Crude protein and supplemental dietary tryptophan effects on growth and tissue neurotransmitter levels in the broiler chicken*

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Indian River male broiler chickens growing from 7 to 28 d of age were fed on diets containing 120, 210 and 300 g crude protein/kg diet and 0, 1·67 or 16·7 g added tryptophan (TRP)/kg diet. The hypothesis tested was that crude protein levels and TRP would affect both growth and neurotransmitter metabolism. Heart, brain and pancreatic neurotransmitter (noradrenaline (NA), dopamine (DA), serotonin (5-HT) and 5-hydroxy-indole-3-acetic acid (5-HIAA)) concentrations were determined by HPLC separation and electrochemical detection. Malate dehydrogenase (2-oxoglutarate decarboxylating) (NADP+) (MDH(NADP+); *EC* 1.1.1.40), isocitrate dehydrogenase (NADP+) (ICD(NADP+); *EC* 1.1.1.42) and aspartate aminotransferase (AAT; *EC* 2.6.1.1) activities were also measured. Supplemental TRP decreased growth and feed intake. Increasing dietary crude protein decreased MDH(NADP+), but increased (ICD(NADP+) and AAT activities. Additional dietary TRP decreased MDH(NADP+) activity, but had no effect on other enzyme activities. Cardiac NA concentrations were directly related to dietary crude protein levels while pancreatic levels were inversely related. An increase in dietary crude protein decreased both brain NA and DA. Supplemental dietary TRP increased both 5-HIAA and 5-HT. Changes in feed intake caused by different levels of both dietary crude protein and TRP are accompanied by altered levels of neurotransmitters. The present study indicates that much larger amounts of TRP are required to make simultaneous changes in feed intake and neurotransmitters.

Protein: Tryptophan: Neurotransmitters: Chicken

The central nervous system (CNS) of rats responds to changes in dietary regimens and regulates eating behaviour (Young & Landsberg, 1977*a*). The addition of both carbohydrates and fats to diets without changing the total energy level activates the sympathetic nervous system (Young & Landsberg, 1977*b*; Schwartz *et al.* 1983). Later studies suggested that substitution of either sucrose or glucose for dietary protein would also stimulate neural activity (Kaufman *et al.* 1984; Vander Tuig & Romsos, 1984; Young *et al.* 1985). A more recent study indicated that dietary energy components might also change the rates of dopamine (DA) and noradrenaline (NA) turnover in rats (Kaufman *et al.* 1989). The addition of casein raised urinary DA excretion while carbohydrate or fat supplementation decreased urinary DA.

A relationship also exists between consumption of protein and carbohydrate and the synthesis of serotonin (5-hydroxytryptamine; 5-HT) by the brain (Fernstrom, 1983). Serotonin is synthesized from the essential amino acid L-tryptophan (TRP) and may regulate feed intake in rats (Nielsen *et al.* 1992). The initial step involves the hydroxylation

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of TRP to form 5-hydroxytryptophan (5-HTP), which is catalysed by TRP hydroxylase (*EC* 1.14.16.4) (Fernstrom, 1979). The major 5-HT metabolite in the brain is 5-hydroxyindole-3-acetic acid (5-HIAA) and its relationship to 5-HT is indicative of 5-HT turnover (Colmenares *et al.* 1975).

There is a shortage of information concerning the effect of dietary components on the metabolism of the CNS in birds. Smith & Waldroup (1988) found no effect of TRP on growth variables. It should be noted that this experiment was designed primarily to determine a dietary requirement of TRP for growth and may not have been optimal for studying CNS responses. In a later study, Denbow *et al.* (1993) reported that supplemental TRP increased brain 5-HT, 5-HIAA and NA concentrations in young turkeys without affecting body-weight gain, feed efficiency or feed intake. In the latter study the levels of supplemental TRP were only slightly greater than those used in the former study (1–3 g/kg diet). It could be deduced from these two studies that small additions of TRP to diets will probably have little or no effect on feed intake even though additions change brain biogenic amines.

