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## Effect of Restricted Access to Feed on the Serum and Liver Lipids of Swine. (28924)

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The pattern of food intake has been reported by several investigators to exert an important influence on the manner in which it is metabolized. Cohn and co-workers(1) found that chicks permitted access to food for an hour in the morning and for another hour in the afternoon after 5 weeks had higher serum cholesterol levels than chicks allowed *ad libitum* access to food. In addition, the thoracic aortas of the chicks eating twice a day had a higher incidence of atherosclerosis, which was also more severe.

Okey, *et al*(2) and Lis and Okey(3) reported that changes in the feeding pattern affect the composition of both plasma and liver lipids of rats, with most of the alterations noted in the plasma lipids. However, the total amounts of serum and liver cholesterol, triglyceride and phospholipid were not appreciably altered by feeding method.

The alterations in lipid metabolism observed in these studies as a consequence of restricted feeding are of particular interest in human nutritional studies since the dietary habits of many humans resemble the pattern of food intake observed in the restricted-fed laboratory animal. Because of the physiological similarities between the pig and the human, the following experiment was conducted to determine what effect the pattern of food intake would have on the blood and liver lipids of swine.

*Materials and methods.* One hundred eight weanling pigs of mixed sex and breeding were randomly assigned to 12 concrete feeding floors each with identical feeding and watering facilities. All pigs received a balanced growing ration containing 16.4% protein and a fat content of 2.7%, of which 85% was unsaturated fat from a plant source (ground milo). Four pens of 9 animals each were assigned to each of the following feeding schedules: 1) *Ad libitum* access to feed, 2) One 2-hour feeding period (8:00 a.m.-10:00 a.m.), 3) Two one-hour feeding periods (8:00-9:00 a.m., 4:00-5:00 p.m.).

The first phase of the experiment was terminated at the end of 84 days, at which time all animals were bled *via* heart puncture and the blood analyzed for serum lipids. Six animals from each of the 3 feeding schedules were selected to continue the experiment for another 90 days, with care being taken to equalize sex and breed between treatments.

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TABLE I. Effect of Feeding Method, Breed and Sex on Serum Lipid Levels of 106 Growing-Fattening Pigs Fed for 84 Days.

| Variable | No./group | Total serum cholesterol | Serum triglyceride | Serum phospholipid |
|---|---|---|---|---|
| Feeding method | | Means (mg/100 ml) with standard errors | | |
| *Ad libitum* | 36 | 97.0 ± 1.92* | 37.0 ± 1.96 | 124.4 ± 3.05* |
| 2 one-hr | 35 | 90.0 ± 1.95 | 36.4 ± 1.99 | 115.9 ± 3.09 |
| 1 two-hr | 35 | 91.2 ± 1.95 | 35.2 ± 1.99 | 115.2 ± 3.09 |
| Breed | | Means (mg/100 ml) | | |
| Minnesota #1 | 31 | 103.6 | 34.6 | 128.4 |
| " #3 | 30 | 87.0 | 38.4 | 109.8 |
| Landrace × Minnesota #1 | 37 | 84.3 | 33.3 | 113.5 |
| Hampshire × Minnesota #1 | 8 | 111.4 | 47.8 | 136.7 |
| Sex | | | | |
| Barrow | 36 | 96.0 | 37.8 | 122.4 |
| Gilt | 47 | 94.7 | 38.7 | 120.8 |
| Boar | 23 | 83.8 | 28.6 | 108.0 |

* $P < .05$.

At the end of the 90-day period, the 18 pigs were killed and blood and liver samples obtained for lipid analysis.

Serum cholesterol was determined by the method of Anderson and Keys(4), serum triglyceride by the method of Van Handel and Zilversmit(5) and serum phospholipid according to the procedure of Zilversmit and Davis(6).

Because of the imbalance in breeds and sexes of pigs available for the first phase of this study, the statistical analysis for this phase was carried out by the method of least squares to correct for this imbalance.

*Results and discussion.* After 84 days, serum cholesterol and phospholipid levels of the *ad libitum*-fed pigs were significantly higher ($P<0.05$) than either of the restricted-fed groups (Table I). Serum triglyceride levels were essentially unaltered by feeding method. These data do not support the finding of Cohn *et al*(1) that serum cholesterol levels were raised in restricted-fed chicks, but are in agreement with the observation of Okey *et al*(7) that *ad libitum*-fed rats had higher serum cholesterol levels than rats with access to a cholesterol-containing diet for 3 hours each day.

