INFLUENCE OF LEVEL OF DIETARY FAT ON ADIPOSE TISSUE LIPOGENESIS AND ENZYMATIC ACTIVITY IN THE PIG

G. L. ALLEE, D. H. BAKER AND G. A. LEVEILLE

University of Illinois at Urbana-Champaign, Urbana 61801

ONE of the foremost goals of swine research is the development of methods to decrease the amount of fat in the pork carcass. We have reported previously (O’Hea, Leveille and Sugahara, 1970; Allee, Baker and Leveille, 1971a; Allee et al., 1971b) that the addition of fat to pig diets induces metabolic responses in adipose tissue resulting in a depression in the capacity for fatty acid synthesis. Although the addition of 12% dietary fat resulted in a marked depression of in vitro fatty acid synthesis, carcass fat was not reduced, presumably because the dietary fat was deposited as such in the carcass. Hill et al. (1958) have reported that lipogenesis in rat liver slices was reduced by feeding as little as 2.5% dietary fat. In the present study we have tested the hypothesis that at a certain minimal level of dietary fat, fatty acid synthesis would be markedly reduced. And, although some of the dietary fat would be deposited, the depression in the conversion of carbohydrate to fat could be of such a magnitude that total body fat might conceivably be reduced.

Experimental Procedures

Metabolic Studies. Biopsy adipose tissue samples (approximately 2 g) were obtained as previously described by Allee et al. (1971b). All samples were placed in 0.9% NaCl in a 37°C water bath during transportation to the laboratory. Duplicate adipose tissue slices were prepared using a Stadie-Riggs hand microtome. The incubation and extraction procedures have been previously described (Allee et al., 1971b). The incubation medium contained 5 μmoles of glucose, 0.3 μC glucose-U-14C and 0.1 unit of insulin per milliliter.

Enzyme Assays. Homogenates for the enzyme assays were prepared as previously described (Allee et al., 1971b). Malic enzyme (EC.1.1.1.40) was assayed by the method of Ochoa (1955) and citrate cleavage enzyme (EC.4.1.3.8) according to the method of Cot tam and Sreere (1969). The protein content of the tissue homogenates used for enzyme assays was determined by the procedure of Lowry et al. (1951). Enzyme activities are expressed as units per milligram protein where a unit is the amount of enzyme which will catalyze the utilization of one nanomole of substrate per minute at 25°C.

Plasma Analysis. Blood was obtained from the vena cava using a 10-ml heparinized syringe. Plasma was collected after centrifugation and was frozen at −20°C until analyzed. Plasma free fatty acids were determined by the method of Ko and Royer (1967). Total plasma cholesterol was determined by a modification (Leveille, Shockley and Sauberlich, 1962) of the method of Searcy and Bergquist (1960).

Experimental Animals. In experiment 1, 40 crossbred pigs averaging 44 kg initially were randomly assigned from outcome groups based on litter and sex to a split-plot design consisting of sex as the main plot and five dietary treatments as subplots. The dietary treatments consisted of 1, 4, 7, 10 and 13% dietary fat (corn oil). The composition of the semi-purified diets is shown in table 1. Dietary fat replaced cornstarch, and sand was added as a nonnutritive diluent to make the diets isocaloric, using as a basis the metabolizable energy values of Diggs et al. (1965). Pigs were individually fed to appetite twice daily at 8:00 am and 3.30 pm for a 60-min period at each feeding. Water was added to the feed in a 1:1 ratio weight ratio to encourage consumption. Between feedings the animals were confined to group pens having concrete floors, and water was provided ad libitum. Body weight was determined bi-weekly prior to the 8:00 am feeding. Biopsy adipose tissue samples were obtained on days 35 and 50 of the experiment to determine fatty acid synthesis. 14CO2 production, and
DIETARY FAT FOR SWINE

TABLE 1. COMPOSITION OF DIETS

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Level of dietary fat, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soybean meal (50% protein)</td>
<td>24.0 24.0 24.0 24.0 24.0</td>
</tr>
<tr>
<td>DL-methionine</td>
<td>0.1 0.1 0.1 0.1 0.1</td>
</tr>
<tr>
<td>Cornstarch</td>
<td>71.4 65.9 60.5 55.0 49.4</td>
</tr>
<tr>
<td>Trace-mineralized salt</td>
<td>0.5 0.5 0.5 0.5 0.5</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>2.0 2.0 2.0 2.0 2.0</td>
</tr>
<tr>
<td>Vitamin mix</td>
<td>1.0 1.0 1.0 1.0 1.0</td>
</tr>
<tr>
<td>Sand</td>
<td>0.0 2.5 4.9 7.4 10.0</td>
</tr>
<tr>
<td>Antibiotic</td>
<td>+ + + + +</td>
</tr>
<tr>
<td>Kcal/kg</td>
<td>3828 3828 3828 3828 3828</td>
</tr>
</tbody>
</table>

a Each value represents mean ± SEM of eight pigs.

