THE EFFECTS OF DIETARY PROTEIN LEVELS ON GROWTH, NITROGEN BALANCE AND REPRODUCTIVE PERFORMANCE IN GILTS

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Bachelor of Science

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1969

Submitted to the Faculty of the Graduate College
of the Oklahoma State University
in partial fulfillment of the requirements
for the Degree of
MASTER OF SCIENCE
May, 1973

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ACKNOWLEDGMENTS

The author wishes to express his appreciation to Dr. C.V. Maxwell, Assistant Professor of Animal Science, for his suggestions and counsel during the preparation of this manuscript.

The author is grateful to Dr. I.T. Omtvedt and Dr. R.H. Thayer for assisting in the proofing of this manuscript and to Rodger K. Johnson, Animal Breeding Graduate Student, for his guidance in analyses of the data. Appreciation is extended to fellow graduate students for their aid in completing this study.

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CHAPTER I

INTRODUCTION

For maximum productivity, it is desirable to produce breeding gilts that exhibit fertile estrous cycles early in life. It is also desired that these gilts conceive promptly, bear large, healthy litters and produce adequate milk for these offspring. Recent research on the effects of protein nutrition on reproduction has failed to include the development of replacement gilts. Accepted management procedures have generally suggested that prospective breeding gilts be limit fed from approximately 50 kg. body weight to breeding at the third estrus. This fact, combined with a tendency to reduce feed intake to prevent excessive conditioning, may indicate a reduced daily protein intake. Little is known about what levels of protein are desirable for developing breeding gilts. Most studies involving levels of protein intake during gestation have not revealed any marked differences in litter size, birth weight or pig survival.

It should be noted that gilts in most of the previous studies received an optimum protein level until breeding or immediately before breeding. The protein stores in these animals, therefore, should have been at a maximum at the initiation of gestation. Little information is available on the effect of low protein diets initiated well before breeding on the reproductive performance of gilts.

Nitrogen retention can be used to indicate the level of dietary protein which maximizes body protein synthesis; however, this technique does not differentiate between protein synthesis that is required for optimal reproduction and that which is simply stored protein. More work is needed to determine the relationship of nitrogen retention to reproduction.

This study was initiated to determine the effect of extended feeding of widely varying levels of crude protein on growth of the gilt, nitrogen balance, estrual regularity and reproductive performance evaluated at 30 days post-breeding. The results of this study should provide evidence for establishing the protein requirements of young breeding gilts.

CHAPTER II

LITERATURE REVIEW

The influence of dietary protein intakes on reproductive performance in swine might be divided into four related subject areas. This review of literature is organized into units concerning (1) puberty and the estrous cycle, (2) gestational weight gain, (3) nitrogen balance of breeding gilts and sows, and (4) reproductive performance at parturition.

Puberty and the Estrous Cycle

The age at puberty and the regularity of fertile estrus are both economically important factors in swine production. The literature offers only limited data on the effects of protein on these two factors. Robertson *et al.* (1951) showed no significant differences in age at puberty in gilts in dry lot which were full or limit fed either a 15 or 11.25% crude protein ration. Gilts fed the high protein ration on pasture were significantly older at puberty ($P < .05$) than gilts on the low protein intake.

Several researchers have noted a higher incidence of reproductive failures in gilts and sows on low protein intakes. Adams *et al.* (1960) fed prepuberal gilts six rations with varying protein quantity and quality and found that gilts fed a "protein free" diet failed to cycle normally or conceive. Boaz (1962) reported a greater irregularity in the length of time from weaning to first estrus and a reduced conception rate associated with low levels of dietary protein. In these experiments, the rations contained 10.31, 14.29 or 18.10% crude protein and were fed at 6 to 7 pounds daily. Other supporting evidence was noted when Pond <u>et al</u>. (1968) placed five gilts on each of three treatments allowing 1.82 kg. daily of 0, 9 or 12% crude protein diets before breeding. Two gilts on the protein-free diet became anestrous, whereas all five gilts on the 9 and 12% protein rations were mated. Numbers were not adequate to associate treatments with reproductive failures.

These scattered reports of irregular estrual cycling, attributed to low protein intake, indicated an area which warranted more research. Svajgr <u>et al</u>. (1972) pursued the problem further and observed that severe protein restriction during gestation and lactation in first litter gilts would significantly reduce percentage of sows returning to estrus and increase average interval from weaning to estrus. In this study, diets fed at 1.82 kg. per day contained 2 or 17% crude protein during gestation and 5 or 17% protein during lactation. In a second trial, Svajgr found that anestrous gilts on the 5% protein ration responded to injections with 1500 i.u. PMS (pregnant mare serum) and 750 i.u. HCG (human chorionic gonadotropin) by exhibiting estrus within 36 hours after injection. All eight sows ovulated after injection. The authors suggested that low protein diets may result in inadequate gonadotropin production and/or release.

The literature also contains evidence of a decrease in conception rate due to low protein intake. A significantly reduced ($P<.05$) farrowing percentage (60.9%) was observed by Baker <u>et al</u>. (1969) in bred gilts fed 144 g. of crude protein daily. The higher protein intakes (224, 304, 384 and 480 g.) resulted in greater farrowing percentages (71.0, 82.3, 71.4 and 76.0%, respectively). The authors speculated that since treatments were imposed after conception, failure of embryos to implant may have resulted from the reduction of daily feed intakes to 0.9 kg. (144 g. protein). Widely varying energy intakes prevent any conclusions as to the simple effects of protein level changes. Other work (Hawton and Meade, 1971) showed that gilts fed 154, 250 or 341 g. of protein daily during gestation exhibited an apparent decrease in conception rate and an increase in resorption of feti associated with the two lower protein levels. The authors suggested that low protein quantity and quality may have reduced the productive life of the sows.

