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# Protein and energy relations in the broiler chicken

## 8. Comparison involving protein- and lysine-adequate and inadequate diets on lipid metabolism

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**Chickens were fed on diets containing either 12·8 MJ, 150 g crude protein (nitrogen × 6·25)/kg or 12·8 MJ, 200 g crude protein/kg to determine differences in metabolism. The diet containing 12·8 MJ, 150 g crude protein/kg contained either 8 or 12 g lysine/kg. Treatment variables examined in vitro were lipogenesis, glucose production and hepatic enzyme activities to compare metabolism in chicks fed on a low-protein, lysine-supplemented diet and a diet formulated to contain the required amount of lysine from intact protein. Growth was similar in chicks fed on diets containing either 12·8 MJ, 154 g crude protein with 12 g lysine/kg or 12·8 MJ, 200 g crude protein/kg. Net glucose production was greater ($P < 0·05$) in liver explants from chickens fed on diets containing either 12·8 MJ, 154 g crude protein with 12 g lysine/kg or 12·8 MJ, 200 g crude protein/kg than in explants from chickens fed on 12·8 MJ, 150 g crude protein with 8 g lysine/kg. Pyruvate use for glucose production was greater ($P < 0·05$) in chickens fed on a diet containing 12·8 MJ, 150 g crude protein with 8 g lysine/kg. The findings from the present study suggest that crystalline and 'natural' lysine additions to chick diets may influence metabolism differently.**

**Lysine: Protein: Lipogenesis: Chicken**

Diets containing large energy: protein ratios (> 72 MJ/kg crude protein (nitrogen × 6·25)) promote high rates of in vitro lipogenesis (Rosebrough & Steele, 1985*a*) as well as *de novo* carcass lipid synthesis by the liver of chickens (Donaldson, 1985). Diets with small energy: protein ratios (< 56 MJ/kg crude protein) promote lean broiler carcasses (Donaldson *et al.* 1956; Thomas & Combs, 1967). We have recently examined energy: protein ratios and have found different possible interpretations of results (Rosebrough & Steele, 1985*a*). For example, giving a very small energy: protein diet (43 MJ/kg crude protein) will result in a very lean carcass (crude protein as a proportion of dry matter) when compared with results obtained by giving a larger energy: protein diet (65 MJ/kg crude protein). When the findings were analysed on the basis of g N deposited over the course of the experiment, it became obvious to us that the diet containing a larger energy: protein ratio favoured the deposition of more carcass protein as well as lipid. Thus, diets containing very small energy: protein ratios may promote lean carcasses by naturally restricting feed intake. Bartov (1979) has also proposed that the excretion of excess amino acid-N would require energy.

Both the quantity (crude protein concentration) and quality (amino acid composition relative to the required balance) of the dietary protein affect the body composition of chicks. If lysine is the limiting amino acid the effective protein level can be calculated according to the lysine content relative to the crude protein level. It was assumed that a completely effective protein contained 60 g lysine/kg protein. Although the crude protein

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

| | Dietary crude protein (nitrogen × 6·25) (g/kg) | | |
|---|---|---|---|
| | 150 | 154 | 200 |
| Ingredient | | | |
| Soya-bean protein* | | | 120 |
| Soya-bean meal | 166 | 166 | 55 |
| Maize meal | 727 | 727 | 727 |
| Maize oil | 20 | 15 | |
| Sand | 27 | 22 | 33 |
| L-Lysine hydrochloride† | | 5 | |
| L-Methionine‡ | 5 | 5 | 5 |
| Dicalcium phosphate | 40 | 40 | 40 |
| Limestone | 10 | 10 | 10 |
| 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) | 12·9 | 12·8 | 12·9 |
| Lysine (g/kg) | 8·1 | 12·1 | 12·3 |
| Sulphur-amino acids (g/kg) | 9·9 | 9·9 | 9·9 |

* Soya-bean protein grade II (21726); US Biochemicals, PO Box 22400, Cleveland, Ohio 44122.
† L-Lysine hydrochloride (18585); US Biochemicals.
‡ L-Methionine (18915); US Biochemicals.
§ 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 0·075, biotin 1, vitamin E 10, riboflavin 10, pantothenic acid 20, choline 2 g, niacin 100, thiamin 10, vitamin $B_6$ 10, menadione sodium bisulphite 1·5, cyanocobalamin 0·1, folic acid 2, ethoxyquin 150.

