^{ab}
35% CP	1.0 \pm 0.52	2.8 \pm 0.54 ^a	2.3 \pm 0.56 ^a
70% CP	1.0 \pm 0.31	3.0 \pm 0.31 ^a	2.0 \pm 0.38 ^a
P-value	0.58	0.005	0.05

¹ Values are means \pm SEM, $n = 5-7$ birds. Means within columns with different superscripts differ, $P < 0.05$.

² All lesions were scored on a 5-point scale, with 1 being least severe and 5 being most severe.

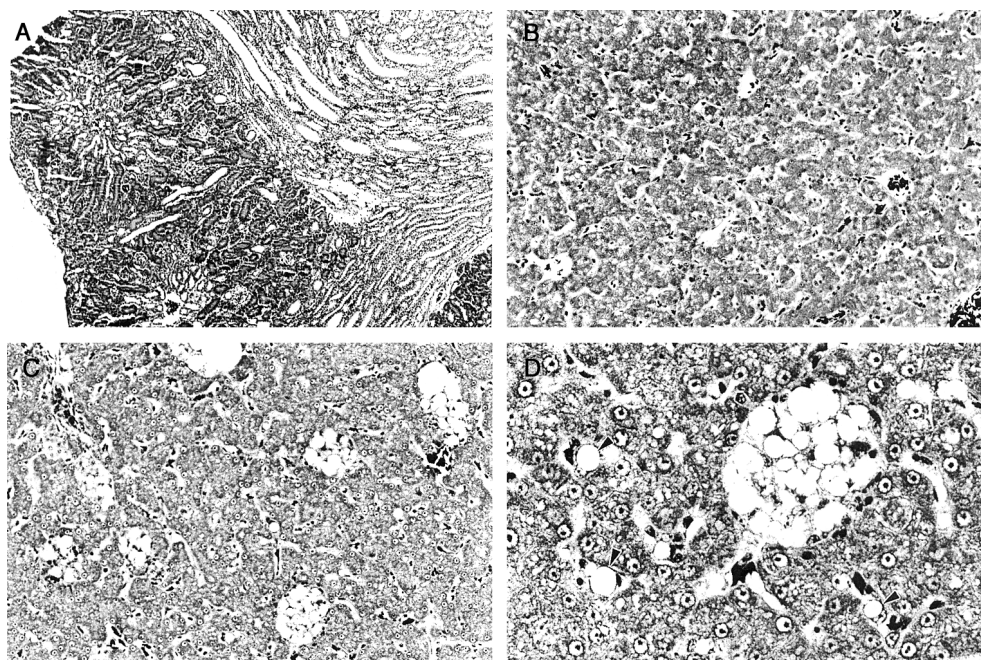


FIGURE 3 Effect of dietary crude protein (CP) level on renal and hepatic histopathology in adult male cockatiels. Birds were fed diets varying from 11 to 70% CP for 11 mo. Liver, kidney and pericardium were taken at necropsy at the termination of the experiment and processed routinely for histologic examination. (A) Kidney section (X20 magnification) from a cockatiel that consumed 70% CP for 11 mo, with no evidence of pathology. (B) Liver section (X20 magnification) from a cockatiel that consumed 11% CP for 11 mo, with no evidence of pathology. (C) Liver section (X20 magnification) from a cockatiel that consumed 70% CP for 11 mo, with multiple lipogranulomas. (D) Liver section (X40 magnification of Fig. 3C) with single vacuolated cell in the sinusoidal/perisinusoidal space (arrows).

with a genetic predisposition for the condition; although high dietary protein aggravates the pathology of the disease, it does not cause it (33–34). These data indicate that susceptibility to gout is genetic, rather than a result of dietary protein levels.

In contrast to low lesion severity in kidneys, liver samples had increasing lesion severity with increasing dietary protein, and lesions were associated with an increased incidence of lipogranulomas. Rates of amino acid deamination and uric acid production are higher in liver than in kidney (35). If rates of deamination exceeded the capacity of uric acid synthesis in the liver, locally high levels of ammonia might have induced the observed pathology. In humans, hyperammonemia due to a variety of genetic disorders causes aberrant hepatic lipid storage (36). However, we did not observe hyperammonemia, and it is not clear whether the changes in liver histology should be considered an indication of protein toxicity. Serum chemistry values indicative of liver function (albumin, total protein) were within normal limits, indicating no severe functional outcome of the lipidosis. Further research is warranted to evaluate the incidence of liver lipogranulomas on the basis of dietary protein level.

