Quantitation of the Bioenergetics of a Tuberculosis Infection in Chicks

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Interaction of an avian tuberculosis infection with a known metabolizable energy yield of dietary corn oil in chicks was used to quantitate total host energy expenditure necessitated by the infectious process. Three trials in which two doses of inoculum were used resulted in mild and severe involvements. Trial 1 (mild) indicated that 6% and trials 2 and 3 (severe) that 96 and 93% of the energy supplied by known quantities of corn oil were utilized by the tuberculosis process. In the birds given the low level of inoculum, the degree of tuberculosis involvement, as measured by increased liver size, was correlated with increased total quantities of hepatic ribonucleic acid, monoglycerides, free fatty acids, free cholesterol, and glucose. All of these effects were observed prior to manifestations of clinical symptoms or failure of the chicks to consume all food offered.

A recent comprehensive review (2) on the relationship between lipid metabolism and infectious illness emphasizes that it is not yet possible, from the data available, to define specific changes in the rates of synthesis, mobilization, peripheral utilization, or degradation of lipid moieties during infection. Research in our laboratories related to protein metabolism (9, 10) also has indicated the need to assess the role of energy utilization during infectious processes.

An avian model for estimating the metabolizable energy of foodstuffs described by Squibb (11) was believed to offer the possibility of casting an infection, avian tuberculosis (TB), into direct interaction with the known metabolizable energy yield of a dietary fat. This combination of experimental models, it was reasoned, would permit the first estimation of total host energy expenditure necessitated by an infectious process.

The results of three trials with such a model are reported here, along with a description of biochemical changes in nucleic acids and several lipid moieties in the liver, one of the principal target organs during disseminated avian TB.

MATERIALS AND METHODS

The procedure for determining the metabolizable energy of foodstuffs with an avian model (11) served as the basis of determining the dietary energy expended during avian TB. A rigidly controlled daily feed restriction schedule was used to restrict chick growth to 40 to 60% of the genetic potential normally achieved with ad libitum feeding. The restricted diet contained adequate quantities of balanced proteins, but was low in total calories. Within the limits used, growth of the chicks exhibited a highly reproducible, direct linear relationship to the availability of calories in the diet. The gain in weight of chicks over a 12-day interval, when compared with an established reference curve (11), was used to provide a readout of total energy expenditure.

The model specifically monitors growth response from 9 to 21 days of age (11). Therefore, 1-day-old broiler chicks averaging 40 g in weight were randomly assigned upon arrival from the hatchery to control and TB-infected groups. The chicks in the latter category were injected that day via the left jugular vein with an 0.5-ml suspension of Mycobacterium avium strain Kirchberg. The bacterial suspension used for injection was a 1:10 dilution of a 10- to 14-day-old culture in Tween-albumin medium, adjusted to an optical density of 0.45 or 0.60 at 550 nm (7). All birds were kept in separate but similar quarters with constant artificial light and a temperature of 22 ± 2 C. A commercial diet and water were provided ad libitum for the first 8 days after delivery and inoculation of the chicks.

The procedure from 9 to 21 days, the end of a trial, was the same as in the published model (11). Quantitation of the amount of energy used was based upon the concept that: (i) by feeding restricted amounts of a balanced 26% protein, low-energy diet to rapidly growing chicks, protein synthesis (one of the principal components of rapid growth) was limited by a single variable, the need for added energy; (ii) any change in energy utilization due to the presence of the infection...
would alter the growth-related yield of metabolizable energy; and (iii) the percentage change in metabolizable energy values could serve as the basis for quantitating the amount of energy expended because of the infectious process.

The 26% protein, low-energy basal ration contained: soybean oil meal (50% protein), 40%; fish meal (60% protein), 5%; alfalfa meal, 4%; ground corn, 35%; complete mineral mix, 4%; vitamin mix, 2%; and, to limit calories, non-nutritive fiber, 10%. To restrain protein synthesis, growth was deliberately held to approximately 55% of that expected under ad libitum feeding by providing each chick in each experimental group with a total of 244 g of diet (11). Since protein intake and body reserves were calculated to be more than adequate, the growth of the controls fed the low-energy basal diet became the point of reference for determining metabolizable energy utilization. Substituting 10% corn oil (known to contain metabolizable energy of 9 kcal/g) for the 10% non-nutritive fiber in the basal diet fueled growth to the extent of the availability of the calories in the corn oil. As previously demonstrated, the resulting synthesis of all components of body mass (weight gain) was linear and, under normal conditions, could be equated to metabolizable energy by reference to the previously published curve (11).