level (g/kg diet), as well as the effective dietary protein level (percentage of the requirement of the first limiting amino acid present in the diet), may regulate lipogenesis, it is unknown if the effect is due to the presence of a limiting amino acid at the ribosomal level or to a shift in cellular metabolism caused by a need to process excess amino acid-carbon. In addition, in the process of converting gluconeogenic amino acid-C into glucose-C, reducing equivalents (NADH, NADPH) normally required for the addition of acetyl residues during *de novo* lipogenesis are utilized and may be unavailable for lipogenesis (Yeh & Leveille, 1969).

The purpose of the experiments described in the present report was to examine lipid and carbohydrate metabolism in chickens fed on additional amounts of the limiting amino acid lysine as either lysine hydrochloride or soya-bean meal. The null hypothesis tested was that lysine supplementation of a low-protein diet would influence metabolism similarly to a diet containing a greater amount of crude protein. In the former case, the effective protein level (g lysine/kg crude protein) would increase and in the latter case, the effective level would remain at 60 g lysine/kg crude protein. A maize–soya-bean meal basal diet was formulated to be marginally adequate for all essential amino acids with the exception of lysine. Crystalline lysine hydrochloride was then added to give a total of 12 g/kg diet.

## EXPERIMENTAL PROCEDURES

### *Animals*

Ross male broiler chicks (7-d-old) were assigned to one of three dietary treatments (150 g crude protein with 8 g lysine, 154 g crude protein with 12 g lysine and 200 g crude protein

with 12 g lysine/kg diet). The latter diet which depended on meeting the lysine requirement (12 g/kg diet and 60 g lysine/kg crude protein) for the 7- to 28-d-old chicken (National Research Council, 1984) was used as a control diet. The diets are described in Table 1. Chickens were housed in heated battery brooders in a light- and temperature-controlled room maintained at 22° with 24 h of light. Each battery was considered a block and treatments were randomly assigned to pens in each battery. There were a total of four replicate pens containing eight chickens for each dietary treatment. For purposes of statistical analyses, the observation was the pen mean.

*In vitro metabolic studies*

Two 28-d-old chickens were randomly selected from each pen replicate treatment and killed by cervical dislocation at 09.00 hours. The livers were rapidly excised, 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). A portion of the liver was sliced with a Stadie–Riggs hand microtome (50–75 mg/explant) and duplicate explants were incubated for 2 h at 37° in 25-ml Erlenmeyer flasks containing 3 ml Hanks' balanced salts (Hanks & Wallace, 1949) supplemented with 20 mM-HEPES (pH 7·4) and 10 g bovine serum albumin/l. Under the described assay conditions, we have found that reactions are linear from 1 to 4 h with substrate concentrations from 5 to 40 mM (Rosebrough & Steele, 1987). Explants were incubated in the presence of 20 mM-[2-$^{14}$C]pyruvate (37 disintegrations/min per nmol), 20 mM-[3-$^{14}$C]pyruvate (37 disintegrations/min per nmol) or 20 mM-[2-$^{14}$C] sodium acetate (37 disintegrations/min per nmol). Following a 2 h incubation period, 0·1 ml 5 M-potassium hydroxide was injected onto filter papers lining wells suspended in each of the flasks to trap carbon dioxide, and 0·3 ml 2 M-sulphuric acid was injected into the media to stop metabolic processes and liberate $CO_2$ produced during the incubation. The incubation was continued for an additional 1 h to ensure adequate $CO_2$ trapping. The liver explants were extracted for 24 h in 15 ml chloroform–methanol (2:1, v/v) and fractionated with 3 ml 155 mM-potassium chloride (Folch *et al.* 1957). The lower phase was then evaporated to dryness and saponified with 2 M-methanolic KOH. The non-saponified fraction was removed with two 10 ml hexane washes and the fatty acids were removed into 10 ml hexane following acidification with 6 M-hydrochloric acid and evaporated to dryness. This fraction was then dispersed in Scintiverse® (Fisher Scientific, Pittsburgh, PA, USA) and counted with a liquid-scintillation counter. Product radioactivity was then divided by the specific activity of the respective radiolabelled precursor to establish the quantity of precursor utilized. Activity was noted as the quantity of labelled acetate or pyruvate used in the incubation and recovered as $CO_2$ or fatty acids. This value was then expressed as $\mu$mol/kg body-weight on the basis of relative liver size of the chicken. This calculation was obtained by using the following formula: $\mu$mol/g liver $\times$ total liver weight $\div$ body-weight $\times$ 1000.

