INFLUENCE OF DIETARY AMINO ACIDS ON THE AGE AT PUBERTY OF YORKSHIRE GILTS 1, 2

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Summary

FIVE gilts in each of four lots were individually and limited-fed from about 115 days of age. The experimental diets comprised a basal (B) to which was added 0.31% L-lysine HCl and 0.08% DL-methionine, singly or combined. Visual signs of first estrus, confirmed with a boar, were taken as the onset of puberty. Gilts fed the basal diet supplemented with methionine were younger (P < 0.05) at puberty than those fed the lysine-supplemented diet. In general, the gilts fed the two lysine-supplemented diets (L and LM) appeared to reach puberty later than the gilts in the other two lots (B and M). These data suggested that a dietary imbalance, due to lysine supplementation, was involved.

In a second experiment, there were four replicates, each of five dietary treatments with gilts fed ad libitum in groups of three per pen. All diets, except a corn-soybean positive control, were supplemented equally with 0.08% DL-methionine. The diets comprised a basal (B) to which was added 0.31% L-lysine HCl 0.05% DL-tryptophan, singly or combined. Gilts fed either the basal or lysine-supplemented (L) diets reached puberty from 12 to 24 days later than those fed the control, the tryptophan (T) or the lysine + tryptophan (LT) supplemented diets. Differences in body weight at puberty were not significant although control pigs average 98 ± 19 kg compared with 87 ± 10 kg for the (L) gilts. Rate of body weight gain was significantly greater (P < 0.01) for the control gilts than for gilts in the other four lots; and greater (P < 0.05) for the (LT) than for the (L) and the (T) gilts. In this study, there was some indication that the faster growing gilts reached puberty at an earlier age than the slower growing gilts.

Introduction

Age at first estrus, or puberty in gilts can vary considerably, with a reported range from 136 days (Sumption, Rempel and Winters, 1961) to 398 days (Salmon-Legagneur, 1970). It appears to be genetically determined but modified by various environmental influences (Joubert, 1963). For example, age at puberty was found to be earlier in Poland China than in Yorkshire gilts (Clark et al., 1970), and age at first conception was less (246 vs. 278 days) for September to February born Landrace gilts (Sviben et al., 1969). Results of a study by Jensen et al. (1970) suggest that even space restriction might influence the onset of estrus.

That age at puberty might be influenced by nutritional factors was suggested, probably first, by McKenzie (1928) on the evidence of postmortem examination of a gilt which had been maintained on a low plane of nutrition. Lodge and McPherson (1961) reviewed the somewhat conflicting literature concerning the relationships between levels of nutrition and body weight and age at puberty in gilts and concluded that fast growing gilts were heavier at puberty than those restricted in feed or energy intake. Age at puberty was either unaffected or reduced when moderate (60 to 70% of full-feed) restriction was imposed and only severe underfeeding (50% restriction) caused a marked delay.

More specifically, Witz and Beeson (1951) reported a retardation of sexual maturity in pigs fed a fat-free diet compared with controls receiving dietary fat. Further, Davidson (1930) fed gilts a protein deficient diet and found they "would not grow, would not breed and, paradoxically, would not even die." These gilts failed to reach sexual maturity at from 12 to 16 months of age.

The nature of the dietary protein seems to have a pronounced effect on age puberty. Fowler and Robertson (1954) demonstrated...
sexual precocity in gilts fed an animal protein rather than a vegetable protein supplement. Johnsen, Moustgaard and Olsen (1952) fed gilts skim milk or vitamin B12 as supplements to a vegetable protein basal diet; age at first estrus for the 20 gilts in each lot averaged 197, 207 and 207 days, respectively. Evans and Bishop (1922) observed a disturbance to the estrus cycle of rats fed wheat as the sole source of dietary protein, and Pearson (1937) was able to restore the estrus cycle in rats by adding “0.6% d-lysine dihydrochloride” to a diet containing gliadin which, previously, had caused cessation of estrus. In spite of this evidence, indicating an amino acid influence, there appears to be only one published report on the effects of dietary amino acid supplementation on reproductive maturity in female swine, that of Larsson, Nilsson and Olsson (1966), who advanced the time of ovulation in gilts by supplementing a commercial diet with 0.10% L-lysine and 0.05% DL-methionine.