In contrast to the above feeding experiments that changed brain biogenic amines without altering feed intake, biogenic amines injected into chicken brains have been shown to change feeding responses, depending on the site of injection. For example, injection of NA into the preoptic and paraventricular areas of the brain increased feed intake, while injection of NA into other areas of the brain decreased feed intake (Denbow & Sheppard, 1993). An intracerebroventricular injection of 5-HT decreased feed intake in birds that had been fasted for 24 h. Moreover, applying this protocol to lines of birds selected for rapid body-weight gain revealed that selection for weight gain also sensitized birds to the inhibitory effect of 5-HT on feed intake (Lacy *et al.* 1987). At least in rats, it can be shown that 5-HT antagonizes NA-stimulated feeding by reducing meal size and frequency (Leibowitz & Shor-Posner, 1986). It has not been shown that birds selected for rapid weight gain have an enhanced noradrenergic-mediated appetite control mechanism that would account for results in the Lacy *et al.* (1987) study. A previous study by this group (Denbow, 1983) did reveal that feeding responses of egg-type chickens following the injection of biogenic amines were different from those of broiler-type chickens.

We have reported several times that broiler chickens eat less of a diet containing 120 g crude protein/kg diet than of one containing 180 g crude protein/kg (Rosebrough & Steele, 1985; Rosebrough *et al.* 1992; Rosebrough & McMurtry, 1993). Birds fed on diets containing low levels of crude protein also grow poorly and are proportionately fatter than are birds fed on diets containing higher levels of crude protein. In this respect, feeding a diet containing a low level of crude protein produces bird that is metabolically similar to the egg-laying bird. Might it also be possible that tissue biogenic amine concentrations would also be similar to those in the egg-laying bird? The present experiment was designed to test the effects of crude protein and TRP on growth and neurotransmitter metabolism. The activities of certain enzymes involved in the control of intermediary metabolism were also monitored to assess the effects of TRP on pathways distinct from the CNS.

MATERIALS AND METHODS

Animals

Male, Indian River broiler chicks growing from 7 to 28 d of age were assigned to one of nine dietary treatments. These treatments consisted of three levels of crude protein (120, 210 and 300 g/kg) and three levels of added TRP (0, 1·67 or 16·7 g/kg diet) arrayed in a 3×3 factorial arrangement. Some characteristics of the diets are described in Tables 1 and 2. The chickens were housed in battery-brooders in an environmentally controlled room

Table 1. *Composition of the basal diets (g/kg diet)*

	Dietary crude protein (N × 6·25) (g/kg protein)		
	120	210	300
Ingredient			
Soyabean meal	100	325	550
Maize meal	790	552	315
Maize oil	30	40	50
Sand	20	20	20
Dicalcium phosphate	40	40	40
Limestone	10	10	10
L-Methionine*	—	2·5	5
Selenium premix†	1	1	1
Mineral premix‡	1	1	1
Vitamin premix§	5	5	5
Iodized salt	3	3	3
Calculated composition			
Metabolizable energy (MJ/kg)	13·5	12·8	12·1
Lysine (g/kg)	6·0	11·4	16·8
Sulphur amino acids (g/kg)	10·3	11·3	12·3

* L-Methionine (18915), US Biochemicals, PO Box 22400, Cleveland, Ohio 44122, USA.
† Provided 0·2 mg Se/kg diet.
‡ Provided (mg/kg diet): manganese 100, iron 100, copper 10, cobalt 1, iodine 1, zinc 100 and calcium 89.
§ Provided (mg/kg diet): retinol 3·6, cholecalciferol 0·075, biotin 1, α-tocopherylacetate 10, riboflavin 10, pantothenic acid 20, choline 2 g, niacin 100, thiamine 10, pyridoxine 10, menadione sodium bisulphite 1·5, cyanocobalamin 0·1, pteroylmonoglutamic acid 2 and ethoxyquin 150.

