NOTICE

The quality of this microform is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us an inferior photocopy.

Reproduction in full or in part of this microform is governed by the Canadian Copyright Act, R.S.C. 1970, c. C-30, and subsequent amendments.

AVIS

La qualité de cette microforme dépend grandement de la qualité de la thèse soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopie de qualité inférieure.

La reproduction, même partielle, de cette microforme est soumise à la Loi canadienne sur le droit d'auteur, SRC 1970, c. C-30, et ses amendements subséquents.

UNIVERSITY OF ALBERTA

THE INFLUENCE OF NUTRITION, GROWTH AND BODY COMPOSITION ON ENDOCRINE STATUS AND REPRODUCTIVE DEVELOPMENT OF GILTS

BY

EDUARDO BELTRANENA MEJICANO

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfilment of the requirements for the degree of DOCTOR OF PHILOSOPHY.

IN

ANIMAL NUTRITION / REPRODUCTION

DEPARTMENT OF ANIMAL SCIENCE

EDMONTON, ALBERTA

SPRING, 1992

ISBN 0-315-73088-9

UNIVERSITY OF SASKATCHEWAN

DEPARTMENT OF ANIMAL
AND POULTRY SCIENCE
Animal Science (306) 966 4128
Poultry Science (306) 966 6594
Fax (306) 966 4151

SASKATOON, CANADA
S7N 0W0

5 March, 1992

Eduardo Beltranena
Department of Animal Science
University of Alberta
Edmonton, Alta
T6G 2P5

Dear Eduardo:

I hereby grant permission for full and unrestricted use of data generated from studies in which I was involved

Sincerely

Dr. R.N. Kirkwood

Faculty of Graduate Studies & Research
University of Alberta
Edmonton

1992, April 6

To whom this may concern:

Permission is hereby granted to Eduardo Beltranena to include in his Ph.D. thesis research data from the following publications or manuscripts submitted for publication, in which I have participated as co-author:

Beltranena, E., F. X. Aherne, G. R. Foxcroft, and R. N. Kirkwood. 1991. Effects of pre- and post-pubertal feeding on production traits at first and second estrus in gilts. J. Anim. Sci. 69:886.

Beltranena, E., G. R. Foxcroft, F. X. Aherne, and R. N. Kirkwood. 1991. Endocrinology of nutritional flushing in gilts. Can. J. Anim. Sci. 71:1063.

Beltranena, E., F. X. Aherne, and G. R. Foxcroft. Innate variability in sexual development irrespective of body fatness in gilts. Submitted to the J. Anim. Sci. in March 1992.

Beltranena, E., A. L. Schaefer, F. X. Aherne, and G. R. Foxcroft. Metabolic and reproductive status of pre-pubertal gilts treated with exogenous porcine somatotropin. Submitted to the J. Anim. Sci. in March 1992.

Dr. George R. Foxcroft
Professor
Department of Animal Science
University of Alberta

Faculty of Graduate Studies & Research
University of Alberta
Edmonton

1992, April 6

To whom this may concern:

Permission is hereby granted to Edu[illegible]lo Beltranena to include in his Ph.D. thesis research data from the following manuscript submitted for publication, in which I have participated as co-author:

Beltranena, E., A. L. Schaefer, F. X. Aherne, and G. R. Foxcroft. Metabolic and reproductive status of pre-pubertal gilts treated with exogenous porcine somatotropin. Submitted to the J. Anim. Sci. in March 1992.

Dr. Al. L. Schaefer
Adjunt Professor
Department of Animal Science
University of Alberta

Faculty of Graduate Studies & Research
University of Alberta
Edmonton

1992, April 6

To whom this may concern:

Permission is hereby granted to Eduardo Beltranena to include in his Ph.D. thesis research data from the following publications or manuscripts submitted for publication, in which I have participated as co-author:

Beltranena, E., F. X. Aherne, G. R. Foxcroft, and R. N. Kirkwood. 1991. Effects of pre- and post-pubertal feeding on production traits at first and second estrus in gilts. J. Anim. Sci. 69:886.

Beltranena, E., G. R. Foxcroft, F. X. Aherne, and R. N. Kirkwood. 1991. Endocrinology of nutritional flushing in gilts. Can. J. Anim. Sci. 71:1063.

Beltranena, E., F. X. Aherne, and G. R. Foxcroft. Innate variability in sexual development irrespective of body fatness in gilts. Submitted to the J. Anim. Sci. in March 1992.

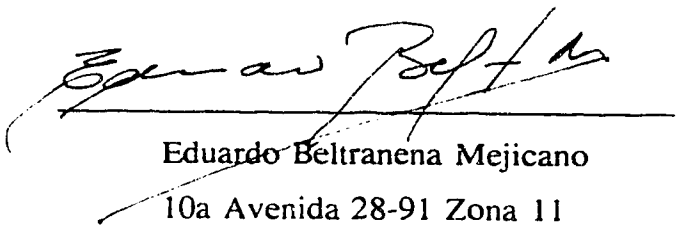
Beltranena, E., A. L. Schaefer, F. X. Aherne, and G. R. Foxcroft. Metabolic and reproductive status of pre-pubertal gilts treated with exogenous porcine somatotropin. Submitted to the J. Anim. Sci. in March 1992.

Dr. Frank X. Aherne
Professor
Department of Animal Science
University of Alberta

UNIVERSITY OF ALBERTA

RELEASE FORM

NAME OF THE AUTHOR:	Eduardo Beltranena Mejicano
TITLE OF THE THESIS:	The Influence of Nutrition, Growth and Body Composition on Endocrine Status and Reproductive Development of Gilts
DEGREE:	Doctor of Philosophy
YEAR THIS DEGREE GRANTED:	1992



Eduardo Beltranena Mejicano
10a Avenida 28-91 Zona 11
Guatemala, Guatemala 01011
Central America

1992, April 22

UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled THE INFLUENCE OF NUTRITION, GROWTH AND BODY COMPOSITION ON THE ENDOCRINE STATUS AND REPRODUCTIVE DEVELOPMENT OF GILTS submitted by EDUARDO BELTRANENA MONCANO in partial fulfilment of the requirements for the degree of DOCTOR OF PHILOSOPHY in ANIMAL NUTRITION / REPRODUCTION

Supervisor: Dr. George R. Foxcroft

Supervisor: Dr. Frank X. Aherne

Committee: Dr. Al. L. Schaefer

Committee: Dr. Robert J. Christopherson

Committee: Dr. Vicki Harber

External: Dr. Ray H. King

1992, April 6

ABSTRACT

The effects of feeding level on body weight (BW), growth rate (GR), fatness (BF/BW) and ovulation rate (OR) at first (puberty) and second estrus were examined in 145 gilts. From 47.2 kg to second estrus, gilts were fed 2.0 kg/d (LL) or were allowed ad libitum access to feed (HH). From first (puberty) to second estrus, half the LL gilts were fed 2.8 kg/d (LH). A quadratic negative relationship between GR and age at puberty indicated that age at puberty was minimum at a lifetime GR of .60 kg/d. It was concluded that maximizing GR in replacement gilts does not hasten the attainment of puberty. Flush-feeding of LL (LH) gilts only normalized OR to that in HH gilts. Gilts in LH and HH groups had a greater proportion of samples >1 ng/mL PLH suggesting that episodic pLH secretion was increased compared with LL gilts. Increased plasma insulin in HH gilts suggested that insulin may be an important metabolic cue, which in association with gonadotropins and IGF-I, mediates the ovulatory response to flushing.

To separate the effects of fatness from those of lean tissue growth on sexual development, 52 gilts (33.2 kg) were allowed ad libitum access to feed (HE; n = 21) or were fed restrictively (LE; n = 31) until 160 d of age. Sixteen LE gilts were then realimented until 175 d (LER). Carcass fat depth was lower in LE gilts than in HE or LER gilts, but carcass lean depth was similar. Treatment did not affect pLH or GH secretion, plasma insulin or IGF-I concentrations, reproductive tract weight, follicular volume, plasma or follicular fluid estradiol-17β. Differences in fatness, in the presence

of similar lean accretion rates therefore did not influence sexual development in the pre-pubertal gilt.

To study the effects of pST administration on reproductive development, 23 gilts (61.7 kg) served as untreated controls (n = 8), or were injected (2 mg/d; n = 8) or implanted (2 mg/d; n = 7) with pST, for 28 d and allowed ad libitum access to feed. Although pST administration had no effect on GR, feed conversion or carcass characteristics, basal and episodic GH secretion were suppressed while plasma insulin and plasma and follicular IGF-I were elevated. Secretion of pLH, reproductive tract weight, follicular volume, plasma and follicular fluid estradiol-17β were similar among groups. Therefore, administration of pST did not impair reproductive development in pre-pubertal gilts.

ACKNOWLEDGEMENTS

I am greatly indebted to Dr. George R. Foxcroft and Dr. Frank X. Aherne for their guidance, patience and support throughout my graduate program and for showing me what excellence in research means. Also, I would like to thank Dr. Roy N. Kirkwood and Dr. Al L. Schaefer for their constructive criticism of my work and their camaraderie. Thanks are due also to Dr. Ray H. King, Dr. Robert J. Christopherson and Dr. Vicki Harber, members of my examination committee, and to Dr. John P. Bowland, who reviewed the final manuscript of this thesis; their comments were fair and constructive.

Without the tenacious assistance of Ms. Cheryl Streeter, Ms. Shirley Shostak, Ms. Madhu Garg, Ms. Dorothy Payne, and Ms. Lynn Wright, I would not have gotten through all those RIA hormone assay tubes. A special acknowledgement is extended to Mr. Gordon Grant and Ms. Nancy and Dr. Frank Robinson for their guidance during my initiation to radioactive isotopes and RIA.

The terrific aid of Mr. Bob Mascarin, Mr. Keith Moss and Mr. Ed Maycher in feeding and caring of all animals involved in my many experiments at the University's Piggery, is greatly appreciated. Similarly, I acknowledge Ms. Laverne Holt, Ms. Lynda Nicholls and the personnel of the Piggery and Meat Lab at the Agriculture Canada Lacombe Research Station for their assistance with the pST experiment (Chapter V). Thanks are due also to Ms. Dorothy Irwin who cared for the cannulated gilts, and whose cheerful temperament kept us going, making the long hours of bleedings much shorter. For their skilful assistance during surgery, I thank Ms. Brenda Tchir and Ms. Charlene Gorsak, and to Mr. Jack Francis, who built our surgery tables and recovery pens.

For showing me not only the how, but also the why around the lab, I am grateful to Mr. Terry and Ms. Mijarna Fenton, Mr. Brian Turner and the lab personnel, and to Ms. Anya Nowinska for ordering all research supplies and her constant bugging. To Mr. Ray Weingardt who with unlimited patience taught me the secrets of biometrics, and to Mr. Brian Kerrigan and Mr. Gavin Godby for nourishing my love for computers and software, I express my thanks.

Because we went through together and because they supported and advised me, I thank my research group colleagues Dr. Andrew Pharazyn, Ms. Sue Charlton, Ms. Emma Clowes, Dr. John Cosgrove, Ms. Teresa Wiesak and Mr. Jianchi Ding. I also thank my friends Dr. Erasmus Okine, Mr. Sam Harbison, Dr. Sam Baidoo, Dr. Tomas A. Scott and Mr. Gordon Grant who were there to listen and advise me throughout these years. And to Dr. Alfredo Newell and Dr. Enrique Hernandez whose erudition steamed my initial interest in science and research.

With great perseverance and oblation, my parents have continuously supported and encouraged my pursuit for higher education; to them, I will be eternally grateful. Also to my Agueli and clan Beltranena, I felt them always close although they were far away. And last, but always with me, to Cathy whose advocation got me to conclude this degree.

Finally, the financial support for these studies by the Natural Science and Engineering Council of Canada, the Alberta Pork Producers Development, the Alberta Agriculture Research Institute, the Department of Animal Science and Faculty of Graduate Studies and Research, is greatly appreciated.

TABLE OF CONTENTS

LIST OF TABLES

Table Page

LIST OF FIGURES

I. INTRODUCTION

A. Problem Demarcation

Management of the replacement gilt and her successful integration into the breeding herd continue to present severe problems to the swine industry. Presently, sow culling rates are high, with most sows being culled before they achieve their potential optimum performance. Sow culling rates ranging from 45 to 70% (Dial, 1992), indicate that an equivalent number of replacement gilts must be brought into the herd annually. However, replacement gilts have lower productivity, and at best will merely maintain herd performance. Thus, when the goal is to optimize the herd's reproductive performance, productivity of the replacement gilt is crucial to the herd's profitability. Nonetheless, several factors affect productivity of the replacement gilt, and therefore, the efficiency with which they are brought into the herd:

Sexual Precocity. Ideally, the replacement gilt must be pregnant at that age equivalent to the herd's average age at market weight. The number of days between mean age at market weight and age at which the replacement gilt first becomes pregnant, has housing, management and feeding costs associated with it that ought to be minimized. This period of time is comparable to the weaning to mating interval, but even in the majority of well managed, established herds, it exceeds the weaning to mating interval, not merely by several days, but by several weeks. Minimizing age at the attainment of puberty provides the opportunity for an equivalent reduction in age at

first mating. Achievement of this objective, however, is limited by factors that influence the attainment of puberty in the replacement gilt. Some of these factors or cues are intrinsic or genetically-determined, henceforth referred as "internal" and include body weight, body fatness, and growth rate. Others, in contrast, are environmental cues, henceforth referred as "external", which influence the expression of the genetically-determined internal factors and include nutrition, boar stimulation, confinement, photoperiod, etc. The interaction between external factors, mainly nutrition, and the genetically-determined internal factors are the main topic of this dissertation; their importance will be further emphasized later in this chapter.

Reduced Litter Size. Primiparous sows tend to have smaller litters than older, multiparous sows (Aherne and Kirkwood, 1985). A reduced litter size suggests that replacement gilts may have lower ovulation rates, larger embryo mortality or a combination of these two (Aherne and Kirkwood, 1985; Dyck, 1988). Nutritional management to minimize embryo mortality in the first parity gilt in particular, requires careful consideration (see Pharazyn, 1992). The subject of ovulation rate and nutritional flushing are also part of this dissertation and will be further emphasized later in this chapter.

Longevity and Productivity. Modern sow productivity is based on longevity rather than annual productivity. Nonetheless, a large component of a herd's annual culling rate, comprises culling of first and second parity sows. High removal rates after the

first or second parity are frequently associated with low birth rates, reduced milk production, excessive weight loss during lactation and extended weaning to mating interval. The physiological correlates of these phenomena will not be discussed here, but require careful consideration and the development of management practices to avoid such apparent metabolic exhaustion early in the productive life of the sow (see Lythgoe, 1987; Williams and Mullan, 1989; Baidoo, 1989).

B. Sexual Development Leading to Puberty in the Replacement Gilt

Puberty is associated with the development and activation of the hypothalamic-hypophysial-gonadal axis. In order to place in context the genetically-determined traits and environmental factors that influence sexual development and the attainment of puberty, it is necessary, first, to briefly describe the endocrine correlates leading to the natural attainment of puberty. Several reviews have recently examined this subject in the replacement gilt (Elsaesser, 1982; Ellendorff and Parvizi, 1982; Baird, 1985; Dyck, 1988; Short et al., 1988; Foxcroft et al., 1989); based on these, a brief summary is as follows:

Ovarian Follicular Development. Around day 30 of gestation, the porcine fetal gonads differentiate to an ovary, which contains egg nests. The number of egg nests however, declines with the advance of fetal development, and they are seldom observed in the 20 day-old female. From approximately day 70 of gestation to day 70 after birth, primordial follicles are the dominant oogonic structures in the ovary. Primary follicles

are first observed by day 70 of gestation. Secondary follicles are usually observed by the time of birth, and constitute about 30% of the total oogonic structures by day 90 after birth. Tertiary or antral follicles, which are gonadotropin-sensitive, are not commonly present in the 70 day-old growing gilt (see Pressing et al., 1992). Measurable concentrations of estradiol and progesterone observed in the gilt at birth are not of follicular origin, but from maternal-placental sources, and are usually undetectable five days after birth.

Developmental Pattern of Gonadotropin Secretion. Prior to day 80 of fetal life, gonadotropin secretion is undetectable; thereafter both circulating FSH and LH concentrations increase and are highest about the time of birth. During infancy and the early juvenile periods, they follow divergent patterns; FSH remains elevated while LH declines to very low levels. It appears that exposure to comparatively high levels of FSH results in the development of gonadotropin receptors in antral, tertiary follicles. Once the gonadotropin receptors have been established, maturation of the first wave of follicles appears to be dependent upon the frequency of episodic LH secretion. The decline in circulating FSH concentrations observed in the mid pre-pubertal period, appears to be related to a follicular selective suppressive mechanism, possibly inhibin produced by the growing follicles; however, this decline in FSH secretion has also been reported in the ovariectomized gilt, which suggests the involvement of a central rather than a follicular suppressive mechanism. Opioid peptides have been suggested as

possible central selective regulators of gonadotropin secretion (see Trudeau et al., 1988; Trudeau et al., 1989).