Weight Gain

Since reproductive processes seem to have higher priority for protein than growth processes (Pond, *et al*., 1969), measurement of growth in the gilt or sow should give some evidence of the adequacy of a diet to supply, firstly, the protein needed for reproduction and, secondly, the extra protein needed for growth of body tissues.

Clawson *et al*. (1963) fed gilts either 136 or 544 g. of protein in either 1.36 kg. or 2.73 kg. of daily ration and found a highly significant ($P<.01$) weight gain advantage in gilts fed the high protein rations. Average weight gain during gestation for gilts fed the low protein ration was 52.3 and 49.1 kg., whereas gilts fed the high protein level gained 58.6 and 53.6 kg. for trials 1 and 2, respectively. Total litter weight at farrowing was slightly higher in gilts fed the higher protein ration.

Pond *et al*. (1968) fed 1.82 kg. daily of a protein free diet, a 9% corn diet, or a 12% protein corn-soybean meal diet to gilts before and during pregnancy. Gilts on the protein free diet lost 2 kg. by day 78 of pregnancy, while gilts on the 9 and 12% rations gained 17 and 31 kg., respectively. Litter weights at farrowing, indicating relative protein deposition for reproductive processes, were 7.99, 11.70 and 12.35 kg. from lowest to highest protein intakes. In yet another study, Pond et al. (1969) observed that gilts fed a protein free diet from day 24 to parturition lost 5.5 kg., whereas gilts fed a 12% protein diet gained 22.4 kg. This indicated that protein storage was not adequate before and during early pregnancy to maintain weight gain during the latter part of pregnancy.

Gestation weight gain for gilts fed graded levels of a 16% protein ration (Baker et al., 1969) increased quadratically ($P<.01$) with increasing feed intake. Weight gain tended to plateau at daily intakes above 1.9 kg. indicating that the energy and protein (304 g.) needs were probably met.

During the last half of gestation, gilts gained significantly ($P<.01$) more when fed a 16% crude protein corn-soybean meal ration as compared to a 5% protein diet (Rippel et al., 1965a). In another work (Baker et al., 1970b) gestation was divided into trimesters and four schemes were devised for feeding either a corn diet (8.7% protein) and/or a corn-soybean meal diet (16% protein) during gestation. Weight gains of gilts fed corn for the first two-thirds and corn-soybean meal during the last third of pregnancy, were as great or greater than gains of gilts fed the corn-soybean meal diet throughout gestation. From this fact it was indicated that either the corn had adequate protein to maximize growth of the gilt during early gestation or that the corn diet depleted protein "stores" and stimulated more efficient nitrogen retention late in gestation to replenish the lost "stores". Low protein intake (corn) early in gestation showed no permanent reduction in gestation weight gain.

Studies by Baker et al. (1970a) on protein quality and quantity using corn, opaque-2 corn and corn-soybean meal diets showed that gilts fed a corn diet (8.8% protein) during gestation gained less ($P<.05$) than the average of all other gilts on trial. Gilts fed opaque-2 corn gained less ($P<05$) than gilts fed corn-soybean meal diets. Level of protein from corn-soybean meal diets (12, 16 and 20% protein) did not affect gestation gain, suggesting that 1.90 kg. of the 12% protein diet was adequate to maximize gain. These same relationships were reflected in litter weights at birth. Similar results were noted by Hesby et al. (1970) who also studied quality and quantity of protein and found that gilts fed a corn diet (9.6% protein) gained less ($P<.05$) during gestation than gilts fed opaque-2 corn (11.2% protein) or corn-soybean meal diets (11.2 or 16.2% protein). Litter weight was increased by feeding opaque-2 corn or the high protein corn-soybean meal diets.

Hawton and Meade (1971) fed pregnant gilts 341, 250 or 154 g. of protein daily during gestation and observed weight gains of 46.6, 42.1 and 36.1 kg., respectively. When these gilts were rebred and fed the same rations during the second pregnancy, weight gains were significantly different ($P<.05$) on 341 and 154 g. of protein. Values were 47.8, 43.8 and 33.8 kg. for high, medium and low protein intakes, respectively. This study may indicate that extended protein restriction may increase the differences in gestational weight gain attributed to protein intake differences.

Differences in gestational gain due to the pregnancy state have been observed by using pregnant and non-pregnant gilts fed 309 g. of crude protein daily and slaughtered at day 111 after breeding of pregnant gilts (Elsley et al., 1966). When adjusted to equal beginning live weight, pregnant gilts gained 25 kg. more than non-pregnant controls. The increased weight was more than accounted for by increases in blood, mammary tissue, empty reproductive tract and contents of the reproductive tract. These workers suggested that differences in live weight early in gestation were due to "pregnancy anabolism". Kline et al. (1972) also reported greater body weight gain in pregnant gilts versus non-pregnant gilts on 1.82 kg. of a 14% protein ration. Ham muscles of pregnant gilts had significantly less protein ($P<.01$) and significantly more water ($P<.01$) than non-pregnant controls. The authors suggested that protein was mobilized from muscular tissues to develop the fetal tissues.

Nitrogen Balance

Nitrogen balance has been used to determine the dietary level of crude protein which maximizes nitrogen retention or protein synthesis in the gilt. Although nitrogen balance tends to over-estimate nitrogen retention, this technique is still valuable for investigating the relationship of protein intake to protein synthesis in the body.

