THE EFFECT OF FEED LEVEL DURING OESTRUS ON OVULATION RATE IN THE GILT

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Summary. The effect of feed intake during the oestrous period and during previous oestrous cycles on ovulation rate, pituitary weight and pituitary LH potency, has been investigated. Control gilts were fed 1.8 kg food/day from puberty to the third oestrus. Increasing feed intake to 3.6 kg for 1 day, on the day following mating did not significantly affect ovulation rate, pituitary weight or residual LH potency. Increasing feed intake to 3.6 kg on the day of mating significantly ($P < 0.05$) increased ovulation rate by 1.3 ± 0.15 ova, but did not affect pituitary weight or residual LH potency. Gilts fed 3.6 kg food/day from puberty to the third oestrus had significantly higher ovulation rates than gilts fed 1.8 kg food/day (13.2 versus 11.1 ova; $P < 0.05$), and significantly heavier dried pituitary glands (47.8 versus 38.3 mg; $P < 0.01$). Residual pituitary LH potency was not affected by feed level.

INTRODUCTION

The experiments described in this paper were designed to study the effects of plane of nutrition between puberty and the third oestrus, and of feed intake during the third oestrous period on ovulation rate and early embryo mortality in the gilt.

A number of studies have shown that the ovulation rate of gilts is influenced by the plane of nutrition before oestrus, gilts allowed free access to food for 3 or more weeks before oestrus having higher ovulation rates than those on restricted feeding (Robertson, Casida, Grummer & Chapman, 1951; Christian & Nofziger, 1952; Self, Grummer & Casida, 1955). Further, it has been shown that increasing the feed intake for as short a period as 6 days before oestrus increased ovulation rate (Zimmerman, Spies, Self & Casida, 1960).

More recently, the work of Heap, Lodge & Lamming (1967) indicated that, in the sow, ovulation rate may be influenced by changing the plane of nutrition during the oestrous period. Subsequently, Lodge & Hardy (1968) showed that doubling the feed intake of gilts from 1.8 to 3.6 kg for 1 day only (at the first feed following service) significantly ($P < 0.05$) increased the mean number of piglets born from 8.9 to 10.8. They also found that increasing the feed allowance of gilts from 1.4 to 4.1 kg for a single feed given 12 hr after the onset of oestrus significantly ($P < 0.05$) increased the mean ovulation rate from 12.14 to 14.71.

In a further experiment (Hardy & Lodge, 1969), there was some evidence that ovulation rate at the post-lactational oestrous period was higher when sows were fed an extra 1.8 kg. (Both groups received 2.3 kg food/day between weaning and mating.) These workers suggested that increasing feed intake during the oestrous period was rectifying the depressant effect of previous feed restriction on ovulation rate.

A similar effect has been reported in the rat. Cooper, Haynes & Lamming (1970) found that reproductive capacity decreased with increases in the degree of nutrient restriction imposed, but that unrestricted feeding for 12 hr immediately before the estimated time of ovulation significantly increased ovulation rate, but not the number of viable fetuses.

MATERIALS AND METHODS

Animals and management

The gilts used in these experiments were by Landrace boars and born to Landrace × Large White sows. The gilts were selected at approximately 170 days of age, and were housed in groups of twelve in deep-strawed yards. All the gilts were individually fed 1.8 kg of a breeding sow ration/day at a single feed

TABLE 1
COMPOSITION AND CHEMICAL ANALYSIS OF THE DIET

<i>Composition (%)</i>	
Ground barley	50
Ground wheat	40
White fishmeal	5
Soya bean meal	5
Plus mineral vitamin supplement providing/kg diet	
Ca 1.9 g; P 1.0 g; NaCl 2.5 g; Fe 60 mg; Zn 50 mg;	
Vitamin A 6000 i.u.; Vitamin D 250 i.u.; Riboflavin	
2.5 mg; Nicotinic acid 10 mg; Pantothenic acid 2.5 mg;	
Vitamin B ₁₂ 10 µg; Vitamin E 10 mg.	
<i>Chemical analysis</i>	
Moisture	11.49% of air-dried feed
Nitrogen × 6.25	17.03% of dry matter
Ash	4.05% of dry matter
Ether extract	1.58% of dry matter
Fibre	3.37% of dry matter
Ca	1.25% of dry matter
P	0.57% of dry matter
Energy (determined)	4604 kcal/kg dry matter

given in the morning. The composition of the ration is given in Table 1. The gilts had unlimited access to water.

All gilts were tested daily for oestrus using vasectomized boars. Puberty was defined as the day on which intromission by a vasectomized boar was permitted. The 1st day of oestrus as determined by boar acceptance was designated Day 1 of the cycle, or of pregnancy.