Episodic LH secretion in the gilt, as in other species, closely corresponds to the hypothalamic release of GnRH, which is regulated by the "pulse generator centre" located in the medio-basal hypothalamus (Knobil, 1989). During the early pre-pubertal period, there appear to be well-defined, large LH episodes occurring at a rate of 1/1.5 hours. However, during the late pre-pubertal period, there appears to be an increase in episodic frequency and a decline in episodic amplitude, which appear to be more evident during the night time (see Cosgrove, 1991). Another characteristic of the late pre-pubertal period in the gilt is a diurnal rhythm in episodic LH secretion; night-time frequency is increased with respect to day-time frequency (see Booth 1990b; Cosgrove, 1992).

The Estrogen/Progesterone Feedback Mechanism. As the growing follicles mature, circulating levels of estrogens increase, suppressing LH pulse amplitude. This elevation in circulating estrogens, mainly estradiol-17β, ultimately acts on the central nervous system (CNS) to cause behaviourial pubertal estrus and on the combined hypothalamic-hypophysial axis to cause the pre-ovulatory LH "surge" and first ovulation. This stimulatory effect of ovarian steroids on the hypothalamic-hypophysial axis has been termed "positive feedback". The mature follicles in turn respond to this LH surge by ovulating, while the remaining granulosa and theca tissue of the follicle undergoes luteinization to form the corpora lutea and the synthesis and release of progesterone

increases. Progesterone modulates gonadotropin release by the hypothalamic-hypophysial axis, preventing the development of highly estrogenic follicles, an essentially "negative feedback" effect. Finally, if fertilization and implantation do not take place, luteal function is terminated by uterine prostaglandins, which induce luteolysis of the corpora lutea thereby removing the inhibitory effect of progesterone on the hypothalamic-hypophysial axis.

Theories on the Onset of Puberty. Two hypotheses have been commonly proposed as explanations for the mechanism involved in the control of the onset of puberty (see Paterson, 1982). Firstly, since a smaller dose of estrogen suppresses circulating levels of gonadotrophin in immature rats as compared with adults, a theory of "differential sensitivity to ovarian steroids" or "gonadostat theory" was proposed. According to this theory, puberty occurs when the hypothalamic pulse generator centre that controls gonadotrophin release becomes desensitized to steroid inhibition during sexual maturation. The second theory postulates, that "establishment of the positive feedback system" is responsible for the onset of puberty. This theory is based upon evidence that estrogen treatment does not induce an LH surge in pre-pubertal monkeys and humans. Neither of these theories explains the increase of gonadotrophins observed in neonatally-gonadectomized female animals at the time at which puberty would normally occur and during adolescence in agonadal humans with Turner's {X_} and Klinefelter's syndrome {XXY} (see Adams and Steiner, 1988).

Accumulating evidence in the literature suggests that the key events controlling the onset of puberty are not solely dependent on changes in gonadal steroid feedback but on the maturation of the more complex hypothalamic mechanisms controlling the release of GnRH (see Karsch, 1987). Pulsatile administration of GnRH induces precocious puberty in immature gilts (see Pressing et al., 1992). When the pituitaries of immature rats are transplanted to mature hypophysectomized-rats, or the gonads of immature rats are transplanted to mature gonadectomized-rats normal reproductive activity has been reported. The anterior pituitary gland or adenohypophysis and the ovary or testis, therefore, are capable of becoming functional before puberty. Consequently it has been proposed that, it is indeed the hypothalamus which regulates the onset of puberty (Clarke, 1988). Besides, lesions of the posterior hypothalamus advance the onset of menarche and first ovulation, while neither pregnant-mare serum gonadotrophin (PMS) and/or human-chorionic gonadotrophin (hCG) treatment, nor repeated estrogen injections or combinations of these exogenous hormonal treatments, resulted in precocious puberty in rhesus macaque monkeys (Terasawa et al., 1983).

That gonadal steroid feedback affects the hypothalamic centre controlling GnRH release must not be ruled out however (see Karsch, 1987). The lack of negative feedback of ovarian steroids during the neonatal and infancy periods may be due to a lack of ovarian secretory activity per se. Species and sex differences do, in fact, exist. In lambs, the feedback mechanism seems to develop early in life as compared with other species (Foster et al., 1988). Young male pigs differ from their female contemporaries in that the testes appear to be active at an earlier age than the ovaries. Maturation of

the positive feedback mechanism early in life may not be essential; nonetheless, during the peri- and post-pubertal periods such a mechanism may indeed affect the hypothalamic-hypophysial release of gonadotrophin.

To conclude, while the initial stage of sexual development may be solely dependent on the onset of pulsatile release of GnRH, the establishment of a gonadal steroid positive feedback mechanism at puberty is imperative for the acquisition of full reproductive capacity.

C. Effects of Body Weight, Body Composition and Growth Rate on Sexual Development and the Attainment of Puberty

As mentioned earlier, several environmental cues or "external" factors interact with genetically-determined "internal" factors to influence sexual development and the attainment of puberty. The objective of this section is not to present an exhaustive review, but rather to summarize relevant information on how body weight, body composition and growth rate may influence or signal maturation of the reproductive axis. An attempt will be made to dissociate and examine individual factors, although the complex and additive interactions among them, must be kept in mind.

Body Weight and Growth Rate. Various studies have shown either a direct, an inverse or no relationship between body weight or growth rate and the attainment of puberty (see Anderson and Melampy, 1972; den Hartog and van Kempen, 1980; Hughes, 1982; Knott et al., 1984). Because most of these studies (see Anderson and

Melampy, 1972; den Hartog and van Kempen, 1980; Hughes, 1982; Knott et al., 1984) used different planes of feeding and different age at boar stimulation, the relationship between body weight or growth rate and attainment of puberty are totally confounded. Thus it can be expected that gilts which have attained a certain degree of sexual maturity, irrespective of body weight or growth rate, may respond equally fast to b stimulation. It has been suggested, however, that there is a threshold or minimum weight, probably associated with a certain degree of somatic growth, that gilts need to achieve in order to attain puberty (Kirkwood and Aherne, 1985; Kirkwood et al., 1987). However, once gilts surpassed this yet undetermined threshold value, body weight becomes relatively unimportant to the attainment of puberty, and other factors appear to withhold the attainment of puberty (i.e., boar stimulation, stress). Thus, neither body weight nor age at puberty, are reliable indicators of sexual development.

Body Composition. Frisch (see Frisch, 1988) suggested that there was a relationship between body weight and the age at which girls achieved menarche. Later, it was suggested that body fatness explained the onset of menarche better than did body weight, and this theory was subsequently refined to a ratio of fat:lean body mass, which not only appeared to explain onset of menarche, but also amenorrhea observed in women suffering anorexia nervosa and women who exercised strenuously (Frisch, 1988). Based on the Frisch model, Kirkwood and Aherne (1985) extrapolated the theory to swine. They suggested that a minimum level of fatness may be required for the attainment of puberty in the replacement gilt. They also suggested that the level of

fatness could be a better indicator of reproductive development than age, body weight or growth rate. Kirkwood et al. (1987) suggested later that, as for body weight, a minimum level of fatness or fatness threshold may be required or permissive for puberty to occur, but was not, in itself, sufficient to trigger the onset of puberty. Several reports are consistent with this theory in swine, since it appears that fast-growing, lean gilts may attain puberty at an older age than their slow-growing, fatter counterparts (Reutzel and Sumption, 1968; Price et al., 1981). Although not proven yet, concern has been expressed that fast-growing gilts may also have a decreased propensity to fatten, therefore, sexual development and the attainment of puberty could be delayed, even though the age and weight thresholds have been exceeded (Kirkwood and Aherne, 1985). Further concern has been expressed that there has been a considerable reduction in fatness in market weight hogs (see Aherne and Kirkwood, 1985), and this could be indicative of future problems linking body fatness and reproductive activity in gilts and sows.

Body fatness may also influence reproduction through steroid metabolism. It appears that body fatness may hold a significant pool of progesterone, which is capable of aromatizing androgens to estrogens, and altering the ratio of free steroid:sex hormone-binding globulins (Kirkwood and Aherne, 1985; Kirkwood et al., 1987; Frisch, 1988; I'Anson et al., 1991). Most of these studies have been conducted in adult and(or) obese humans, and therefore the possibility exists that, because of reduced body fatness in the pre-pubertal animal, fatness may have little influence on steroid metabolism. Other reports suggest that in the gilt, body weight and fatness are also associated with an

alteration in the excretion of estrogens, and as the gilt matures, there is a decrease in the metabolic clearance rate of estradiol (Elsaesser et al., 1982).

Tissue-Repartitioning Agents and Sexual Development. Driven by a demand for leaner pork, modern swine production will probably involve the use of tissue-repartitioning agents such as beta-agonists and recombinant porcine somatotropin (pST). Administration of such drugs can result in drastic reduction of body fatness with a concomitant increase in lean tissue accretion (Chung et al., 1985; Campbell et al., 1989). Concern has been expressed, that if replacement gilts have previously been treated with these drugs, achievement of the permissive fat:lean ratio required for sexual development and attainment of puberty will be even more difficult to achieve. Furthermore, it has not been established what effects these drugs may have irrespective of changes in body composition. Reports have already been published suggesting that pST-treated gilts may not achieve puberty while receiving the drug, but that following withdrawal, normal attainment of puberty is possible (Bryan et al., 1989; Bryan et al., 1990). Other reports have suggested that pST treatment may impair follicular steroidogenesis (Hagen et al., unpublished observations; Hagen et al., 1990). Thus, there is an urgent need to clarify the effects of tissue-repartitioning agents on sexual development.

Nutrition, Metabolic State and Sexual Development. It has long been recognized in several species that because the reproductive axis has a low priority for nutrients, sexual

development and attainment of puberty can be delayed by poor nutrition (Steiner et al., 1983; Foster et al., 1988; I'Anson et al., 1991). Nutritional insult results in alterations in metabolic state: Armstrong and Britt (1987) have shown that pulsatile secretion of GnRH in the gilt may be switched on and off by feed restriction and realimentation; Booth (1990b) has also shown that acute feed restriction followed by realimentation in the absence of changes in body composition results in alteration in the frequency of episodic LH secretion, and finally, Cox et al. (1987) have shown that adminstration of exogenous insulin increased ovulation rate. Although not confirmed in swine, Steiner et al. (1983) and Downing and Scaramuzzi (1991) have shown that dietary intake of certain amino acids may alter episodic LH secretion in the Macaque monkey and insulin concentrations in sheep, respectively. Thus, plane of feeding and diet composition can alter certain metabolic signals that can influence the maturation of the hypothalamic-hypophyseal-ovarian axis. Roles for plasma amino acids, free fatty acids, glucose, growth hormone, thyroxine and insulin-like growth factor I (IGF-I) have been proposed as mediators or indirect regulators of the nutritional effects on the reproductive axis in swine (Booth, 1990a). Thus, it appears that the hypothalamus of the growing animal is capable of monitoring some metabolic parameters, which are used to time the onset of puberty (I'Anson et al., 1991).

Nutrition and Ovulation Rate. Restricting feed or energy intake before puberty, or between estrous cycles, reduces ovulation rate in gilts; however, short-term, high-level feeding during the first estrous cycle (flushing), increases ovulation rate (Anderson and

Melampy, 1972; den Hartog and van Kempen, 1980). It is not clear, however, whether flushing merely reverses the negative effect of nutritional restriction on ovulation rate or induces superovulation (Aherne and Kirkwood, 1985). The physiological mechanisms, by which an increase in feeding restores ovulation rate, have yet to be established (see Rhodes et al., 1987; Hughes and Pearce, 1989). Since it has been shown that chronic, severe feed restriction impairs the hypothalamic GnRH pulse generator (Armstrong and Britt 1987) and that a rapid increase in pulsatile LH secretion occurs after realimentation (Flowers et al., 1988; Booth 1990b; Cosgrove, 1992), it appears that gonadotropins mediate the restoration of ovulation rate. Cox et al. (1987) have established that administration of exogenous insulin increases ovulation rate, and provides preliminary evidence that insulin affects both gonadotropin secretion and the ovary directly by altering follicular atresia.

D. Objectives

The objective of the studies described herein were:

In the first study, to further examine the effects of pre-pubertal feeding level on body weight, fatness, growth rate, and age at the attainment of puberty. Also, to evaluate the effect of pre- and post-pubertal feeding level on ovulation rate at first and second estrus in gilts.

In the second study, to compare the endocrine status of gilts fed restrictively, flushed and allowed ad libitum access to feed throughout the second peri-estrus period, and thereby provide additional in vivo confirmation of potential mediators of the ovulatory response observed in flushing.

In the third study, to examine the effects of manipulation of body fatness on the activity of the hypothalamic-hypophyseal-ovarian axis in relation to the attainment of puberty in littermate gilts achieving maximal protein deposition rates, but in which fat deposition rate was altered.

And in the fourth study, to examine the physiological mechanism(s) by which porcine somatotropin (pST) might affect sexual development and the attainment of puberty in the replacement gilt.

E. Literature Cited

Adams, L. A. and R. A. Steiner. 1988. Puberty. In: Oxford Reviews of Reproductive Biology. J. R. Clarke (ed). Oxford University Press. pp. 1.

Aherne, F. X. and R. N. Kirkwood. 1985. Nutrition and sow prolificacy. J. Reprod. Fert. Suppl. 33:169.

Anderson, L. L. and R. M. Melampy. 1972. Factors affecting ovulation rate in the pig. In: Pig Production. D. J. A. Cole (ed). Butterworth, London. pp. 329.

Armstrong, J. D. and J. H. Britt. 1987. Nutritionally-induced anestrus in gilts: metabolic and endocrine changes associated with cessation and resumption of estrous cycles. J. Anim. Sci. 65:508.

Baidoo. S. K. 1989. The effect of weight and body fat loss on the reproductive performance and endocrinological status of the lactating and post-weaning sow. Ph.D. thesis, University of Alberta.

Baird, D. T. 1985. The Ovary. In: Hormonal Control of Reproduction. C. R. Austin and R. V. Short (ed). Cambridge University Press. 2nd ed. pp. 101.

Booth, P. J. 1990a. Metabolic influences on hypothalamic - pituitary - ovarian function. J. Reprod. Fert. Suppl. 40:89.

Booth, P. J. 1990b. Physiological mechanisms mediating nutrition-reproduction interactions in the pre-pubertal gilt. Ph.D. thesis, University of Nottingham.

Bryan, K. A., J. M. Hammond, S. Canning, J. Modschein, D. E. Carbaugh, A. M. Clark and D. R. Hagen. 1989. Reproductive and growth responses to exogenous porcine pituitary growth hormone. J. Anim. Sci. 67:196.

Bryan, K. A., A. M. Clark and D. R. Hagen. 1990. Effect of treatment with and subsequent withdrawal of exogenous porcine somatotropin on growth and reproductive characteristics of gilts. J. Anim. Sci. 68:2357.

Campbell, R. G., N. C. Steele, T. J. Caperna, J. P. McMurtry, M. B. Solomon and A. D. Mitchell. 1989. Interrelationship between sex and exogenous growth hormone adminstration on performance, body composition and protein and fat accretion on growing pigs. J. Anim. Sci. 67:177.

Chung, C. S., T. D. Etherton and J. P. Wiggins. 1985. Stimulation of swine growth by porcine growth hormone. J. Anim. Sci. 60:118.

Clarke, I. J. 1988. GnRH and ovarian hormone feedback. In: Oxford Reviews of Reproductive Biology. J. R. Clarke (ed). Oxford University Press. pp. 54.

Cosgrove, J. R. 1992. Endocrine responses to feed-restriction and realimentation in pre-pubertal gilts. Ph.D. thesis, University of Nottinghan.

Cox, N. M., M. J. Stuart, T. G. Althen, W. A. Bennett and H. W. Miller. 1987. Enhancement of ovulation rate in gilts by increasing dietary energy and administering insulin during the follicular growth. J. Anim. Sci. 64:507.

den Hartog, L. A. and van Kempen, G. J. M. 1980. Relation between nutrition and fertility in pigs. Neth. J. Agric. Sci. 28:211.

Dial, G. D. 1992. The replacement gilt: 3. Integrated management and economic considerations. In: Advances in Pork Production; Proc. of the Banff Pork Seminar. G. R. Foxcroft (ed). The University of Alberta, Department of Animal Science. pp. 157.

Downing, J. A. and R. J. Scaramuzzi. 1991. Nutrient effects on ovulation rate, ovarian function and the secretion of gonadotrophic and metabolic hormones in sheep. J. Reprod. Fert. Suppl. 43:209.

Dyck, G. W. 1988. Factors influencing sexual maturation, puberty and reproductive efficiency in the gilt. Can. J. Anim. Sci. 68:1

Ellendorff, F. and N. Parvizi. 1982. The central nervous system and the control of pituitary hormone release in the pig. In: Control of Pig Reproduction. D. J. A. Cole and G.R. Foxcroft (eds). Butterworths, London. pp. 179.

Elsaesser, F. 1982. Endocrine control of sexual maturation in the female pig and sexual differentiation of the stimulatory oestrogen feedback mechanism. In: Control of Pig Reproduction. D. J. A. Cole and G.R. Foxcroft (eds). Butterworths, London. pp. 93.