Rippel et al. (1965b) investigated nitrogen balance during two five-day periods starting at day 82 and day 102 post-breeding. Nitrogen retention plateaued at approximately 12.5% (228 g.) crude protein when feeding 1.82 kg. of feed daily from day 70 of pregnancy. Nitrogen retention was greater during the second collection period. An intake of approximately 55 g. of nitrogen daily appeared to be slightly in excess of maintenance needs. Miller et al. (1969) also studied nitrogen balance during late pregnancy (day 95). Regression of nitrogen retention on dietary protein was significantly linear (P<.01) but not significantly quadratic, indicating that retention had not plateaued at the higher protein intakes. Gilts receiving 114 to 342 g. of protein daily had mean daily nitrogen retentions of 4.45 to 15.40 g., when measured at day 95 of gestation. The authors stated that maximal nitrogen retention during late pregnancy was not necessary for optimum reproductive perfor- as measured by number and weight of pigs farrowed and weaned.

Experiments were conducted by Hesby et al. (1971) to determine the effects of dietary protein from various sources on nitrogen balance. Gilts fed a corn-soybean meal control diet (15% protein) and those fed an opaque-2 corn diet (11.2% protein) retained significantly more nitrogen (P<.01) than gilts fed a corn-soybean meal diet (11.2% protein) or a normal corn diet (9.2% protein). Daily retentions were 16.91, 15.19, 11.41 and 7.15 g. for the respective diets. Percent retained nitrogen was significantly greater (P<.01) for gilts fed opaque-2 corn than for those fed other diets.

Theoretically, addition of a purified source of a ration's first limiting amino acid should increase retention of nitrogen. Holden et al. (1971) therefore supplemented an 8% crude protein ration with four levels of methionine but observed no significant effect on nitrogen balance of gilts before or during gestation. Nitrogen retentions before breeding and at 30, 68 and 108 days of gestation were 10.40, 9.98, 9.52 and 9.13 g., respectively. The reduction of nitrogen retention over the four stages of pregnancy was not significant and was not in agreement with other workers' results.

Shearer et al. (1971) studied nitrogen retention in the gilt from pre-puberty through parturition. Nitrogen retention expressed in mg./kg. body weight/day decreased markedly with the onset of puberty, 245.7 ± 2.8 versus 124.5 ± 8.8 mg. Throughout early gestation, retention remained rather constant (73.5 ± 5.9 mg.) then rose to approximately 90.0 ± 5.8 mg. by day 90 and remained constant to day 110. The authors stated that the significant fall in retention and greater variability in the cyclic animal as compared to the pre-puberal animal suggests a hormonal regulation of nitrogen retention instigated at puberty which is catabolic in nature. However, attempts to relate progesterone levels to nitrogen retention were unsuccessful.

Elsley et al. (1966) found that pregnant gilts fed 309 g. of crude protein daily retained 16.7 g. of nitrogen as compared to non-pregnant controls which retained 12.4 g. daily. Percent retention was 33.6% for pregnant gilts and 24.8% for open gilts.

While observing nitrogen retention of pregnant, cyclic, hysterectomized and overiectomized gilts, Kline et al. (1972) observed that pregnant gilts retained more nitrogen as pregnancy progressed. Retention for pregnant and cyclic gilts were 0.04 ± 6.2 and 4.67 ± 4.02 g., respectively, at 15 to 20 days post-breeding. At days 107 to 112, retention had increased to 11.93 ± 6.91 and 7.74 ± 5.41 g., respectively. Ham muscles of pregnant gilts had significantly ($P<.01$) less protein and significantly more water than cyclic gilts. The authors suggested that protein was mobilized from the dam to develop the fetuses.

Reproductive Performance

Reproductive performance is evaluated in this section as ovulation rate, number of embryos or pigs, birth weight and subsequent growth and viability of offspring.

Robertson *et al.* (1951) reported that protein levels of 20, 15 or 11.5% of the diet did not significantly affect ovulation rate at first or second estrus in gilts. There were slight advantages for high protein levels in gilts on pasture as opposed to advantages for low protein levels in gilts in dry lot. No differences in ovulation rate or number of embryos at 22 to 28 days post-breeding could be shown by Tribble *et al.* (1956) when feeding 12 or 16% protein rations. Different conclusions were arrived at by Adams *et al.* (1960) who placed gilts on six treatment rations with various protein quantities and qualities. Ovulation rate and number of embryos present at 25 days post-breeding were greatest in groups fed a 32% corn-soybean meal diet or a 15% corn-sesame meal diet plus 0.25% L-lysine hydrochloride, as opposed to a "protein free" diet, a corn diet or a 16% corn-soybean meal diet.

Evidence of the importance of early pregnancy protein nutrition was presented by Pond *et al.* (1968) who studied the effects of a "protein free" diet on the reproductive performance of gilts and the subsequent growth of the offspring. Control diets were a 12% corn-soybean meal ration and a 9% corn ration. The authors concluded that gilts can maintain pregnancy in the absence of dietary protein by mobilizing maternal tissue stores of amino acids for fetal development. Inception of the protein free treatment at day 24 to 28 of gestation apparently had a less adverse effect on performance than inception before breeding. Six-week weights of pigs indicated a decreased milk production in gilts on the protein free diet. Further work by Pond *et al.* (1969) showed that protein deprivation has a greater detrimental effect on reproductive performance during certain periods of gestation than others. Birth weight and post-natal growth rate of progeny of gilts fed protein at days 16 to 20 of gestation (implantation) were greater ($P<.05$) than those for progeny of gilts fed a protein free diet throughout gestation. The authors indicated that this supports the concept of the importance of protein in early gestation. Litter size was not significantly affected by dietary treatment.

