Protein and energy relations in the broiler chicken

Chronic or acute effects of alternating protein or intermittent feeding regimens on broiler lipid metabolism*

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1. Broiler chickens growing from 7 to 28 d of age were given: (1) a 210 g protein/kg control diet for the entire experimental period, (2) an intermittent feeding regimen (210 g protein/kg diet for either 1 or 2 d followed by a 1 d fast), or (3) a daily change in the dietary protein level from 120 to 300 g/kg diet. Treatment variables examined were lipogenesis and glucose production in vitro, and circulating concentrations of insulin, triiodothyronine (T_3) and thyroxine (T_4) to determine the effects of chronic or acute dietary treatments.

2. Giving the 300 g protein/kg diet or withholding feed for 1 d decreased ($P < 0.05$) lipogenesis in vitro compared with controls.

3. Giving the 120 g protein/kg diet or refeeding with a 210 g protein/kg diet for 1 or 2 d increased ($P < 0.05$) lipogenesis in vitro compared with controls. Glucose production was affected in the same manner.

4. Fasting decreased ($P < 0.05$) plasma insulin and T_3 and increased T_4. Both refeeding and a low-protein diet increased T_3. Refeeding increased and a low-protein diet decreased insulin.

5. Chronic use (7–28 d of age) of either an alternating protein or intermittent feeding regimen caused greater responses compared with acute bouts (single cycle) of either of the regimens.

There are findings characterizing physiological responses to meal feeding and intermittent feeding in rodents. Both these feeding regimens increase lipogenesis *de novo*, carcass and liver fat, and improve dietary energy utilization. Early reports (Leveille, 1966; Yeh & Leveille, 1971) showed that periodic feeding regimens increase lipid metabolism in vitro in chickens but do not improve energetic efficiency. We found that intermittent feeding cycles or daily changes in the dietary protein level also change lipid metabolism (Rosebrough & Steele, 1985). The alteration in metabolism is similar to the intermittent feeding response noted in older chickens (Leveille & Yeh, 1972).

An overall comparison between the effects of diet composition and feeding regimens reveals that giving low-protein diets as a part of an alternating high–low-protein feeding cycle and refeeding fasted chickens result in similar responses. In both cases, lipid synthesis *de novo* increases. In contrast, giving high-protein diets and feeding fasting chickens decrease lipogenesis *de novo*. It is of interest if both these types of nutritional regimens can be used on a chronic basis to adapt intermediary metabolism in the broiler to increase lipogenesis further during the respective refeeding and low-protein phases of the two feeding programmes.

The purpose of the present study was to examine lipogenesis and glucose production in vitro in chickens subjected to chronic or acute bouts of either an intermittent or alternating protein feeding regimen. The overall hypothesis was that an alternating protein diet series would result in a metabolic response similar to the refeeding response noted during the intermittent feeding regimen. Circulating metabolic hormone levels were also measured

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during chronic and acute dietary treatment regimens. We were especially interested in the adaptations made by chickens subjected to repeated bouts of high–low-protein feeding and fasting–refeeding, compared with those made by chickens subjected to single bouts of these two regimens.

MATERIALS AND METHODS

Animals. Male broiler chickens (Ross) were purchased from ISA Poultry Services, Gainesville, GA. Chickens were fed on a common starter diet (230 g crude protein (nitrogen × 6·25) and 13 MJ metabolizable energy/kg) to 7 d of age and then randomly assigned to dietary treatment groups. Experiments were planned with these chickens (average weight 160 g) to determine the effects of chronic or acute bouts of either intermittent feeding or cyclic levels of dietary protein on metabolism in broiler chickens. Chickens were raised in Petersime chick batteries in an environmentally controlled room maintained at 22°, and given their respective diets until the termination of the experiment. A 12 h light–12 h dark cycle (06·00–18·00 hours light) was maintained, and water was available *ad lib*.