Flowers, B., M. J. Martin, T. C. Cantley and B. N. Day. 1988. Endocrine changes associated with a dietary-induced increase in ovulation rate (flushing) in gilts. J. Anim. Sci. 67:771.

Foster, D. L., F. J. P. Ebling, L. A. Vannerson, D. C. Bucholtz, R. I. Wood and A. F. Micka. 1988. Modulation of gonadotropin secretion during development by nutrition and growth. Proc. of the 11th International Congress on Animal Reproduction and Artificial Insemination. Dublin, Ireland. pp. 100.

Foxcroft, G. R., P. J. Booth, F. Elsaesser, R. T. Lancaster and A. M. Paterson. 1989. Physiological mechanism controlling sexual maturation in the pig. In: Control of

the Onset of Puberty III. H. A. Delemarre-van de Waal et al. (eds). Elsevier Science Publishers B. V. pp. 205.

Frisch, R. E. 1988. Fatness and fertility. Scient. Am. 258 3:88.

Hagen, D. R., K. A. Bryan, A. M. Clark, R. A. Graboski and J. M. Hammond. 1990. Effects of duration of treatment with growth hormone (GH) and pregnant mare serum gonadotropin (PMSG) challenge on growth factors and ovarian steroids in gilts. Biol. Reprod. 42(Suppl. 1):155 Abstr.

Hughes, P. E. 1982. Factors affecting the natural attainment of puberty in the gilt. In: Control of Pig Reproduction. D. J. A. Cole and G. R. Foxcroft (eds). Butterworths, London. pp. 117.

Hughes, P. E. and G. P. Pearce. 1989. The endocrine basis of nutrition - reproduction interactions. Manipulating Pig Production II. J. L. Barnett and D. P. Hennessy (eds). Australian Pig Science Association. Victoria, Australia. pp.290.

I'Anson, H., D. L. Foster, G. R. Foxcroft and P. J. Booth. 1991. Nutrition and reproduction. Oxford Reviews of Reproductive Biology. S. R. Milligan (ed). Oxford University Press. pp. 240.

Karsch, F. J. 1987. Central actions of ovarian steroids in the feedback regulation of pulsatile secretion of luteinizing hormone. Ann. Rev. Physiol. 49:365.

Kirkwood, R. N. and F. X. Aherne. 1985. Energy intake, body composition and reproductive performance of the gilt. J. Anim. Sci. 60:1518.

Kirkwood, R. N., D. C. Cumming and F. X. Aherne. Nutrition and puberty in the female. Proc. Nutr. Soc. 46:177.

Knobil, E. 1989. The GnRH pulse generator. In: Control of the Onset of Puberty III. H. A. Delemarre-van de Waal et al. (eds). Elsevier Science Publishers B. V. pp. 11.

Knott, R. E., D. C. England and W. H. Kennick. 1984. Estrus, ovulation, conception and embryo survival in confinement-managed gilts of three weight groups. J. Anim. Sci. 58:281.

Lythgoe, E. S. 1987. Metabolic and endocrinological consequences of nutritional stress imposed over successive lactations in primiparous and multiparous sows. Ph.D. thesis, University of Alberta.

Paterson, A. M. 1982. The controlled induction of puberty. In: Control of Pig Reproduction. D. J. A. Cole and G.R. Foxcroft (eds). Butterworths, London. pp. 139.

Pharazyn, A. 1992. Nutritional effects on embryo survival in the gilt. Ph.D. thesis, University of Alberta.

Pressing, A., G. D. Dial, K. L. Esbenshade and C. M. Stroud. 1992. Hourly administration of GnRH to pre-pubertal gilts: Endocrine and ovulatory responses from 70 to 190 days of age. J. Anim. Sci. 70:232.

Price, M. A., F. X. Aherne, J. I. Elliot and G. A. Lodge. 1981. The effect of age at puberty on growth and carcass quality at market weight in the gilt. Anim. Prod. 33:159.

Reutzel, L. F. and L. J. Sumption. 1968. Genetic and phenotypic relationships involving age at puberty and growth rate of gilts. J. Anim. Sci. 27:27.

Rhodes, M. T., J. E. Minton, J. S. Stevenson, and D. L. Davis. 1987. Endocrine changes associated with flushing and altrenogest treatment in gilts. J. Anim. Sci. 65:154.

Short R.V. 1980. The hormonal control of growth at puberty. Growth in Animals. T. L. J. Lawrence (ed). Butterworths, London. pp. 25.

Short, R. E., R. B. Staigmiller and R. A. Bellows. 1988. Hormonal treatments to induce ovulation. Proc. of the 11th International Congress on Animal Reproduction and Artificial Insemination. Dublin, Ireland. pp. 146.

Steiner R. A., J. L. Cameron, T. H. McNeill, D. K. Clifton, W. J. Bremner. 1983. Metabolic signals for the onset of puberty. Neuroendocrine Aspects of Reproduction. Proc. of the 2nd ORPRC Symposium on Primate Reproductive Biology, Beaverton, Oregon. R. L. Norman (ed). Academic Press, Inc. New York. pp. 183.

Terasawa, E., T. E. Nass, R. R. Yeoman, M. D. Loose and N. J. Schultz. 1983. Hypothalamic control of puberty in the female rhesus macaque. In: Neuroendocrine Aspects of Reproduction. R. L. Norman (ed). Academic Press, New York. pp. 149.

Trudeau, V. L., J. C. Meijer, D. F. M. van de Wiel, M. M. Bevers and J. H. F. Erkens. 1988. The effects of morphine and naloxone on plasma levels of LH, FSH, prolactin, and growth hormone in the immature male pig. J. Endocrinol. 119:501.

Trudeau, V. L., A. Pharazyn, E. Beltranena and F. X. Aherne. 1989. Naloxone elevates plasma follicle stimulating hormone but not luteinizing hormone levels in the immature male pig. Can. J. Anim. Sci. 69:1095.

Williams, I. H. and B. P. Mullan. 1989. Nutritional influences on sows. Manipulating Pig Production II. J. L. Barnett and D. P. Hennessy (eds). Australian Pig Science Association. Victoria, Australia. pp.285.

II. EFFECTS OF PRE- AND POST-PUBERTAL FEEDING ON PRODUCTION TRAITS AT FIRST AND SECOND ESTRUS IN GILTS[1]

A. Introduction

Demand for leaner pork has forced swine breeders to continuously select pigs for reduced body fatness while continuing to select for improvements in growth rate. Consequently, the ratio of lean tissue to fat is being altered. If a permissive lean:fat ratio is required for the onset of puberty (Kirkwood and Aherne, 1985; Kirkwood et al., 1987), such a selection trend is likely to retard the attainment of puberty because this ratio will take longer to be achieved. Reproductive efficiency is also affected in that leaner gilts farrow at an older age (Johansson and Kennedy, 1983). Furthermore, this selection trend may lead to females with a large mature body size, with a consequent increase in energy maintenance requirements. Thus, the associations among growth rate, body weight and fatness and the attainment of puberty in genetically lean gilts need to be re-evaluated.

Restricting feed or energy intake before puberty, or between estrous cycles, reduces ovulation rate in gilts. Nonetheless, short-term, high-level feeding during the first estrous cycle (flushing), increases ovulation rate (Anderson and Melampy, 1972; den Hartog and van Kempen, 1980). It is not clear, however, whether flushing merely

---

[1]A version of this chapter has been published. E. Beltranena, F. X. Aherne, G. R. Foxcroft and R. N. Kirkwood. J. Anim. Sci. 1991. 69:886.

reverses the negative effect of nutritional restriction on ovulation rate or induces superovulation (Aherne and Kirkwood, 1985).

The objectives of this study were, first, to examine the effects of pre-pubertal feeding level on body weight, fatness, growth rate, and age at the attainment of puberty; and second, to evaluate the effect of pre- and post-pubertal feeding level on ovulation rate at first and second estrus in gilts.

B. Experimental Procedure

Animals and Treatments. One hundred and forty-five (Yorkshire x Landrace) crossbred pre-pubertal gilts were used in three replicate experiments conducted during the summer and fall of consecutive years. The gilts were weaned at 28 days of age, thereafter penned in groups and allowed ad libitum access to commercial starter and grower diets. After allocation to the experimental treatments, gilts were fed a barley, wheat, and soybean meal-diet formulated to provide a minimum of 16% crude protein, .95% lysine, 13.1 MJ DE/kg and to exceed the NRC (1979) recommended requirements for growing pigs for amino acids, vitamins and minerals. Gilts fed restrictively were penned in groups of four but were individually fed three meals of approximately equal size at 0630, 1130 and 1530 h. Gilts with ad libitum access to feed also were penned in groups of four but had continuous access to the diet from self-feeders.

From 47.2 SD .6 kg body weight and 98.2 SD .5 days of age, until three days after the attainment of first estrus (puberty), sets of gilts with similar post-weaning growth rates (calculated as current body weight minus birth weight divided by age using only

animals within ± 1.8 x SD), were assigned randomly to be fed either 2.0 kg/day (L, n = 86) or were given ad libitum access to feed (H, n = 59). From three days after the attainment of first estrus until five days after the attainment of second estrus, the feed allowance of one-half of the gilts in the L group was increased to 2.8 kg/day (LH, n = 43); the other L gilts continued to be fed 2.0 kg/day (LL, n = 41). All gilts in the pre-pubertal H group continued feeding ad libitum (HH, n = 46) until five days after the attainment of second estrus.

From an average pen body weight of 65 kg until five days after the individual attainment of second estrus, gilts were moved daily as a group into one of three or four mature boar pens on a rotational basis for stimulation and detection of estrus. Gilts were in full body contact with boars for approximately 20 minutes and subsequently were returned to their home pens. Any gilt showing estrus was removed immediately from the boar's presence in order to prevent mating.

Measurements. Body weight was recorded weekly for all gilts in the three experiments. Backfat thickness was measured (Scanoprobe II, Ithaca, NY) at the same time as body weight in all gilts in experiments 2 and 3. Probe sites were aligned with the apex of the curve of the last rib, at both sides, 5 centimetres off the midline and were permanently marked with a tattoo for subsequent readings. Gilts that showed normal estrous activity were slaughtered five to seven days after the onset of their second estrus. Any gilt that had not shown behaviourial second estrus 30 days after the date of onset of first estrus was slaughtered. After slaughter, reproductive tracts were collected and ovaries were removed at the hilus. The ovaries were then sliced in

approximately 1-mm sections. Ovulation rate at first and second estrus was established by counting corpora albicantia (CA) and corpora lutea (CL), respectively. In experiment 1, in order to substantiate this method for determining ovulation rates in consecutive estrous cycles in the same animal, the number of corpora albicantia counted at slaughter after the second estrus in HH gilts was compared with counts of corpora lutea following the slaughter of 11 H gilts five days after their pubertal estrus. Hereafter, this will be referred as the "CA*CL test".

Statistical Analyses. Statistical analyses were performed using the General Linear Models procedures of SAS (1988). Differences in age, body weight, backfat thickness and ovulation rate were assessed by unbalanced analysis of variance of a split-plot design. Overall treatment effects were established using the error term of gilts nested within year x treatment. Multiple comparison for interactions (feeding level x time [first to second estrus]) were performed according to the method of Harter (1970). Tukey's studentized range test was used to compare means. Associations between variables were explored using Pearson correlation and regression analyses with correlation coefficients first calculated separately for each year and treatment and then pooled. Student's t-test was conducted to examine the hypothesis of difference in the estimation of pubertal ovulation rate by counting corpora lutea versus corpora albicantia for the CA*CL test.

C. Results

Animals. Four of the 145 gilts were withdrawn from the experiments before the attainment of puberty due to unsatisfactory growth rate (2 L) and leg weakness (2 H). In addition, the data from 11 H gilts involved in the CA*CL test, data from three gilts injured by the boars (2 LH, 1 HH) and 12 gilts slaughtered 30 days after puberty having not shown second estrus, were excluded from statistical analysis of traits at second estrus. Post-mortem examination of the reproductive tract morphology of these anestrous gilts revealed a gravid uterus in four (2 LL, 2 HH) and cystic follicles in the ovaries of two (1 LL, 1 LH); the presence of corpora lutea and corpora albicantia in the ovaries of one (1 LH) gilt suggested that a second estrus had gone undetected. The absence of corpora lutea in the ovaries of the remaining five gilts indicated true post-pubertal anestrus (4 LL, 1 LH). Consequently, data from 141 gilts (84 L, 57 H) were used in regression and correlation analysis of traits at puberty. However, complete data sets from only 115 gilts were available for a statistical analysis of changes in a variable from first to second estrus (34 LL, 38 LH and 43 HH).

Feed Intake. The pooled pattern of voluntary feed intake for the HH gilts is show in Figure II.1. Feed intake for the H gilts at initiation of the study was similar to the feeding allowance of the L gilts. At first estrus, feed intake for the H gilts was 3.4 kg/day, equivalent to 170% of the feeding allowance of the L gilts. And at second estrus, feed intake for the HH gilts reached 3.6 kg/day, equivalent to 190 and 135% of the feeding allowance for the LL and LH gilts, respectively.

Production Traits. Table II.1 presents the least-square means of the pooled data for age, body weight, ovulation rate and backfat thickness. These data were pooled because there was no significant effect of year nor were there significant interactions for year x feeding level, year x time (first, second estrus) or year x feeding level x time ($P > .05$).

The main effect of time (first, second estrus), irrespective of feeding level, was significant for all studied traits. Gilts were older, heavier, fatter and had higher ovulation rate at second than at first estrus.

The main effect of feeding level, irrespective of time, was significant for age, backfat thickness and ovulation rate. The HH gilts were younger and fatter than either the LL or LH gilts. Gilt in the L group that had their feed allowance increased (LH) and HH gilts had greater ovulation rate than LL gilts.

Feeding level x time interactions were significant for body weight, backfat thickness and ovulation rate only. Body weight gain between first and second estrus for the HH gilts was almost twice that of the LL gilts; the LH gilts exhibited intermediate body weight gain. Gain of backfat thickness between first and second estrus was greater for the HH gilts than for either LL or LH gilts. Increase in ovulation rate between first and second estrus was greater for HH and LH gilts than for LL gilts.

*CA*CL Test.* Pubertal ovulation rate, estimated by counting corpora lutea following slaughter of 11 H gilts after first estrus, was not different from that estimated by counting corpora albicantia following slaughter of their contemporary HH gilts after the second estrus ($P > .05$).

Correlations Between Production Traits at Puberty. Overall and within each group, there was a linear and positive correlation between age and body weight at first estrus (Table II.2, Figure II.2a) and younger gilts were lighter at puberty. In contrast to body weight, there was overall a highly significant quadratic and negative relationship between age at first estrus and lifetime growth rate (Table II.2, Figure II.2b). This relationship was even stronger for the L gilts (Table II.2) and the greater the growth rate, the younger the gilt at puberty. This relationship was, however, nonsignificant ($P > .05$) for the H gilts.

The correlation between age and backfat thickness at first estrus was low and nonsignificant ($P > .05$). However, when fatness was expressed as backfat thickness/body weight at first estrus, a significant negative linear relationship with age was observed. Overall (Table II.2, Figure II.2c), and for each group, the fatter the gilt the younger it was at puberty.

Correlations between age and ovulation rate at first estrus were low and nonsignificant ($P > .05$).

Overall, body weight at first estrus was positively and highly correlated to lifetime growth rate and backfat thickness at first estrus. For the L gilts, however, there was no correlation between body weight and backfat thickness at first estrus ($P > .05$).

Correlations between lifetime growth rate and backfat thickness at first estrus and backfat thickness/body weight at first estrus were positive and significant overall and for the H gilts, yet were nonsignificant for the L gilts ($P > .05$).

Overall, ovulation rate was positively and highly correlated with fatness at first estrus (r = .27), yet this relationship was nonsignificant for the individual groups ($P > .05$).

Correlations Between the Increase in Ovulation Rate from First to Second Estrus and Age, Body Weight, Backfat Thickness, Growth Rate and Fatness at Second Estrus or Their Change Between First and Second Estrus. Overall, the increase in ovulation rate from first to second estrus was positively correlated with the increase in body weight between first and second estrus (r = .25) and with lifetime growth rate from birth to second estrus (r = .27). However, significant correlations were not established for the individual treatment groups ($P > .05$).

D. Discussion

Feed Restriction and the Attainment of Puberty. Whether moderate feed restriction (60 to 85% of ad libitum) affects age at puberty in gilts has been the subject of controversy (reviewed by Brooks and Cole, 1974; Hughes, 1982; Aherne and Kirkwood, 1985). In the present study, feed restriction delayed, but did not prevent the onset of puberty. After puberty was attained, the length of the first estrous cycle was neither affected by feeding level nor by indirect changes in growth rate, body weight or fatness over the first estrous cycle. Thus, mild feed or energy restriction delays attainment of puberty primarily by affecting growth rate.

Body Weight at Puberty. The close proximity in body weight at puberty between treatments groups is consistent with the suggestion that body weight may be an important determining factor in the attainment of reproductive activity (Frisch, 1988).