The purpose of the present study was to determine with prepuberal gilts the effect on age at puberty of dietary supplementation with lysine, methionine and tryptophan, either singly or in combination.

**Experimental Procedure**

**Experiment 1.** A basal corn diet calculated to contain 0.24% lysine and 0.10% methionine was supplemented with 0.31% L-lysine HCl (0.25% free base) and 0.08% DL-methionine, both singly and combined. The levels of supplementation were based on estimated requirements (N.R.C. 1968) by the bred gilt for 0.49% lysine and 0.35% methionine in the diet. Cystine was assumed to satisfy 50% of this need for methionine. Sand, as an inert filler (Baker et al., 1965), was used for the amino acid premixes (table 1).

Twenty Yorkshire gilts, born April 13 to 26, 1971, were started on experiment when averaging 115 days of age (107 to 119 days). There were five replicates of each of the four dietary treatments, comprising a basal diet to which was added the supplements. The gilts were allocated at random to individual pens and diets, but because it is known (Dyck, 1971) litter-mates tend to reach puberty at a similar age, no two gilts from the same litter were allocated to the same replicate nor to the same dietary treatment. The concrete-floored pens were bedded with wood shavings and provided with automatic waterers. Each gilt was offered her respective diet up to a maximum of 2 kg in two equal feedings daily.

The date of the first observed estrus was taken to be the onset of puberty. Daily testing by an attendant using one of two entire boars housed with the gilts, supplemented any visual signs of estrus. The presence and use of these boars probably induced earlier estrus than would have occurred in their absence (Du Mesnil du Buisson and Signoret, 1972; Brooks and Cole, 1970). Estrus was confirmed by the gilt “standing” to the boar but coitus was not permitted. After the first estrus, the experimental diet was then no longer fed; the gilt remained in the same pen and was fed a standard diet for an additional 21 days to record a second estrus. The occurrence of this estrus was taken as evidence of a normal reproductive cycle. Records were kept of weekly body weights, individual feed refusals and the date of and body weight at puberty. A sample of the basal corn diet was analysed for lysine, methionine and cystine content using a Jeol type JLC-SAH Amino Acid Analyser according to the mercaptoethanol method of Keutmann and Potts (1969).

**Experiment 2.** Tryptophan was used in this experiment to correct a suspected amino acid imbalance and was added, assuming an 80%
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utilization (Baker et al., 1971), as a dietary supplement to a basal diet calculated to contain 0.10% tryptophan.

There were four replicates selected on the basis of age. Each replicate consisted of five dietary treatments comprising a positive control corn-soybean diet, a basal corn diet and this basal diet to which was added either 0.31% L-lysine HCl 0.05% DL-tryptophan or 0.31% L-lysine HCl plus 0.05% DL-tryptophan (table 2). All diets, other than the control, were supplemented equally with 0.08% DL-methionine.

Sixty Yorkshire gilts, born October 1 to 25, 1971, were started on experiment at an average age of 119 days (107 to 131 days). The gilts were allocated to their dietary treatments within replicates, to provide groups of three per pen. They were fed a standard diet ad libitum for 1 week before starting the experimental diets. This preliminary period allowed better detection of any chance incidence of estrus provoked by the sudden change in material and social environment (Du Mesnil du Buisson and Signoret, 1962). Pens were bedded with wood shavings and each provided with an automatic waterer and a self-feed hopper. The pens of gilts were fed the experimental diets ad libitum and any feed wastage was accounted for when determining weekly fed consumption for each pen. Weigh-backs were taken immediately after a gilt was removed from the experiment so that average feed consumption per gilt could again be calculated. Gilts were weighed weekly and completed the experiment when they had exhibited an estrus confirmed by the boar. Three entire boars, penned in the same building as the gilts, were available for daily detection of estrus. As in the first experiment, a gilt was retained in her pen until a second estrus established a normal reproductive cycle, but records of body weight and feed were completed 1 recording week from the occurrence of first estrus. The experiment ended after 139 days of feeding the test diets. Animals not having exhibited estrus by that time, or having abnormal cyclic behavior, were slaughtered and the ovaries examined for follicular growth and corpora luteal development.