Diets and feeding regimens. Three diets were formulated to contain 120, 210 and 300 g crude protein and approximately 13 MJ metabolizable energy/kg. In addition, the diets were also formulated to contain 68–70 % of the total energy as carbohydrate. This was accomplished by the energy contribution for fat and protein and assuming the remainder to be carbohydrate. Maize starch was assumed to be 100 % carbohydrate energy and isolated soya-bean protein was assumed to be carbohydrate-free. The differences in energy from fat among the diets were considered to be insignificant because the greatest contribution from fat (< 5 %) to dietary energy was in the 120 g crude protein/kg diet. This level of fat energy probably does not affect intermediary metabolism (Hillard *et al.* 1980). The diets are shown in Table 1.

For the first experiment, 192 chickens were placed in three battery brooders (eight chickens per pen and eight pens per brooder) and assigned to one of three dietary treatments (eight pen replicates per dietary treatment). Replicates were arranged as blocks within batteries with all treatments appearing at a particular tier level among batteries. Previous research in our laboratory has established a slight effect of pen height. In contrast we cannot show variation among batteries so this factor was discounted as a source of variation. The first treatment was a 3 d intermittent feeding regimen (1 d fast–2 d refeed). The second treatment was a daily alternating dietary protein regimen (120 g protein/kg on day 1 and 300 g protein/kg on day 2). The control treatment was a 210 g protein/kg diet for the entire 21 d experimental period. These cycles were repeated until the chickens were 28 d of age. Chickens fed on the control diet were also placed on either the first or second treatment for one cycle to determine acute treatment effects. On day 28 and on successive days, two chickens were selected from each pen replicate at 09.00 hours to minimize diurnal variation. Chickens were bled by cardiac puncture into combination syringe-collection tubes containing EDTA as an anticoagulant (Sarstedt Corp., Princeton, NJ). The blood samples were centrifuged at 600 × ***g***. Plasma samples were collected with individual Pasteur pipettes and were stored at −70° for later analyses of metabolic hormones. The chickens were then weighted and killed by cervical dislocation. The livers were weighed and placed in individual vessels containing 10 mM-HEPES (*N*-2-hydroxyethyl piperazine-*N*′-2-ethane sulphonic acid) and 155 mM-sodium chloride (pH 7·5).

Expt 2 was similar to the first experiment although the intermittent feeding regimen was modified to a 1 d fast followed by a 1 d refeed. In addition, assays in vitro were expanded to include an estimate of net glucose production by liver explants. Plasma insulin,

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

Ingredient	Dietary crude protein (nitrogen × 6·25) (g/kg)		
	120	210	300
Maize meal	800	600	400
Soya-bean meal (490 g protein/kg)	100	50	
Soya-bean protein*		150	300
Maize starch	40	140	240
Dicalcium phosphate	40	40	40
Limestone	10	10	10
Selenium premix†	1		
Mineral premix‡	1	1	1
Vitamin premix§	5	5	5
Iodized salt	3	3	3
Calculated analyses			
Metabolizable energy (MJ/kg)	12·9	13·2	13·0
Carbohydrate (MJ/kg)	9·7	9·3	8·9

* Soya-bean protein grade II (21726), US Biochemicals, PO Box 22400, Cleveland, Ohio 44122

† Provided 0·2 mg Se/kg diet.

‡ Provided (mg/kg diet): manganese 100, iron 100, copper 10, cobalt 1, iodine 1, zinc 100, calcium 89.

§ Provided (mg/kg diet): retinol 3·6, cholecalciferol 75 μg, vitamin E 10·0, riboflavin 10, pantothenic acid 20, choline 2 g, niacin 100, thiamin 10, vitamin B_6 10, menadione sodium bisulphite 1·5, vitamin B_{12} 100 μg, folic acid 2, ethoxyquin 150.

triiodothyronine (T_3) and thyroxine (T_4) concentrations were also measured during chronic and acute dietary treatments.

Enzyme activities. A portion of each liver was homogenized in 50 mM-HEPES–3·3 mM-mercaptoethanol (pH 7·5) and centrifuged for 60 min at 50000 × ***g***. The supernatant fraction was stored frozen (−80°) until the activities of malate dehydrogenase (oxaloacetate-decarboxylating) ($NADP^+$) (malic enzyme) (*EC* 1.1.1.40, ME; Hsu & Lardy, 1969), isocitrate dehydrogenase ($NADP^+$) (*EC* 1.1.1.42, ICD; Cleland *et al.* 1969) and fatty acid synthase (*EC* 2.3.1.85, FAS; Mersmann *et al.* 1973) were measured. Activities are expressed as μmol of product formed/min under the assay conditions (Rosebrough & Steele, 1985).