Similarly, van Lunen and Aherne (1987) observed no difference in body weight at puberty between gilts reared on different feeding regimens, even though growth rate and age at puberty were different.

The observed positive correlation between age and body weight at puberty (Figure II.2a) indicated that the heavier the gilt at boar stimulation, the heavier it was at puberty. This is likely a function of most gilts achieving a level of development permitting a response to boar contact. This suggestion is in agreement with the data of Burnett et al. (1988) and Knott et al. (1984). Therefore, a weight or age difference at commencement of boar contact would still be evident at puberty.

Growth Rate and the Attainment of Puberty. Of great relevance in this study is the negative quadratic relationship between age and lifetime growth rate to puberty. In contrast to this quadratic relationship, a linear and negative relationship has been reported previously (Reutzel and Sumption, 1968; Cunningham et al., 1974; Hutchens et al., 1981; Price et al., 1981; den Hartog and Noordewier, 1984; Mabry et al., 1985). The manner in which those results were analyzed, added to variability in experimental design, may have masked the existence of a quadratic relationship, in that the existence of a linear relationship may be limited to gilts fed restrictively. No relationship between age at puberty and lifetime growth rate was evident from these data (Table II.2), or those of Young et al., (1990), for gilts with ad libitum access to feed. Hence, the negative quadratic relationship observed in this study is interpreted as follows: There are at least two components in the relationship between lifetime growth rate and age at puberty (Figure II.2b). In the first, with a negative slope and mainly associated with

feeding restrictively, the greater the growth rate, the younger the gilts would be at puberty. In the second, with a slope no different from zero and mainly associated with gilts given ad libitum access to feed, growth is not limiting and gilts reach puberty at the youngest age possible. Accordingly, this minimum age at puberty coincided with a lifetime growth rate of .60 kg/day ($x = -B/2C$). From the equation generated with these data, a third component seems feasible, in which the slope becomes positive and corresponds to the fastest growing gilts; in this situation age at puberty again becomes delayed as growth rate increases. Caution must be exercised however, because the number of gilts in that component was small due to the allocation procedure used in this study (only gilts within $\pm$ 1.8 x SD of post-weaning growth rate were considered). Hence, whether the slope remains zero, in which case faster-growing gilts will be heavier at puberty, or becomes positive, in which case gilts will not only be heavier but also older at puberty, is not clear.

Body Fatness and the Attainment of Puberty. Linear and positive relationships among lifetime growth rate, body weight and fatness, were noted as significant overall and for the H gilts but not for the L gilts (Table II.2). Thus, lifetime growth rate was associated with body weight at puberty but not necessarily with a certain backfat thickness or degree of fatness. The reason for this may be in the quality of tissue growth. For L gilts with a low growth rate, extremely lean growth occurred and no correlation was observed; in contrast, for H gilts, a greater growth rate may have been associated with greater fat deposition. This rationale does not, however, rule out the possibility that puberty was delayed until a certain amount of body fat or a certain

degree of fatness had been achieved. Thus, for gilts within the first component of the relationship between age at puberty and lifetime growth rate, growth or some aspect of body state probably limited attainment of puberty. Similarly, for those gilts within the third component, with the fastest and possibly also the leanest growth, achievement of a certain degree of fatness also may be the factor limiting activation of the hypothalamic-hypophysial-ovarian axis. Both situations would be in agreement with the Frisch model of puberty attainment (Frisch, 1988). In contrast, for gilts within the second component, for which fat deposition is greater, neither fatness nor body weight limits attainment of puberty, because these are guaranteed by the rate and quality of growth. This seems in agreement with the conclusions of Young et al. (1990) that body weight and fatness did not limit attainment of puberty in gilts with ad libitum feed intake. Growth-independent effects on the maturation of the hypothalamic-hypophysial-ovarian axis seem, therefore, to be limiting puberty attainment in gilts within the second component.

Nutritional Flushing. Dyck (1971) suggested that only the number of corpora lutea will provide accurate estimates of ovulation rate. It is possible that when the ovary is examined superficially, follicular growth and new corpora lutea may mask the regressing corpora albicantia from the ovarian surface. Slicing of ovarian tissue accordingly is a more precise method of establishing ovulation rate in the same animal over two consecutive estrous periods; the accuracy of this method was confirmed here.

King (1989) reported that heavier and fatter gilts had higher ovulation rates at puberty. In the present study, however, a higher ovulation rate was associated with greater fatness, but not body weight, at puberty.

It has been previously reported (reviewed by Anderson and Melampy, 1972; Brooks and Cooper, 1972; den Hartog and van Kempen, 1980) that feed restriction followed by realimentation (flushing) increases ovulation rate in swine. Because of the lack of adequate control groups, however, it never has been clear whether flushing induces superovulation or merely normalizes ovulation rate from a previously depressed level (Knott et al., 1984; Aherne and Kirkwood, 1985). From the present results the following four conclusions can be made: 1) continuous feed restriction to 50 to 65% of ad libitum (1.7 - 2 x maintenance; ARC, 1981) adversely affects ovulation rate at both first and second estrus; 2) in gilts with ad libitum access to feed (2.8 - 3.0 x maintenance), ovulation rate is higher and it increases from first to second estrus; 3) flushing (2.4 x maintenance) of gilts restrictively fed previously only increases the ovulation rate to that observed in gilts with ad libitum intake and does not stimulate superovulation; and 4) the lack of a significant correlation between the increase in ovulation and change in any production trait from first to second estrus for the LH gilts (flushing), indicates that realimentation may exert a short-term effect on ovulation rate more related to an overall improvement of metabolic status than to a dependence on changes in body weight, backfat thickness or fatness (see Booth, 1990).

E. Implications

Maximizing growth rate in replacement gilts with ad libitum access to feed may not always hasten the attainment of puberty. Indeed, excessively lean growth may delay attainment of puberty and increase mature body size and, therefore, maintenance costs. Therefore, it may be practical to manipulate growth rate by feed restriction in order to attain a target body weight at boar stimulation ($\approx$90 kg), at which minimum age ($\approx$ 155 d) and body weight at puberty ($\approx$97 kg) would coincide. Nutritional flushing during the first estrous cycle then could be used to normalise ovulation rate at mating at second estrus in gilts that were fed restrictively when pre-pubertal.

Table II.1. The effect of pre- and post-pubertal level of feeding on production traits[a]

Item	LL[b]	LH[b]	HH[b]	Main effect of time[c]	$P > F$	SE
Feeding level, kg/d						
47 kg to first estrus	2.0	2.0	ad lib			
n	41	43	57			
First to second estrus	2.0	2.8	ad lib			
n	34	38	43			
Age, d						
First estrus	187.0	184.5	170.1	180.5[h]	---	---
Second estrus	208.0	205.9	191.4	201.8[i]	.0001	SEM .19
Main effect of feeding level[c,d]	197.5[j]	195.2[j]	180.8[k]	---	.0004	SEM 2.94
Feeding level x time interaction[c]	21.0	21.4	21.3	---	.7367	SED .37
Body weight, kg						
First estrus	96.6	95.7	98.9	97.1[h]	---	---
Second estrus	104.4	107.8	114.3	108.9[i]	.0001	SEM .33
Main effect of feeding level[c,d]	100.5	101.8	106.6	---	.0350	SEM 1.61
Feeding level x time interaction[c]	7.9[x]	12.1[y]	15.4[z]	---	.0001	SED .67
Backfat thickness, mm						
First estrus	11.2	10.9	13.1	11.7[h]	---	---
Second estrus	12.0	12.6	16.5	13.7[i]	.0001	SEM .17
Main effect of feeding level[c,d]	11.6[j]	11.8[j]	14.8[k]	---	.0001	SEM .38
Feeding level x time interaction[c]	.8[x]	1.7[x]	3.4[y]	---	.0001	SED .34
Ovulation rate						
First estrus (CA)[e]	11.1	11.5	12.1	11.6[h]	---	---
Second estrus (CL)[e]	12.0	14.0	14.7	13.5[i]	.0001	SEM .16
Main effect of feeding level[c,d]	11.6[j]	12.7[k]	13.3[k]	---	.0001	SEM .26
Feeding level x time interaction[c]	.9[x]	2.4[y]	2.6[y]	---	.0009	SEM .31

[a]Least-square means for the pooled data of three consecutive years.

[b]LL = gilts that remained on a constant diet; LH = gilts that were given an increased feed allowance; HH = gilts with ad libitum access to feed.

[c]Means within a row lacking a common superscript letter differ; h, i main effect of time irrespective of feeding level; j, k main effect of feeding level irrespective of time; x,y,z feeding level x time interaction expressed by the difference between first and second estrus.

[d]Gilts (year x treatment) as error term.

[e]CA and CL = corpora albicantia and corpora lutea, respectively.

[f]Standard error of the mean (SEM) and difference (SED).

Table II.2. Correlation coefficients (r) between various production traits at puberty

Traits	No. of gilts	Mean	± SE	Age at first estrus, d	Body weight at first estrus, kg	Lifetime growth rate[a], kg/d
				r	r	r
Age at first estrus,d						
L[b]	84	185.1	2.4	---	---	---
H[c]	57	170.5	2.6	---	---	---
L + H	141	179.2	1.9	---	---	---
Body weight at first estrus, kg						
L	84	96.1	1.0	+.68**	---	---
H	57	99.1	1.8	+.76**	---	---
L + H	141	97.3	1.0	+.61**	---	---
Lifetime growth rate[a] kg/d						
L	84	.51	.01	-.57**	+.21*	---
H	57	.57	.01	-.09 NS[e]	+.57*	---
L + H	141	.53	.01	-.47**	+.41**	---
Backfat thickness at first estrus, mm						
L	59	11.1	.23	+.10 NS	+.16 NS	+.07 NS
H	34	13.4	.59	+.01 NS	+.39*	+.61**
L + H	93	12.0	.28	-.12 NS	+.28**	+.51**
Fatness[d] at first estrus, mm/kg						
L	59	.12	.01	-.32**	---	-.07 NS
H	34	.14	.01	-.49**	---	+.24*
L + H	93	.13	.01	-.48**	---	+.29**

[a]Birth to first estrus.
[b]L gilts were given ad libitum access to feed from birth to 47 kg body weight, and then 2.0 kg/d to first estrus.
[c]H gilts were given ad libitum access to feed from birth to first estrus.
[d]Backfat thickness, mm/body weight, kg
[e]NS = nonsignificant ($P > .05$);
*$P < .05$; **$P < .01$.

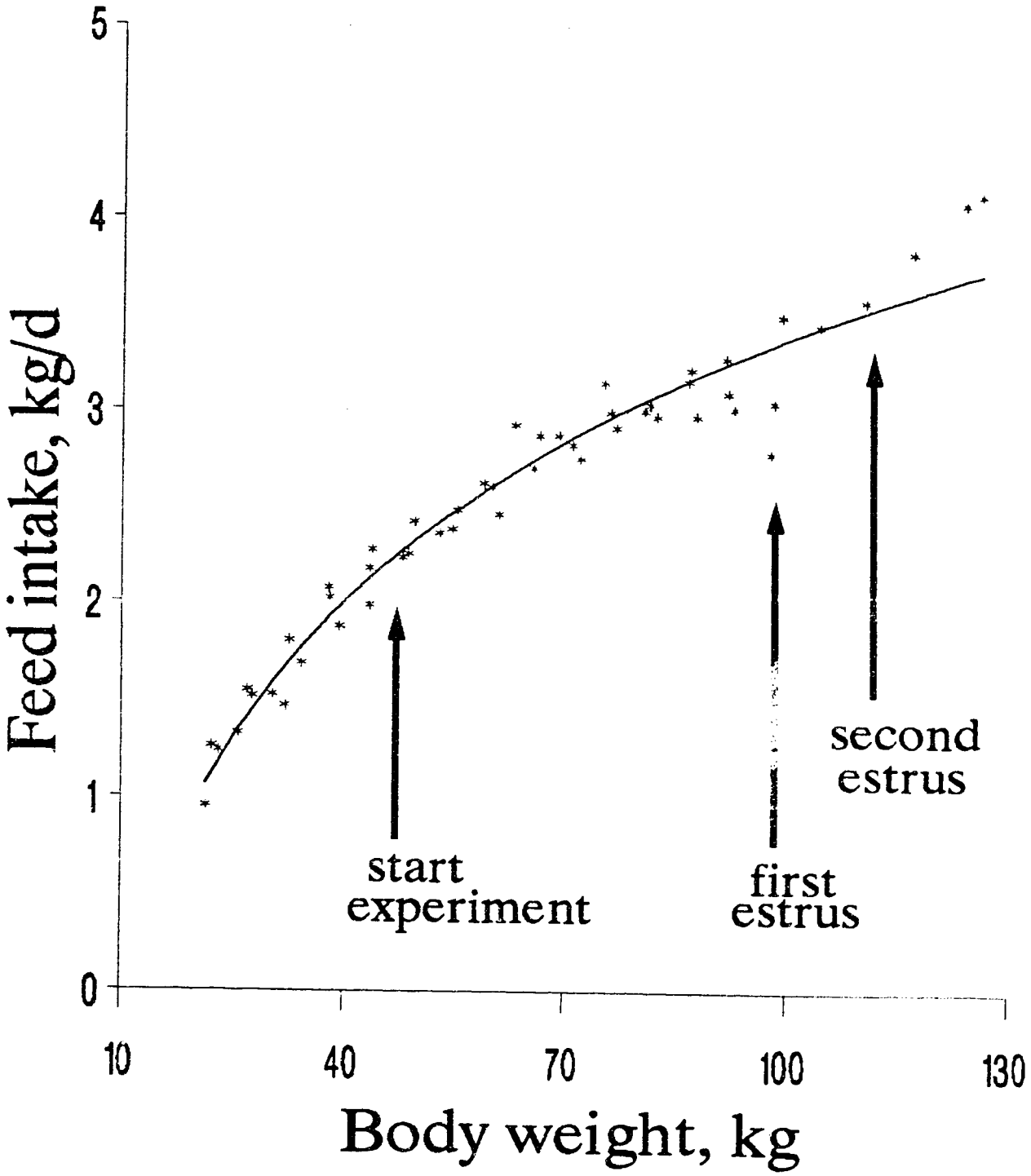


Figure II.1. Pooled daily feed intake for HH gilts in experiments 1, 2, and 3 (n = 57; Y = .4696(± .1795) + .0413(± .0043)WT - .00012(± .0003)WT^2.

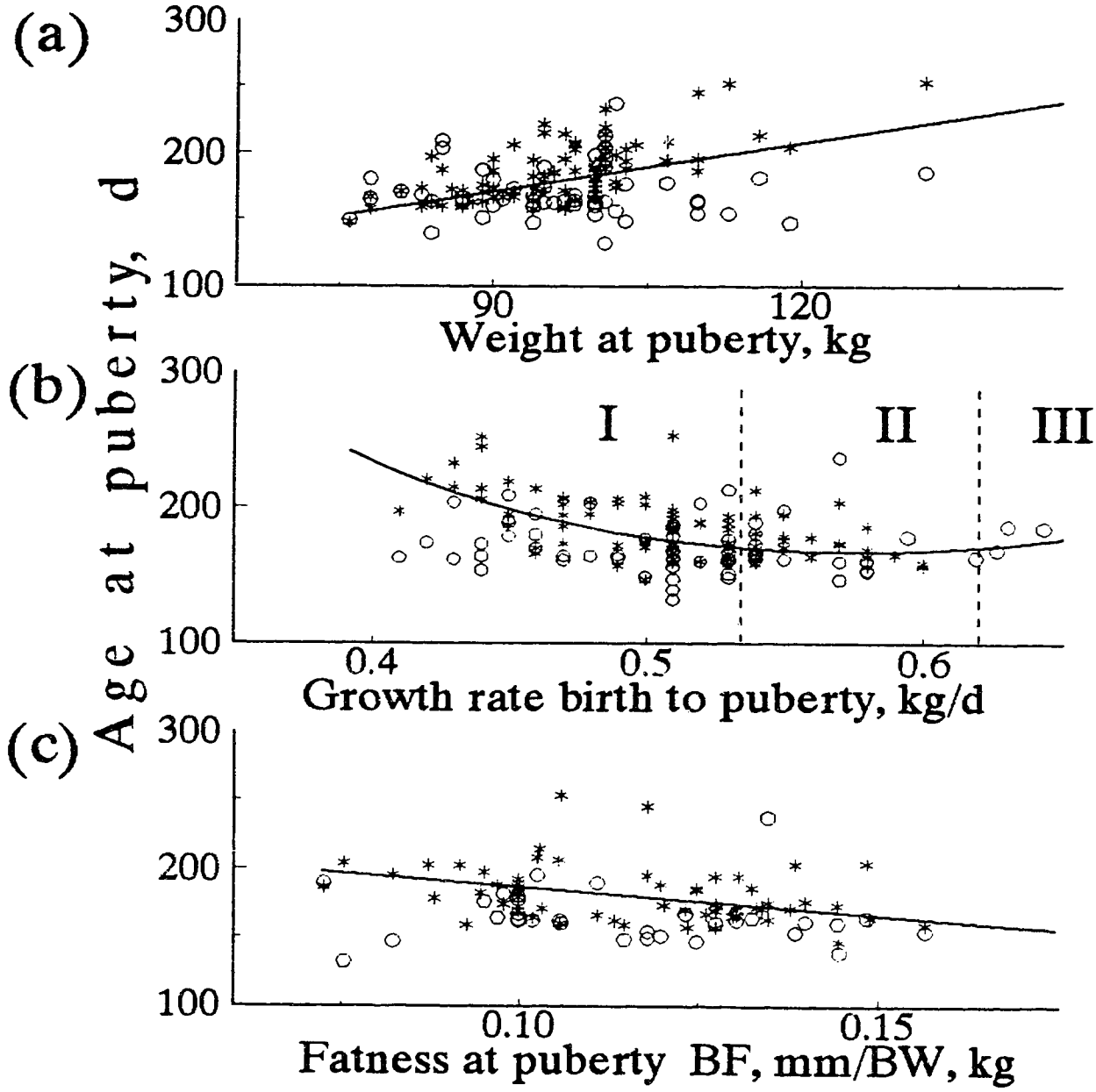


Figure II.2. Relationship between age at puberty and several production traits at puberty: (a) body weight (BW) at puberty (n = 141; Y = 62.86{± 17.74} + 1.20{± .13}BW; $P < .0001$), (b) growth rate (GR) from birth to puberty (n = 141; Y = 721.27{± 18.75} - 1835.37{± 410.07}GR + 1520.53{± 377.83}GR^2; $P < .0001$) and (c) fatness at puberty (n = 93; Y = 220.72{± 18.54} - 364.11{± 69.32}BF/BW; $P < .0001$) for the pooled data from gilts in experiments 1, 2 and 3. Feeding levels from 47 kg body weight to puberty were 2.0 kg/d(*) or ad libitum(○). Refer to the Discussion section for an explanation of the different components (I, II, and III) of the regression line in (b).