A 1 ml portion of the 3 ml incubation volume containing either 20 mM-[2-$^{14}$C]pyruvate or 20 mM-[3-$^{14}$C]pyruvate was applied to a 7 $\times$ 30 mm column containing AG 1-X8, $HCOO^-$ (Yeh & Leveille, 1969). Glucose was eluted with 5 ml water and measured with glucose oxidase (EC 1.1.3.4)–peroxidase (*EC* 1.11.1.7). Radioactivity in the fraction containing glucose was also determined. The specific activity of glucose was then divided by the specific activity of the respective precursor to derive the mass of product formed. Net glucose production was determined by measuring glucose in samples of medium following the incubation. The difference attributed to pyruvate was noted as net glucose production.

Remaining liver tissue was homogenized (1:10, w/v) in 100 mM-HEPES (pH 7·5)–3·3 mM-mercaptoethanol and centrifuged at 50000 $\times$ ***g*** for 60 min. The supernatant fractions were kept at 0° until analysed for malic enzyme (*EC* 1.1.1.40; ME), isocitrate dehydro-

genase (NADP) (*EC* 1.1.1.42; ICD) and aspartate aminotransferase (*EC* 2.6.1.1; ASPT). ME activity 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-manganese chloride and the substrate, 2·2 mM-L-malate (disodium salt). A 50 $\mu$l portion of the 50000 × *g* supernatant fraction was pre-incubated for 15 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 was found to proceed in a linear manner for at least 60 min providing that the reaction contained nor more than 100 $\mu$g supernatant protein.

ICD 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. A 25 $\mu$l portion of the 50000 × *g* supernatant fraction was pre-incubated for 15 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 was found to proceed in a linear manner for at least 60 min providing that the reaction contained no more than 50 $\mu$g supernatant protein. ASPT 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 malic dehydrogenase (*EC* 1.1.1.37)/l and the substrate, 15 mM-2-oxoglutarate. A 25 $\mu$l portion of the 50000 × *g* supernatant fraction was pre-incubated for 15 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 was found to proceed in a linear manner for at least 30 min providing that the reaction contained no more than 50 $\mu$g supernatant protein. Activities of all enzymes are expressed as $\mu$mol product formed/min under the assay conditions (Rosebrough & Steele, 1985*a*).

Data were analysed as a randomized block design. The model included dietary treatments randomized within blocks (batteries). Significance of pairwise comparisons was determined with a Student's *t* test (Kirk, 1968).

## RESULTS

Body-weights and feed consumption are presented in Table 2. Chickens fed on the diet containing 150 g crude protein and 8 g lysine/kg were lighter ($P < 0·05$) than chickens fed on the diet containing either 154 g crude protein and 12 g lysine/kg or 200 g crude protein and 12 g lysine/kg. Feeding the latter two diets resulted in chickens nearly equal in weight. Efficiencies of food utilization were similar among all treatment groups.

The relative rates of net glucose production as well as the utilization of pyruvate for glucose production in vitro are presented in Table 3. An increase in dietary lysine (154 g crude protein with 12 g lysine/kg diet) and crude protein status (200 g crude protein/kg diet) increased ($P < 0·05$) net glucose production compared with feeding a diet containing 150 g crude protein and 8 g lysine/kg. An examination of the use of pyruvate by liver explants from chickens consuming these diets revealed that, in contrast to pyruvate-dependent glucose production noted previously, the use of pyruvate as a glucose precursor was greater ($P < 0·05$) in liver explants from chickens receiving the diet containing 200 g crude protein/kg than for either of the other treatments. A calculation of the ratios for utilization of [2-$^{14}$C]- and [3-$^{14}$C]pyruvate showed that both label positions appeared in glucose. A comparison of rates of utilization showed that [3-$^{14}$C]pyruvate appearance exceeded that for [2-$^{14}$C]pyruvate in chicks given the required amount of dietary lysine whether from additional soya-bean meal or from lysine hydrochloride.

