THE USE OF DIFFERENT ANABOLIC AGENTS IN GILTS

BY

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DECLARATION

I declare that the dissertation hereby submitted by me for the M.Sc. Degree at the University of the Orange Free State is my own independent work and has not previously been submitted by me at another University/Faculty. I furthermore cede copyright of the dissertation in favour of the University of the Orange Free State.

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CONTENTS

CHAPTER 1

GENERAL INTRODUCTION

The need and demand for nutrients, mainly in the 3rd World (Africa, Central and South America and Asia) is ever increasing, from limited resources. Due to the world population growth rate, the resources are diminishing with a resultant food shortage (Aykroyd, 1964). This shortage is described to be more an aspect of less protein intake rather than energy deficiencies which are supplied by carbohydrates and fats (Deatherage, 1975). As regards plant protein, Gillin and Krane (1989) found increases in the yield per hectare and a variation in the crops produced as the main reasons for the increased crop production between 1961 and 1988. Watanabe (1976) who studied vegetable protein as a source for the human diet, concluded that its use as an extender of animal protein is now 10% of all meat consumption. The future supply and demand of human and animal requirements is described as critical and the promotion of plant protein (now used in animal diets) for human consumption is further advocated. However, Aykroyd (1964) emphasised that the deficiencies in some essential amino-acids, absence of vitamin B_{12} and the poor digestibility of plant protein could restrict its use in human nutrition. An impaired immune response and thus increased susceptibility to diseases is the result of protein-calorie malnutrition (Muñoz *et al.* 1994), so measures to upgrade the public health makes more sense if made together with a programme to produce more animal protein (Aykroyd, 1964).

The per capita protein consumption in Mozambique decreased from 35.9g/day in the 1964-1966 period to 31.0g/day in the 1988-1990 period, while in the Republic of South Africa there was a slight increase from 70.6g to 79.3g/day for the corresponding periods (FAO, 1992a). For these two countries, animal products have been the lowest source of protein supplied - 12 and 13%, 24 and 35% - in the above mentioned periods, for the two countries respectively (FAO, 1991, 1992b). This would indicate that livestock, as a source of animal protein, has the potential and an important role to play in an attempt to enhance the protein supply for human consumption.

Amongst the farm animals, the pig has been shown to be the most efficient in the conversion of feed to body energy and is ranked second only to the chicken and fish in the efficiency of the conversion of feed energy to body protein. Pork as such is an excellent source of high quality protein, B-vitamins and trace elements (Pond & Maner, 1974). The trend towards the consumption of white, rather than red meat makes the potential of increased meat production from pigs in the developing world a distinct reality (Holness, 1991).

The pig, an actual fast growing animal and efficient in converting feed to meat (protein) (Boatfield, 1983) has been domesticated from the wild boar (*Suis Scrofa*) and developed to the almost 300 different breed lines of pork used throughout the world - except for a few cultures where pork is banned for religious reasons (Deatherage, 1975). The swine's inability to utilise roughage is a definite limitation and it limits their commercial production to regions that produce an abundance of concentrates (Krider & Carroll, 1971). However they have the potential to be highly productive -

large litters after a relatively short gestation period, short inter-furrowing periods, rapid growth, their output in terms of meat yield per ton of breeding females live weight per year (six times that of cattle) and quicker turnover rate on the investment (Holness, 1991). The fact that the market value of old sows which have done service in a breeding herd is higher than it is for cows or ewes and the evidence that pork can be more successfully cured and stored than other meats, makes the pig a good option in the attempt to supply more animal protein for human diets (Smith & Hutchings, 1952). Feed is the greatest single item in the imput-chain of producing pork, representing 70 to 80% of the total production costs (Krider & Carroll, 1971; Gordijn, 1993). The fattening (finishing) unit is the most important part of the commercial pig farmer's operation, since his income is derived from the number and live weight of the pigs sold. The profitability is largely determined by the feed costs per unit of live weight gain, growth rate and grading results. Thus, the longer weaners are kept, the higher the feeding costs and the higher the risk of mortalities (Gordijn, 1993). Traditionally pig diets contain, amongst others, fish meal as a protein source, which is limited and expensive. One of the possible ways to solve the situation of high costs of pork production, is to shorten the finishing period.