Lipogenesis. Another portion of each liver was then sliced (50–75 mg explants) with a Stadie-Riggs hand microtome. Duplicate explants were incubated at 37° for 2 h in Hanks' balanced salts (HBSS; Hanks & Wallace, 1949) supplemented with 10 mM-HEPES, bovine serum albumin (10 g/l) and 20 mM-sodium[2-^{14}C]acetate (37 disintegrations/min per nmol). After 2 h, the slices were extracted and treated according to Folch *et al.* (1957). Lipogenesis was noted as that amount of sodium[2-^{14}C]acetate incorporated into hepatic fatty acids during the incubation period. This value is expressed per g liver and per kg body-weight.

Glucose production. Duplicate slices were also incubated at 37° for 2 h in HBSS supplemented with 10 mM-HEPES and bovine serum albumin (10 g/l) in the presence and absence of 10 mM-pyruvate. Glucose was then measured in the medium with a coupled hexokinase (*EC* 2.7.1.1) + glucose-6-phosphate dehydrogenase (*EC* 1.1.1.49) reaction (Stein, 1963).

Plasma metabolites. Plasma insulin concentration was estimated with a homologous avian radioimmunoassay system which uses chicken insulin as both the standard and ^{125}I-labelled tracer. The primary antiserum has been described previously (McMurtry *et al.*

Table 2. *Expt 1. Effect of intermittent feeding (1 d fast–2 d refeed) or alternating protein feeding (120 g protein/kg on day 1 and 300 g protein/kg on day 2) regimens on chicken growth from 7 to 28 d of age**

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

Diet, days 7–28	28-d wt (g)		Food intake (g)		Food conversion efficiency
	Mean	SE	Mean	SE	
Control	1137	15[c]	1753	19[c]	0·574
Intermittent feeding:	932	16[a]	1437	9[a]	0·558
Alternating protein:	1072	18[b]	1638	17[b]	0·592
120 g/kg			860	9	
300 g/kg			778	8	

[a, b, c] Values within a vertical column with different superscript letters were significantly different ($P < 0·05$).

* Chickens (7-d-old; average wt 160 g) were assigned to these dietary treatments: (1) control (210 g protein/kg diet for the entire experimental period), (2) intermittent feeding (210 g protein/kg diet for 2 d followed by a 1 d fast) or (3) a daily alternating dietary protein regimen (120 g protein/kg on day 1 and 300 g protein/kg on day 2). These cycles were continued for 20 d and then chickens were selected from each treatment to determine the effects of dietary treatments on intermediary metabolism.

1983). Both T_3 and T_4 concentrations were estimated with commercial, solid-phase kits (Immuchem Corp, Carson, CA). These assays were validated for avian samples by dispersing standards in charcoal-stripped chicken serums and by noting recovery of added T_3 and T_4 (94%). All hormone assays were conducted as single batches to minimize intra-assay variation. The inter-assay coefficients of variation averaged 2·7 and 1·3% for T_3 and T_4 respectively.

Statistical analyses. Metabolic activities are expressed per g of liver and per unit relative liver size (liver as percentage of body-weight × metabolic activity per g liver). The values for chickens from a particular pen were averaged to derive a pen mean which was considered as the experimental unit. Values were analysed as a randomized block design with tier position pooled across batteries as the blocking factor. Significance of differences between control and experimental treatment means was tested with Student's *t* test at the 0·05 level of probability (Remington & Schork, 1970).

RESULTS

Expt 1. Body-weights and feed consumption are presented in Table 2. Chickens fed on an intermittent basis were lighter ($P < 0·05$) than chickens fed on the alternating protein diets. Both these two groups were lighter ($P < 0·05$) than controls (given a diet containing 210 g/kg protein diet throughout the 21-d experimental period). Voluntary feed intake for both of these groups was also less ($P < 0·05$) than that for controls. We also found that chickens fed on the alternating protein diets ate more of the diet containing 120 g protein/kg than of the diet containing 300 g protein/kg.