A comparison of relative rates of substrate usage showed that pyruvate utilization exceeded ($P < 0·05$) that for acetate as both a lipogenic (Table 4) and oxidative substrate

Table 2. *Dietary crude protein (nitrogen × 6·25) level, lysine supplementation and chick growth**

(Mean values are average individual bird weights for pens treated alike)

| Crude protein (g) | Lysine content (g) | 28 d body-wt (g) | 7–28 d performance | |
|---|---|---|---|---|
| | | | Wt gain (g) | Food eaten (g) |
| 150 | 8 | 872 | 727[a] | 1112[a] |
| 154 | 12 | 945 | 800[b] | 1280[b] |
| 200 | 12 | 941 | 796[b] | 1257[b] |
| Pooled SEM | | | 20 | 43 |

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

* Chickens (7-d-old; average of 145 g) were assigned to these dietary treatments: (1) control, 200 g crude protein and 12 g lysine/kg diet, (2) 150 g crude protein and 8 g lysine/kg diet or (3) 154 g crude protein and 12 g lysine/kg diet for a 21 d experimental period. Chickens were then selected from each treatment to determine the effects of dietary treatments on intermediary metabolism.

Table 3. *Effect of dietary crude protein (nitrogen × 6·25) level and lysine supplementation on the use of pyruvate as a substrate for in vitro glucose metabolism (incorporation of [2-$^{14}$C]- and [3-$^{14}$C]pyruvate into media glucose and total media glucose change) by liver explants from broiler chickens**

(Mean values for four pen means per dietary treatment are expressed as μmol substrate utilized/kg body-weight)

| Crude protein (g) | Lysine content (g) | NGP† | Apparent pyruvate utilization for in vitro glucose metabolism | |
|---|---|---|---|---|
| | | | [2-$^{14}$C]-pyruvate | [3-$^{14}$C]-pyruvate |
| 150 | 8 | 724[a] | 208[a] | 245[a] |
| 154 | 12 | 961[b] | 211[a] | 321[a] |
| 200 | 12 | 969[b] | 404[b] | 626[b] |
| Pooled SEM | | 90 | 96 | 85 |

NGP, net glucose production.

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

* Chickens (7-d-old; average of 145 g) were assigned to these dietary treatments: (1) control, 200 g protein and 12 g lysine/kg diet, (2) 150 g protein and 8 g lysine/kg diet or (3) 154 g protein and 12 g lysine/kg diet for a 21 d experimental period. Chickens were then selected from each treatment to determine the effects of dietary treatments on intermediary metabolism.

† Calculated by incubating explants with or without 20 mM-pyruvate and determining the change in media glucose. Values are expressed as μmol glucose produced/kg body-weight.

(Table 5). An increase in crude protein decreased ($P < 0·05$) *de novo* fatty acid synthesis from both pyruvate and acetate. A slight, though significant ($P < 0·05$) decrease in pyruvate utilization for lipogenesis was noted when lysine hydrochloride was added to the lower-protein diet. In contrast to the apparent differences in pyruvate-C appearing in glucose, both of the two labelled pyruvate-carbons appeared at the same rate in the lipid product, indicating a common pathway before incorporation into a final product.

Diets did not influence $CO_2$ production although, at all protein levels, utilization of pyruvate surpassed ($P < 0·05$) that for acetate and indicated unequal competition between the two substrates for metabolic pathways. The [2-$^{14}$C]pyruvate : [3-$^{14}$C]pyruvate utilization

Table 4. *Effect of dietary crude protein (nitrogen × 6·25) level and lysine supplementation on the use of either acetate or pyruvate as substrates for in vitro lipogenesis (incorporation of [2-$^{14}$C]acetate, [2-$^{14}$C]pyruvate and [3-$^{14}$C]pyruvate into hepatic fatty acids) by liver explants from broiler chickens**

(Mean values for four pen means per dietary treatment are expressed as μmol substrate utilized/kg body-weight)

| Crude protein (g) | Lysine content (g) | Apparent pyruvate and acetate utilization for in vitro lipogenesis | | |
|---|---|---|---|---|
| | | [2-$^{14}$C]-acetate | [2-$^{14}$C]-pyruvate | [3-$^{14}$C]-pyruvate |
| 150 | 8 | 960[b] | 2431[c] | 2392[c] |
| 154 | 12 | 945[b] | 2000[b] | 1880[b] |
| 200 | 12 | 448[a] | 1083[a] | 1040[a] |
| Pooled SEM | | 116 | 119 | 121 |

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

* Chickens (7-d-old; average of 145 g) were assigned to these dietary treatments: (1) control, 200 g protein and 12 g lysine/kg diet, (2) 150 g protein and 8 g lysine/kg diet or (3) 154 g protein and 12 g lysine/kg diet for a 21 d experimental period. Chickens were then selected from each treatment to determine the effects of dietary treatments on intermediary metabolism.