Feeding various levels and qualities of protein during the last 50 days of pregnancy had no significant effect on total number of pigs farrowed, live pigs farrowed, birth weight or livability of pigs at two weeks post-partum (Rippel et al., 1965a). Rations fed at 1.82 kg. daily were 5% crude protein gelatin, 5% sesame meal, 5% corn-soybean meal, 8% corn and 16% corn-soybean meal. Holden et al. (1968) found no significant differences in number of pigs farrowed alive, birth weight or number of pigs weaned when sows were fed 146, 218, 291 or 364 gm. of crude protein during pre-breeding and gestation. Crude protein levels of sows' milk and pig gain from birth to weaning increased linearly ($P<.05$) with increasing protein intakes.

Baker et al. (1970b) reported that gilt and sow litters from dams fed a corn diet (8.7% crude protein) throughout pregnancy gained less from birth to 21-day weaning than litters from dams fed a 16% protein corn-soybean meal diet. Litter growth rate was not improved by feeding the 16% protein diet during the first trimester of gestation, but was improved by feeding the corn-soybean meal diet during the last third of gestation. No differences were observed between treatments in number of pigs farrowed (total or live) or birth weights.

Hawton and Meade (1971) fed three levels of protein (154, 250 and 341 g./day) to gilts and sows during two successive gestation periods.

Protein treatments did not significantly affect total or live pigs per litter, birth weight or weaning weight in the first reproductive cycles.

A summary by Boaz (1962) of three experiments on protein effects on reproduction stated that only small and statistically non-significant effects on prolificacy could be shown when protein levels were varied from approximately 280 to 493 g. of crude protein daily. Sows confined to concrete yards showed a tendency to have smaller litters when fed the low protein levels. All treatments were imposed after the animals were well grown and in good body condition.

Clawson et al. (1963) reported no significant differences in numbers of pigs born to gilts fed 136 or 544 g. of crude protein daily. Both protein levels were fed at feed intake levels of 1.36 and 2.72 kg. per day. Level of protein fed during gestation had no significant effect on birthweight, pig survival or litter weight at weaning. In other work, Frobish et al. (1966) compared daily crude protein intakes of 182 or 364 g. in combination with energy intakes of either 5,400 or 10,800 K cal of metabolizable energy per day. No significant differences in number of pigs farrowed alive, birthweight of live pigs or pig gain from birth to weaning were observed between gilts fed 182 or 364 g. of protein daily. However, more ($P \leq .05$) pigs were weaned from gilts on high protein intakes than on low intakes. Reproductive performance of gilts and subsequent progeny development were studied by Baker et al. (1969) when feeding five intake levels of a 16% crude protein ration. When allowed a daily intake of 0.9, 1.4, 1.9, 2.4 or 4.0 kg. of the experimental ration, number of pigs farrowed or weaned were not significantly affected by intake level. Birth weight and weaning weight increased as feed intake increased, but plateaued at 1.9 and 2.4 kg.

intakes, respectively.

The influence of protein quality on reproduction was investigated by Hesby et al. (1970) with opaque-2 corn, normal corn, and two corn-soybean meal diets. No differences were shown among treatments for live and total pigs per litter, birth weight, number of stillborn pigs or pig weights at 21 and 35 days. The vitamin and mineral-fortified opaque-2 corn diet (11.2% crude protein) compared favorably to the 15% corn-soybean meal diet with respect to all reproductive parameters. A similar study by Baker et al. (1970a) with 269 first-litter gilts showed no dietary treatment effect on number or birth weight of pigs farrowed. An opaque-2 corn (9.7%) diet was equal to corn-soybean meal diets (12, 16 or 20% protein) and superior to normal corn (8.8%) in number of pigs weaned per litter, litter gain and litter weight at weaning (21 days of age).

Assuming that 1.82 kg. of an 8% protein corn-soybean meal diet was adequate to meet gross requirements for pregnancy, Holden et al. (1971) added DL-methionine to the diet to determine minimum requirements for gestation. DL-methionine levels of 0.18, 0.26, 0.39 or 0.58% of the diet gave average litter sizes of 5.9, 8.1, 7.7 and 8.3 pigs, respectively. Within treatment variation was high and no significant differences could be shown. Plasma free methionine increased quadratically ($P < .05$) with increasing dietary methionine indicating that the 0.18% level was adequate for pregnancy.

CHAPTER III

MATERIALS AND METHODS

Data utilized in this experiment were collected from 90 Yorkshire gilts in three trials conducted from January, 1970 through October, 1972. Table I gives the distribution of gilts by treatment, trial and disposition. Gilts were allotted by weight to three protein level groups at an overall average age of 146 days and average weight of 66.8 kg. Age and weight on test for the three treatments were: high protein, 145.5 days, 66.8 kg.; medium protein, 146.3 days, 67.5 kg.; low protein, 146.3 days, 66.0 kg. Each of the three treatment groups was randomly assigned to one of three dirt lots. Each lot was equipped with an automatic waterer, sow house, sprinkler system and 10 individual feeding stalls.

Protein Treatments

Three rations were formulated to contain 8% (low), 14% (medium), or 20% (high) crude protein (Table II). The 20% milo-soybean meal ration was diluted with adequate amounts of cornstarch to give 14% and 8% protein rations. This procedure maintained constant amino acid ratios across treatments. Dietary calcium and phosphorus levels were maintained at 0.75 and 0.50% of the ration, respectively. Vitamins and trace minerals were supplemented by the premix described in Table II.