Data were examined statistically by analysis of variance and comparisons between sets of means were tested by the Student-Newman-Keuls method (Steel and Torrie, 1960). Missing values were calculated from the means of the known values for the respective replicate and treatment containing each missing value (Steel and Torrie, 1960). In the analysis of variance, error degrees of freedom were reduced by the number of missing values.

**TABLE 2. COMPOSITION OF THE CONTROL, BASAL AND AMINO ACID SUPPLEMENTED DIETS FED TO YORKSHIRE GILTS IN EXPERIMENT 2**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Control (C)</th>
<th>Basal (B)</th>
<th>Lysine (L)</th>
<th>Tryptophan (T)</th>
<th>Lysine+Tryptophan (LT)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn, ground</td>
<td>84.0</td>
<td>92.5</td>
<td>92.5</td>
<td>92.5</td>
<td>92.5</td>
</tr>
<tr>
<td>Soybean meal (49%)</td>
<td>12.0</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td>Dical. phos.</td>
<td>2.0</td>
<td>3.5</td>
<td>3.5</td>
<td>3.5</td>
<td>3.5</td>
</tr>
<tr>
<td>Limestone</td>
<td>1.0</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Salt (iodized)</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Vitamin premix*</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Amino acid premix:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-lysine HCl d</td>
<td>0.00</td>
<td>0.00</td>
<td>0.31</td>
<td>0.00</td>
<td>0.31</td>
</tr>
<tr>
<td>DL-tryptophan e</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>DL-methionine f</td>
<td>0.00</td>
<td>0.08</td>
<td>0.08</td>
<td>0.08</td>
<td>0.08</td>
</tr>
<tr>
<td>Sand f</td>
<td>0.00</td>
<td>0.42</td>
<td>0.11</td>
<td>0.37</td>
<td>0.37</td>
</tr>
</tbody>
</table>

* The control and basal diets contained, respectively, by analysis (as fed): DM, 88.9 and 90.3; ash, 5.0 and 5.6; CP, 15.9 and 9.8%; GE, 3.64 and 3.77 kcal/g and were calculated to contain about 0.50 and 0.22% lysine; 0.15 and 0.10% methionine; 0.15 and 0.10% cystine and 0.15 and 0.10% tryptophan.

* Containing 18.5% Ca and 20.5% P.

* Supplying per kilogram of the control and basal diets: vitamin A, 5000 IU; vitamin D₃, 500 IU; vitamin E, 11 IU; vitamin B₁₂; riboflavin, 4.4 and 6.6 mg; Ca pantothenate, 8.8 and 13.2 mg; niacin, 13.2 and 19.8 mg; choline chloride, 270.0 and 330.0 mg plus 2.75 and 2.25 g ground corn as filler.

* Purchased from Dawes Laboratories of Canada Ltd., Weston, Ontario.

* Purchased from Philipp Bros. Canada Ltd., Montreal 109, Quebec (agent for Ajinomoto Co. of New York, N. Y. 10022).

* Silica sand, No. 40 grade, fine.
TABLE 3. AGE, BODY WEIGHT AND FEED DATA FOR GILTS IN EXPERIMENT 1 *

<table>
<thead>
<tr>
<th>Experimental diets</th>
<th>Supplements to basal diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Item</td>
<td>Basal (B)</td>
</tr>
<tr>
<td><strong>Puberty:</strong></td>
<td></td>
</tr>
<tr>
<td>Age, days b</td>
<td>178 ± 24</td>
</tr>
<tr>
<td>Body weight, kg</td>
<td>80 ± 12</td>
</tr>
<tr>
<td>Weight gain, kg/day</td>
<td>0.43 ± .02</td>
</tr>
<tr>
<td>Feed intake, kg/day</td>
<td>1.91 ± .03</td>
</tr>
</tbody>
</table>

a Each value is the mean±SD for five gilts. b Difference between the lysine (L) and the methionine (M) diet significant (P<.05).