Table 2. *Calculated composition of the diets*

CP (g/kg diet)	Added TRP (g/kg diet)	LNAA* (g/kg diet)	TRP:LNAA	Total TRP (g/kg diet)
120	0	32·6	0·038	1·24
120	1·7	32·8	0·089	2·91
120	16·7	32·8	0·549	17·91
210	0	53·8	0·045	2·43
210	1·7	53·8	0·076	4·09
210	16·7	53·8	0·355	19·09
300	0	73·7	0·048	3·57
300	1·7	73·7	0·070	5·24
300	16·7	73·7	0·274	20·24

CP, crude protein; TRP, tryptophan; LNAA, large neutral amino acids.
* Tyrosine, phenylalanine, leucine, isoleucine and valine.

maintained at 23° with a 12 h light–dark cycle (06.00–18.00 hours light). Treatments were randomly assigned to pens in each battery. The experiment was repeated twice for a total of eight pens of six chickens each for each dietary treatment. Both feed and water were apportioned on an *ad libitum* basis. At 28 d of age, one bird from each pen was randomly selected and killed. In the first repetition, livers were removed into liquid N_2 and stored at −70°. In the second repetition, brains, hearts, livers and pancreata were removed into liquid N_2 and stored at −70°.

Neurotransmitters

Organs from the second experimental repetition were homogenized in 0·1 M-$HClO_4$ (PCA) (1:10, w/v) and centrifuged at 12000 *g* for 15 min. For NA and DA analyses, a 2 ml portion of the resultant supernatant fraction was added to 2 ml 1·5 M-Tris–50 mM-EDTA (pH 8·6) containing 100 mg acid-washed alumina (Anton & Sayre, 1962). Dihydroxybenzylamine (DHBA; 5 ng) was added to each sample as an internal standard. Then, each sample was mixed for 2 min to trap catechols on the alumina. The alumina was then washed five times with 10 mM-Tris and catechols were then eluted with 1 ml 0·1 M-PCA.

Analyses of catecholamines were performed by modifications of the method of Eriksson & Persson (1982). The chromatography system consisted of a pump (PM-48: Bioanalytical Systems, West Lafayette, IN, USA), a reverse-phase column (100 × 3·2 mm, ODS Phase II: Bioanalytical Systems) and a glassy-carbon amperometric detector (LC-4B: Bioanalytical Systems). The mobile phase was a monochloroacetate buffer (150 mM) containing 1 mM-EDTA and 1 mM-sodium octyl sulphate at pH 3·0. The organic modifier was acetonitrile (50 ml/l). The electrochemical detectors were set at +0·65 V *v.* Ag/AgCl reference electrode with ranges of 5 and 50 nA for NA, DHBA and DA.

Analyses of indolamines were achieved by injecting portions of the PCA extract into the HPLC without any intermediary trapping step. The mobile phase was a monochloroacetate buffer (150 mM) containing 1 mM-EDTA and 1 mM-sodium octyl sulphate at pH 3·0. The organic modifiers were acetonitrile (40 ml/l) and tetrahydrofuran (10 ml/l) respectively. The detectors were set at +0·80 V and +0·65 V *v.* a Ag/AgCl reference electrode and 5 and 10 nA for 5-HT and 5-HIAA.

Enzyme assays

Livers from both experimental repetitions were homogenized (1:10, w/v) in 50 mM-HEPES (pH 7·5)–3·3 mM-β-mercaptoethanol and centrifuged at 50000 *g* for 60 min (Rosebrough & Steele, 1985). The supernatant fractions were kept at 0° until analysed for the activities of malate dehydrogenase ((oxaloacetate decarboxylating) (NADP+)) (MDH(NADP+); *EC* 1.1.1.40), isocitrate dehydrogenase (NADP+) (ICD(NADP+); *EC* 1.1.1.42) and aspartate aminotransferase (AAT; *EC* 2.6.1.1).

MDH(NADP+) was determined by a modification of the method of Hsu & Lardy (1969). The reaction contained 50 mM-HEPES (pH 7·5), 1 mM-NADP, 5 mM-$MnCl_2$ and the substrate, 2·2 mM-L-malate (disodium salt) in a total volume of 1 ml. A 50 μl portion of the 50000 *g* supernatant fraction (diluted 1:10) was incubated for 5 min in the presence of the first three ingredients. The reaction was initiated by adding the substrate and following the rate of reduction of NADP at 340 nm at 25°. The reaction rate was linear for at least 60 min providing that the reaction contained no more than 100 μg supernatant protein.