The greatest differences in serum lipid levels were noted between breeds of pigs (Table I). The Minnesota #3 breed had markedly lower serum cholesterol and phospholipid levels but slightly higher serum triglyceride levels than the Minnesota #1. It is interesting to note the effects of crossbreeding on serum lipid levels of the Minnesota #1. The breed influence of the Landrace lowered while that of the Hampshire raised all of the serum lipid parameters measured in the crossbred pigs compared to the purebred Minnesota #1. The difference in serum lipid levels observed between breeds is thought to be of a true genetic nature but because of the imbalance in the number of animals of each breed available for the experiment no attempt will be made to report the statistical significance of these values. Although the estimated averages were properly adjusted by the least squares solution, decisions concerning such differences were not the initial objective of this experiment. Thus, it is preferred to call such finding suggestions for future investigation.

Sex appeared to have a pronounced effect on serum lipid levels with the intact males having consistently lower values than either the females or castrated males. In the human serum cholesterol levels in the premenopausal female are usually lower than in the male, although the reverse is true in the female rat and rabbit(8). In this respect swine resemble these laboratory animals more than the human.

The unadjusted treatment means are presented in Table II to show the breed and sex response to feeding method.

The effect of restricted access to food for a period of 173 days as measured by serum and

TABLE II. Effect of Feeding Method on Serum Lipid Levels by Breed and Sex.

| Variable | No./ group | Total serum cholesterol | Serum tri-glyceride | Serum phospho-lipid |
|---|---|---|---|---|
| *Ad lib* Feeding | | Unadjusted means (mg/100 ml) | | |
| Minnesota #1 | | | | |
| Barrow | 6 | 117.4 | 38.8 | 133.3 |
| Gilt | 5 | 116.1 | 51.4 | 145.6 |
| Boar | 0 | — | — | — |
| Minnesota #3 | | | | |
| Barrow | 0 | — | — | — |
| Gilt | 7 | 94.4 | 42.1 | 114.0 |
| Boar | 3 | 72.3 | 25.0 | 104.0 |
| Landrace × Minnesota #1 | | | | |
| Barrow | 1 | 82.6 | 19.0 | 114.1 |
| Gilt | 5 | 87.7 | 42.4 | 135.2 |
| Boar | 7 | 76.3 | 21.4 | 102.3 |
| Hampshire × Minnesota #1 | | | | |
| Barrow | 1 | 128.9 | 32.1 | 159.0 |
| Gilt | 0 | — | — | — |
| Boar | 1 | 108.1 | 33.0 | 134.8 |
| 2 One-Hr Feedings | | | | |
| Minnesota #1 | | | | |
| Barrow | 5 | 101.6 | 37.8 | 141.0 |
| Gilt | 4 | 103.0 | 33.0 | 112.8 |
| Boar | 0 | — | — | — |
| Minnesota #3 | | | | |
| Barrow | 2 | 92.5 | 39.5 | 116.5 |
| Gilt | 4 | 79.5 | 34.2 | 92.8 |
| Boar | 4 | 88.0 | 37.8 | 117.5 |
| Landrace × Minnesota #1 | | | | |
| Barrow | 5 | 89.4 | 36.2 | 118.0 |
| Gilt | 6 | 87.0 | 33.6 | 111.6 |
| Boar | 2 | 62.5 | 31.0 | 85.0 |
| Hampshire × Minnesota #1 | | | | |
| Barrow | 1 | 106.0 | 41.0 | 131.0 |
| Gilt | 2 | 112.5 | 52.1 | 141.3 |
| Boar | 1 | 112.0 | 54.2 | 120.0 |
| 1 Two-Hr Feeding | | | | |
| Minnesota #1 | | | | |
| Barrow | 7 | 99.2 | 28.1 | 120.2 |
| Gilt | 4 | 99.6 | 32.5 | 141.2 |
| Boar | 0 | — | — | — |
| Minnesota #3 | | | | |
| Barrow | 2 | 81.0 | 42.5 | 120.2 |
| Gilt | 5 | 86.3 | 37.0 | 102.0 |
| Boar | 3 | 81.3 | 36.4 | 101.4 |
| Landrace × Minnesota #1 | | | | |
| Barrow | 5 | 87.0 | 42.2 | 114.0 |
| Gilt | 6 | 86.6 | 30.7 | 114.3 |
| Boar | 1 | 90.1 | 30.0 | 94.9 |
| Hampshire × Minnesota #1 | | | | |
| Barrow | 1 | 116.1 | 88.1 | 164.2 |
| Gilt | 0 | — | — | — |
| Boar | 1 | 80.0 | 24.3 | 92.0 |

liver lipids is shown in Table III. Serum triglyceride was significantly depressed and the C/P ratio significantly elevated ($P<0.05$) in the *ad libitum*-fed pigs compared with pigs receiving one 2-hour feeding. No statistically significant differences were noted in any of the other serum lipids.