F. Literature Cited

ARC. 1981. The Nutrient Requirements of Pigs. Commonwealth Agricultural Bureaux, Slough, UK.

Aherne, F. X. and R. N. Kirkwood. 1985. Nutrition and sow prolificacy. J. Reprod. Fert. Suppl. 33:169.

Anderson, L. L. and R. M. Melampy. 1972. Factors affecting ovulation rate in the pig. In: D. J. A. Cole (ed) Pig Production. Butterworths, London. pp. 329.

Booth, P. J. 1990. Metabolic influences on hypothalamic-pituitary-ovarian function in the pig. J. Reprod. Fert. Suppl. 40:89.

Brooks, P. H. and K. J. Cooper. 1972. Short term nutrition and litter size. In: D. J. A. Cole (ed) Pig Production. Butterworths, London. pp. 385.

Brooks, P. H. and D. J. A. Cole. 1974. The effect of nutrition during the growing period and the oestrous cycle on the reproductive performance of the pig. Livest. Prod. Sci. 1:7.

Burnett, P. J., N. Walker and D. J. Kilpatrick. 1988. The effect of age and growth traits on puberty and reproductive performance in the gilt. Anim. Prod. 46:427.

Cunninghan, P. J., C. H. Naber, D. R. Zimmerman and E. R. Peo Jr. 1974. Influence of nutritional regime on age at puberty in gilts. J. Anim. Sci. 39:63.

den Hartog, L. A. and G. J. M. van Kempen. 1980. Relation between nutrition and fertility in pigs. Neth. J. Agric. Sci. 28:211.

den Hartog, L. A. and G. J. Noordewier. 1984. The effect of energy intake on age at puberty in gilts. Neth. J. Agric. Sci. 32:263.

Dyck, G. W. 1971. Ovulation rate and weight of the reproductive organs of Yorkshire and Lacombe swine. Can. J. Anim. Sci. 51:141.

Frisch, R. E. 1988. Fatness and fertility. Scient. Am. 258 3:88.

Harter, H. L. 1970. Multiple comparison procedures for interactions. Am. Statistn. 24:30.

Hughes, P. E. 1982. Factors affecting the natural attainment of puberty in the gilt. In: D. J. A. Cole and G. R. Foxcroft (ed) Control of Pig Reproduction. Butterworths, London. pp. 117.

Hutchens, L. K., R. L. Hintz and R. K. Johnson. 1981. Genetic and phenotypic relationships between pubertal and growth characteristics in gilts. J. Anim. Sci. 53:946.

Johansson, K. and B. W. Kennedy. 1983. Genetic and phenotypic relationships of performance test measurements with fertility in Swedish Landrace and Yorkshire sows. Acta Agric. Scand. 33:195.

King, R. H. 1989. Effect of live weight and body composition of gilts at 24 weeks of age on subsequent reproductive efficiency. Anim. Prod. 49:109.

Kirkwood, R. N. and F. X. Aherne. 1985. Energy intake, body composition and reproductive performance of the gilt. J. Anim. Sci. 60:1518.

Kirkwood, R. N., D. C. Cumming and F. X. Aherne. 1987. Nutrition and puberty in the female. Proc. Nutr. Soc. 46:177.

Knott, R. E., D. C. England and W. H. Kennick. 1984. Estrus, ovulation, conception and embryo survival in confinement managed gilts of three weight groups. J. Anim. Sci. 58:281.

Mabry, J. W., W. M. Weaver, L. L. Benyshek and M. A. Marks. 1985. Phenotypic and genetic parameters for growth, puberty and composition traits in gilts. Growth 49:282.

NRC. 1979. Nutrient Requirements of Swine (8th Ed.). National Academy Press, Washington, DC.

Price, M. A., F. X. Aherne, J. I. Elliot and G. A. Lodge. 1981. The effect of age at puberty on growth and carcass quality at market weight in the gilt. Anim. Prod. 33:159.

Reutzel, L .F. and L. J. Sumption. 1968. Genetic and phenotypic relationships involving age at puberty and growth rate of gilts. J. Anim. Sci. 27:27.

SAS. 1988. SAS User's Guide: Statistics. SAS Inst., Inc. Cary, NC.

van Lunen, T. A. and F. X. Aherne. 1987. The effect of long-term feed restriction on age at puberty of gilts. Can. J. Anim. Sci. 67:797.

Young L. G., G. J. King, J. S. Walton, I. McMillan and M. Klevorick. 1990. Age, weight, backfat and time of mating effects on performance of gilts. Can. J. Anim. Sci. 70:469.

III. ENDOCRINOLOGY OF NUTRITIONAL FLUSHING IN GILTS[2]

A. Introduction

Female prolificacy is determined genetically by the number of ova released at ovulation. Nutrition is among the environmental factors that may constrain ovulation rate. It has been long established that nutritional distress reduces lambing rate in ewes (Youatt, 1837; Coop, 1962) and litter size in pigs (Robertson et al., 1951; Christian and Nofziger, 1952; Clark et al., 1973). Nevertheless, short-term improvements in nutritional state preceding mating increases twinning rates in ewes (Nichols, 1924), and similarly, short-term, high-level feeding during the estrous cycle increases ovulation rate in gilts (Robertson et al., 1951). This phenomenon has been named "nutritional flushing" (reviewed by Anderson and Melampy, 1972; den Hartog and van Kempen, 1980; Aherne and Kirkwood, 1985 in pigs and Downing and Scaramuzzi, 1991 in sheep). In the pig, it appears that flushing increases ovulation rate but does not induce superovulation; the data presented in Chapter II corroborated the results of Self et al. (1955) in that nutritional flushing merely restored ovulation rate of gilts fed restrictively to that of contemporaries with ad libitum access to feed.

The physiological mechanisms by which an increase in feeding restores ovulation rate requires further elucidation. Since chronic, severe feed restriction impairs the hypothalamic gonadotropin-releasing hormone (GnRH) pulse generator (Armstrong and

---

[2]A version of this chapter has been published. Beltranena, E., G. R. Foxcroft, F. X. Aherne and R. N. Kirkwood. 1991. Can. J. Anim. Sci. 71:1063.

Britt 1987) and a significant and rapid increase in luteinizing hormone (pLH) secretion occurs after realimentation (Flowers et al., 1988; Booth, 1990), it appears that gonadotropins mediate the restoration of ovulation rate. Whether a comparable hypothalamic-hypophyseal response occurs during realimentation after mild-restriction (i.e., flushing) however, needs clarification. Although Cox et al. (1987) have established a role for insulin as a mediator of nutritional effects on ovulation rate, in vivo evidence suggesting a role for other hormones or metabolites is limited. Roles for plasma amino acids, free fatty acids, glucose, growth hormone, thyroxine and insulin-like growth factor I (IGF-I) have been proposed mainly by extrapolation of results obtained from in vitro studies of cultured ovarian tissue (for review see Booth, 1990). The objective of the present study was to compare the endocrine status of gilts fed restrictively, flushed or given ad libitum access to feed throughout the second peri-estrus period, and thereby provide additional information of potential mediators of the ovulatory response to flush feeding.

B. Experimental Procedure

The detailed experimental procedure used in this study is described in the preceding chapter (II.B); therefore, only a brief description of the overall experimental treatments and modifications are therefore presented here.

Animals and Treatments. Forty-two gilts, progeny of Camborough dams x Canabrid sires (Pig Improvement Canada, Acme, AB) were fed a barley, wheat and soybean meal diet, formulated to provide a minimum of 16% crude protein and 12.5 MJ ME/kg

(NRC, 1979). From 45.3 SD 3.1 kg body weight and 103.4 SD 5.7 days of age until three days after the attainment of first estrus, the gilts were assigned to be fed 2.0 kg/day (L, n = 28) or had ad libitum access to feed (H, n = 14). From three days after the attainment of first estrus (puberty) until five days after the onset of second estrus, one-half of the gilts in the L group had their feed allowance increased to 2.8 kg/day (LH, n = 14), a level of realimentation calculated to be equivalent to 80% of ad libitum intake. Gilts in the H group continued to be have ad libitum access to feed (HH, n = 14). Gilts fed restrictively (LL, LH) were individually fed three meals of approximately equal size at 0630, 1130 and 1530 hours; HH gilts, in contrast, had continuous access to the diet from self-feeders.

Cannulation and Bleeding. Twelve to fourteen days after the onset of first estrus, 30 of these gilts (10 per treatment) had an indwelling catheter (i.d. 1.58 mm, o.d. 3.18 mm; Silastic, Dow Corning Corporation, Midland, MI) surgically implanted via the external jugular vein and exteriorised on the dorsal side of the neck under general anaesthesia (Fluothane, Ayerst Laboratories, Montreal, QE). These gilts were penned individually after surgery and stimulation and detection of estrus was done on an individual basis. Beginning on approximately day 14 of the first estrous cycle, until three days after the onset of second estrus, blood samples were collected at 0300, 0700, 1100, 1500, 1900 and 2300 hours into heparinized tubes. All gilts were slaughtered five to seven days after onset of their second estrus and ovulation rates were established as described in the previous chapter (II.B).

Radioimmunoassays. Plasma follicle stimulating hormone (FSH) was assayed by the homologous double antibody radioimmunoassay of Bolt et al. (1981) and Guthrie and Bolt (1983) with modifications. Porcine FSH (USDA-pFSH-PP1B; USDA Animal Hormone Program, Beltsville, MD) was used as radioiodinated (^{125}I) antigen (see Kraeling et al., 1982) and USDA-pFSH-B-1 (USDA Animal Hormone Program, Beltsville, MD) as standard (.5 to 512 ng/tube). The rabbit anti-porcine FSH serum (USDA-10-1010; USDA Animal Hormone Program, Beltsville, MD) was used at a final dilution of 1:18000. Assay sensitivity (2.5 x SD of zero standard), was 1 ng/tube. Lack of plasma interference was confirmed by demonstrating parallelism between the standard curve and increasing concentrations of a control pooled plasma (100, 200, and 300 μL/tube). Recovery of 2, 8, 18, 48, and 96 ng FSH added to 300 μL of control pooled plasma, and corrected for endogenous FSH, was 105.7%. Plasma samples were quantified in six assays with intra- and inter-assay coefficients of variation of 12.3 and 22.4%, respectively, calculated from eight to ten replicates per assay of two control-pooled plasma samples.

Plasma luteinizing hormone (pLH) was assayed using the heterologous radioimmunoassay described by Kraeling et al. (1982). Assay sensitivity was .1 ng/tube. Recovery of purified standard added to control pooled plasma averaged 103.4%. Dose response curves for different volumes of pooled plasma and increasing concentrations of hormone (.1 to 6 ng/mL; USDA-pLH-B-1; USDA Animal Hormone Program, Beltsville, MD) were parallel. The plasma samples were quantified in six

assays with intra- and inter-assay coefficients of variation of 6.7 and 12.1%, respectively, calculated from six replicates per assay of a control, pooled plasma.

Plasma insulin was assayed using the homologous double antibody radioimmunoassay procedure reported by Ray et al. (1983). Purified porcine insulin (Calbiochem, La Jolla CA) was used as radioiodinated antigen and standard and IN-40 (Endocrine Science, Tarzana CA) as antiserum. Additions of .25, 2.5, 5 and 7.5 ng of hormone and 50, 100, 150 and 300 μL of pooled plasma yielded parallel inhibition curves. Plasma samples were analyzed in five assays with intra- and inter-assay coefficients of variation of 12.5 and 15.4%, respectively, calculated from four replicates each of plasma obtained from a single gilt either pre- and post-prandially.

Plasma progesterone was assayed in all 1900 hours daily samples by the method of Rawlings et al. (1977, 1980) using an antiserum raised against 4-Pregnen-11α-ol-3,20-dione hemisuccinate:BSA (N. C. Rawlings, University of Saskatchewan, Sask.). Assay sensitivity was .05 ng/tube. Intra- and inter-assay coefficients of variation for the three assays were 9.6 and 13.8%, respectively, calculated from three duplicates of pooled plasma. Inhibition curves with purified progesterone standards and various dilutions of pooled plasma were parallel. Extraction efficiency using 2 mL of double-distilled petroleum ether and 50 μL of plasma sample and determined by recovery of [1,2-^{3}H(N)]-progesterone (Dupont Canada Inc., NEN Products, Mississauga, ON) from barrow plasma, averaged 86.3%; plasma potencies were corrected for recovery.

Plasma estradiol-17β in the 0700 and 1900 hours daily samples was assayed by the method of Wettemann et al. (1972) using an antiserum raised against 1,3,5(10)-

estratrien-3,17β-diol-6-one-6CMO:BSA (N. C. Rawlings, University of Saskatchewan, Sask.). Plasma samples (1 mL) were extracted twice using 7 mL of glass-distilled diethyl ether, and extraction efficiency, as determined by the recovery of [2,4,6,7-^{3}H(N)]-17β estradiol (Dupont Canada Inc., NEN Products, Mississauga, ON) from barrow plasma averaged 91.3% in nine assays; plasma potencies were corrected for recovery. Intra- and inter-assay coefficients of variation were 11.4 and 13.9%, respectively, calculated from six replicates of pooled plasma. Initially, it was not possible to obtain parallelism between the unextracted standard curve and extracts of increasing amounts of control pooled plasma and purified estradiol-17β added to control pooled plasma. Nevertheless, when the standards were extracted from 1 mL of charcoal stripped barrow plasma, parallelism was obtained; therefore all standards were extracted from barrow plasma as described for sample tubes.

Plasma insulin-like growth factor I (iGF-I) was assayed in all 1900 hours samples corresponding to the time of individual pre-ovulatory pLH surges using a nonequilibrium, double antibody radioimmunoassay as described by Kirkwood et al. (1988), following the method of Furlanetto et al. (1977). All samples were quantified in a single assay with sensitivity and intra-assay coefficient of variation of .4 mU/mL and 2.3%, respectively.

Statistical Analyses. Statistical analyses were performed by unbalanced analysis of variance using the General Linear Models procedures (SAS, 1988) using a split-plot design that included feeding level, time and its interaction as main effects. Treatment differences were established using the error term of gilts nested within feeding level.

Differences in ovulation rate for all 42 gilts were assessed as described in the previous chapter (II.B). For all cannulated gilts, analyses for pLH, FSH, estradiol-17β and progesterone were conducted for three peri-estrous time-periods based on the time of individual pLH surges, where time zero was taken as the time of maximal pLH concentrations during the pre-ovulatory pLH surge. The pre-surge period included samples from -144 to -28 hours (day -6 to -2), the surge period from -24 to +24 hours (day -1 to +1), and the post-surge period from +28 to +72 hours (day +2 to +3). Hormonal concentrations for individual samples out of the -144 to +72 hours range were excluded from statistical analyses; uneven replication at the start of the sampling period was accounted for by the model. For the pre- and post-surge periods, sequences of individual means of six consecutive samples, representing 24-hours blocks (time), were used to assess the effect of time, and feeding level by time interaction. For the surge period, in contrast, the effect of time, and feeding level by time interaction on pLH and FSH, were assessed using individual samples taken every 4 hours. The variance of individual daily samples during the pre- and post-surge periods and the proportion of individual samples taken every 4 hours that were greater than 1 ng/mL (chi-squared; Elsaesser and Foxcroft, 1978) were also examined as indicators of the basal episodic secretion of pLH and FSH. One-way analyses of variance were conducted for the maximum pLH concentration (amplitude) and duration of the pLH surge. Duration of the pLH surge was determined as the period over which pLH concentrations were >1 ng/mL. Fisher's least-significant-difference was used to establish mean differences.