Values relating to the effect of intermittent feeding and alternating protein regimens on lipogenesis are presented in Table 3. Values shown are expressed on a g liver and a kg body-weight basis to estimate whole-body lipogenic potential. When compared with values for controls, the 1 d fast decreased ($P < 0·05$) lipogenesis in chickens; however, refeeding these same chickens increased ($P < 0·05$) lipogenesis 100% relative to controls on both days of refeeding. A single fast–refeed bout also gave similar results although the size of the

Table 3. *Expt 1. Effect of intermittent feeding (1 d fast–2 d refeed) or alternating protein feeding (120 g protein/kg on day 1 and 300 g protein/kg on day 2) regimens on lipogenesis in vitro ([2-^{14}C]acetate incorporation into hepatic fatty acids) by liver explants from broiler chickens*†

(Mean values with their standard errors for four pens per dietary treatment)

		Cycled days 7–28†		Control days 7–28‡	
		Mean	SE	Mean	SE
μmol/g liver:					
Control		25·1	2·3	25·1	2·3
Intermittent feeding:	Fed 1 d	53·3[a]*	2·8	35·0[b]*	2·8
	Fed 2 d	64·6[a]*	1·6	28·9[b]*	4·1
	Fasted 1 d	3·0[a]*	0·2	1·7[b]*	0·1
Alternating protein:	120 g/kg	51·8[a]*	1·7	42·3[b]*	2·4
	300 g/kg	21·9[a]*	2·3	28·1[b]*	1·2
μmol/kg body-wt:					
Control		510	50	510	50
Intermittent feeding:	Fed 1 d	1830[a]*	110	1060[b]*	11
	Fed 2 d	2260[a]*	90	760[b]*	110
	Fasted 1 d	60*	10	30*	10
Alternating protein:	120 g/kg	1480[a]*	80	1160[b]*	80
	300 g/kg	920[a]*	50	690[b]*	40

[a,b] Values within a horizontal row with unlike superscript letters were significantly different ($P < 0·05$).

* Mean value was significantly different from control value ($P < 0·05$).

† Chickens (7-d-old; average wt 160 g) were assigned to these dietary treatments: (1) control (210 g protein/kg diet for the entire experimental period), (2) intermittent feeding (210 g protein/kg diet for 2 d followed by a 1 d fast) or (3) a daily alternating dietary protein regimen (120 g protein/kg on day 1 and 300 g protein/kg on day 2). These cycles were continued for 20 d and then chickens were selected from each treatment to determine the effects of dietary treatments on intermediary metabolism.

‡ Pens of controls were subjected to a single intermittent feeding or alternating protein feeding.

response was not nearly as great. A comparison between chronic and acute treatment groups showed that the rebound in lipogenesis was greater ($P < 0·05$) in the chronic than in the acute treatment group. Switching from 300 to 120 g protein/kg diet also increased lipogenesis in both the chronic and acute treatment groups, although the increase did not approach that noted by refeeding.

Expt 2. This experiment was designed to explore further extremes in metabolism caused by a 1 d on–1 d off intermittent feeding regimen compared with the alternating protein regimen. The 1 d on–1 d off regimen was chosen because the regimen involved a 2 d cycle like the alternating protein regimen. Chickens fed on the control diet were heavier ($P < 0·05$) than those in either of the two experimental groups (Table 4). The chickens fed on the alternating protein regimen were heavier ($P < 0·05$) than chickens fed on an intermittent basis. There were no significant differences in gain per unit feed intake.

The alternating protein regimen, either on an acute or chronic basis, resulted in patterns of lipogenesis similar to those in Expt 1 (Table 5). Shortening the intermittent feeding regimen accentuated the previously noted rebound in lipogenesis following refeeding. The chronic treatment increased lipogenesis following refeeding; moreover, the response was nearly twice that of the acute treatment group.