Experiment 1

The experiment was a randomized block design. Thirty-six animals were arranged in three blocks of twelve on the basis of age at selection. Within each block of twelve animals, individuals were randomly allocated to one of three treatment groups at puberty. The treatments were as follows.

Control. These animals were fed 1.8 kg food/day from puberty to slaughter. This level is similar to that given in commercial practice.

High plane. Gilts in the 'high plane' group were fed 3.6 kg food/day from puberty to slaughter.

Extra nutrient intake (ENI). Gilts in the ENI group were fed 1.8 kg food/day from puberty to slaughter. However, on Day 2 (i.e. day after first service) of the third oestrous period, the allowance was increased to 3.6 kg for 1 day only.

Each treatment group was further subdivided into two slaughter groups. Following the third oestrus, gilts were slaughtered either on Day 4 or Day 20. All gilts slaughtered on Day 20 were mated with a fertile boar at the third oestrus. Two matings, on consecutive days were given if possible.

Experiment 2

This was also of a randomized block design. Thirty-six animals (three blocks of twelve allocated on the basis of age) were used. Within each block, four animals were allocated to the Control group and eight to the ENI group at puberty. The two treatments were as follows.

Control. The animals were fed as in Exp. 1, i.e. 1.8 kg food/day from puberty to slaughter.

ENI. Gilts on this treatment were fed as the controls, but the feed level was increased from 1.8 kg to 3.6 kg on Day 1 of the third oestrous period. The increased intake was achieved by giving the normal 1.8 kg daily feed and a second 1.8 kg feed following successful mating.

As in Exp. 1, gilts on each treatment were subdivided into two slaughter groups, and following the third oestrus were slaughtered on Day 4 or Day 20. All animals slaughtered on Day 20 were mated with fertile boars.

Slaughter procedure. The animals were stunned using a captive bolt pistol and killed by exsanguination from the jugular vein. Gilts killed on Day 4 were decapitated immediately, and their anterior pituitary glands were removed within 5 min of stunning. The pituitaries were freeze-dried and retained for subsequent assay for LH potency. The reproductive tracts were obtained on evisceration of the carcass and the ovaries were removed and stored in formol saline for histological examination. The uteri of gilts slaughtered on Day 20 were dissected and the number of viable embryos present recorded.

Ovarian histology. The ovaries were cut into 1-mm sections and the number of CL (and in those pigs slaughtered on Day 4, the number of corpora albicantia remaining from the previous ovulation) were recorded. A random selection of ovaries from all treatment groups were serially sectioned at 7 μ m and examined for the presence of retained ova.

Anterior pituitary assays. The assay method used was the ovarian ascorbic acid depletion assay of Parlow (1958) employed as modified by Schmidt-Elmendorff & Loraine (1962) with further minor modifications. The assay animals were

intact, immature rats of Wistar origin, 28 to 35 days old. Each animal received a subcutaneous injection of 50 i.u. PMSG and 72 hr later, a subcutaneous injection of 30 i.u. HCG. Assay of anterior pituitary LH potency was carried out 7 days after the injection of HCG. The standard and unknown materials were dissolved in 0.9% NaCl solution and 1 ml was injected intravenously into the jugular vein of each assay animal. The animals were killed in ether 4 hr $\pm$ 5 min after the intravenous injection and the left ovary was removed. Each ovary was weighed, and stored overnight in the dark at -15° C. The following day, each ovary was homogenized with 10 ml of a freshly prepared solution of 2.5% metaphosphoric acid and centrifuged to remove tissue debris. Duplicate 3.5-ml aliquots of the supernatant were taken and each aliquot added to a tube containing 4 ml of dye solution. This solution was a mixture of 0.03% 2,6-dichlorophenolindophenol and 4.53% sodium acetate (adjusted to pH 7.0 with acetic acid) in the ratio of 1:7. The optical density of the resulting solution was measured colorimetrically at 520 $m\mu$ with a 1.0-cm glass cell and a blue filter. The ascorbic acid concentration of each ovary was calculated from a standard curve.

Quantitative assays were carried out with pooled anterior pituitaries using a four-point design, employing two doses of standard (S) and unknown (U), respectively. In each assay, six animals were used for each dose level of S and U. The log dose interval was $\log_{10} 5$. The doses of S used were 0.8 and 4.0 μ g NIH-LH-S14 and the doses of U were 0.5 and 2.5 mg dried pituitary tissue.

Statistical analysis. All data apart from assays were analysed using the method of Kempthorne (1952) for a two-way classification with unequal numbers. In Exp. 1, where significant treatment effects were obtained, individual differences were examined by Multiple Range Test (Duncan, 1955) using the critical values calculated by Harter (1960).

For each assay, the ascorbic acid content of each ovary was adjusted by covariance analysis, as recommended by Sakiz & Guillemin (1963). The validity, index of precision (λ), relative potency, and fiducial limits at $P = 0.95$ were calculated for each assay using standard methods.