Normal animal growth is regulated by a hormonal complex including, amongst others, growth hormone (somatotropin), insulin, somatomedins, thyroid hormones, glucocorticoids, epinephrine, androgens and oestrogens. The main effects of some hormones are associated with fat growth, that is usually enhanced by positive encouragement of fatty acid oxidation, or with muscle growth that is oriented to active glucose metabolism for the creation of the high levels of energy needed, together with a high level of stimulation of amino acid anabolism and protein accretion (Whittemore, 1993).

Growth promoters are agents used as feed additives, slow-release implants or injections to enhance the growth rate of the animal. Armstrong (1986) defined these growth promoters as additives that fullfil the role of enhancement of the animal's performance in terms of increased growth rate and/or feed conversion rate in clinically healthy and nutritionally normal animals, fed a balanced diet (adequate in all known nutrients). It is accepted that trials in the field of growth promoters in monogastric animals has been far more extensive in poultry and rather neglected in pigs (Armstrong, 1986). The fact that growth promoters tend to increase the lean (muscle) to fat ratio of the carcass makes the pig an obvious choice for treatment with anabolic agents - to avoid the problem of excessive fat deposition (Sheridan *et al.* 1990).

The aim of this trial was to compare the effect of three growth promoters on the growth rate, feed conversion rate and carcass qualities in pigs. By shortening the time to reach a live weight of 80 to 100 kg live weight (baconer), with a more consumer oriented carcass composition, the input costs can be decreased and the financial gains increased. With the potential productivity of the porcine specie and the potential consumption of pork in a rapidly growing Africa, the aspect of using growth promoters in pigs holds great promise.

CHAPTER 2

LITERATURE REVIEW

2.1 Growth

According to Reeds *et al.* (1993), there are as many definitions of animal growth as there are workers that carry out research on this process. However, it can be broadly described as a multifactorial process under the passage of time, in which take place dimensional, compositional and functional changes. Growth is usually understood to relate to gain in weight, brought about by cell multiplication in prenatal cleavage, and cell enlargement in a post natal phase. The latter is more a function of increase in cell size and of filling, than of an increase in cell number. So, growth must be distinguished from the concept of development, in which the changes in the shape and function are the main processes (Whittemore, 1993).

If the liveweight gains of healthy and well fed pigs from the birth up to the maturity are plotted against their ages, a sigmoidal curve of growth is obtained, and this means that in the earlier stages of growth there is a moderate weight gain, followed by rapid growth until the mature weight is attained (McMeekan, 1959; Goodwin, 1973). The growth curve has been described by Whittemore (1993) as being comprised of accelerated growth from birth to the point of inflexion, as a half age of the animal, before a decelerated growth starts towards the mature size of the animal.

According to Lindsay (1983), growth isn't simply an increase in the size of an animal, so that it is convenient to define it in terms of an increase in the amount of body protein, which is maximal at the earlier stages of age and decreases substantially by the time that fat deposition become apparent, later in life. Murray and Oberbauer (1992) postulate that growth of an animal is a complex interaction of genetic, environmental, nutritional and hormonal influences, inter-related at some level. But according to Goodwin (1973) and Boatfield (1983), there is a characteristic sequence in which the heart, gut, bones, skeletal muscles, and fat tissue are successively developed during the growth of the pig. In agreement to this, Reeds *et al.* (1993), postulated that there is a general relationship between function and mass. Organs that are concerned with the absorption and metabolism of nutrients, and the elimination of their catabolic end-products, such as the gastrointestinal tract, the heart and the liver, show a high rate of growth during the neonatal period, and mature relatively early, while the skeleton and the skeletal musculature show a relatively slower growth rate towards attainment of mature size. The last system to mature is the fat mass of the body.

Growth is dependent on the development of muscle tissue (Lindsay, 1983), as the greatest ratio of the body composition from a certain liveweight is found in muscle tissue (Lindsay, 1983; Whittemore, 1993; Blasco *et al.* 1994). Thus,

the skeleton, as a firm supporting structure of the body grows relatively more in the earlier stages, followed by the increase in lean tissue growth, and visible fat appears later in the growth (Lindsay, 1983). Growth occurs through the medium of the accretion of bone, lean and fatty tissues in the body, and is a result of a positive difference between the continuous anabolic and catabolic processes associated with tissue turnover. A hormonal complex which includes growth hormone (somatotropin), insulin, somatomedins, thyroid hormones, glucocorticoids, epinephrine, androgens and oestrogens, amongst others, is mentioned to act as regulator of normal growth (Whittemore, 1993). Besides this regulation, growth is influenced by heredity, nutrition, freedom from diseases, environment and management (McMeekan, 1959; Goodwin 1973).