Table 5. *Effect of dietary crude protein (nitrogen × 6·25) level and lysine supplementation on the use of either acetate or pyruvate as substrates for in vitro oxidation (incorporation of [2-$^{14}$C]acetate, [2-$^{14}$C]pyruvate and [3-$^{14}$C]pyruvate into $CO_2$) by liver explants from broiler chickens**

(Mean values for four pen means per dietary treatment are expressed as μmol substrate utilized/kg body-weight)

| Crude protein (g) | Lysine content (g) | Apparent pyruvate and acetate utilization for in vitro oxidation | | |
|---|---|---|---|---|
| | | [2-$^{14}$C]-acetate | [2-$^{14}$C]-pyruvate | [3-$^{14}$C]-pyruvate |
| 150 | 8 | 145 | 880 | 461 |
| 154 | 12 | 124 | 1020 | 545 |
| 200 | 12 | 126 | 891 | 462 |
| Pooled SEM | | 16 | 49 | 41 |

Values within a column were not significantly different ($P > 0.05$).

* Chickens (7-d-old; average of 145 g) were assigned to these dietary treatments: (1) control, 200 g protein and 12 g lysine/kg diet, (2) 150 g protein and 8 g lysine/kg diet or (3) 154 g protein and 12 g lysine/kg diet for a 21 d experimental period. Chickens were then selected from each treatment to determine the effects of dietary treatments on intermediary metabolism.

ratio for $CO_2$ was similar for all dietary treatments; however, apparent utilization of [2-$^{14}$C]pyruvate was nearly twice the rate for [3-$^{14}$C]pyruvate.

The activities of certain hepatic enzymes are presented in Table 6. The activities of ASPT and ICD were increased by an increase in the dietary crude protein level but not in the lysine level. In contrast, ME activity was decreased by an increase in the crude protein, but not the lysine level.

Table 6. *Effect of dietary crude protein (nitrogen × 6·25) level and lysine supplementation on liver enzyme activities (one unit is that amount of enzyme resulting in the production of 1 μmol oxidized or reduced NAD(P)/min at 25°) in broiler chickens**

(Mean values for four pen means per dietary treatment are expressed as units/kg body-weight)

| Crude protein (g) | Lysine content (g) | ASPT | ICD | ME |
|---|---|---|---|---|
| 150 | 8 | 1843[a] | 277[a] | 249[a] |
| 154 | 12 | 1791[a] | 320[ab] | 264[a] |
| 200 | 12 | 2371[b] | 415[b] | 177[b] |
| Pooled SEM | | 143 | 53 | 15 |

ASPT, aspartate aminotransferase (*EC* 2.6.1.1); ICD (NADP), isocitrate dehydrogenase (NADP) (*EC* 1.1.1.42); ME, malic enzyme (*EC* 1.1.1.40).

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

* Chickens (7-d-old; average of 145 g) were assigned to these dietary treatments: (1) control, 200 g protein and 12 g lysine/kg diet, (2) 150 g protein and 8 g lysine/kg diet or (3) 154 g protein and 12 g lysine/kg diet for a 21 d experimental period. Chickens were then selected from each treatment to determine the effects of dietary treatments on intermediary metabolism.

## DISCUSSION

There are many reports concerning the relationship between dietary energy and protein and subsequent effects on intermediary metabolism; few studies attempt to explain the basis of changes in the lean:fat ratio in the animal carcass and the energy:protein relationship in the diet. Generally, as the dietary crude protein intake increases, transamination of amino acids, in excess of those required for growth, increases the demand for 2-oxoglutarate provided by mitochondrial citrate. Clark *et al.* (1979) proposed that avian acetyl CoA-carboxylase (*EC* 6.4.1.2) was more sensitive than mammalian acetyl CoA-carboxylase to activation by citrate.