The chronic, intermittent feeding regimen lowered ($P < 0·05$) net glucose production on both days of the cycle compared with the respective controls (Table 6). The diet containing 300 g progein/kg, as a component of the alternating protein regimen, also lowered

Table 4. *Expt. 2. Effect of intermittent feeding (1 d fast–1 d refeed) or alternating protein feeding (120 g protein/kg on day 1 and 300 g protein/kg on day 2) regimens on chicken growth from 7 to 28 d of age**

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

Diet, days 7–28	28-d wt (g)		Food intake (g)		Food conversion efficiency
	Mean	SE	Mean	SE	
Control	1072[c]	9	1508[b]	19	0·625
Intermittent feeding	621[a]	5	791[a]	38	0·625
Alternating protein:	971[b]	9	1426[b]	33	0·592
120 g/kg			746	21	
300 g/kg			680	13	

[a, b, c.] Values within a vertical column with different superscript letters were significantly different ($P < 0·05$).

* Chickens (7-d-old; average wt 160 g) were assigned to these dietary treatments: (1) control (210 g protein/kg diet for the entire experimental period), (2) intermittent feeding (210 g protein/kg diet for 1 d followed by a 1 d fast) or (3) a daily alternating dietary protein regimen (120 g protein/kg on day 1 and 300 g protein/kg on day 2). These cycles were continued for 20 d and then chickens were selected from each treatment to determine the effects of dietary treatments on intermediary metabolism.

Table 5. *Expt 2. Effect of intermittent feeding (1 d fast-1 d refeed) or alternating protein feeding (120 g protein/kg on day 1 and 300 g protein/kg on day 2) regimens on lipogenesis in vitro ([2-^{14}C]acetate incorporation into hepatic fatty acids) by liver explants from broiler chickens*†

(Mean values with their standard errors for four pens per dietary treatment)

		Cycled days 7–28†		Control days 7–28‡	
		Mean	SE	Mean	SE
μmol/g liver:					
Control		13·6	1·8	13·6	1·8
Intermittent feeding:	Fed 1 d	79·6[a]*	5·0	33·4[b]*	7·5
	Fasted 1 d	3·6[a]*	0·5	1·7[b]*	0·1
Alternating protein:	120 g/kg	66·4[a]*	8·1	49·0[b]*	5·3
	300 g/kg	25·0[a]*	5·2	16·4[b]*	2·1
μmol/kg body-wt:					
Control		290·0	40·0	290·0	40·0
Intermittent feeding:	Fed 1 d	2500·0[a]*	160·0	1002·0[b]*	180·0
	Fasted 1 d	80·0*	14·0	90·0*	10·0
Alternating protein:	120 g/kg	1760·0[a]*	223·0	1206·0[b]*	130·0
	300 g/kg	580·0[a]*	121·0	439·0[b]*	50·0

[a, b] Values within a horizontal row with unlike superscript letters were significantly different ($P < 0·05$).

* Mean value was significantly different from control value ($P < 0·05$).

† Chickens (7-d-old; average wt 160 g) were assigned to these dietary treatments: (1) control (210 g protein/kg diet for the entire experimental period), (2) intermittent feeding (210 g protein/kg diet for 1 d followed by a 1 d fast) or (3) a daily alternating dietary protein regimen (120 g/kg protein on day 1 and 300 g/kg protein on day 2). These cycles were continued for 20 d and then chickens were selected from each treatment to determine the effects of dietary treatments on intermediary metabolism.

‡ Pens of controls were subjected to a single intermittent feeding or alternating protein feeding.

Table 6. *Expt. 2. Effect of intermittent feeding (1 d fast–1 d refeed) or alternating protein feeding (120 g protein/kg on day 1 and 300 g protein/kg on day 2) regimens on glucose production (glucose produced in the presence of pyruvate) by liver explants from broiler chickens*†

(Mean values with their standard errors for four pens per dietary treatment)

		Cycled days 7–28†		Control days 7–28‡	
		Mean	SE	Mean	SE
μmol/g liver:					
Control		156·0	11·7	156·0	11·7
Intermittent feeding:	Fed 1 d	89·4[a]*	5·6[a]	138·4[b]	10·2
	Fasted 1 d	15·7*	0·81[a]	18·9	2·6
Alternating protein:	120 g/kg	125·8	15·7	147·3	14·5
	300 g/kg	89·3	7·7[a]	78·2	10·3
μmol/kg body-wt:					
Control		3320·0	250·0	3320·0	250·0
Intermittent feeding:	Fed 1 d	2800·0[a]	180·0	4150·0[b]	310·0
	Fasted 1 d	340·0[a]*	180·0	380·0[a]*	50·0
Alternating protein:	120 g/kg	3330·0[a]	420·0	3610·0[a]	360·0
	300 g/kg	2070·0[a]*	180·0	1730·0[a]*	230·0

[a, b] Values within a horizontal row with unlike superscript letters were significantly different ($P < 0·05$).