At the end of a 12-day trial, by calculation, each chick on the basal ration received a total of 927 calories, and those which received the substitution of 10% corn oil for the inert material in the basal ration received 1,147 calories. A chick given the same basal diet on ad libitum feeding will consume approximately 1,685 calories during this 12-day period and weigh approximately 445 g at 21 days of age. It was assumed that, if the early tuberculous process placed an energy demand on the chick, with the interaction limited to the 220 calories supplied by the corn oil, a comparison of the metabolizable energy yield of corn oil in the control and infected groups would serve as the basis for calculating energy expenditure due to the TB. The assumption required that the infectious process under investigation be regulated so that its energy demands would be less than the 220 calories supplied by the 10% corn oil. An illustration is presented in the Results.

Trial 1 consisted of the following groups: basal diet; basal diet plus 10% corn oil, with and without TB (optical density, 0.45); and basal diet plus 15% corn oil, with and without TB (optical density, 0.45). Although not a part of the model, the latter two groups were included to evaluate adequacy of the host reservoir of free amino acids while, at the same time, recording the effect of a higher intake of energy on the TB process. The TB groups had double the number of chicks to allow for possible mortality and to permit classifying biochemical data of the individual chicks in the TB groups according to low or high degrees of severity (12). At the end of the trial, all chicks were weighed and killed, and their livers were weighed and frozen for later analyses. Hepatic nucleic acid and protein content were determined according to modifications of Wannemacher et al. (13); hepatic lipid fractions were separated according to Biezenski et al. (3); and the method of Goodwin (4), which uses the o-toluidine reaction, was used to determine hepatic glucose.

Trials 2 and 3 were replicates of trial 1 except that: (i) the chicks were inoculated with a greater concentration of TB organisms (optical density, 0.60); (ii) the 15% corn oil diet was discontinued; and (iii) no biochemical observations were made.

RESULTS

In trial 1 (Table 1), as expected, the TB inoculum used resulted in a comparatively mild infection. The efficiency of feed utilization (grams of gain in body weight/total feed consumed, i.e., 244 g) in the 15% corn oil control group was somewhat higher than that in both 10% corn oil groups and significantly higher than that in the 15% corn oil TB group.

The following calculations of metabolizable energy utilization in the 10% corn oil groups of trial 1 will serve as examples for trials 2 and 3 also. (i) The final weight of basal controls was 190 g. (ii) The final weight of 10% corn oil controls was 245 g, an increase of corn oil controls over basal controls of 129%. According to the reference curve (11), this 129% increase was equivalent to and confirmed the metabolizable energy yield to be 9 kcal/g, i.e., that of the corn oil. (iii) The final weight of the 10% corn oil TB group was 240 g, an increase of TB over basal control of 127%. According to the reference curve (11), this 127% increase was equivalent to a utilization of metabolizable energy of 8.4 kcal/g of corn oil or 0.6 kcal/g less than the corn oil controls (see above). This difference in growth-related yield of metabolizable energy calculates to be 6.6% (0.6 kcal/g divided by 9 kcal/g) and indicates that 15 kcal of the extra 220 kcal provided were expended in some way as a result of the infectious process, which in this illustration was relatively mild.

Liver weights, liver/body weight ratios, and biochemical data of trial 1 (Table 2) were arrayed according to an index of TB involvement previously reported (12), which uses liver weight of tuberculous chicks as an indication of degree of infection. Within each diet, those with the least increase in liver weight were designated group A and those with the greatest increase, group B. Compared to their respective controls, chicks in the B groups had liver weights and liver/body weight ratios which were significantly higher, indicating that the degree of infection was greater in these groups than in the A groups, i.e., those with a low level of infection.

The arrayed data of trial 1 showed that in the 10% corn oil group A, with a low level of TB
TABLE 1. Trials 1, 2, and 3: body weights, efficiencies of feed utilization, and metabolizable energy yields during a tuberculosis infection in chicks

| Treatment                        | Final body wt          | Wt gain (g/g of food) | Metabolizable energy |
|----------------------------------|------------------------|-----------------------|----------------------|
|                                  | Actual (g) | Percentage of basal control |                      |                      |
|                                  | 1  | 2  | 3  | 1  | 2  | 3  | 1  | 2  | 3  |
| Controls                         |              |                      |                      |                      |
| Basal diet                       | 190 | 189 | 185 | —   | —  | —  | .28 | .27 | .35 |
| +10% corn oil                    | 245 | 244 | 243 | 129 | 129 | 131| .50 | .49 | .55 |
| +15% corn oil                    | 252 | —   | 133 | —   | —  | —  | .53 | —  | —  |
| Tuberculosis-infected Basal diet |              |                      |                      |                      |
| +10% corn oil                    | 240 | 191 | 189 | 127 | 101 | 102| .50 | .37 | .34 |
| +15% corn oil                    | 250 | —   | 131 | —   | —  | —  | .55 | —  | —  |

* = Treatment 1 = 6 chicks in each control group, 15 in each tuberculosis group; trial 2 = 6 chicks in each control group, 12 in each tuberculosis group; trial 3 = 6 chicks in all groups.