Results

Experiment 1. At the start of the experiment, the overall age and body weight were 115 ± 4 days and 51 ± 7 kg, respectively. All gilts had exhibited an estrus, confirmed by the boar, by 110 days from the start of the experiment. At puberty, body weight differences between pigs were not significant but gilts fed the basal diet supplemented with methionine (M) were younger (P<.05) at puberty than those fed the lysine-supplemented (L) diet (table 3). However, these two lots were, respectively, the lightest and heaviest in body weight at puberty.

The basal corn diet contained by analysis: 0.26% lysine, 0.15% methionine and 0.17% cystine. Average daily body weight gains and feed intakes from the start of the experiment to the attainment of puberty, did not differ significantly. Feed intakes were affected mostly by the incidence of lameness which caused temporary lack of appetite or locomotor facility. Two cases occurred in the L lot, three in the LM lot and one each in lot B and lot M.

Experiment 2. A total of five gilts did not conclude the experiment successfully and missing values were calculated to complete the data. One gilt in the control (C) lot diet, the cause of death being diagnosed (Agriculture Canada, Health of Animals Branch, Animal Diseases Research Institute) as degenerative arthropathy. Two gilts in lot L were discarded from the experiment due to acute lameness. A third gilt from this lot and one from lot B failed to show any sign of sexual maturity; post-mortem examination revealed immaturity of ovaries and follicles. Reproductive development and cyclic regularity was confirmed in three other gilts for which insufficient evidence had been obtained previous to slaughter. At the start of this experiment, the overall age and body weight was 119 ± 8 days and 60 ± 8 kg, respectively (table 4). Gilts fed either the basal (B) or lysine-supplemented (L) diet, reached puberty from 21 to 24 days later than those fed the control (C) or the lysine plus tryptophan supplemented (LT) diet. In spite of this later age, gilts fed the L, T and the LT diets were about 10 kg lighter in body weight at puberty than those fed the control (C) diet. Total feed consumption by gilts fed the C, L and T diets was similar, whereas the B gilts consumed about 10 kg more and the LT gilts about 13 kg less. The differences in age, weight and feed intakes were not significant (P>.05).

Daily gain in body weight was significantly (P<.01) less for gilts fed the basal corn (B) and the amino acid supplemented (L, T and LT) diets than for the controls (C) fed the corn-soybean diet. Rates of gain were significantly (P<.01) less for gilts fed diet L and just significantly (P<.05) less for diet T than for gilts fed diet LT. The lower daily gain for gilts fed diet B compared with those fed diet LT approached, but did not reach, statistical significance (P>.05).

The daily intake of diet C was the greatest (P<.01). The high rate of gain by gilts fed diet C resulted in their having the highest feed efficiency which, however, was significantly (P<.01) different only from that of gilts fed diet L.

Discussion

The basal diet fed to gilts in experiment 1 was ostensibly deficient in lysine and methionine for finishing pigs and for bred sows and gilts, assuming dietary requirements (N.R.C.
TABLE 4. AGE, BODY WEIGHT AND FEED DATA FOR GILTS IN EXPERIMENT 2 *