TABLE 2. EFFECT OF LEVEL OF DIETARY FAT ON RATE AND EFFICIENCY OF GAIN OF PIGS FED ISOCALORIC DIETS (EXPERIMENT 1)*

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Level of dietary fat, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1  4  7  10  13</td>
</tr>
<tr>
<td>Initial weight, kg</td>
<td>44.3 ±1.3 43.7 ±0.8 43.4 ±1.0 43.5 ±1.2 44.4 ±1.4</td>
</tr>
<tr>
<td>Final weight, kg</td>
<td>93.0 ±1.3 95.9 ±0.8 93.0 ±1.0 97.1 ±1.2 92.5 ±1.4</td>
</tr>
<tr>
<td>Daily gain, kg</td>
<td>0.671 ±0.03 0.832 ±0.03 0.790 ±0.04 0.759 ±0.04 0.761 ±0.04</td>
</tr>
<tr>
<td>Gain/feed</td>
<td>0.346 ±0.01 0.361 ±0.01 0.371 ±0.01 0.367 ±0.01 0.360 ±0.01</td>
</tr>
</tbody>
</table>

* Each value represents mean ± SEM of eight pigs.
the diet resulted in a softer belly as measured by subjective analysis.

In experiment 1 the effect of sex could be evaluated statistically since the experiment was a split-plot with sex serving as the main plot. The effects of sex are shown in table 5. Barrows gained significantly (P<.01) faster and tended to have a lower gain/feed ratio than gilts. Females had a significantly (P<.01) lower rate of glucose-U-14C incorporation into fatty acids and oxidation to 14CO2 than did castrated males. And the activity of malic enzyme was significantly (P<.05) lower in adipose tissue from females than in tissue from castrated males. Gilt carcasses contained less backfat and perirenal fat but a greater percentage of lean cuts and a larger L. dorsi area than did the carcasses from barrows.

The second experiment was designed to study the response of young pigs to graded levels of dietary fat (tallow). Pigs fed 4, 7 and 10% dietary fat tended to gain faster and more efficiently than those consuming the 1% fat diet, but, these trends were not statistically significant (table 6). Plasma free fatty acids increased linearly (P<.01) with increasing levels of dietary fat and there was a trend toward increased plasma cholesterol levels as the fat content of the diet was increased.

Increasing the level of dietary fat resulted in a significant (P<.01) linear depression in glucose-U-13C incorporation into fatty acids and oxidation to 14CO2 (figure 1). The activities of malic enzyme and citrate cleavage enzyme were also decreased linearly (P<.01) as dietary fat level increased (figure 2).

Discussion

The addition of corn oil or tallow to the basal diet (1% fat) increased daily gain and gain per unit of energy consumed in both the finishing pig and the young pig. Several reports (Barrick et al., 1953; Gesler, Ensminger and Elam, 1957; Abernathy, Sewell and Tarpley, 1958; Kennington, Perry and Beeson, 1958; Kennington, Perry and Beeson, 1958; Pond, Kwong and Loosli, 1960; Kuryvial, Bowland and Berg, 1962; Clawson et al., 1962; Wagner et al., 1963) have shown that
fat addition to finishing pig diets results in an increase in daily gain. Other reports (Kropf, Pearson and Wallace, 1954; Baird, McCampbell and Neville, 1958; Noland and Scott, 1960) indicated no difference in daily gain due to the addition of dietary fat. In most reports efficiency of feed utilization has been improved by adding fat to the diet. This would be expected based upon the higher caloric value of fat than of carbohydrate.

Numerous workers (Asplund, Grummer and Phillips, 1960; Eusebio et al., 1965; Frobish et al., 1969; Frobish et al., 1970) have reported that the young pig is unable to utilize dietary fat efficiently. However, our recent results (Allee et al., 1971a) suggest that the young pig can utilize fat (corn oil) calories as efficiently as carbohydrate calories. Data presented in table 6 confirm this observation and suggest that fat calories may be used even more effectively than carbohydrate calories.

In attempting to explain the mechanisms by which dietary fat depresses fatty acid synthesis the inverse relationship between the rate of fatty acid synthesis and the level of free fatty acids in plasma is noted. Increasing the level of dietary fat resulted in a linear decrease in fatty acid synthesis and a linear increase in plasma free fatty acids. An inhibition of fatty acid synthesis by the in vitro addition of free fatty acids to crude preparations of liver (Langdon, 1960; Bortz et al., 1962) and adipose tissue (Cahill, LeBoeuf and Flinn, 1960) has been reported. Free fatty acids or their CoA derivatives have been reported (Bortz and Lynen, 1963a, b; Tubbs and Garland, 1964; Korchak and Masoro, 1964) to inhibit acetyl CoA carboxylase, the rate limiting enzyme in fatty acid synthesis (Chakrabarty and Leveille, 1969). More recent data (Dorsey and Porter, 1968; Pand and Mead, 1968), however, argue against ascribing a regulatory role to long-chain acyl CoA derivatives on the basis of their inhibitory effect on a wide range of enzymes.
enzymes. Increased levels of long-chain acyl CoA derivatives have been found in livers of fasted rats (Bortz and Lynen, 1963b) and in diabetic and fat-fed rats (Wieland and Weiss, 1963). Under these conditions circulating free fatty acid levels are elevated and the capacity for fatty acid synthesis is depressed. The mechanism responsible for the observed depression in fatty acid synthesis remains to be determined.