* Mean values were significantly different from control values ($P < 0·05$).

† Chickens (7-d-old; average wt 160 g) were assigned to these dietary treatments: (1) control (210 g protein/kg diet for the entire experimental period), (2) intermittent feeding (210 g protein/kg diet for 1 d followed by a 1 d fast) or (3) a daily alternating dietary protein regimen (120 g/kg protein on day 1 and 300 g/kg protein on day 2). These cycles were continued for 20 d and then chickens were selected from each treatment to determine the effects of dietary treatments on intermediary metabolism.

‡ Pens of controls were subjected to a single intermittent feeding or alternating protein feeding.

($P < 0·05$) glucose production compared with the control diet. Compared with the control diet, a single intermittent feeding regimen also decreased ($P < 0·05$) glucose production following fasting and restored production following the refeeding phase. A comparison between chronic and acute treatment groups showed a lower rate of glucose production following the refeeding of the chronic than of the acute treatment group. Switching from 300 to 120 g protein/kg diet also decreased ($P < 0·05$) glucose production in the acute group. The absolute values were similar for both treatment groups following this period of treatment.

Compared with values for controls, the 1 d fast decreased ($P < 0·05$) plasma insulin in chickens; however, refeeding these same chickens increased ($P < 0·05$) insulin 300% relative to controls (Table 7). A single intermittent feeding regimen also gave similar results, although the magnitude of the response was not nearly as great. A comparison between chronic and acute treatment groups showed that the rebound in plasma insulin was greater ($P < 0·05$) in the chronic than in the acute treatment group.

When compared with values for controls, the 1 d refeed increased ($P < 0·05$) plasma T_3 and decreased ($P < 0·05$) plasma T_4 in chickens. We also noted the same trend when chickens were switched from a diet containing 300 g protein/kg to one containing 120 g protein/kg.

In the context of this experiment (altered feeding behaviour in all treatment groups compared with the *ad lib.*-feeding regimen), we found that an increase in protein intake decreased ($P < 0·05$) both ME and FAS activities (Table 8). The higher protein intake and fasting increased ICD activity and the higher energy intake increased FAS activity.

Table 7. *Expt 2. Effect of intermittent feeding (1 d fast–1 d refeed) or alternating protein feeding (120 g protein/kg on day 1 and 300 g protein/kg on day 2) regimens on certain metabolic hormone concentrations (ng/ml) in chickens*†

(Mean values with their standard errors for four pens per dietary treatment)

		Cycled days 7–28†		Control days 7–28‡	
		Mean	SE	Mean	SE
Insulin:					
Control		0·56	0·04	0·56	0·04
Intermittent feeding:	Fed 1 d	1·84[a]*	0·16	0·60[b]	0·03
	Fasted 1 d	nd		nd	
Alternating protein:	120 g/kg	0·47[a]	0·03	0·39[a]	0·03
	300 g/kg	0·82[a]*	0·01	0·22[b]*	0·02
Triiodothyronine:					
Control		5·08	0·26	5·08	0·26
Intermittent feeding:	Fed 1 d	6·41[a]*	0·38	4·11[b]*	0·20
	Fasted 1 d	4·53[a]	0·31	4·63[a]	0·20
Alternating protein:	120 g/kg	7·35[a]*	0·27	7·33[a]*	0·42
	300 g/kg	5·28[a]	0·17	6·40[a]*	0·38
Thyroxine					
Control		7·45	0·27	7·45	0·27
Intermittent feeding:	Fed 1 d	6·45[a]	0·49	8·14[a]	0·25
	Fasted 1 d	12·03[a]*	0·59	11·14[a]*	0·39
Alternating protein:	120 g/kg	4·70[a]*	0·38	5·45[a]*	0·30
	300 g/kg	8·71[a]	0·38	9·50[a]*	0·31

nd, Not determined.