ICD(NADP+) activity was determined by a modification of the method of Cleland *et al.* (1969). The reaction contained 50 mM-HEPES (pH 7·5), 1 mM-NADP, 5 mM-$MnCl_2$ and the substrate, 4·4 mM-DL-isocitrate in a total volume of 1 ml. A 50 μl portion of the 50000 *g* supernatant fraction (diluted 1:10) was incubated for 5 min in the presence of the first three ingredients. The reaction was initiated by adding the substrate and following the rate of reduction of NADP at 340 nm at 25°. The reaction rate was linear for at least 60 min providing that the reaction contained no more than 50 μg supernatant protein.

AAT was determined by a modification of the method of Martin & Herbein (1976). The reaction contained 50 mM-HEPES, 200 mM-L-aspartate, 0·2 mM-NADH, 1000 units/l malate: NAD+ oxidoreductase (*EC* 1.1.1.37) and the substrate, 15 mM-2-oxoglutarate in a total volume of 1 ml. A 25 μl portion of the 50000 *g* supernatant fraction (diluted 1:10)

was incubated for 5 min in the presence of the first four ingredients. The reaction was initiated by adding the substrate and following the rate of oxidation of NADH at 340 nm at 25°. The reaction rate was linear for at least 30 min providing that the reaction contained no more than 50 μg supernatant protein. Enzyme activities are expressed as μmol product formed/min under the assay conditions (Rosebrough & Steele, 1985).

Statistical analyses

The experimental design was considered as 3×3 factorial arrangement of treatments, with TRP and crude protein being the main treatments. Each treatment was replicated four times. The replicate was a pen of six birds. The experiment was also repeated twice for a total of seventy-two pens and 432 birds. Observations for body-weight changes and feed consumption were derived from data for an entire pen of birds for both repetitions (n 8). Observations for enzyme activities were derived from data for one bird randomly selected from each pen for both experimental repetitions (n 8). In the case of neurotransmitter data this observation was derived from one bird per pen for the second repetition of the experiment (n 4). Data were analysed by a two-way ANOVA (Kirk, 1968). The main treatment effects of crude protein level and TRP and their interaction were tested against the residual.

RESULTS

Growth and feed consumption

Table 3 summarizes the effects of dietary crude protein and added TRP on broiler growth and feed consumption. Data are based on total feed consumption and weight gain per pen of birds divided by the number of birds in the respective pens. Birds consuming the diets containing 120 g crude protein were smaller than birds consuming either of the other two levels of dietary crude protein ($P < 0{\cdot}0001$). When analysing the effect of added TRP, it was noted that the addition of 16·7 g TRP/kg diet resulted in smaller birds than did the other levels of TRP ($P < 0{\cdot}001$). The interaction between crude protein and TRP was not statistically significant ($P = 0{\cdot}500$). The same dietary effects were also noted for feed consumption as well as efficiency of feed utilization.

Hepatic enzyme activities

There was a significant effect of dietary crude protein on the activities of all three enzymes (Table 4; $P < 0{\cdot}001$). An increase in crude protein decreased MDH(NADP+) enzyme activity ($P = 0{\cdot}000$) while an increase in TRP level decreased enzyme activity ($P = 0{\cdot}001$). It should be noted that a significant crude protein $\times$ TRP interaction may complicate interpretation of these findings. In contrast, an increase in crude protein increased both ICD(NADP+) and AAT activities ($P = 0{\cdot}000$). Dietary TRP levels did not affect the activities of either ICD(NADP+) or AAT ($P = 0{\cdot}245$ and $0{\cdot}092$ respectively).