RESULTS

Experiment 1

The number of animals providing data at various stages is shown in Table 2. All the selected animals reached puberty. Two gilts became acyclic following their second oestrus and were discarded. At slaughter, thirteen of the seventeen animals mated were found to be pregnant. However, one of these animals was found to be a pregnant intersex (Scofield, Cooper & Lamming, 1969) and was therefore excluded from the data.

The results obtained are summarized in Table 3. There was no significant difference in the mean age and weight of the three treatment groups at puberty. At mating, the animals on a high plane of feeding were 12 kg heavier than the control group and 15 kg heavier than the ENI group ($P < 0.05$). Serial sectioning of the ovaries selected from all treatment groups failed to reveal any ova retained in CL. The CL counts were therefore taken as a reliable estimate of

TABLE 2
NUMBERS OF ANIMALS PROVIDING DATA AT THE VARIOUS STAGES IN EXPERIMENT 1

<i>Treatment:</i> <i>Slaughter group:</i>	<i>Control</i>		<i>High plane</i>		<i>ENI</i>	
	<i>Day 4</i>	<i>Day 20</i>	<i>Day 4</i>	<i>Day 20</i>	<i>Day 4</i>	<i>Day 20</i>
No. of gilts at puberty	6	6	6	6	6	6
No. of gilts reaching third oestrus	5	6	6	6	6	5
No. mated	—	6	—	6	—	5
No. pregnant at Day 20	—	4	—	4	—	4*

ENI = Extra nutrient intake.

* One pregnant intersex gilt excluded from data.

TABLE 3
EFFECT OF PLANE OF NUTRITION AND FEED LEVEL AT OESTRUS ON REPRODUCTION IN THE GILT (EXPERIMENT 1)

	<i>Treatment</i>			<i>Standard error of differences between means*</i>
	<i>Control</i>	<i>High plane</i>	<i>ENI</i>	
Mean pubertal age (days)	177.7	210.8	199.5	19.63
Mean pubertal weight (kg)	91.7	92.0	87.8	2.18
Mean weight at third oestrus (kg)	106.5 ¹	118.2 ^{1,2}	103.1 ²	3.94
Mean percentage weight change from first to third oestrus	15.0	30.3	15.5	—
Mean ovulation rate of second oestrus	9.9	11.8	10.3	0.96
Mean ovulation rate of third oestrus	11.1 ¹	13.2 ¹	11.7	0.34
Mean number of viable embryos at Day 20	9.3	10.0	10.8	2.33
Embryo mortality†	14.8	15.5	8.8	—
Mean dried pituitary weight (mg)	38.3 ¹	47.8 ^{1,2}	37.7 ²	2.2
Mean pituitary LH potency/mg (μ g S)	2.0 (1.3 to 3.2)	2.5 (1.6 to 3.9)	2.6 (1.2 to 4.4)	—
Total pituitary LH potency/mg (μ g S)	77.6 (50.9 to 146.8)	117.8 (77.7 to 184.5)	96.6 (45.4 to 166.8)	—
Index of precision (λ)	0.22	0.22	0.22	—

ENI = Extra nutrient intake.

S = Standard (see text).

* Due to unequal subclass numbers, there are slight variations in the standard errors of differences between means. An average figure is therefore presented.

† Ratio of embryos to CL expressed as a percentage.

^{1,2} Means with the same suffix differ at $P < 0.05$.

ovulation rate. The ovulation rate of the 'high plane' animals was significantly greater than that of controls ($P < 0.05$, see Table 3). The higher ovulation rate of the 'high plane' gilts was apparent at second oestrus although in this case the difference was not significant.

TABLE 4
NUMBERS OF ANIMALS PROVIDING DATA AT THE VARIOUS STAGES IN
EXPERIMENT 2

<i>Treatment:</i> <i>Slaughter group:</i>	<i>Control</i>		<i>ENI</i>	
	<i>Day 4</i>	<i>Day 20</i>	<i>Day 4</i>	<i>Day 20</i>
No. of gilts at puberty	6	6	12	12
No. of gilts reaching third oestrus	5	5	10	10
No. mated	—	5	—	10
No. pregnant at Day 20	—	3	—	10

ENI = Extra nutrient intake.