Unlike analyses for gonadotropins and steroid hormones, insulin concentrations were assessed using the daily sampling times as main effect of time. All individual samples for a given sampling time were pooled and were then related to feeding. The 0300- and 2300-hour sample concentrations were considered as estimates of pre-prandial insulin and generally corresponded to the nadir in insulin in all groups. The post-prandial response to feeding was estimated from insulin concentrations at 0700, 1100, 1500 and 1900 hours. One-way analysis of variance was conducted to establish a feeding level effect for IGF-I plasma concentrations during the pLH surge period. Fisher's least-significant-difference was used to establish mean differences.

C. Results

Feed Intake. Feed intake at initiation of the experiment was similar for the L and H groups. At puberty, however, HH gilts consumed 3.5 $\pm$ .3 kg/day or 170% more feed than the L gilts. At second estrus, feed intake for the HH gilts reached 3.8 $\pm$ .4 kg/day, equivalent to 193 and 138% that of the LL and LH gilts, respectively.

Ovulation Rates. HH and LH gilts exhibited not only a greater mean ovulation rate (main effect of feeding level irrespective of time), but also a greater increase in ovulation rate (feeding level x time interaction) between first and second estrus, compared with LL gilts (Table III.1).

Blocked cannulas from two gilts (LH, n = 1; HH, n = 1) resulted in lost plasma samples from these animals.

Changes in Plasma pLH and FSH. Changes in pLH and FSH mean plasma concentrations are shown in Figure III.1. There was no effect of feeding level, irrespective of time, on mean plasma pLH or FSH concentrations for the pre-surge, surge or post-surge periods ($P > .05$): however, a significant effect of time, irrespective of feeding level, was observed for both gonadotropins for the three peri-estrous periods ($P < .0001$; Figure III.1). During the pre-surge period, both mean pLH and FSH concentration declined. During the surge period, mean pLH concentrations increased, peaked and then decreased as is characteristic for this gonadotropin during the pre-ovulatory period. Furthermore, there was no effect of feeding level ($P > .05$) on the amplitude (LL 5.27, LH 4.72, HH 3.79 $\pm$ .41 ng/mL) or duration (LL 30.5, LH 31.2, HH 29.4 $\pm$ 3.2 hours) of the pLH pre-ovulatory surge. Mean FSH concentrations during the surge period initially increased to a peak at time 0, declined to a nadir at between +16 and +20 hours, then increased again by +24 hours. In the later post-surge period, FSH concentrations increased to a second peak by +56 hours and then declined. Variability in the episodic release of pLH during the pre-surge period was the only characteristic of gonadotropin secretion affected by feeding level. Sample variance was greater ($P < .05$) for LH and HH gilts than for LL gilts on days -6, -5 and -4 of the pre-surge period (Figure III.2, see error bars). Similarly, during the pre-surge period, LH and HH gilts had a greater ($P < .05$) proportion of samples >1 ng/mL (LL 1.1, LH 8.9, HH 10.1%)

Changes in Plasma Estradiol-17β and Progesterone. As for the gonadotropins, no effect of feeding level on plasma estradiol-17β or progesterone concentrations was

observed, although a time effect was evident for both estradiol-17β and progesterone ($P < .01$). Estradiol-17β concentrations gradually increased from day -5 (17 $\pm$ 1.3 pg/mL), attaining a maximum by day -2 (39.4 $\pm$ .8 pg/mL) and returned to basal levels (7.6 $\pm$ 2.3 pg/mL) during the post-surge period. Progesterone concentrations decreased from day -1 to day 1 (5.0 $\pm$ 2.3 to 1.5 $\pm$ .7 ng/mL) and then increased with the advent of the luteal phase (28.9 $\pm$ 3.2 ng/mL by day +7).

Changes in Plasma Insulin and IGF-I. There was an effect of feeding level on overall, pre-prandial and post-prandial plasma insulin; HH gilts showed greater ($P < .05$) concentrations than LL gilts with those of LH gilts being intermediate (Figure III.3). Furthermore, an effect of time was evident also, as insulin concentrations at 0300 and 2300 hours were lower than at any other time ($P < .0001$). Plasma concentrations of IGF-I were different between the LL and HH groups ($P < .05$), with the LH group being intermediate (LH versus LL, $P = .06$; LH versus HH, $P = .07$; Figure III.4).

D. Discussion

Gonadotropin Involvement in Flushing. With the exception of possible differences in pLH pre-surge secretion, there was a lack of a significant effect of feeding level on mean plasma gonadotropins, estradiol-17β or progesterone. It has been suggested that the main effect of feeding level on ovarian activity is mediated by an alteration of plasma gonadotropin concentrations. Armstrong and Britt (1987) fed post-pubertal gilts at approximately .125 x maintenance (ARC, 1981) until they became anestrous. They

noted a reduction in the frequency of pLH episodes as gilts became anestrous whereas realimentation was associated with an increased frequency of pLH episodes. As pulsatile administration of GnRH and pLH induced follicular growth and ovulation in gilts fed restrictively, the authors concluded that the long-term effect of feed restriction on ovarian function was mediated by suppression of hypothalamic release of GnRH. Furthermore, in a contemporary study by Flowers et al. (1988), increasing dietary energy from 22.6 MJ ME/day (1.4 x maintenance) to 46 MJ ME/day (2.8 x maintenance), increased ovulation rate and the number of pLH pulses but did not affect pLH pulse amplitude. Similarly, Cox et al. (1987), feeding a dietary energy level of 41.7 MJ ME/d (2.3 x maintenance) compared with 24.1 MJ ME/day (1.3 x maintenance), with or without insulin administration, augmented ovulation rate and increased pLH pulsatile release as well as basal plasma pLH and FSH concentrations. Nevertheless, in a second experiment in the same study, feeding the same high dietary energy level, accompanied by long- or short-acting insulin administration, increased ovulation rate but had no significant effect on plasma gonadotropin concentrations. As a distinct pattern of episodic LH release above a low baseline would be expected in cyclic gilts in the late luteal to early follicular phase, significant differences in the variance in pLH levels, and in the proportion of samples assayed as being >1 ng/mL, is interpreted as indirect evidence for differences in episodic pLH release in this study. If this interpretation is accepted, then these data support the concept that acute (as in flushing), as well as chronic, long-term, feed restriction followed by realimentation directly affect the hypothalamic GnRH pulse generator.

Insulin and IGF-I Involvement in Flushing. A common pattern in the three studies referred to above in the pig, and a recent report in sheep (Downing and Scaramuzzi, 1991), is that elevated plasma insulin levels were associated with an increased ovulatory response. Similar responses were seen in this study, although the differences in insulin and IGF-I concentrations between the LH and LL groups did not reach significance ($P < .1$). The relatively minor difference between the two levels of intake (2.0 and 2.8 kg/day), and the fact that the availability of gilts limited allocation of littermates across treatment, may have contributed to this lack of a significant feeding level effect.

The results of the present study are therefore consistent with previous suggestions that in the pig, increased episodic pLH secretion and elevated plasma insulin and IGF-I concentrations, acting independently or synergistically, play an important role in mediating the restoration of ovulation rate observed in response to nutritional flushing. The extent of these interactions may be determined by the severity and length of the restriction and the pattern of realimentation. Insulin appears to be the primary and, perhaps, the most important metabolic signal in each of the mechanisms proposed. First, increased plasma insulin during flush-feeding may influence both GnRH release, and hypophyseal sensitivity to GnRH, and hence gonadotropin release. Second, insulin may exert a direct effect at the ovarian level as reviewed by Booth (1990). It is pertinent to emphasize that flushing did not induce superovulation but merely restored ovulation rate in the present study (see Self et al., 1955; see Chapter II.D also). The observation of Britt et al. (1988) that insulin diminished atresia without altering the total number of follicles present, suggests that increased plasma insulin during realimentation

may have also reduced the number of follicles undergoing atresia in this study, although an increased rate of follicular recruitment can not be ruled out. Third, insulin may interact with other hormones or growth factors at both the hepatic and the follicular level (see Clemmons et al., 1988; Davis, 1988). During realimentation or after growth hormone or insulin administration, synthesis of IGF-I appears to be augmented. Periods of nutritional deprivation are, in contrast, associated with decreased plasma IGF-I levels, even though plasma growth hormone levels are higher than those observed during realimentation (Buonomo and Baile, 1991). Moreover, an exogenous growth hormone challenge results in no change in plasma IGF-I concentrations under conditions of feed restriction in cattle (Elsaesser et al., 1989). Therefore, it appears that even though growth hormone is responsible for hepatic IGF-I production, hepatic tissue becomes insensitive to growth hormone due to the absence of high-affinity receptors in steers fed restrictively (Breier et al., 1987; Buonomo and Baile, 1991). An insulin threshold governs the dissociation or association of the growth hormone/IGF-I regulatory mechanism (Baxter et al., 1980).

Finally, it is suggests that the mechanisms that mediate the flushing phenomenon must be seen as responses to short-term nutritional modulation of the reproductive axis, since flushing induces a fluctuation in the energy status of the animal in the absence of major changes in body weight or composition.

E. Implications

The results of this study suggest that increased episodic pLH secretion and elevated plasma insulin and IGF-I concentrations, acting independently or synergistically, play an important role in mediating the restoration of ovulation rate observed in response to nutritional flushing.

Table III.1. The effect of pre- and post-pubertal level of feeding on ovulation rate at first and second estrus[a]

Treatment	LL[b]	LH[b]	HH[b]	Main effect		
n	14	13	14	of time[c]	$P > F$	SE[d]
Feeding level kg/d:						
45 kg to first estrus	2.0	2.0	ad lib			
First to second estrus	2.0	2.8	ad lib			
Ovulation rate:						
First estrus(CA)[e]	10.4	10.8	11.1	10.8[u]		
Second estrus(CL)[e]	11.1	13.5	14.2	12.9[v]	.0001	SEM .16
Main effect of feeding level[e,f]	10.7[w]	12.1[x]	12.7[x]		.0078	SEM .29
Feeding level x time interaction[c]	.7[y]	2.7[z]	3.1[z]		.0028	SED .41

[a]Least square means.

[b]LL = gilts that remained on a constant diet; LH = gilts that were given an increased feed allowance; HH = gilts with ad libitum access to feed.

[c]Means lacking a common superscript letter differ: u,v main effect of time irrespective of feeding level; w,x main effect of feeding level irrespective of time; y,z feeding level x time interaction expressed by the difference between first and second estrus.

[d]Standard error of the mean (SEM) and difference (SED).

[e]CA and CL = corpora albicantia and corpora lutea, respectively.

[f]Gilts nested within feeding level as error term.

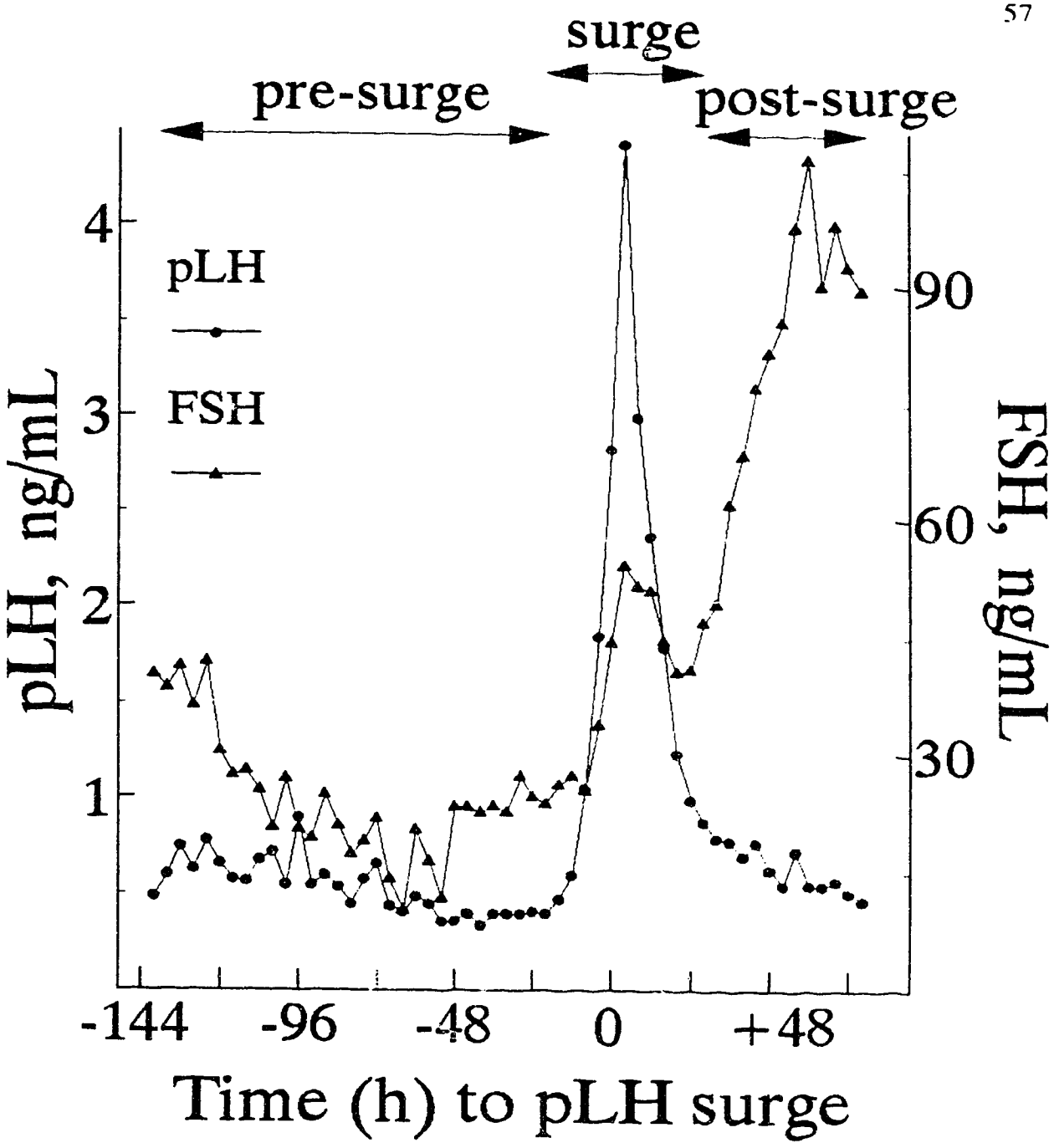


Figure III.1. Least-square means for the main effect of time irrespective of feeding level on gonadotropin plasma concentrations through the second peri-estrous period. Standard error of the mean for the pre-surge period (30 samples at 4 h intervals) were pLH ± .04, FSH ± 1.92; Surge (13 samples at 4 h intervals) pLH ± .18, FSH ± 2.83; Post-surge (12 samples at 4 h intervals) pLH ± .08, FSH ± 8.19.