<table>
<thead>
<tr>
<th>Item</th>
<th>Control b (C)</th>
<th>Basal c (B)</th>
<th>Lysine+ (L)</th>
<th>Tryptophan (T)</th>
<th>Lysine+tryptophan (LT)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Puberty:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, days</td>
<td>170 ± 30</td>
<td>192 ± 28</td>
<td>194 ± 26</td>
<td>180 ± 25</td>
<td>171 ± 32</td>
</tr>
<tr>
<td>Body weight, kg</td>
<td>98 ± 19</td>
<td>91 ± 14</td>
<td>87 ± 10</td>
<td>88 ± 11</td>
<td>89 ± 15</td>
</tr>
<tr>
<td>Feed intake, kg</td>
<td>145 ± 17</td>
<td>166 ± 67</td>
<td>149 ± 54</td>
<td>145 ± 61</td>
<td>123 ± 76</td>
</tr>
<tr>
<td>Weight gain, kg/day d</td>
<td>0.74± .12</td>
<td>0.42± .10</td>
<td>0.35± .14</td>
<td>0.44± .10</td>
<td>0.55± .11</td>
</tr>
<tr>
<td>Feed intake, kg/day *</td>
<td>2.69± .19</td>
<td>2.16± .20</td>
<td>1.93± .18</td>
<td>2.23± .13</td>
<td>2.27± .14</td>
</tr>
<tr>
<td>Feed efficiency f</td>
<td>3.7 ± 0.5</td>
<td>5.4 ± 1.7</td>
<td>6.3 ± 2.8</td>
<td>5.3 ± 1.2</td>
<td>4.3 ± 1.0</td>
</tr>
</tbody>
</table>

a Each value is the mean±SD for 12 gilts.
b Corn-soybean diet (13.9% CP).
c Corn diet (9.8% CP).

Only the difference in age (201 vs. 168 days) at puberty between gilts fed the lysine and the methionine supplemented diet was significant (P<.05), but the data also indicated that, compared with gilts fed diets to which lysine had been added (diets L and LM), those fed diets without supplementary lysine (diets B and M) reached puberty in about 12% less time (173 vs. 196 days).

The data suggested that a dietary amino acid imbalance, due to lysine supplementation of the corn diet, was involved. This phenomenon, in which lysine and not tryptophan assumed the role of the second-limiting amino acid in swine diets, has been reported by Baker et al. (1969) for growing pigs.

In experiment 2 there was some indication, though not supported statistically, that the combined addition of lysine and tryptophan to the basal corn diet (B) restored it, with respect to age at puberty, to a performance potential comparable to that of the corn-soybean control diet (C). Such a response parallels that reported by Cromwell, Picket and Beeson (1967) who obtained similar, though not equal performances from pigs fed either opaque-2 corn or normal corn supplemented with lysine and tryptophan combined; the effect was not apparent when the supplements were fed singly.

There are too few experiments with simple diets to determine, confidently, the effect of amino acid deficiency or imbalance on sexual maturity and development in swine. Robertson et al. (1951) fed high protein (20% then 15%) and low protein (15% then 11.25%) diets to gilts and reported that the low protein gilts reached puberty 6 days earlier (P<.05) than the high protein group. Adams et al. (1960) found that gilts on a “protein-free” diet failed to cycle normally or conceive; ovulation rate and number of embryos were greatest in gilts fed either a 32% corn-soybean diet or a 15% corn-sesame (methionine-rich) diet plus 0.25% L-lysine HCl, compared with a 16% corn-soybean and a 15% corn-sesame diets. Unfortunately, the effect of these diets on early maturity was not reported.

Fowler and Robertson (1954) conducted an experiment in which 10 gilts fed an all-plant protein, basal diet (milo, soybean and alfalfa meals with vitamin B12 and mineral supplements) reached puberty 16.9 days later (P<.05) than a similar group fed the basal diet in which 10.4% then 8.0% meat scrap replaced the same quantity of soybean meal. Meat scrap has a similarly high proportion of lysine and arginine as does soybean meal, but has slightly more methionine and distinctly less tryptophan. Calculations show little difference in arginine, lysine and methionine content between the vegetable and animal protein diets used, although the latter contained a slightly lower proportion of tryptophan.

Much earlier work with poultry (Prentice, Baskett and Robertson, 1930) gave similar results; pullets fed an all-cereal diet laid their first egg 49 days later than those receiving...
the same diet plus a supplement of separated milk. However, the addition of only a mineral mixture to the cereal diet also decreased age of maturity, by 40 days. Davidson (1930) perceived too, a need for dietary mineral supplementation to overcome delayed post-weaning estrus in gilts fed a protein-deficient diet.