Gilts were individually fed 2.27 kg. of the assigned ration once daily until average gilt age was 180 days, then the daily allowance was lowered to 1.82 kg. Rations were sampled at the time of mixing and Kjeldahl nitrogen determinations (A.O.A.C., 1960) were conducted on each sample. Calculated and actual nitrogen and crude protein intakes are shown in Table III.

TABLE I

DISTRIBUTION AND DISPOSITION OF GILTS

	Protein Level					
	20%		14%		8%	
Trial	No. Allotted	No. Mated	No. Allotted	No. Mated	No. Allotted	No. Mated
Trial 1	10	9	10	10	10[1]	8
Trial 2	10[1]	8	10	8	10	7
Trial 3	10[2]	7	10	5	10	4
Totals	30	24	30	23	30	19

[1] One died.

[2] Crippled gilt removed.

Gilts were weighed every two weeks to monitor growth and were checked daily for signs of estrus by introducing a teaser boar into each pen and observing behavior and appearance of each gilt. Ages of gilts at first and second estrus were recorded and gilts were handmated to Yorkshire boars at the third estrus. An attempt was made to mate gilts on the first and second days of the second post-puberal estrus with different boars to help eliminate boar influence on reproduction. Gilts returning to estrus after the first mating were rebred in the same manner.

TABLE II

COMPOSITION OF EXPERIMENTAL RATIONS

Ingredients, %	20% Protein	14% Protein	8% Protein
Milo (8.0%)	68.52	47.96	27.41
Soybean meal (49.0%)	28.50	19.95	11.40
Cornstarch	-----	28.61	57.39
Dicalcium phosphate	0.64	1.30	1.89
Limestone	1.34	1.18	0.91
Vitamin-trace mineral premix[1]	0.50	0.50	0.50
Salt	0.50	0.50	0.50
Total	100.00	100.00	100.00

[1] Vitamin-trace mineral premix supplied 3300 I.U. vitamin A, 330 I.U. vitamin D_3, 4.4 mg. riboflavin, 33 mg. niacin, 22 mg. pantothenic acid, 1100 mg. choline, 16.5 mcg. vitamin B_{12} per kg. of feed, 0.22 ppm. iodine, 99 ppm. iron, 22 ppm. manganese, 11 ppm. copper and 99 ppm. zinc.

TABLE III

DAILY NITROGEN AND CRUDE PROTEIN INTAKES[1]

	Protein Level		
	High 20%	Medium 14%	Low 8%
Calculated N	58.2	40.8	23.3
Actual N	55.4	40.6	22.8
Calculated crude protein	364.0	254.8	145.6
Actual crude protein	346.2	253.8	142.5

[1] All values given in g./day when feeding ration at 1.82 kg./day.

Two five-day nitrogen balance studies were conducted with as many randomly selected gilts as facilities would allow. Six metal metabolism stalls were equipped for collection of urine and feces. Animals were placed in the stalls on day 11 after the second estrus and again on day 21 after breeding with a two-day adjustment period allowed before collection began. Urine was collected in polypropylene bottles with 20 ml. of concentrated hydrochloric acid added to prevent loss of NH_3. Daily urine volumes and fecal weights were recorded and 10% of each daily collection was frozen for duplicate Kjeldahl nitrogen determinations.

Gilts were slaughtered at approximately 30 days post-breeding at Ralph's Packing Company in a twice-weekly slaughter schedule. This resulted in embryo ages ranging from 28 to 35 days. The reproductive tracts were recovered and evaluated in the Physiology Laboratory. Each corpus luteum on the ovaries was counted and dissected to prevent counting the same ones twice. Each uterine horn was cut open, starting from the cervical end to obtain the embryos. Crown to rump length was measured with calipers on all viable embryos while encased in the amnionic sac. Viable embryos were considered to be those which showed no signs of atrophy and were of reasonably normal size. Anestrous gilts were slaughtered at the end of each trial and tracts were examined for evidence of estrual cycling.

Statistical analysis was carried out following the procedure described by Harvey (1960). Embryo numbers and percent survival were adjusted for unequal numbers of corpora lutea and embryo length was adjusted for unequal age of embryos. These covariables were fitted to the following mathematical model:

$$Y_{ijk} = \mu + \beta(X_{ijk} - \bar{X}_{\cdot\cdot}) + t_i + p_j + (tp)_{ij} + e_{ijk}$$

where,

Y_{ijk} = individual observation of number of live embryos, percent survival or average embryo length.

μ = mean number of live embryos, percent survival or average embryo length.

β = a regression coefficient for the effect of either number of corpora lutea or days pregnant, X_{ijk} covariables, with $\bar{X}_{\cdot\cdot}$ being the overall mean associated with the appropriate covariable.

t_i = an effect of the ith trial.

p_j = an effect of the jth protein level.

$(tp)_{ij}$ = an effect for the interaction of the ith trial with the jth protein level.

e_{ijk} = the failure of the stated model to estimate number of live embryos, percent survival or average embryo length.

The resulting analysis of variance is shown in Table IV.

All other analyses in this study utilized the model shown by the following example:

$$Y_{ij} = \mu + t_i + p_j + (tp)_{ij} + e_{ij}$$

where,

Y_{ij} = individual observation of age at first estrus.

μ = mean age at first estrus.

t_i = an effect of the ith trial.

p_j = an effect of the jth protein level.

$(tp)_{ij}$ = an effect for the interaction of the ith trial with the jth protein level.

e_{ij} = failure of the model to estimate age at first estrus.