The addition of fat to the diets of finishing pigs has in most cases resulted in an increase in carcass fatness. In the present study the addition of dietary fat resulted in an increase in backfat thickness and a smaller l. dorsi area. Other carcass characteristics were not significantly affected by increasing the level of dietary fat. Pigs fed the basal diet containing 1% fat gained at a slower rate and consequently were approximately 10 days older at slaughter than were the pigs fed diets containing higher levels of dietary fat. This difference in age at slaughter could have influenced some of the carcass parameters, particularly l. dorsi area.

In the present study fat was added to the diets at the expense of cornstarch with sand being added as a nonnutritive diluent. Therefore, the concentration of each nutrient in the diet was kept in a constant ratio to metabolizable energy. In most of the previous studies conducted to assess the effects of dietary fat on performance and carcass characteristics in the pig, fat has been substituted for an equal weight of carbohydrates (usually corn), thereby altering the calorie:protein ratio and the amino acid pattern in the diets. Sand has been employed successfully as a nonnutritive diluent without any deleterious effects on performance (Baker et al., 1967).

Numerous studies (Cahill et al., 1960; Allen and Bray, 1964; Doornenbal, 1967; Baker et al., 1967; Bruner and Swiger, 1968) have shown the effects of sex on performance and carcass characteristics and our results are in agreement with these well-established effects. We were also able to show that adipose tissue of females had a lower rate of glucose-U-14C incorporation into fatty acids and oxidation to CO2 than did adipose tissue of castrated males. Malic enzyme activity was also lower in females than in male castrates. Thus, with regard to sex differences it appeared that in vitro rates of lipogenesis are indicative of total body fat as measured by carcass analysis.

Increasing the level of fat (tallow) in the diet of the young pig resulted in an increase

Figure 1. Effect of level of dietary fat on glucose-U-14C incorporation into fatty acids and oxidation to CO2 by pig adipose tissue. Increasing the level of dietary fat resulted in a significant (P<.01) linear depression in fatty acid synthesis and CO2 formation. Each point represents the mean±SEM of eight pigs.

Figure 2. Effect of level of dietary fat on the activity of malic enzyme and citrate cleavage in pig adipose tissue. Increasing the level of dietary fat resulted in a significant (P<.01) linear depression in the activities of malic enzyme and citrate cleavage enzyme. Each point represents the mean±SEM of eight pigs.
in plasma cholesterol level. Similar results have been reported for the finishing pig (Jurgens and Peo, 1970) and for the adult pig (Barnes et al., 1959). It has been reported by Dupont (1965) that when beef tallow was fed to rats in increasing amounts (20 to 80% of the calories) there was a linear increase in cholesterol biosynthesis. The relationship between fat feeding and cholesterol biosynthesis in the rat and the possible regulatory mechanisms have been discussed (Bortz, 1967). Whether these same mechanisms are of significance in the pig remains to be demonstrated.

Summary

Biopsy samples of porcine adipose tissue were employed to investigate in vitro fatty acid synthesis and the activities of malic enzyme and citrate cleavage enzyme in pigs fed graded levels of dietary fat. The effects of level of dietary fat on performance and carcass characteristics were also evaluated.

Increasing the level of dietary fat resulted in a linear depression of glucose-U-14C incorporation into fatty acids and oxidation to 14CO2. The activities of malic enzyme and citrate cleavage enzyme also decreased linearly as dietary fat level increased. Adipose tissue slices from females had a lower rate of glucose-U-14C incorporation into fatty acids and oxidation to 14CO2 than did that from castrated males. The activity of malic enzyme was also lower in females than castrated males.

Addition of corn oil or tallow to the basal diet (1% fat) increased daily gain and gain per unit of energy consumed in both the finishing pig and the young pig. Pigs fed diets containing 4, 7, 10 and 13% dietary fat had more backfat than the pigs consuming the 1% fat diet. However, there were no differences in perirenal fat, fat trim or percent four lean cuts due to level of dietary fat.

Increasing the level of dietary fat resulted in a linear increase in plasma free fatty acids. The inverse relationship between the rate of fatty acid synthesis and the level of free fatty acids in the plasma suggest a role for free fatty acids in the control of fatty acid synthesis.

Literature Cited