TABLE 5
EFFECT OF FEED LEVEL AT OESTRUS ON REPRODUCTION IN THE GILT (EXPERIMENT 2)

	<i>Treatment</i>		<i>Standard error of differences between means</i>
	<i>Control</i>	<i>ENI</i>	
Mean pubertal age (days)	224.8	229.1	2.61
Mean pubertal weight (kg)	94.8	102.6	4.07
Mean weight at third oestrus (kg)	108.9	117.7	4.18
Mean percentage weight change from first to third oestrus	15.4	14.2	—
Mean ovulation rate at second oestrus	11.1	11.4	0.47
Mean ovulation rate at third oestrus	11.9*	13.2*	0.15
Mean number of viable embryos at Day 20	10.7	10.6	—
Embryo mortality†	5.8	14.9	—
Mean dried pituitary weight (mg)	43.4	42.9	3.50
Mean pituitary LH potency/mg (μ g S)	3.2 (2.3 to 4.6)	2.5 (1.8 to 3.6)	—
Total pituitary LH potency (μ g S)	138.9 (99.8 to 199.6)	107.3 (77.2 to 154.4)	—
Index of precision (λ)	0.19	0.19	—

ENI = Extra nutrient intake.

S = Standard (see text).

* Significantly different at $P < 0.05$, all other differences between means were not significant.

† Ratio of embryos to corpora lutea expressed as a percentage.

Notwithstanding the significant differences in ovulation rate at third oestrus, the number of viable embryos at Day 20 was not significantly influenced by treatment. However, the number of animals providing data on embryo numbers was small (Table 2). 'High plane' gilts had a significantly greater mean dried pituitary weight, but the differences in LH potency/mg pituitary weight and total pituitary LH content were not significant.

Experiment 2

The number of animals providing data at the various stages is shown in Table 4. Six of the selected animals failed to reach puberty and hence provided no data. Examination of these animals at autopsy showed that five had ovaries containing CL suggesting that they had cycled without showing psychic heat. Thirteen of the fifteen gilts mated were pregnant at slaughter.

The results are summarized in Table 5. There were no significant differences in pubertal weights or ages, nor was there any significant difference in the weight gain between puberty and the third oestrus on the two treatments. Although there was no significant difference in ovulation rate at the second oestrus, the ENI group had a significantly higher ($P < 0.05$) ovulation rate at the third oestrus than the controls (13.2 versus 11.9), but this difference was not reflected in the number of viable embryos at Day 20. Treatment did not significantly affect either mean dried pituitary weight, pituitary LH potency/mg pituitary weight or total pituitary LH content.

DISCUSSION

Serial sectioning of selected ovaries from all treatment groups in both experiments supported the results of Perry (1961), who found no polyovular or anovular follicles and no CL with retained ova. Therefore, an accurate determination of the number of CL during early pregnancy was taken to be representative of ovulation rate since each CL can, for practical purposes, be assumed to represent one ovulated ovum. The results of Exp. 1 confirm previous reports (Robertson *et al.*, 1951; Christian & Nofziger, 1952; Self *et al.*, 1955) that a high plane of nutrition following puberty increases ovulation rate but not necessarily litter size. Increasing feed intake from 1.8 to 3.6 kg/day between puberty and the third oestrus significantly ($P < 0.05$) increased ovulation rate. Furthermore, there were indications that a high plane of nutrition for one cycle length only, that is from puberty to the second oestrus, resulted in a higher ovulation rate, though the increase was not significant in this experiment.

Increasing feed intake on Day 2 of the cycle only (ENI group) did not produce a significant increase in ovulation rate, but here the extra feed intake probably preceded ovulation by only a few hours and thus was possibly too close to the time of ovulation. In Exp. 2, the feed intake was increased on Day 1, allowing a longer period between the time of increased feed intake and ovulation. This resulted in a significant increase in ovulation rate, confirming the results obtained by Lodge & Hardy (1968) for the number of ova released but not for litter size. In the experiments described here, the number of animals on which estimates of embryo mortality were based was too small to draw firm conclusions but the data available revealed no significant effect of treatment for this aspect of the study.

The pituitary LH potencies recorded in the two experiments represent the amount of LH remaining in the anterior pituitary approximately 70 hr after the preovulatory release of gonadotrophins. The residual levels of LH found in these experiments were unaffected by nutrition and remained constant at a basal concentration of between 2 and 4 μg of standard/mg pituitary tissue.

Further studies will be needed to elucidate whether an alteration of pituitary hormone secretion is implicated in the ovarian response to transient increases in feed intake.

The increase in ovulation rate following ENI on Day 1, but not on Day 2, of oestrus suggests that the timing of the increased feed intake is critical. The ovulatory release of gonadotrophins takes place at the onset of behavioural oestrus (Niswender, Reichert & Zimmerman, 1970). Further, the final maturation of the follicles destined to rupture occurs during the period between the release of the ovulatory hormones and ovulation. Since the effect of a single increment of extra nutrients on the pituitary and ovary is likely to be of a limited duration, the maximum increase in ovulation rate will only be obtained when the ENI is supplied close to ovulation while still allowing sufficient time for any pituitary or ovarian changes to take place. The optimum time appears to be around the time of the ovulatory release of gonadotrophins in the pig. However, while the number of ova released determines the maximum potential litter size, increase in ovulation rate will not necessarily result in increases in the number of piglets born.

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