Table: 2. Trial 1: effect of low dosage of tuberculosis inoculum and two levels of calorie intake on liver weights and total quantities of liver nucleic acids and lipids fractions

| Liver component       | Percentage change | Tuberculosis effect | Diet and tuberculosis effect |
|-----------------------|-------------------|---------------------|----------------------------|
|                       | 10%, group A*     | 10%, group B       | 15%, group A*             | 15%, group B       | 10%, group A*     | 10%, group B       | 15%, group A*             | 15%, group B       |
| Weight                | +1                | +35**               | +9                        | +41*               | +1                | +10                 | +35*                   | +43*               |
| Liver/body weight     | 0                 | +39*                | +11                       | +44*               | 0                 | +7                  | +39*                   | +39*               |
| DNA                   | -8                | +6                  | -6                        | +17                | -8                | +3                  | +6                     | +6                |
| RNA                   | +4                | +25*                | +15*                      | +47*               | +4                | +13                 | +25*                   | +44*               |
| Protein               | -9                | +21                 | +7                        | +27*               | -9                | +4                  | +21                    | +23                |
| Monoglycerides        | +6                | +38*                | +8                        | +46*               | +6                | +16                 | +38*                   | +57*               |
| Diglycerides          | -5                | +22                 | -2                        | +31*               | -5                | -5                  | +22                    | +27*               |
| Triglycerides         | -19               | +42                 | -15                       | +76*               | -19               | -19                 | +42                    | +68*               |
| Free fatty acids      | +6                | +31*                | +8                        | +39*               | +6                | +7                  | +31*                   | +38*               |
| Free cholesterol      | +2                | +34*                | +19                       | +47*               | +2                | +18                 | +34*                   | +45*               |
| Cholesterol esters    | -5                | +35                 | +3                        | +24                | -5                | +4                  | +35                    | +26                |
| Glucose               | +78               | +169*               | +50                       | +133*              | +78               | +153                | +169*                  | +294*              |

* = Tuberculosis groups compared with respective controls; six to eight chicks per group.

All groups compared with 10% corn oil control; six to eight chicks per group.

Corn oil level.

*Group A = chicks with least increase in liver weight. Group B = chicks with greatest increase in liver weight.

Asterisks indicate $P < 0.05$.

Intrials, there were irregular and nonsignificant disease effects on total quantities of nucleic acids and lipid moieties; chicks with greater TB involvement (group B) showed significantly increased total quantities of liver ribonucleic acid (RNA), monoglycerides, free fatty acids, free cholesterol, and glucose. The increased energy input from the 15% corn oil not only increased the foregoing liver components but also total quantities of protein and the di- and triglycerides. A comparison of all TB and diet group interactions (Table 2) with the normal 10% corn oil control group showed that the observed increases in total quantities of various hepatic constituents were also related to severity of the infectious process, with the least effect noted for the mild TB-10% corn oil group and the greatest in the TB chicks fed the excess 15% corn oil.

In trials 2 and 3 (Table 1), which used a higher
inoculum of TB organisms, body weight gains and feed efficiencies were significantly depressed during the infectious process. The expected 9 kcal of metabolizable energy yield per g of corn oil was reduced in chicks with TB of greater severity to 0.4 kcal/g in trial 2 and to 0.6 kcal/g in trial 3. Calculated as above, these reductions in metabolizable energy values were 96 and 93%, respectively. Thus, energy cost of the infection amounted to 211 and 205 calories of the total 220 calories provided by the 10% corn oil, or to 18.4 and 17.8%, respectively, of the total dietary intake of calories.