The most consistent effects due to feeding method were observed in the liver lipids. Okey *et al*(2) and Lis and Okey(3), however, noted that, in contrast to the liver lipids, the composition of the plasma lipids was affected much more by changes in feeding pattern. Most of the variation in plasma and liver lipids reported by these workers was in the composition of fatty acids and in the proportion of free *vs* esterified cholesterol rather than overall changes in triglyceride, phospholipid or total cholesterol levels.

Total cholesterol, triglyceride and the C/P ratio were significantly higher in the livers of the *ad libitum*-fed pigs compared with either of the restricted-fed groups (Table III). The increase in total cholesterol was slight but the variation within treatments was so small that the difference was highly significant statistically ($P<0.01$). The elevated plasma and liver C/P ratios of the *ad libitum*-fed pigs may be a reflection of an elevated concentration of beta-lipoprotein relative to the alpha group, which does not vary greatly in concentration and usually transports less cholesterol than the beta group.

The results of this feeding experiment with swine do not appear to support the findings of other workers(1) that plasma and liver lipids are elevated as a consequence of restricted access to feed. *Ad libitum*-fed pigs exhibited consistently higher serum and liver lipid levels than the restricted-fed animals except for the serum triglyceride and phospholipid values of the pigs fed for 173 days. Alterations in hepatic lipid content due to feeding method appeared to be of greater magnitude and to be less variable than serum lipid content.

It is possible that swine do not adapt to a restricted feeding schedule as readily as do other laboratory animals since the restricted-fed pigs in this study consumed approximately 10% less feed and gained 10% less weight

TABLE III. Effect of Restricted Access to Food on Serum and Liver Lipids of 18 Pigs Fed for 173 Days.

| Variable | Ad libitum | One 2-hr feeding | Two 1-hr feedings |
|---|---|---|---|
| Serum Lipids (mg/100 ml) | | | |
| Total cholesterol | 104.5 ± 2.3* | 100.6 ± 2.3 | 102.2 ± 2.3 |
| Triglyceride | 30.5 ± 4.1 | 48.2 ± 4.1† | 41.8 ± 4.1 |
| Phospholipid | 100.6 ± 7.6 | 127.3 ± 7.6 | 106.3 ± 7.6 |
| C/P ratio | 1.04 ± .06 | .79 ± .06† | .96 ± .06 |
| Liver lipids (mg/g) | | | |
| Total cholesterol | 2.99 ± .09‡ | 2.38 ± .09 | 2.20 ± .09 |
| Triglyceride | 9.62 ± 1.59† | 3.56 ± 1.59 | 2.57 ± 1.59 |
| Phospholipid | 3.34 ± .13 | 3.09 ± .13 | 2.95 ± .13 |
| C/P ratio | .90 ± .03‡ | .75 ± .03 | .77 ± .03 |

* Means with standard errors. † $P < .05$. ‡ $P < .01$.

than the *ad libitum*-fed animals. Since the restricted-fed chickens used in the work of Cohn *et al*(1) ate one-third less feed and gained one-third less body weight than *ad libitum*-fed controls it may be that species differences exist between chickens, pigs and rats in the adjustment to restricted feeding and in the way that limited access to food influences the manner in which the food is metabolized.

*Summary.* Serum cholesterol and phospholipid levels were significantly elevated ($P<0.05$) in *ad libitum*-fed pigs compared with the restricted-fed animals at the end of 84 days. After 173 days of restricted feeding pigs fed *ad libitum* had significantly higher liver cholesterol, triglyceride and C/P ratios than pigs with limited access to feed. The serum C/P ratio was significantly higher but serum triglyceride significantly lower ($P<0.05$) in the *ad libitum*-fed pigs when compared with the group receiving one 2-hour feeding. Marked variations in the serum lipid levels between different breeds of pigs and between sexes were observed.

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## Effect of Triiodothyronine Administration on Erythrocyte Radioiron Incorporation in Rats. (28925)

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Thyroxine or triiodothyronine has produced an augmentation of erythropoiesis with a resultant erythrocythemia in both dogs and rats(1,2,3). An increase in oxygen requirement as a result of thyroid hormone administration reportedly acts as the stimulus to the enhanced red blood cell production. This hypothesis is consistent with the concept that the red cell mass is related to total tissue

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