Differences in performance demonstrated in the present study using amino acid supplements, might be explained most readily in terms of amino acid balance and imbalance, as suggested by Baker et al. (1969), rather than in terms of absolute deficiencies, since the supplementary lysine level, for example, brought the dietary total within the range of estimated requirements. The experiment of Larsson et al. (1966) involved, in essence, only four gilts in each of two lots totalling 17 pigs. The control lot were fed a basal diet (88% cereal, 6.6% vegetable protein feeds, 3.3% meat meal and 2.1% minerals), whereas the treated lot received the basal diet supplemented with lysine and methionine. Examination of the ovaries of the control gilts revealed that only one ovulation per gilt had taken place compared with at least three ovulations in the case of gilts fed the lysine-methionine supplement. The growth rate and feed efficiency of all pigs was improved by the addition of amino acids.

In the present study, with gilts fed a corn-soybean diet or a basal corn diet with or without lysine and tryptophan, body weight within each dietary treatment was positively correlated (.001<P<.05) with age at puberty (table 5); except for the lysine-supplemented diet, the reason for which was not clear. In general, therefore, the heaviest gilts within a treatment lot were the oldest at puberty. But, body weight differences among treatments were not significant (table 4), even though, for example, the control (C) gilts were 13% heavier at puberty than those fed diet (L). O’Bannon et al. (1966) found that gilts given high energy diets were 13 days younger, 21 kg heavier and gained faster than those given low energy (52% alfalfa supplement) diets (P<.01). But differences (P<.05) in age at puberty of Palouse gilts (Hafez, 1960) indicated that gilts restricted to 70% of full-feed reached puberty earlier than did full-fed controls.

According to Lodge and McPherson (1961), puberty in gilts appeared to be influenced less by body weight than by age. Certainly, in their study, all gilts regardless of level of feed intake, reached puberty at about the same average age (176, 176 and 178 days for low, medium and high, respectively) but differed markedly (P<.001) in body weight and rate of growth. All the gilts were moved together, at about 6 months of age, from concrete-floored pens to deep-littered yards and it is conceivable (Du Mesnil du Buisson and Signoret, 1962) that the relatively early and similar age at puberty might be attributable partly to this environmental change, especially since the onset of estrus was established by visual observation only.

Results from the present study (table 5) showed a negative correlation (P<.05) between growth rate and age at puberty only within the control lot of gilts. However, the trend in this study appeared to be (table 4), for the faster growing gilts to reach puberty at an earlier, but not significantly different, age than the slower growing gilts.

Daily feed intake (table 4), was lowest (P<.01) for pigs fed diet (L) which, in view of the significantly (P<.01) greater intake by the controls, indicated that diet rather than high incidence of lameness was responsible for the recorded inappetence, typical of an amino acid imbalance (Harber, 1967; Rérat, 1972). Differences in sexual maturity might be due, in part at least, to differences

<table>
<thead>
<tr>
<th>TABLE 5. CORRELATION COEFFICIENTS WITHIN DIETS FOR DAILY RATE OF GAIN, BODY WEIGHT AND AGE AT PUBERTY (EXPERIMENT 2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experimental diets</td>
</tr>
<tr>
<td>Item</td>
</tr>
<tr>
<td>Daily rate of gain</td>
</tr>
<tr>
<td>Body weight at puberty</td>
</tr>
</tbody>
</table>

*, ** Statistical significance (df=10) at the 5% and the 1% levels of probability, respectively.
in rate of development of the reproductive tract and associated glands as well as in body growth and body weight. It is not unreasonable to anticipate some influence of a protein inadequacy on sexual maturity since evidence is available (Platt and Stewart, 1967) suggesting that dietary protein plays an important role in maintaining a delicate endocrine balance in the pig.

Literature Cited