Brain neurotransmitters

The effects of both dietary crude protein and TRP levels on brain neurochemical levels are presented in Table 5. An increase in dietary crude protein decreased both brain NA and DA ($P = 0{\cdot}006$ and $0{\cdot}014$ respectively). The addition of TRP to the diets had no effect on either NA or DA ($P = 0{\cdot}077$ and $0{\cdot}887$ respectively). The DA:NA ratio was not affected by either crude protein or TRP. Although both crude protein and TRP influenced 5-HT, these main treatment effects were confounded by a crude protein $\times$ TRP interaction ($P = 0{\cdot}013$). In contrast, only TRP influenced 5-HIAA ($P = 0{\cdot}000$). Both dietary crude protein and its interaction with TRP had little effect on 5-HIAA ($P = 0{\cdot}661$ and $0{\cdot}368$ respectively). Both crude protein and TRP influenced the 5-HT:5-HIAA ratio ($P = 0{\cdot}013$ and $0{\cdot}000$

Table 3. *Effect of diets containing different levels of crude protein (CP) and added tryptophan (TRP) on growth and feed intake of broiler chickens**

(Mean values with their standard errors for eight pen means per dietary treatment)

CP (g/kg diet)	Added TRP (g/kg diet)	Body weight (g)		Feed intake (g)		F/G†	
		Mean	SE	Mean	SE	Mean	SE
120	0	651	28·9	1147	24·4	2·25	0·12
120	1·7	636	45·3	1103	22·8	2·37	0·25
120	16·7	458	38·9	887	72·3	2·90	0·31
210	0	975	21·8	1384	50·0	1·65	0·06
210	1·7	964	16·3	1312	51·2	1·58	0·08
210	16·7	789	56·1	1291	24·2	2·12	0·02
300	0	956	32·8	1391	25·9	1·72	0·02
300	1·7	911	32·3	1365	30·7	1·78	0·02
300	16·7	843	39·8	1312	39·0	1·88	0·02
ANOVA							
CP ($P=$)		0·000		0·000		0·000	
TRP ($P=$)		0·000		0·000		0·001	
TRP × CP ($P=$)		0·500		0·069		0·435	

* For details of diets and procedures, see Table 1 and pp. 88–89.

† Feed intake over 21 d (g)/body-weight gain over 21 d (g).

Table 4. *Effect of diets containing different levels of crude protein (CP) and added tryptophan (TRP) on hepatic enzyme activities (units/g liver*) of broiler chickens†*

(Mean values with their standard errors for eight pen means per dietary treatment)

CP (g/kg diet)	Added TRP (g/kg diet)	MDH(NADP+)		ICD(NADP+)		AAT	
		Mean	SE	Mean	SE	Mean	SE
120	0	28·5	1·23	25·5	2·01	55·8	2·90
120	1·7	31·4	0·88	33·1	3·48	52·2	2·32
120	16·7	25·3	1·06	29·8	3·44	49·8	3·73
210	0	17·0	2·96	41·1	3·44	83·4	7·51
210	1·7	14·1	1·22	34·8	3·98	9·3	9·07
210	16·7	11·4	0·93	37·2	5·24	70·4	10·07
300	0	4·8	0·62	63·5	5·96	105·3	8·33
300	1·7	5·1	1·05	43·8	5·17	77·7	9·91
300	16·7	4·1	0·72	48·4	3·74	84·8	9·42
ANOVA							
CP ($P=$)		0·000		0·000		0·000	
TRP ($P=$)		0·001		0·244		0·092	
TRP × CP ($P=$)		0·017		0·060		0·069	

MDH(NADP+), malate dehydrogenase (oxaloacetate decarboxylating) (NADP+) (*EC* 1.1.1.40), ICD(NADP+) isocitrate dehydrogenase (NADP+) (*EC* 1.1.1.42), AAT, aspartate aminotransferase (*EC* 2.6.1.1).

* One unit is that amount of enzyme resulting in the production of 1 μmol oxidized or reduced NAD(P)/min at 25°.

† For details of diets and procedures, see Table 1 and pp. 88–91.