Finally, the serum concentrations of several amino acids were affected by dietary crude protein level. In chickens fed an isolated soy protein-based diet containing 21 or 64% CP, plasma amino acids were altered in a manner similar to that seen in the present experiment (31). Interestingly, dietary protein levels did not affect serum arginine concentrations, and this lack of change can be attributed to the nearly three-fold increase in activity of kidney arginase. This evidence indicates that the granivorous cockatiel is as capable of disposing of surfeit amino acids as the omnivorous chicken.

On the basis of the data, it seems that cockatiels, a granivorous avian species, are quite capable of adapting to high dietary protein concentrations. To do so, these birds change activity of enzymes for amino acid catabolism, as well as rates of gluconeogenesis. In addition, at high dietary protein concentrations, cockatiels increase uric acid production to excrete excess nitrogen. These data suggest that unlike the lack of metabolic plasticity in faunivorous species, granivores that have evolved consuming low protein diets are able to adapt to changing protein concentration.

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LITERATURE CITED

1. Diamond, J. M. (1991) Evolutionary design of intestinal nutrient absorption: enough but not too much. *News Physiol. Sci.* 6: 92–96.
2. Smith, R. H. (1980) Comparative amino acid requirements. *Proc. Nutr. Soc.* 39: 71–78.
3. Baker, D. H. & Speer, V. C. (1983) Protein-amino nutrition of nonruminant animals with emphasis on the pig: past, present and future. *J. Anim. Sci.* 57 (suppl. 2): 284–299.
4. Chandra, M., Singh, B., Soni, G. L. & Ahuja, S. P. (1984) Renal and biochemical changes produced in broilers by high-protein, high-calcium, urea-containing and vitamin A-deficient diets. *Avian Dis.* 28: 1–11.
5. Featherston, W. R. & Freedland, R. A. (1973) Influence of dietary protein and carbohydrate levels on liver enzyme activities in quail. *J. Nutr.* 103: 625–634.
6. Krebs, H. A. (1972) Some aspects of the regulation of fuel supply in omnivorous animals. *Adv. Enzyme Regul.* 10: 397–420.
7. Wergedal, J. E. & Harper, A. E. (1964) Metabolic adaptations in higher animals: IX. Effect of high protein intake on amino acid nitrogen catabolism *in vivo*. *J. Biol. Chem.* 239: 1156–1163.
8. Coulson, R. A. & Hernandez, T. (1983) *Alligator Metabolism. Studies on Chemical Reactions In Vivo*. Pergamon Press, Oxford, UK.
9. Cowey, C. B., Cooke, D. J., Matty, A. J. & Adron, J. W. (1981) Effects of quantity and quality of dietary protein on certain enzyme activities in rainbow trout. *J. Nutr.* 111: 336–345.
10. Migliorini, R. H., Linder, C., Moura, J. L. & Veiga, J. A. (1973) Gluconeogenesis in a carnivorous bird (black vulture). *Am. J. Physiol.* 225: 1389–1392.
11. Myers, M. R. & Klasing, K. C. (1999) Low glucokinase activity and high rates of gluconeogenesis contribute to hyperglycemia in barn owls (*Tyto alba*) after a glucose challenge. *J. Nutr.* 129: 1896–1904.
12. Veiga, J. A., Roselino, E. S. & Migliorini, R. H. (1978) Fasting, adrenalectomy, and gluconeogenesis in the chicken and a carnivorous bird. *Am. J. Physiol.* 234: R115–R121.
13. Klasing, K. C. (1998) *Comparative Avian Nutrition*. CAB International, Wallingford, UK.
14. Jones, D. (1987) Feeding ecology of the cockatiel, *Nymphicus hollandicus*, in a grain-growing area. *Aust. Wildl. Res.* 14: 105–115.
15. Association of Official Analytical Chemists (1975) *Official Methods of Analysis*. AOAC, Washington, DC.
16. Bidlingmeyer, B. A., Cohen, S. A. & Tarvin, T. L. (1984) Rapid analysis of amino acids using pre-column derivatization. *J. Chromatogr.* 336: 93–104.