The formulation of hypotheses concerning the dual role of dietary energy and crude protein and lipogenesis has been hampered by the relatively simple approach taken in the past when diets were formulated. In most cases, the level of crude protein was increased at the expense of dietary carbohydrate. We have examined the role of dietary crude protein (120–300 g/kg) in conjunction with diets that contain the same quantity of carbohydrate (Rosebrough & Steele, 1985*b*, 1986*a*; Rosebrough *et al.* 1988) and have found specific effects of both dietary protein and carbohydrate levels in the regulation of intermediary metabolism in the broiler. For example, a diet containing 230 g crude protein and 700 g carbohydrate equivalents/kg will evoke a greater rate of lipogenesis than will a diet containing 230 g crude protein and 550 g carbohydrate equivalents/kg.

A logical progression in the study of the role of protein *per se* in the regulation of metabolism involves further experiments concerning protein quality (amino acid composition). The present study applies the concept of lysine as a limiting factor in protein quality. The results of the present study concerning metabolism in chickens fed on diets containing a marginal crude protein level complement our work (Rosebrough *et al.* 1982) in feeding similar regimens to turkey poults. In contrast, the turkey poult will eat a greater quantity of a lysine-supplemented diet relative to a control diet than will a chicken. Thus, comparing results between these two species may be difficult because of differences in voluntary feed intake.

The use of either pyruvate or acetate as substrates for lipogenesis permitted us to compare substrate utilization rates in broiler chickens. Pyruvate utilization always exceeded that of acetate. This finding suggests either divergent pathways for mitochondrial

translocation or that both may use a common transport system that differs in its affinity for metabolites. As the dietary crude protein content was increased, the ratio [3-$^{14}$C]-:[2-$^{14}$C]pyruvate appearing in glucose also increased. This finding is not surprising as there is substantial glucose-C recycling in liver. It is of particular interest to compare the effects of lysine supplementation on liver glucose metabolism. Explants from supplemented chickens produced more glucose in the presence of pyruvate than did those from unsupplemented chickens. In contrast, use of pyruvate *per se* as a glucose precursor was not changed by an increase in dietary lysine. The formation of glucose from pyruvate requires partial metabolism through the Krebs' cycle, involving oxidation and subsequent loss of C atoms as well as randomization of product labelling in the formation of fumarate before anaplerotic reactions resulting in glucose synthesis. An examination of C metabolism would, therefore, allow a determination of C recycling during metabolism. For example, a ratio other than one indicates that radiolabelled glucose may have been formed through indirect pathways rather than a more direct route through pyruvate carboxylation. In contrast, hepatic fatty acid labelling ratios are not different from unity, which supports the hypothesis that pyruvate competes with acetate for mitochondrial transport. Once transported, the remaining C fragments are metabolized through citrate in a similar fashion to acetate.

In summary, it can be shown that a diet containing a marginal level of crude protein (150 g/kg) can be supplemented with lysine to give a growth rate similar to that attained with a diet containing a higher level of crude protein (200 g/kg). Changes in intermediary metabolism such as oxidation, lipogenesis and liver glucose production are not equal, however, under these conditions. It is interesting to note the relationship between protein quality and the activities of ICD and ME. Both enzymes catalyse reactions that generate NADPH required for *de novo* lipogenesis and would seem to be 'lipogenic' in nature. Attention to the role of ME in NADPH production is important because the bird cannot produce NADPH through the pentose cycle and must rely on the reaction catalysed by ME to provide NADPH for *de novo* lipogenesis (Raheja *et al.* 1971). The product of the reaction catalysed by ICD, 2-oxoglutarate resulting from citrate via isocitrate, may be important because avian acetyl CoA carboxylase is distinctively sensitive to citrate concentrations, as mentioned previously. Citrate may be depleted by demand for 2-oxoglutarate provided through ICD. The same trend was noted for ASPT which is considered a key enzyme in protein metabolism and requires 2-oxoglutarate as an amine receptor. Also, if protein furnishes acetyl CoA, the cytoplasmic reducing equivalents normally produced from dietary carbohydrate through aerobic glycolysis may be limiting because glucose production from dietary protein places a demand on limited supplies of these reducing equivalents. In the end, it must be stated that modifications in metabolism resulting from changes in the energy: protein ratio cannot be explained on the basis of meeting the need for a single limiting dietary amino acid.

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