Table 5. *Effect of diets containing different levels of crude protein (CP) and added tryptophan (TRP) on brain noradrenaline (NA), dopamine (DA) serotonin (5-HT) and 5-hydroxy-indole-3-acetic acid (5-HIAA) concentrations in broiler chickens**

(Mean values with their standard errors for four observations per dietary treatment, expressed as ng/g tissue)

CP (g/kg diet)	Added TRP (g/kg diet)	NA		DA		DA/NA		5-HT		5-HIAA		5-HT:5-HIAA	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
120	0	398	8·3	446	34·8	1·11	0·08	594	29·9	225	15·6	2·69	0·07
120	1·7	366	29·6	398	30·6	1·11	0·04	536	39·8	237	30·6	2·49	0·18
120	16·7	409	15·8	451	30·5	1·10	0·04	829	44·7	498	37·0	1·72	0·08
210	0	368	18·6	325	9·8	0·91	0·06	447	48·7	224	15·1	2·03	0·08
210	1·7	345	16·0	400	34·2	1·23	0·15	608	40·7	288	43·2	2·36	0·12
210	16·7	401	19·2	373	26·8	0·96	0·09	586	43·7	405	57·6	1·76	0·06
300	0	336	23·7	356	31·0	1·06	0·05	630	59·6	223	39·2	2·87	0·13
300	1·7	352	23·9	357	24·6	1·01	0·03	570	29·8	236	33·1	2·53	0·17
300	16·7	289	28·2	343	29·5	1·22	0·06	633	51·1	416	49·9	1·52	0·09
ANOVA													
CP ($P=$)		0·006		0·014		0·459		0·019		0·661		0·013	
TRP ($P=$)		0·777		0·887		0·325		0·002		0·000		0·000	
TRP × CP ($P=$)		0·088		0·370		0·055		0·013		0·368		0·007	

* For details of diets and procedures, see Table 1 and pp. 88–90.

Table 6. *Effect of diets containing different levels of crude protein (CP) and added tryptophan (TRP) on cardiac and pancreatic noradrenaline (NA) and dopamine (DA) concentrations in broiler chickens**

(Mean values with their standard errors for four pen means per dietary treatment, expressed as ng/g tissue)

CP (g/kg diet)	Added TRP (g/kg diet)	Heart						Pancreas NA	
		NA		DA		DA:NA			
		Mean	SE	Mean	SE	Mean	SE	Mean	SE
120	0	231	9·4	1387	367·1	6·11	1·63	446	21·0
120	1·7	217	33·9	622	269·0	2·54	0·83	481	18·4
120	16·7	311	63·6	1219	150·0	4·45	0·80	430	14·8
210	0	341	72·0	969	325·0	3·40	1·19	328	45·2
210	1·7	363	68·8	1342	342·0	4·08	0·86	322	43·7
210	16·7	322	70·5	1286	251·0	4·33	0·85	266	24·4
300	0	347	59·1	1400	510·0	3·55	1·03	271	29·4
300	1·7	414	70·2	918	498·0	1·97	0·98	260	31·0
300	16·7	386	55·8	855	339·0	2·83	1·44	363	26·0
ANOVA									
CP ($P=$)		0·033		0·304		0·199		0·000	
TRP ($P=$)		0·757		0·296		0·247		0·960	
TRP × CP ($P=$)		0·623		0·307		0·387		0·071	

* For details of diets and procedures, see Table 1 and pp. 88–90.

respectively), but interaction between the two ($P = 0{\cdot}007$) confounds interpretation of main treatment effects.

Heart and pancreas

Table 6 summarizes the effects of both dietary crude protein and TRP on peripheral concentrations of NA and DA. Dietary protein increased ($P = 0{\cdot}033$) cardiac NA, decreased ($P = 0{\cdot}000$) pancreatic NA, but had no effect on cardiac DA ($P = 0{\cdot}304$). Dietary TRP did not affect amines in either hearts or pancreata ($P = 0{\cdot}296$ and $0{\cdot}960$ respectively).