17. Dipietro, D. L. & Weinhouse, S. (1960) Hepatic glucokinase in the fed, fasted, and alloxan-diabetic rat. *J. Biol. Chem.* 235: 2542-2545.
18. Segal, H. L. & Matsuzawa, T. (1970) L-Alanine aminotransferase (rat liver). *Methods Enzymol.* 17A: 153-159.
19. Tamir, H. & Ratner, S. (1963) Enzymes of arginine metabolism in chicks. *Arch. Biochem. Biophys.* 102: 249-255.
20. Opie, L. H. & Newsholme, E. A. (1967) The activities of fructose 1,6-diphosphatase, phosphofructokinase, and phosphoenolpyruvate carboxylase in white and red muscle. *Biochem. J.* 103: 391-398.
21. Gill, J. L. (1978) *Design and Analysis of Experiments in the Animal and Medical Sciences.* University Press, Ames, Iowa.
22. Roudybush, T. E. & Grau, C. R. (1986) Food and water interrelations and the protein requirement for growth of an altricial bird, the cockatiel (*Nymphicus hollandicus*). *J. Nutr.* 116: 552-559.
23. Murphy, M. E. (1993) The protein requirement for maintenance in the white-crowned sparrow, *Zonotrichia-Leucophrys-Gambelii*. *Can. J. Zool. Rev. Can. Zool.* 71: 2111-2120.
24. Angel, R. & Ballam, G. (1995) Dietary effect on parakeet plasma uric acid, reproduction and growth. *Proc. Assoc. Avian Vet.* 27-32.
25. Harper, A. E., Benevenga, N. J. & Wohlhueter, R. M. (1970) Effects of ingestion of disproportionate amounts of amino acids. *Physiol. Rev.* 50: 428-471.
26. Marks, H. L. & Pesti, G. M. (1984) The roles of protein level and diet form in water consumption and abdominal fat pad deposition of broilers. *Poult. Sci.* 63: 1617-1623.
27. Davis, A. J. & Austic, R. E. (1997) Dietary protein and amino acid levels alter threonine dehydrogenase activity in hepatic mitochondria of *Gallus domesticus*. *J. Nutr.* 127: 738-744.
28. Klasing, K. C. (1997) Interactions between nutrition and infectious disease. In: *Diseases of Poultry* (Calnek, B.W., ed.). Iowa State University Press, Ames, IA.
29. Lumeij, J. T. (1988) Avian clinical pathology: some experimental findings of importance to the practitioner. *Proc. Assoc. Avian Vet.* 79-86.
30. Wiggins, D., Lund, P. & Krebs, H. A. (1982) Adaptation of urate synthesis in chicken liver. *Comp. Biochem. Phys. B Comp. Biochem.* 72: 565-568.
31. Featherston, W. R. (1969) Nitrogenous metabolites in the plasma of chicks adapted to high protein diets. *Poult. Sci.* 48: 646-652.
32. Estrom, D. & Degernes, L. (1989) Avian gout. In: *Proceedings of the 1989 Annual Conference of the Association of Avian Veterinarians.* Lake Worth.
33. Cole, R. K. & Austic, R. E. (1980) Hereditary uricemia and articular gout in chickens. *Poult. Sci.* 59: 951-960.
34. Peterson, D. W., Hamilton, W. H. & Lilyblade, A. L. (1971) Hereditary susceptibility to dietary induction of gout in selected lines of chickens. *J. Nutr.* 101: 347-354.
35. Chou, S. T. (1972) Relative importance of liver and kidney in synthesis of uric acid in chickens. *Can. J. Physiol. Pharm.* 50: 936-939.
36. Badizadegan, K., & Perez-Atayde, A. R. (1997) Focal glycogenosis of the liver in disorders of ureagenesis: its occurrence and diagnostic significance. *Hepatology* 26: 365-373.