[a, b] Values within a horizontal row with unlike superscript letters were significantly different ($P < 0·05$).

* Mean values were significantly different from control values ($P < 0·05$).

† Chickens (7-d-old; average wt 160 g) were assigned to these dietary treatments: (1) control (210 g protein/kg diet for the entire experimental period), (2) intermittent feeding (210 g protein/kg diet for 1 d followed by a 1 d fast) or (3) a daily alternating dietary protein regimen (120 g protein/kg on day 1 and 300 g protein/kg on day 2). These cycles were continued for 20 d and then chickens were selected from each treatment to determine the effects of dietary treatments on intermediary metabolism.

‡ Pens of controls were subjected to a single intermittent feeding or alternating protein feeding.

DISCUSSION

Interpreting results of experiments using rodents and extrapolating these results to explain regulation of lipogenesis in chickens is difficult. The liver is the major site of lipogenesis in chickens and only a minor site in young rodents. In addition, the usual method of testing the effect of dietary protein on metabolism involves changing the maize:soya-bean meal value which also alters the carbohydrate content of the diet. We feel that by maintaining a constant quantity of carbohydrate in the diet, we can attribute results to changes in protein intake.

Rats fed on an intermittent basis demonstrate many of the characteristics of meal-fed animals (Leveille, 1970). The switch from a high- to a low-protein diet and from a fasted to a fed state rapidly increased lipogenesis in a fashion similar to the meal-feeding response seen in rodents. The present study indicates that this increase is also greater when chickens are repeatedly fasted and refed or fed alternating high–low-protein diets. Yeh & Leveille (1971) and Tanaka *et al.* (1983) reported similar findings in older chickens. The former group attributed the rapid change to a decrease in fatty acid release from adipose tissue during changes from low to high lipogenic states. This decrease increases CoA availability for the citrate cleavage and acetyl-CoA carboxylase (*EC* 6.4.1.2) reactions. In

Table 8. *Expt 2. Effect of intermittent feeding (1 d fast–1 d refeed) or alternating protein feeding (120 g protein/kg on day 1 and 300 g protein/kg on day 2) regimens on certain liver enzyme activities (one unit is that amount of enzyme resulting in the production of 1 μmol oxidized or reduced NADP/min at 25°) in broiler chickens*†

(Mean values with their standard errors for four pens per dietary treatment)

Enzyme...	FAS				ME				ICD			
	Cycled† days 7–28		Control‡ days 7–28		Cycled† days 7–28		Control‡ days 7–28		Cycled† days 7–28		Control‡ days 7–28	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Units/g liver:												
Control	0·8	0·1	0·8	0·1	2·6	0·2	2·6	0·2	23·9	1·2	23·9	1·2
Intermittent feeding: Fed 1 d	1·4[a]*	0·1	1·8[b]*	0·1	3·4[a]	0·6	9·1[b]*	1·5	23·5[a]	0·8	26·5	1·1
Fasted 1 d	1·0[a]	0·1	0·9[a]	0·1	0·9[a]*	0·1	3·5[b]	0·5	37·4[a]	1·4	45·3[b]*	1·8
Alternating protein: 120 g/kg	2·1[a]*	0·2	2·2[a]*	0·1	6·1[a]*	0·2	7·4[b]*	0·7	20·6[a]	1·0	22·5	0·9
300 g/kg	0·8[a]	0·1	1·1[a]	0·6	6·6[a]*	0·6	7·2[a]*	0·2	27·3[a]	2·5	28·1*	0·9
Units/kg body-wt:												
Control	18·0	1·1	18·0	1·1	56·0	5·5	56·8	5·5	510·0	23·0	510·0	23·0
Intermittent feeding: Fed 1 d	34·0[a]*	4·0	44·0[b]*	3·3	82·0[a]	12·0	216·0[b]*	36·0	556·0	9·1	625·0*	26·0
Fasted 1 d	22·0[a]	1·1	21·0[a]	3·3	19·0[a]*	1·1	74·0[b]	10·0	815·0[a]*	30·0	947·5[b]*	38·0
Alternating protein: 120 g/kg	55·0[a]*	4·4	52·0[a]	2·2	160·0[a]*	24·0	171·0[a]*	17·0	544·0[a]	18·0	515·0	21·0
300 g/kg	19·0[a]	2·2	25·9[a]	3·3	149·0[a]*	24·0	166·3[a]*	6·0	622·0[a]*	56·0	646·0*	21·0