The analysis of variance components are shown in Table V. Significant

TABLE IV

ANALYSES OF VARIANCE FOR NUMBER OF LIVE EMBRYOS, PERCENT SURVIVAL AND AVERAGE LENGTH

Source	df
Total	66
Mean	1
Covariable[1]	1
Trial	2
Treatment (Protein Level)	2
Linear	1
Quadratic	1
Trial X Treatment	4
Error	56

[1] Covariables were either number corpora lutea held constant for number of live embryo and percent survival analyses, or days pregnant held constant for embryo length analysis.

trial effects warranted blocking by trial and trial by treatment interactions were not significant except for fecal nitrogen, collection period I. This allowed comparison of treatments across trials for all other variables.

Student's "t" test was used for testing differences between means, (Snedecor and Cochran, 1967). Due to unequal numbers, the $S_{\bar{d}}$ was calculated as follows:

$$S_{\bar{d}} = \sqrt{\frac{S^2 (N_1 + N_2)}{N_1 N_2}}$$

where,

$S_{\bar{d}}$ is the standard error of the difference.

S^2 is the estimated variance (error mean square).

N_1 is the number of observations in group one.

N_2 is the number of observations in group two.

The "t" test was calculated from the following formula:

$$t = \frac{\bar{d}}{S_{\bar{d}}}$$

where $\bar{d}$ is the difference between treatment least squares means.

TABLE V

ANALYSIS OF VARIANCE FOR
AGE AT FIRST ESTRUS

Source	df
Total	81
Mean	1
Trial	2
Treatment (Protein Level)	2
Linear	1
Quadratic	1
Trial X Treatment	4
Error	72

CHAPTER IV

RESULTS AND DISCUSSIONS

This chapter presents data collected on growth, estrous cycles, nitrogen balance and reproductive performance from the experimental animals with supporting discussion of the results. Tabular data is presented with standard errors when applicable.

Growth and Sexual Maturity

Protein level had a significant linear effect ($P<.01$) on average daily gain to first estrus (Table VI) with the low protein level (8%) gilts gaining significantly slower ($P<.001$) than other gilts. Total weight gain to puberty was not significantly affected by treatment, suggesting a relationship between body weight and age at sexual maturity. Since energy values were similar among treatments, it is apparent that protein was a limiting factor in growth. Observed weight gains on all diets were only slightly less than those cited by Cunha (1957) as being acceptable for developing replacement gilts. Krider and Carroll (1971) suggest that a gain of as much as 0.7 kg. per day may be entirely safe for developing breeding gilts and recommend a daily feed intake of 2.5 to 3.0 kg. per 100 kg. of bodyweight of a 14 or 15% crude protein ration. This would indicate that the feed intake level was adequate at the levels fed before 180 days of age but slightly restricted after daily allowances were reduced to 1.82 kg.

TABLE VI

TOTAL WEIGHT GAIN AND AVERAGE DAILY GAIN TO FIRST ESTRUS[1]

	Protein Level		
	20%	14%	8%
No. of gilts exhibiting estrus	27	28	25
Initial weight, kg.	66.8	67.5	66.0
Total weight gain, kg.	35.1 ± 1.7	34.9 ± 1.7	31.4 ± 1.8
Average daily gain, kg.[2]	.44[a] ± .015	.42[a] ± .014	.34[b] ± .015

[1] Values given are means ± standard error.

[2] Values without a common letter differ significantly ($P<.001$) and linear effect significant ($P<.01$).

TABLE VII

AGE AT FIRST, SECOND AND THIRD ESTRUS[1]

	Protein Level		
	20%	14%	8%
First estrus	226.7 ± 4.3	229.9 ± 4.2	238.0 ± 4.4
Second estrus	248.5 ± 4.2	249.3 ± 4.2	259.6 ± 4.2
Third estrus	268.0 ± 3.6	269.3 ± 4.1	279.0 ± 3.9

[1] Ages given in days ± standard error.

Age at first, second and third estrus (Table VII) tended to increase as protein level decreased, but the effect was not significant ($P<.10$). Robertson _et al_ (1951) found that a 15% protein ration offered no significant reduction in age at puberty when compared to an 11% protein ration, either full or limit fed in dry lot. The overall mean age at puberty in this study, 231.1 days, is quite acceptable when compared to the age reported by Foote et al. (1956) of 267.1 days for purebred Yorkshire gilts. Average estrous cycle length appeared normal on all protein treatments. Average intervals from first to third estrus were 43.2, 39.4 and 41.0 days for high, medium and low protein levels, respectively.

A higher incidence of anestrous gilts tended to be associated with the two lower protein levels; however, the numbers involved in this study were too limited to pick up a significant difference. (Table VIII). The percentage of gilts not exhibiting a third estrus based on the number of gilts finishing the trials were: high level, 3.6%; medium level, 23.3%; and low level, 20.7%. Adams et al. (1960), Boaz, (1962) and Pond et al. (1968) reported similar increases in the number of anestrous gilts and sows when fed low protein diets. Svajgr et al. (1972) produced anestrous first-litter gilts with low protein diets and then corrected the abnormality with PMS and HCG injections.

Nitrogen Balance

Retained, urinary and fecal nitrogen values are given in Table IX with collection period I (before breeding) including 48 gilts and collection period II (after breeding) including 21 pregnant gilts. Actual crude protein intakes (N x 6.25) of 346, 254 and 143 g. gave mean daily retentions of 14.2, 14.9 and 10.2 g., respectively, in collection period I. A linear effect of protein level on nitrogen retention approached significance ($P<.10$), but no quadratic effect was indicated. Elsley

TABLE VIII

ESTROUS IRREGULARITIES OBSERVED

	Protein Level		
	20%	14%	8%
No. gilts on trial [1]	28	30	29
No. gilts never exhibiting estrus	1	2	4
No. gilts exhibiting estrus once	0	1	0
No. gilts exhibiting estrus twice	0	4	2
No. gilts anestrous at breeding	1	7	6
Anestrous rate, %	3.6	23.3	20.7

[1] One gilt died and one crippled gilt was removed from the 20% group and one gilt died in the 8% group.