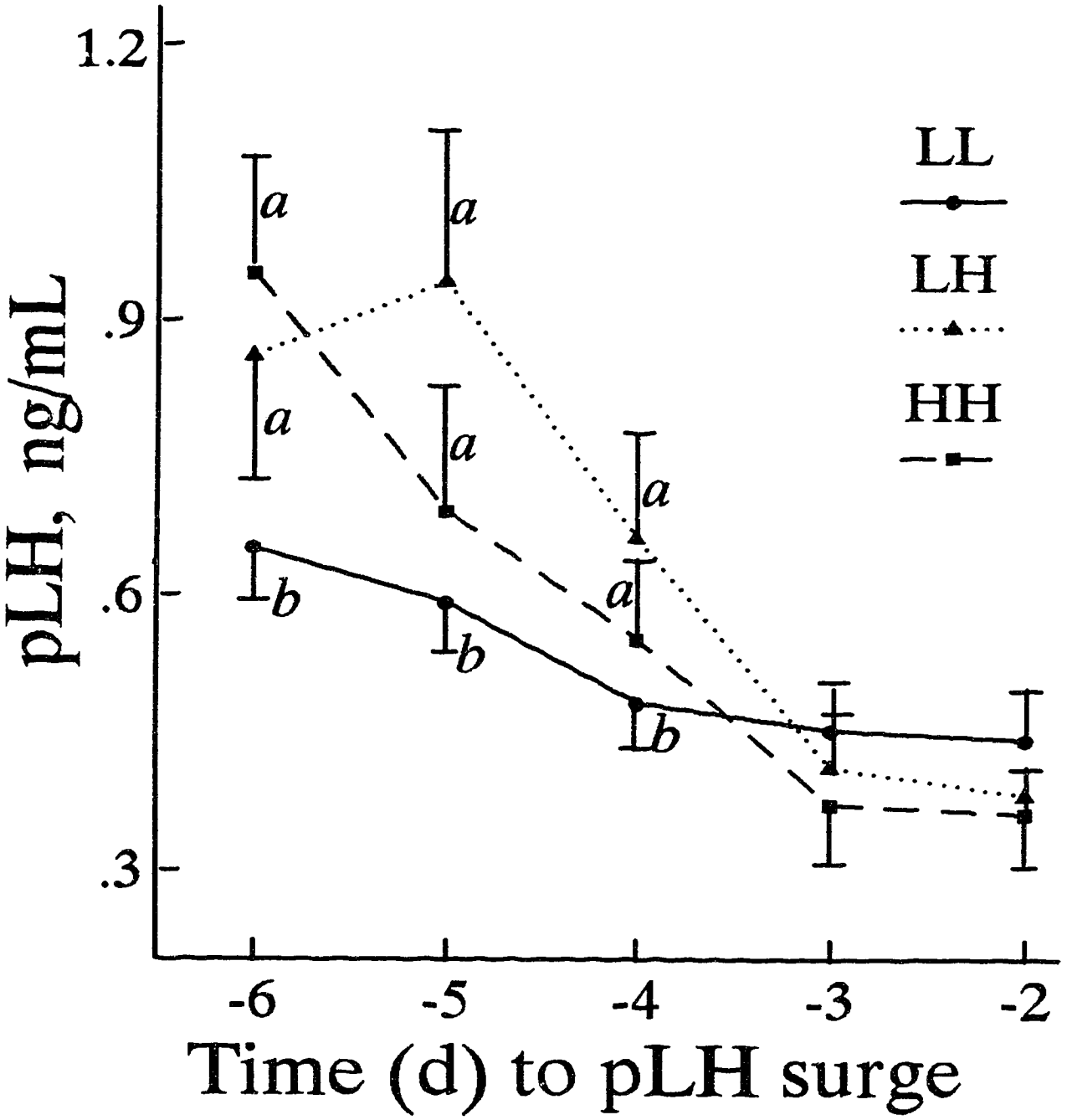


Figure III.2. Least-square means and standard errors for feeding level x time (day) for pLH during the pre-surge period. Standard error bars lacking common superscript letters differ ($P < .05$). From first to second estrus feeding levels were 2.0 kg/day (LL, n = 10), 2.8 kg/day (LH, n = 9) or ad libitum access to feed (HH, n = 9).

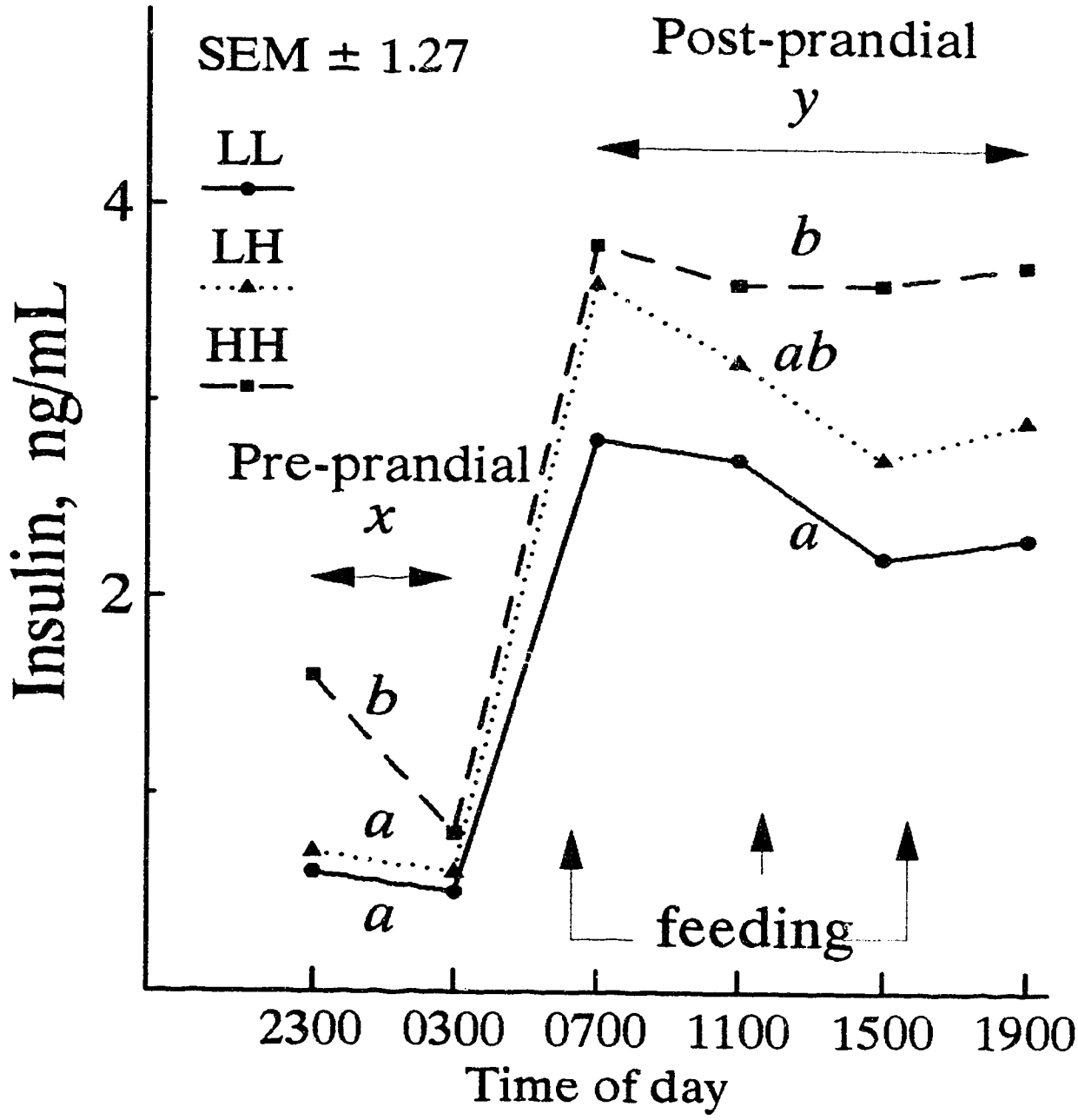


Figure III.3. Least-square means and standard error for feeding level x time of the day on pre- and post-prandial plasma insulin through the second peri-estrous period. From first to second estrus feeding levels were 2.0 kg/day (LL, n = 10), 2.8 kg/day (LH, n = 9) or ad libitum access to feed (HH, n = 9). Feeding times for gilts fed restrictively gilts (LL and (LH) were 0630, 1130 and 1530 h; Gilts in the H group had ad libitum access to feed at all times. Means lacking a common superscript letter differ ($P < .05$): a,b main effect of feeding level within a period; x,y main effect of time of the day.

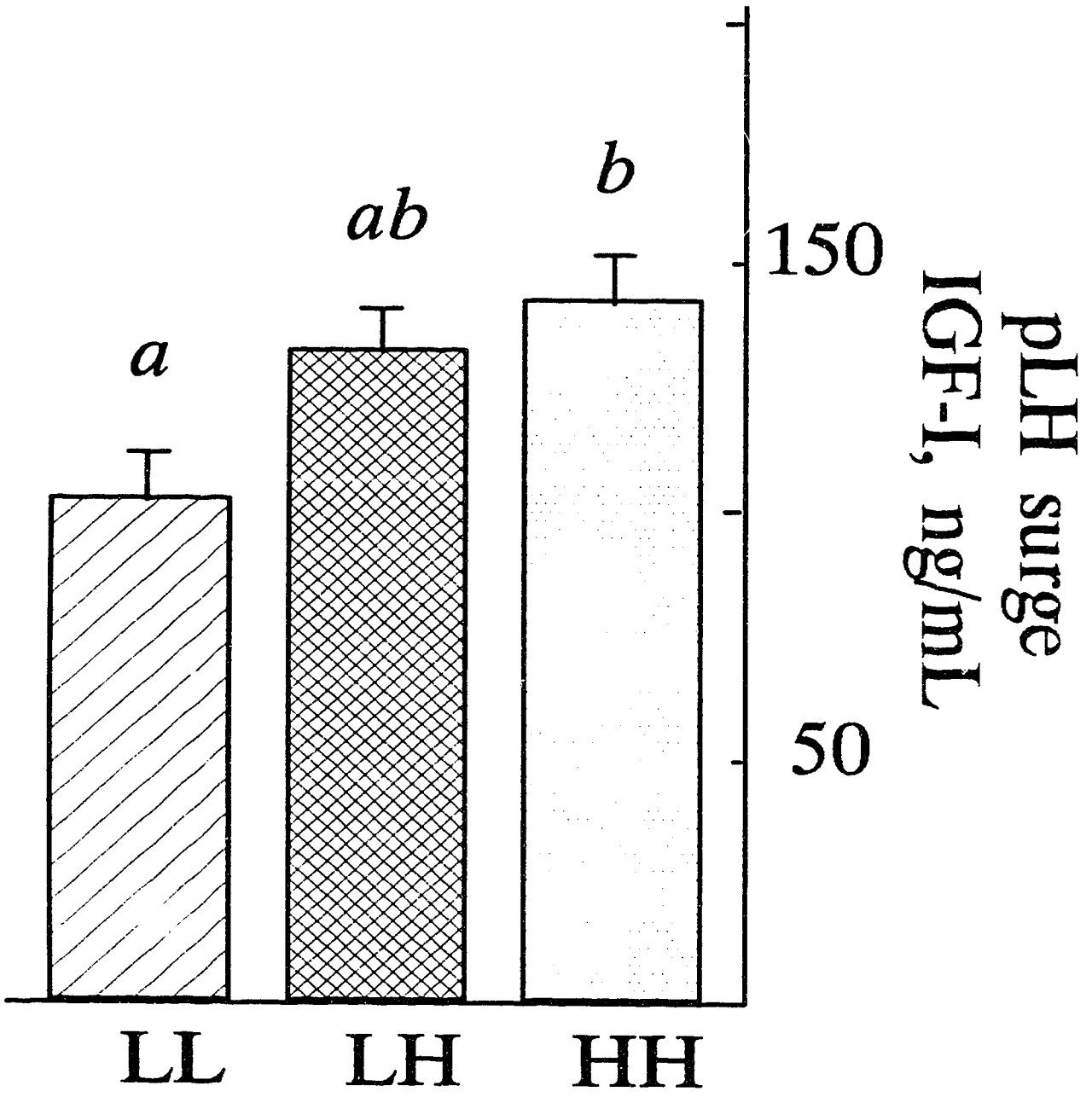


Figure III.4 Least-square means for plasma IGF-I during the pLH surge period. Means lacking a common superscript letter differ ($P < .05$): a,b main effect of feeding level. From first to second estrus feeding levels were 2.0 kg/day (LL, n = 10), 2.8 kg/day (LH, n = 9) or ad libitum access to feed (HH, n = 9).

F. Literature Cited

Agricultural Research Council (ARC). 1981. The Nutrient Requirements of Pigs. Commonwealth Bureaux, Slough.

Aherne, F. X. and R. N. Kirkwood. 1985. Nutrition and sow prolificacy. J. Reprod. Fert. Suppl. 33:169.

Anderson, L. L. and R. M. Melampy. 1972. Factors affecting ovulation rate in the pig. In: D. J. A. Cole (ed) Pig Production. Butterworth, London. pp. 329.

Armstrong, J. D. and J. H. Britt. 1987. Nutritionally-induced anestrus in gilts: metabolic and endocrine changes associated with cessation and resumption of estrous cycles. J. Anim. Sci. 65:508.

Baxter, R. C., Brown, A. S. and J. R. Turtle. 1980. Association between serum insulin, serum somatomedin and liver receptors for human growth hormone in streptozotocin diabetes. Horm. Metab. Res. 12:377.

Beltranena, E., F. X. Aherne, G. R. Foxcroft and R. N. Kirkwood. 1991. Effects of pre- and post-pubertal feeding on production traits at first and second estrus in gilts. J. Anim. Sci. 69:886.

Bolt, D. J., R. Rollins and H. D. Guthrie. 1981. Development of a porcine FSH radioimmunoassay. J. Anim. Sci. 53 (Suppl. 1):298 Abstr.

Booth, P. J. 1990. Metabolic influences on hypothalamic - pituitary - ovarian function. J. Reprod. Fert. Suppl. 40:89.

Breier, B. H., P. D. Gluckman and J. J. Bass. 1987. The somatotrophic axis in young steers: influence of nutritional status and oestradiol-17β on hepatic high- and low-affinity somatotrophic binding sites. J. Endocr. 116:169.

Britt, J. H., J. D. Armstrong and N. M. Cox. 1988. Metabolic interfaces between nutrition and reproduction in pigs. Proc. of the 11th International Congress on Animal Reproduction and Artificial Insemination. Dublin, Ireland. pp. 117

Buonomo, F. C. and C. A. Baile. 1991. Influence of nutrient deprivation on insulin-like growth factor I, somatotropin, and metabolic hormones in swine. J. Anim. Sci. 69:755.

Christian, R. E. and J. C. Nofziger. 1952. Puberty and other reproductive phenomena in gilts as affected by plane of nutrition. J. Anim. Sci. 11:789 (Abstr).

Clark, J. R., T. N. Edey, N. L. First, A. B. Chapman and L. E. Casida. 1973. The effects of four genetic groups and two levels of feeding on ovulation rate and follicular development in pubertal gilts. J. Anim. Sci. 36:1164.

Clemmons, D. R., M. Dehoff, R. McCusker, R. Elgin and W. Busby. 1988. The role of insulin-like growth factor I in the regulation of growth. J. Anim. Sci. 65 (Suppl. 2):168.

Coop, I. E. 1962. Liveweight-productivity relationships in sheep. N. Z. J. Agric. Res. 5:249.

Cox, N. M., M. J. Stuart, T. G. Althen, W. A. Bennett and H. W. Miller. 1987. Enhancement of ovulation rate in gilts by increasing dietary energy and administering insulin during follicular growth. J. Anim. Sci 64:507.

Davis, S. L. 1988. Recent concepts in regulation of growth by growth hormone and IGF. J. Anim. Sci. 66 (Suppl. 3):84.

den Hartog, L. A. and G. J. M. van Kempen. 1980. Relation between nutrition and fertility in pigs. Neth. J. Agric. Sci. 28:211.

Downing, J. A. and R. J. Scaramuzzi. 1991. Nutrient effects of ovulation rate, ovarian function and the secretion of gonadotrophic and metabolic hormones in sheep. J. Reprod. Fert. Suppl. 43:209.

Elsaesser, F. and G. R. Foxcroft. 1978. Maturational changes in the characteristics of oestrogen-induced surges of luteinizing hormone in immature domestic gilts. J. Endocr. 78:455.

Elsaesser, T. H., T. S. Rumsey and A. C. Hammond. 1989. Influence of diet on basal and growth hormone-stimulated plasma concentrations of IGF-I in beef cattle. J. Anim. Sci. 67:128.

Flowers, B., M. J. Martin, T. C. Cantley and B. N. Day. 1988. Endocrine changes associated with a dietary-induced increase in ovulation rate (flushing) in gilts. J. Anim. Sci. 67:771.

Furlanetto, R. W., L. E. Underwood, J. J. Van Wyk and A. J. D'Ercole. 1977. Estimation of somatomedin C level in normals and patients with pituitary disease by radioimmunoassay. J. Clin. Invest. 60:648.

Guthrie, H. D. and D. J. Bolt. 1983. Changes in plasma estrogen, luteinizing hormone, follicle-stimulating hormone and 13, 14-dihydro-15-keto prostaglandin $F_{2\alpha}$

during blockade of luteolysis in pigs after human chorionic gonadotropin treatment. J. Anim. Sci. 57:993.

Kirkwood, R. N., P. A. Thacker, A. D. Gooneratne, B. L. Guedo and B. Laarveld. 1988. The influence of exogenous growth hormone on ovulation rate in gilts. Can. J. Anim. Sci. 68:1097.

Kraeling, R. R., G. B. Rampacek, N. M. Cox and T. E. Kiser. 1982. Prolactin and luteinizing hormone secretion after bromocryptine (CB-154) treatment in lactating sows and ovariectomized gilts. J. Anim. Sci. 54:1212.

Nichols, J. E. 1924. Fertility in sheep. J. Min. Agric. 16:835.

NRC. 1979. Nutrient Requirements of Swine (8th Ed.). National Academy Press, Washington, D.C.

Rawlings, N. C., S. W. Kennedy, C. H. Chang and J. R. Hill. 1977. Onset of seasonal anestrus in the ewe. J. Anim. Sci. 44:791.

Rawlings, N. C., L. Weir, B. Todd, J. Manns and J. H. Hyland. 1980. Some endocrine changes associated with post-partum period of the suckling beef cow. J. Reprod. Fertil. 60:301.

Ray, S. R., W. J. Croom Jr., A. H. Rakes, A. C. Linnerud and J. H. Britt. 1983. Effects of methionine hydroxy analog on milk secretion and ruminal and blood variables of dairy cows fed a low fibre diet. J. Dairy Sci. 66:2084.

Robertson, G. L., L. E. Casida, R. H. Grummer and A. B. Chapman. 1951. Some feeding and managements factors affecting age at puberty and related phenomena in Chester White and Poland China gilts. J. Anim. Sci. 10:841.