FAS, fatty acid synthase (*EC* 2.3.1.85); ME, malate dehydrogenase (oxaloacetate-decarboxylating) ($NADP^+$) (malic enzyme) (*EC* 1.1.1.40); ICD, isocitrate dehydrogenase ($NADP^+$) (*EC* 1.1.1.42)

[a,b] Values within a horizontal row with unlike superscript letters were significantly different ($P < 0.05$).

* Mean values were significantly different from control values ($P < 0.05$).

† Chickens (7-d-old; average wt 160 g) were assigned to these dietary treatments: (1) control (210 g protein/kg diet for the entire experimental period), (2) intermittent feeding (210 g protein/kg diet for 1 d followed by a 1 d fast) or (3) a daily alternating dietary protein regimen (120 g protein/kg on day 1 and 300 g protein/kg on day 2). These cycles were continued for 20 d and then chickens were selected from each treatment to determine the effects of dietary treatments on intermediary metabolism.

‡ Pens of controls were subjected to a single intermittent feeding or alternating protein feeding.

contrast, Tanaka *et al.* (1983) wrote that the supply of reducing equivalents (NADPH) regulates lipogenesis *de novo* in chickens. Based on a noted high correlation between ME activity and lipogenesis *de novo*, Yeh & Leveille (1969) originally proposed that availability of NADPH regulates lipid metabolism in chickens fed on high-protein diets.

The enzyme activities in the present study indicate that ICD functions in both lipid and protein metabolism. The enzyme provides both a residual capacity for the production of reducing equivalents and a co-reactant for transamination (α-ketoglutarate). Competition exists between acetyl-CoA carboxylase and the aconitase–isocitrate dehydrogenase pathway for limited cytoplasmic citrate. Thus, the requirement for α-ketoglutarate as a co-reactant for transamination of excess amino acids depresses citrate levels and the subsequent activation of acetyl CoA carboxylase. Phosphorylation–dephosphorylation did not control avian acetyl-CoA carboxylase to the extent noted in the rat enzyme. Thus, citrate levels appear to control the avian enzyme more than the rat enzyme (Clark *et al.* 1979). Hillard *et al.* (1980) reported that dietary carbohydrate was a potent regulator of avian lipogenesis, possibly through a regulation of the supply of citrate.

Oppenheimer *et al.* (1978) found a positive correlation between T_3 level and hormone action at the cellular level. According to their hypothesis, a decrease in either tissue binding or circulating levels of T_3 (as is the case in the present study) would decrease enzyme

activity. Although ME may provide the necessary NADPH for lipogenesis, findings from the present study do not necessarily show that the enzyme strictly regulates lipogenesis.

The increase in serum insulin accompanying an increase in dietary protein suggests a different method of insulin regulation in chickens than in mammals. Amino acids, rather than glucose, are the major stimuli for insulin release from the islet cells in teleosts (Ablett *et al.* 1983). Ablett *et al.* (1983) suggested that high-protein diets chronically elevate insulin in salmon (*Salmo gairdneri*) and reduce insulin binding by liver and muscle membranes. The down regulation of the insulin receptor by elevated circulating insulin is similar to the response in mammals. It is possible that tests of insulin sensitivity in birds may also require the consideration of the dietary protein level or the availability of certain amino acids.

Two experiments were conducted to compare the effects of repeated cycles of either an alternating low–high-protein or intermittent feeding regimen. Both regimens gave the familiar meal-feeding response. Repeated cycles of either of these regimens gave responses greater than a single cycle. The findings in the present study further reinforce our hypothesis that dietary protein alters lipid metabolism. Dietary carbohydrate does not support a high rate of lipogenesis when combined with a high-protein intake. Although high-protein diets result in a pattern of lipogenesis in vitro similar to fasting, hormone levels are different from fasting values.

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