TABLE IX

NITROGEN BALANCE OF GILTS FED GRADED LEVELS OF PROTEIN[1]

	Protein Level		
	20%	14%	8%
		Collection Period I	
Retained N	14.2 ± 1.67	14.9 ± 1.57	10.2 ± 1.62
Urinary N[2]	33.4[a] ± 1.51	19.2[b] ± 1.42	8.8[c] ± 1.46
Fecal N	7.2 ± .34	6.5 ± .32	3.9 ± .33
		Collection Period II	
Retained N[3]	21.0[a] ± 2.80	9.8[b] ± 3.32	7.2[b] ± 2.93
Urinary N[2]	26.8[a] ± 2.41	25.9[a] ± 2.86	11.6[b] ± 2.52
Fecal N[3]	8.2[a] ± 1.09	6.3[ab] ± 1.29	3.8[b] ± 1.14

[1] Values given in grams per day ± standard error.

[2] Values without a common letter differ significantly ($P<.01$) and linear effect significant ($P<.05$).

[3] Values without a common letter differ significantly ($P<.05$) and linear effect significant ($P<.05$).

et al. (1966) demonstrated a daily retention of approximately 12 g. of nitrogen when feeding 309 g. of crude protein daily to non-pregnant gilts at similar body weights. Using the value of 124.5 mg. retained nitrogen per kg. of body weight per day reported by Shearer et al. (1971), gilts in this study should have retained approximately 12.4 g. per day on the average. Holden et al. (1971) fed gilts 150 g. of protein daily and observed a mean daily retention of 10.4 g. which compares favorably to the 10.2 g. found in this experiment.

Pre-breeding urinary nitrogen increased linearly with increasing protein intake ($P<.01$) and fecal nitrogen was confounded by a significant trial by treatment interaction ($P<.01$). The two higher protein levels gave significantly more ($P<.05$) fecal nitrogen than the low protein level in the first two trials.

When expressed as a percentage of intake, pre-breeding retention of nitrogen increased linearly ($P<.01$) with decreasing protein levels, (Table X). Mean apparent digestibilities of nitrogen tended to increase with increasing dietary source; however, treatment X trial interactions were highly significant ($P<.01$).

Early pregnancy nitrogen balance was determined at 23 to 27 days post-breeding, which is shortly after implantation of the embryos at days 16 to 20 (Pond et al., 1969). Retained nitrogen in this study increased linearly ($P<.01$) as protein intake increased with high-level gilts retaining significantly more nitrogen ($P<.05$) than gilts fed the lower levels, (21.0, 9.8 and 7.2 g., respectively). Using values reported by Shearer et al. (1971), gilts in this study should have retained 8.5 g. daily on the average; whereas Holden et al. (1971) reported a value of 9.98 g. retained at 150 g. protein intake.

Percentage nitrogen retained approached a quadratic effect with increasing dietary protein ($P<.10$).

TABLE X

NITROGEN BALANCE AND APPARENT DIGESTIBILITY OF NITROGEN[1]

	Protein Level		
	20%	14%	8%
		Collection Period I	
% Retained N[2,3]	28.2 ± 3.80[a]	36.4 ± 3.44	44.5 ± 3.55[b]
Apparent digestibility, %[2]	87.2 ± .97	83.7 ± .88	83.0 ± .91
% Urine N[4]	58.2 ± 3.31[a]	47.3 ± 3.00[b]	38.4 ± 3.09[c]
% Fecal N	12.8 ± .97	16.3 ± .88	17.0 ± .91
		Collection Period II	
% Retained N	36.1 ± 5.54	25.7 ± 6.69	43.3 ± 6.69
Apparent digestibility, %	85.5 ± 1.94	84.0 ± 2.34	84.4 ± 2.34
% Urine N	49.2 ± 4.62	58.3 ± 5.58	41.0 ± 5.58
% Fecal N	14.5 ± 1.94	16.0 ± 2.34	15.6 ± 2.34

[1] Values given are means ± standard errors.

[2] Significantly linear ($P<.01$).

[3] Values without a common letter differ significantly ($P<.01$).

[4] Values without a common letter differ significantly ($P<.05$).

Rippel (1967) stated that fetal development exerts its influence on nitrogen efficiency (percentage retained) only when dietary nitrogen levels provide for retention in excess of extra-uterine needs. Retention of more than 8 to 10 g. of nitrogen is needed to give maximal intrauterine development when embryo numbers are normal. In this experiment, gilts fed the 8 and 14% rations retained 7.2 and 9.8 g., respectively, which closely approximated the 8 to 10 g. range cited by Rippel (1967). Apparently, the 20% ration provided sufficient nitrogen intake to capitalize on the efficiency of nitrogen utilization exhibited by the uterus and fetus after extra-uterine needs were met. Efficiency of nitrogen utilization in this study was 36.1% for the high level gilts, 25.7% for the medium level, and 43.3% for low intake gilts. The importance of this increased nitrogen retention with respect to gestation and parturition performance is not well documented. Balance studies in the last third of gestation have failed to show any close association between reproductive performance and nitrogen retained (Rippel, et al., 1965b).