DISCUSSION

Avian TB in the rapidly growing chick is an excellent model for quantitative studies of a chronic infection, even though precise control of the exact number of live bacilli to be injected is not yet available (6). When our standardized method for estimating inoculum dose is used, miliary TB is produced consistently, with the vast majority of tubercles being found in the liver and spleen; regardless of inoculum size, the earliest tissue reaction occurs in the liver during the second week (6). Foci composed of several lymphocytes appear first and become admixed with small epithelioid cells during the third week, the time of termination of the present studies. If the disease process is studied further, weight loss of the chicks may begin; the tubercles increase in size, displacing normal tissue; tubercles eventually develop central necrosis and a peripheral deposition of amyloid; skeletal muscles become severely wasted, and death occurs in 5 to 9 weeks, depending on inoculum size (6). A progressive gain in weight of the liver results from the increase in size and number of tubercles. This initial early reaction, in terms of protein synthesis, was found to be largely anabolic and fueled in the initial stages by nutrients in the diet (12). As the disease progressed beyond the incubation stage, the apparent toxicity of the TB led to depressed diet intake, thus forcing acute catabolism of muscle and other protein stores of the body to continue the reaction. In earlier studies, attempts to aid host resistance by increasing dietary protein (12) and fat (7) above normal requirements resulted in an increased magnitude of the catabolic reactions as well as higher mortality rates. Present data agree with these earlier observations.

The recent report of Squibb (11), describing a new model for determining the metabolizable energy content of foodstuffs, served as the basis here for the quantitation of the energy cost of a TB infection. In attempting to quantitate energy utilization in TB-infected chicks, two distinct protein synthetic processes must be considered, namely, those applicable to the ongoing anabolic processes of normal growth, and, second, the superimposed effects on host metabolism associated with, or initiated by, the invading bacteria.

When the corn oil reference standard, having 9 kcal/g of metabolizable energy, was substituted for the 10% inert material of the basal diet, growth response (increase in body mass) of the controls ranged from 129 to 131% over the basal groups, within the published experimental error of the model (11). When the tuberculous process was interacted with this known amount of added energy (10% corn oil), the infected birds failed to attain predicted body weight. The magnitude of the growth depression was related to the intensity of the infection; i.e., of the energy provided by corn oil, 94% was utilized for some purpose other than growth during the severe disease (trials 2 and 3), and 6% was similarly used during the mild involvement (trial 1). We recognize that the disease process utilizes energy substrates other than that supplied by corn oil. However, present data relate only to the basic assumption made here: with all other experimental conditions identical, any difference in the known metabolizable energy yield of corn oil can be charged to the stress of the infection; i.e., failure to attain predicted body weight is due to the competition of body growth requirements and the disease process.

Although the present report provides, during the period studied, a proximate quantitation of the total energy utilized by the TB-infected host, there is no way to equate such data to the precise amount of overt disease within each bird. Therefore, to relate response to severity, two infecting doses of TB inoculum were used to produce a range of disease effects varying from mild to severe but still remaining within the fiducial limits of the established metabolizable energy reference curve (11). In this regard, the maximal substitution of the reference corn oil for the 10% inert material was further adjusted in one trial to 15% to demonstrate that the regulated intake of the balanced 26% dietary protein had the potential to provide an excess of free amino acids for additional growth. The fact that 4% additional growth was observed in the noninfected 15% corn oil groups confirmed the availability of sufficient free amino acids in the basal ration.

Biochemical calculations were obtained from
data arrayed according to a proximate index of involvement (Table 1). Artificial infection of groups of animals, even though accomplished with measured concentrations of an inoculum, seldom, if ever, result in an entirely uniform course or severity of disease. Averaging data of widely diverse degrees of infection masks changes in biochemical response, especially when the disease is quite mild (8).

The data of the first trial demonstrated that even though the TB inoculum used resulted in a comparatively mild infection during the period of disease studied, liver size and total quantities of RNA, protein and lipid fractions were increased in those chicks with the greatest TB involvement (B groups) compared to those in the mild category (A groups). Serum hyperproteinemia and similar increases in liver size and total quantities of nucleic and free amino acids have been observed in previous studies (12, 14). These changes may be associated with increased nitrogen retention in other infections of chicks (5) or humans (1). On the whole, these early increases in nucleic and free amino acids and lipid fractions do correlate with an increase in the rates of synthesis of certain intracellular hepatic and extracellular serum proteins. As stated previously, any such diversion of necessary precursor materials, as well as the molecular machinery required for the synthesis of proteins specifically involved in host responses to the infectious process, are all components of or relate to weight changes, which is the basis of the metabolizable energy model employed here. However, since such newly synthesized proteins contribute to total body weight in the present model, there is the probability of some degree of underestimation of energy costs due to the infections.

It is recognized that the restricted feeding regimen required by the Squibb model may in itself affect the progress or intensity of the infection. However, the increases in liver size and total quantities of tissue protein and RNA noted here occurred prior to any voluntary restriction of food intake, confirming observations made under ad libitum feeding (12).

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