SAS. 1988. SAS User's Guide: Statistics. SAS Inst. Inc. Cary, NC.

Self, H. L., R. H. Grummer and L. E. Casida. 1955. The effects of various sequences of full and limited feeding on the reproductive phenomena in Chester White and Poland China gilts. J. Anim. Sci. 14:573.

Wettemann, R. P., H. D. Hafs, L. A. Edgerton and L. V. Swanson. 1972. Estradiol and progesterone in blood serum during the bovine estrous cycle. J. Anim. Sci. 34:1020.

Youatt, W. 1837. Sheep: Their breeds, Management and Diseases. Baldwin and Craddock, London. pp. 34.

IV. INNATE VARIABILITY IN SEXUAL DEVELOPMENT IRRESPECTIVE OF BODY FATNESS IN GILTS[3]

A. Introduction

It has been suggested that the onset of puberty in the gilt is delayed until the attainment of a permissive level of fatness or lean:fat ratio (Kirkwood and Aherne, 1985). As modern, hybrid pigs become increasingly leaner, possible detrimental effects of extreme leanness on the attainment of puberty and subsequent reproductive performance of gilts kept as breeding replacements, may be a concern (see Johansson and Kennedy, 1983; Chapter II).

The main effects of diet composition and feeding level are reflected in the ratio of protein and fat deposition. In young pigs up to 45 kg live weight, the rate of protein deposition is linearly and positively related to feed or energy intake (Campbell et al., 1985a). Energy intake or appetite limits further increments in the rate of protein accretion (Dunkin, 1990). In contrast, a curvilinear positive relationship is apparent between maximal protein accretion rate and energy intake for pigs between 40 and 100 kg live weight. A plateau in maximal protein deposition rate is reached between 70 and 90 kg live weight (Campbell et al. 1985b), but differences exist among genotypes. Thus, increases in daily growth rate in heavier animals mostly reflects increases in fat deposition. It is, therefore, possible to use nutritional strategies to manipulate the rate

---

[3]A version of this chapter has been submitted for publication. Beltranena, E., F. X. Aherne and G. R. Foxcroft. 1992. J. Anim. Sci.

of fat deposition beyond the plateau in maximal rate of protein deposition. This approach would offer the opportunity to study the effects of manipulation of body fatness on the activity of the hypothalamic-hypophyseal-ovarian axis in relation to the attainment of puberty. In the present study, therefore, the metabolic and reproductive status were characterized in littermate gilts in which different diets and dietary regimens were used with the objective of achieving maximal protein deposition but different levels of fatness.

B. Experimental Procedure

Animals and Treatments. Fifty-two Camborough x Canabrid gilts (Pig Improvement Canada, Acme AB), comprising 12 sets of three to five littermates, were used. At 75 SD 1.7 days of age and 33.2 SD 4.7 kg body weight, littermate gilts with similar growth rates were allocated in a one:two or two:three ratio to have ad libitum access to either a high- (HE; n = 21) or a low-energy diet (LE; n = 31), respectively. Gilts were initially housed in groups of three according to treatment and body weight, in slatted-floor pens. Upon attainment of a mean pen body weight of 50 kg, all gilts were relocated to individual pens. Gilts in the HE group continued to have ad libitum access to the high-energy diet, whereas gilts in the LE diet were fed restrictively at approximately 80% of ad libitum. At 160 days of age, all HE and 15 LE gilts were slaughtered, while the 16 remaining LE gilts were then allowed ad libitum access to the low-energy diet until slaughtered at 175 days of age (LER).

Diets. With the intention of maximizing protein deposition rate, two ideal protein grower and finisher diets (Table IV.1) were formulated (see Stranks et al., 1988). In order to reduce or increase fat deposition, the diets were also formulated to provide different energy:lysine ratios. However, to prevent excessive energy intake, and because of the difference in energy content of the two diets (Table IV.1), the LE gilts were fed restrictively the low-energy diet. When fed restrictively at approximately 80% of ad libitum access (see Chapter II, figure II.1), the low-energy diet provided similar daily intakes of protein, amino acids, vitamins and minerals to the high-energy diet. Both diets were formulated to exceed the NRC (1988) recommendations for growing pigs. During feed restriction, LE gilts received meals of approximately equal size at 0900, 1130 and 1530 hours. All gilts were initially fed the grower diet; on attainment of an individual body weight of 75 kg, all gilts were then fed the finisher diets.

Cannulation, Bleeding and Slaughter. For the main endocrine study, 12 trios representing each litter allocated across treatment, were bled two days prior to slaughter. These gilts received indwelling jugular catheters (i.d. 1.58 mm, o.d. 3.18 mm; Silastic, Dow Corning Corporation, Midland, MI) under general anaesthesia (12 mg azaperone + 75 mg metomidate hydrochloride per 20 kg body weight, i.v.; Stresnil and Hypnodil, respectively; Jansen Pharmaceutica, Mississauga, ON). Gilts were returned immediately to their pens following surgery and individual feed intake was closely monitored during the next two days. By 1700 hours on the second day after surgery, access to feed was denied and any uneaten feed was removed. After overnight fasting, blood samples were collected at 10 min intervals from 0600 to 1500 hours and

at 1530, 1600 and 1700 hours. Plasma was harvested after centrifugation of samples at 1240 x g for 15 min, and stored at -35 °C until assayed. On the day of bleeding, gilts in the HE and LER groups had their pen self-feeders replaced immediately after the 0900-blood sample was collected, whereas LE gilts had their daily allowance fed at 0905, 1405 and 1730 hours. Cannulation and bleeding of littermate trios was achieved in four batches with treatments equally represented within day of bleeding. The same four batches of bled gilts together with unbled gilts were slaughtered such that treatments were similarly represented within day of slaughter.

Measurements. Individual body weight and feed intake were recorded weekly. Backfat thickness measurements were taken using the ultrasonic Krautkramer USM2 probe (Krautkramer, Cologne, Germany) in the live animal. Sites, at both sides of the midline were defined as: 1) loin, 5 centimetres off the midline, at a point aligned with the root of the curve of the last rib; 2) back, 5 centimetres off the midline and 5 centimetres in front of the stifle joint of the hind leg; and 3) grade, 7 centimetres off the midline, between the third and fourth last ribs.

During evisceration, the kidneys, the omental inter-visceral lard located ventrally to the kidneys, and the head were removed and weighed separately. The hot, split, half carcasses were weighed and graded using the Hennessi grading system (Hennessi and Chong Ltd., New Zealand). Grading site was as defined for the live measurement grade, on the hot, left, half carcass (Anonymous, 1986) and measurements included carcass lean and fat depth, which were then used to calculate carcass lean yield.

Together with carcass weight, carcass lean yield was in turn used to calculate the carcass index.

The uterus and attached ovaries were also collected at slaughter and immediately placed on ice. After transport to the laboratory, the uterus was sectioned immediately posterior to the cervix, dissected from the broad ligament, fallopian tubes and ovaries, and was then weighed. The removed ovaries were then again placed on ice. Within the next hour, follicular fluid from the largest 10 follicles from each ovary, was aspirated and measured using Hamilton micro-syringes, pooled, snap-frozen and stored at -35°C until assayed.

Radioimmunoassays. Plasma samples collected every 10 min from 0600 to 1500 hours were assayed for luteinizing hormone (LH) concentrations using the homologous double antibody radioimmunoassay procedure described by Cosgrove et al. (1991). Samples were quantified in two assays with intra- and inter-assay coefficients of variation of 6.4 and 8.9%, respectively.

All plasma samples were assayed for growth hormone (GH) using the homologous radioimmunoassay procedure of Marple and Aberle (1972) with modifications: Anti-porcine growth hormone (anti-GH-202-8; D. N. Marple, Auburn University, AL) antisera and normal guinea pig serum (Calbiochem, La Jolla, CA) were used at final dilutions of 1:800000 and 1:4000, respectively. Purified porcine growth hormone (USDA-GH-I-2; USDA Animal Hormone Program, Beltsville, MD) was used as serially diluted standard (.04 - 10 ng/tube) and USDA-GH-PPI (USDA Animal Hormone Program, Beltsville, MD) as radioiodinated antigen. The bound antigen was

precipitated using a locally-raised, horse, anti-guinea pig gammaglobulin (final dilution 1:25) added in a 6% polyethylene glycol solution (PEG 8000; Fisher, Nepean, ON). Addition of .15, .3, .6, 1.2 and 2.4 ng of GH standard to 200 μL of control plasma and assay of 10, 25, 50, 100, 250 and 500 μL of the control plasma pool yielded quantitative recoveries of added hormone (103.3 SD 2.8%) and parallel inhibition curves. Sensitivity, henceforth defined as 2.5 x SD of the zero standard, was .03 ng per tube. Plasma samples were quantified in two assays with intra- and inter-assay coefficients of variation of 3.4 and 5.3%, respectively.

Plasma collected pre-prandially at 0600, 0700, 0800 and 0900 hours and post-prandially at 0920, 0940, 1000, 1030, 1100, 1200, 1300, 1400, 1420, 1440, 1500, 1530, 1600 and 1700 hours was assayed for insulin and insulin-like growth factor I (IGF-I) concentrations. Plasma insulin concentrations were determined using a homologous double antibody radioimmunoassay based on the methodology described by De Boer and Kennelly (1989). Porcine insulin (Calbiochem, La Jolla, CA) was used as radioiodinated antigen and standard. Tracer was added in assay buffer containing normal guinea pig serum at a 1:1050 final dilution. The guinea pig, anti-pig first antibody (Calbiochem, La Jolla, CA) and the goat, anti-guinea pig gamma globulin (Calbiochem, La Jolla, CA) were used at final dilutions of 1:168000 and 1:245, respectively. Specificity and accuracy of the assay were demonstrated by obtaining parallelism between additions of 12.5, 25, 50 and 100 μL of a control pooled plasma and the standard curve, and quantitative recovery (99.6 SD 3.1%) of additions of .075, .15, .3 and .6 ng insulin to 200 μL of control pooled plasma. Sensitivity of the assay

was .01 ng per tube. Plasma samples were analyzed in one assay with intra-assay coefficient of variation of 5.6%.

Insulin-like growth factor I concentrations were assayed using the homologous double antibody radioimmunoassay procedure described by Glimm et al. (1988) with modifications. Individual 100 μL aliquots of plasma or 50 μL aliquots of follicular fluid pooled from the largest 10 follicles were extracted by vortexing with 3 and 1.5 mL, respectively, of acid-ethanol (12.5 HCl (2.2 M), 87.5 ethanol {v/v}), and incubated at room temperature for 20 hours before centrifugation at 1522 x g for 30 minutes at 4°C. Supernatant aliquots of 200 μL were neutralized with 800 μL of Tris-base buffer (.0855 M; Sigma Chemical Co., St. Louis, MO), stored at 4°C and assayed within 72 hours. The antiserum anti-hIGF-I (USDA Animal Hormone Program, Beltsville, MD) was used at a final dilution of 1:25164. Normal rabbit serum obtained locally and goat, anti-rabbit gamma-globulin (Calbiochem, La Jolla, CA) were used at final dilutions of 1:3150 and 1:980, respectively. Sensitivity of the assay was .004 ng per tube. Additions of 25, 50, 100, 200 and 400 μL of plasma and 25, 50, 100, 200 and 400 μL of a pool of control follicular fluid produced inhibition curves parallel to the standards, confirming specificity; additions of .014, .028, .057 and .114 ng of IGF-I added to pools of control plasma and follicular fluid prior to extraction yielded 97.4 SD 4.5% recovery, conferring accuracy. All plasma samples were quantified in two assays with intra- and inter-assay coefficients of variations of 5.7 and 7.3%, respectively. All follicular fluid samples were quantified in one assay with an intra-assay coefficient of variation of 3.4%.

Non-esterified fatty acids (NEFA) concentrations were quantified in all pre- and post-prandial plasma samples by an enzymatic colorimetric method (Wako Pure Chemical Industries, Ltd. Osaka, Japan). Plasma samples were analyzed in 3 assays with intra- and inter-assay coefficients of variation of 4.1 and 6.9%, respectively.

Plasma samples collected at 0700 and 1030 hours were assayed for creatinine and urea nitrogen employing enzymatic colorimetric methods (Sigma Chemical Co. St. Louis, MI). Samples were quantified in single assays with intra-assay coefficients of variation of 3.9 and 4.9%, for creatinine and urea nitrogen, respectively.

Individual pooled plasma, prepared from equal volumes of plasma collected at 0700, 1100 and 1400 hours, was assayed for estradiol-17β concentrations using the homologous double antibody radioimmunoassay described by Webb et al. (1985) with minor modifications: Aliquots of 200 μL of plasma sample and 100 μL of standard estradiol-17β (.2 - 400 pg/tube; Sigma Chemical Co. St. Louis, MI) were extracted with 5 mL of diethyl ether (BDH Inc., Toronto, ON) by vortexing for 8 minutes using a multi-tube vortexer (American Scientific Products, McGaw Park, IL). The aqueous phase was frozen by immersion in a methanol-liquid N_2 bath and supernatants were decanted into assay tubes and vacuum-dried (Speedvac concentrator SVC200H, Farmingdale, NY). The ^{125}I-17β-estradiol-11α-TME and anti-estradiol-17β [R 48] antiserum (R. Webb, AFRC I.A.P.G.R. Roslin, Scotland) used at a final dilution of 1:2000000, were then added in assay buffer and tubes were incubated for a 2-hour period. Normal rabbit serum obtained locally and goat, anti-rabbit gamma-globulin (Calbiochem, La Jolla, CA) were used at final dilutions of 1:1250 and 1:3000,

respectively. Specificity of the assay was demonstrated by obtaining parallelism between extractions of 50, 100, 200 and 400 μL of a control plasma and the standard curve; extraction efficiency was estimated by additions of 100 μL ^{3}H-estradiol (30,000 dpm; Dupont Canada Inc., NEN Products. Mississauga, ON) to duplicate control plasma tubes included with each batch of samples vortexed, and gave an overall mean recovery of 93.1%. Sensitivity of the assay was .4 pg per tube. Plasma samples were analyzed in one assay with an intra-assay coefficient of variation of 8.5%.

Individual pooled follicular fluid samples from the largest 10 follicles were assayed for estradiol-17β concentrations as described in Chapter III.B, and validated for assay of follicular fluid as follows: Aliquots of 100 μL of assay buffer-diluted follicular fluid and estradiol-17β standard (.0008 - 1.6 ng/tube; Calbiochem, La Jolla, CA) were added ^{3}H-estradiol-17β (Dupont Canada Inc., NEN Products, Mississauga, ON) and anti-estradiol-17β (rabbit A11; N. C. Rawlings, Western Coll. of Veterinary Medicine, University of Saskatchewan, SA) at a final dilution 1:600000 and were incubated for 24 hours at 4°C. Free ligand was adsorbed by addition of a cold, equilibrated suspension of .1 mg dextran (Calbiochem, La Jolla, CA) and 1 mg activated charcoal (Norit-A; Calbiochem, La Jolla, CA) per tube, vortexed and immediately centrifugated at 1057 x g for 10 minutes at 4°C. Supernatants were decanted in vials containing liquid scintillation fluid (ICN Biomedicals Inc. Irvine, CA), mixed thoroughly, and counted. Specificity of the assay was demonstrated by obtaining parallelism between 100 μL additions of 1:50, 1:100, 1:200, 1:400 and 1:800 dilutions of a pooled, control follicular fluid and the standard curve; accuracy, using direct assay without extraction, was

determined from quantitative rec... (102 SD 2.8%) of 3.13, 6.25, 12.5, 25 and 50 pg ^{3}H-estradiol added to poole... ...l follicular fluid. Sensitivity of the assay was 3.1 pg per tube. Plasma samp... ...e analyzed in one assay with intra-assay coefficient of variation of 5.9%.

Statistical Analyses. St... ...tical analyses were performed using the General Linear Models procedures of SAS (1990). Treatment differences in live body weight, backfat thickness and daily energy and protein intake prior to slaughter and carcass measurements after slaughter, were assessed by unbalanced analysis of variance; for these, live body weight prior to slaughter and carcass weight were initially considered as covariates, but as they proved nonsignificant ($P > .05$), they were removed from the model. In order to quantify the episodic secretion of LH and GH, data were subjected to cluster (Veldhuis and Johnson, 1986) and sliding window (Shaw and Foxcroft, 1985) analyses, using visual appraisal of individual profiles to confirm episodic secretion (Foxcroft et al., 1988). Treatment and litter differences for episodic characteristics of LH and GH, reproductive tract weight, total follicular fluid volume, plasma and follicular fluid estradiol-17β, were also assessed by unbalanced analysis of variance. Treatment differences involving a change over time, for body weight, backfat thickness, energy intake, insulin, IGF-I, NEFA, creatinine and urea nitrogen, were assessed using time, litter and their two-way interactions as subplot effects. The variance of treatment x litter was used as the error term to test treatment effects. Further statistical analyses of insulin, IGF-I, and NEFA plasma concentrations were conducted with respect to time periods defined in relation to the feeding schedule of the LE gilts on the day of