Post-breeding urinary nitrogen increased linearly ($P<.01$) with increasing protein levels; however, a quadratic effect approached significance ($P<.10$) reflecting the low retention efficiency with the 14% ration which was discussed above.

Post-breeding apparent digestibilities of nitrogen were not affected by protein level. Differences in pre-breeding and post-breeding urinary, fecal and retained nitrogen were not significant; however, numbers of observations were small.

Reproductive Performance

The reproductive performance for the 66 gilts which had apparently ovulated and were mated is given in Table XI. Number of corpora lutea tended to increase ($P<.05$) with increasing protein levels to give 12.9, 13.8 and 14.7 corpora lutea for the low, medium and high intakes, respectively. The overall least squares mean number of corpora lutea was 13.8 which agrees with the value reported by Johnson et al. (1972) of 13.8 for purebred Yorkshire gilts under standard management conditions.

TABLE XI

REPRODUCTIVE PERFORMANCE[1]

	Protein Level		
	20%	14%	8%
No. gilts	24	23	19
No. corpora lutea[2]	14.7 ± .51	13.8 ± .55	12.9 ± .60
No. live embryos	11.0 ± .85	9.3 ± .90	9.6 ± 1.00
No. embryos adjusted[3]	10.3 ± .80	9.3 ± .82	10.3 ± .92
Actual survival rate, %	76.7 ± 5.07	68.7 ± 5.37	75.2 ± 5.93
Adjusted survival rate, %[3]	77.4 ± 5.26	68.7 ± 5.41	74.6 ± 6.09
Embryo length, mm.[4]	26.1 ± .35	26.1 ± .38	25.4 ± .40

[1] Values given are means ± standard errors.

[2] Linear effect significant. (P .05)

[3] Values adjusted to overall ovulation rate of 13.8.

[4] Values adjusted to overall embryo age of 30 days.

Adjustment of number of live embryos to a constant number of corpora lutea resulted in a significant reduction ($P<.01$) of the error mean square estimate. However, dietary protein level apparently had no effect on number of live embryos at 30 days post-breeding. Adjusted least squares means for high, medium and low protein intakes were 10.3, 9.3 and 10.3 embryos, respectively. These values seem somewhat low when compared to 11.4 embryos found by Johnson et al. (1972) in Yorkshire gilts, and could indicate that all treatments were somewhat limiting either before breeding or in early pregnancy.

Survival rate or percentage of corpora lutea represented by live embryos at slaughter was not significantly affected by protein levels.

Survival of ova, adjusted to equal ovulation rate, for gilts fed the 14% ration tended to be lower (68.7%) than for gilts fed either the 8% ration (75.2%) or the 20% ration (76.7%). Hafez (1968) reported an average of 30% mortality of embryos by day 25 of gestation, whereas Johnson _et al._ (1972) reported an 83.9% survival in Yorkshire gilts.

Embryo age at slaughter in this study ranged from 28 to 35 days, and embryo length was adjusted to the overall mean, 30 days of age. Protein level had no significant effect on crown-rump length of embryos with lengths of 26.1, 26.1 and 25.3 mm. for high, medium and low protein intakes, respectively. Length of embryo thus gave little evidence as to the utilization of extra nitrogen retained by gilts fed the high protein ration.

CHAPTER V

SUMMARY

A total of 90 pre-puberal Yorkshire gilts were used to study the effects of three levels of crude protein (8%, 14% and 20%), fed for extended periods of time, on growth, estrual cycling, nitrogen balance and reproductive performance. The study included three trials with 10 gilts per treatment per trial. Gilts were fed 178, 317 or 433 g. of crude protein daily from 146 days of age to 180 days; the protein was then reduced to 143, 254 or 346 g. per day for each treatment, respectively. Average daily gain to first estrus increased linearly ($P<.01$) with increasing protein intake, while total weight gain to first estrus was not significantly affected by treatment.

Ages at first, second and third estrus tended to decrease with additional protein intake, but the effect was not significant ($P<.10$). Average estrous cycle length appeared normal on all protein treatments. Slightly more gilts were anestrous on the two lower protein levels. Percentage of anestrous gilts at breeding time were 20.7, 23.3 and 3.6% for low, medium and high protein intakes, respectively.

Nitrogen balance prior to breeding (third estrus) was not significantly affected by treatment, but tended to increase linearly ($P<.10$) with increasing protein intake. Early pregnancy nitrogen retention increased linearly ($P<.01$) and gilts on the high protein level retained significantly more ($P<.05$) nitrogen than gilts on lower levels. This increased retention may indicate that 1.82 kg. of a 14% crude protein diet (255 g. protein) will not support maximal protein synthesis as described by Elsley et al. (1966) for (1) maintenance, (2) growth of the uterus, fetus and mammary tissue, and (3) any pregnancy anabolism that might occur during early pregnancy. Retention efficiency seems to be increased at dietary protein levels which allow retention above 10 g. of nitrogen daily. An increased post-breeding nitrogen retention was observed in gilts fed the 20% protein ration when compared to the pre-breeding values. The opposite effect was observed in gilts fed the 8 and 14% protein rations. Although the differences were not significant, this may indicate that the protein or amino acid requirements were increased by the pregnancy state and the two lower protein rations were not adequate to meet the demands.

The number of corpora lutea increased linearly as protein intake increased ($P<.05$) with values of 12.9, 13.8 and 14.7. Differences in number of live embryos, survival rate and embryo length were not significant for the different protein levels. These data suggest that the reproductive performance for those gilts that conceived was not greatly affected by dietary protein levels.

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