DISCUSSION

The data from the present study illustrate the caution necessary when studying the effect of added TRP on feed intake. Although prior research indicates little or no effect of supplemental dietary TRP on feed intake, little attention has been given to the basal level of crude protein in these studies (Smith & Waldroup, 1988; Denbow *et al.* 1993). In the present study, supplemental TRP decreased feed intake when combined with a low level of crude protein (120 g/kg diet), but not with higher levels (210 and 300 g/kg). It could also be argued that, based on the present study, much higher levels of TRP are required to change feed intake. The highest level of supplementation used in the present study was nearly ten times that used in the studies mentioned previously. Thus, our findings show that TRP supplementation to meet a dietary requirement does not result in changes in neurotransmitter metabolism or feed intake.

The present study shows that changes in hepatic intermediary metabolism are also accompanied by changes in brain catecholamine levels. For example, it was noted that increasing dietary crude protein decreased both brain DA and NA concentrations. Accompanying this reduction was a decrease in MDH(NADP+) and an increase in ICD(NADP+) and AAT activities. Since the activities of these enzymes have been used as indicators of changes in lipid and protein respectively (Rosebrough & McMurtry, 1993), it is possible that a neural-hepatic loop exists to control responses to dietary changes. Pancreatic NA concentrations were inversely related to dietary crude protein. In contrast, cardiac NA concentrations were directly related to dietary crude protein.

The present study, illustrating a relationship between dietary protein intake and cardiac NA levels, agrees with the work of Young *et al.* (1985) who fed rats on two concentrations of dietary protein (70 and 200 g/kg) to study the dynamics of peripheral catecholamine metabolism. They found that cardiac NA concentrations were 15–20% lower in rats fed on a diet containing a low level of crude protein and that NA turnover was nearly twofold greater. Furthermore, urinary NA excretion was 25% greater in the group fed on the low-protein diet. Although tyrosine availability could regulate NA turnover, supplementation of diets with tyrosine did not change turnover rates.

Regretably, these kinds of studies do not hint at the possible mechanisms involved in accelerated catecholamine metabolism in animals fed on low-protein, high-carbohydrate diets. It should be noted that in most studies carbohydrates are substituted for dietary protein sources during diet formulation and that the effect of a high-carbohydrate level cannot always be separated from that of a low protein level. Thus, the argument can always be made that effects are due to an increase in available dietary carbohydrate as well as a decrease in dietary protein. Indeed, Vander Tuig & Romsos (1984) stated that carbohydrate availability, rather than protein *per se* is the dietary variable regulating CNS activity. This statement may not be all encompassing because both protein-free and high-protein diets reduce feed intake. Depressed feed intake of rats fed on either of these regimens is accompanied by changes in free indispensable amino acids but is not accompanied by any changes in either 5-HT or 5-HIAA. In addition, diets containing wide variations in amino

acid quality and balance produce a competition among amino acids for uptake into the brain. This competition results in changes in feeding behaviour but does not change either 5-HT or 5-HIAA (Tackman *et al.* 1990).

Denbow (1983) and Denbow & Sheppard (1993) extended these findings to birds by showing that NA increased feed intake when injected into the ventromedial or paraventricular nucleii. The latter site in rat brain was previously shown to be sensitive to NA (Leibowitz, 1978; Leibowitz *et al.* 1981), possibly through occupancy of α_2-noradrenergic receptors (Goldman *et al.* 1985). Although NA-stimulated feeding behaviour in rats was attenuated by 5-HT (Leibowitz & Shor-Posner, 1986), the role of 5-HT in birds is not well understood. For example, Denbow *et al.* (1993) reported that even though supplemental dietary TRP increased brain NA, 5-HIAA and 5-HT concentrations, there was no effect on either growth or feed intake.

It is possible that feed intake of chickens fed on diets containing very low levels of crude protein may be affected by TRP through the influence of 5-HT. The value to the chicken of increased NA as a function of low-protein diets probably lies in the ability of these kinds of diets to stimulate fat synthesis. If pancreatic glucagon can be shown to be under the influence of the CNS, the lipolytic characteristics of this hormone may be a natural feedback on the production of excess fat in chickens fed on high-carbohydrate diets. Unlike mammalian liver tissue, the avian liver may not respond to the hormone insulin but is more responsive to the catecholamine family of hormones.

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