Growth as such is influenced by a variety of factors.

2.1.1 Heredity

Genetic potential for lean tissue growth in pigs has been improved considerably during the recent decade (Henry, 1993). According to Reeds *et al.* (1993), it seems reasonable to propose that the major genetic determinant of post natal dimensional growth is the genetic program of the skeleton. The length of the skeletal component (bones) determines the length of skeletal muscles to which they are attached, and the skeletal muscle accounts for at least 50% of body weight. Sustained selection towards muscle growth and against fat deposition has resulted in a considerable widening of genetic variability in pig performance, both within breed and between breeds and lines (Henry, 1993). According to Lindsay (1983), and Blasco *et al.* (1994), genetical differences in the growth patterns between different breed leads to differences in physiological age (or maturity level), and thus, to differences in the growth rate and body composition, even relating to equal body weights. So those breeds with a higher proportion of lean tissue are those which take longer to reach maturity.

The steady genetic improvement for lean meat production allows more liberal feeding without adverse effects on carcass quality even at heavier slaughter weights (Cole & Chadd, 1989). This is achieved, according to Whittemore (1993) by a reduction in appetite and more likely a diminished fat ratio than an increase in lean tissue growth rate of those selected animals.

Crossbreeding, as referred to by Boatfield (1983) has been developed with the aim of putting together in the offspring some of the qualities of each parent, and thus produce an animal which is more productive, as well as achieving the "little bit extra" brought about by hybrid vigour. Current pig production schemes are based on a three or four way cross, which gives a good final product (Blasco *et al.* 1994). This practice is so essential and profitable in pig farming, that about 80 to 90% of the commercial pigs produced are crossbreeds (Eusebio, 1980). The conventional selection and breeding, according to Schaefer, *et al.* (1992) is too slow to respond to current market trends, since it removes only about 0.5 mm of backfat from a pig per year.

2.1.2 Nutrition

In economic terms, feed costs account for 70 - 80% of the total costs of pork production in the growing herd (Thornton, 1981). Therefore, feed conversion rate (FCR), as a measure of the amount of feed needed to produce liveweight in the pig, determines the profit or the loss made in growth (Boatfield, 1983; Holness, 1991). It is because of its simple digestive tract, unable to hold and digest bulky feeds such as hay, that the pig is a competitor with humans - insofar as feed stuffs are concerned. Regardless of that, the pig is the most efficient meat-producer of all the farm animals, converting about 20 to 28% of the digested nutrients into edible meat (McMeekan, 1959).

Growth without feed or nutrition is an oxymoron (Baker *et al.* 1993), since it can only occur when appropriate quantities of nutrients (energy, protein, vitamins and minerals) are consumed and absorbed on a daily basis (Thornton, 1981; Reeds *et al.* 1993). The amount of feed eaten by the pig is a balance between the needs of the animal and the ability of the feed to meet these demands (Cole & Chadd, 1989). According to Barber *et al.* (1972), the quality of the diet and the system of feeding determines the rate of intake. Among the components of feed, two fatty acids, 10 amino acids, 12 mineral elements and 13 vitamins must be present in the diet for achievement of maximal growth (Baker *et al.* 1993).

The energy content in the diet is used by the pig for maintenance, which include the various processes and reactions essential to life. After these activities are met, energy is available for muscular work and production in the form of growth or reproduction (Svendsen, 1974; Thornton, 1981). The degree to which nutrients are consumed in excess of maintenance ultimately determines the incremental growth rate or body weight (Reeds *et al.* 1993). As soon as the feed supply fully satisfies maximum potential for lean tissue growth (protein deposition), rapid fattening occurs, and feed conversion efficiency decreases (Whittemore, 1993).

The efficiency of the growing pig in its use of nutrients is influenced by the size and the level of growth. As the liveweight increases, so the maintenance cost and the energy content per gain also becomes greater (McMeekan, 1959). In young pigs the response of protein retention to feed intake is linear up to maximum appetite, while in slightly older animals, protein retention reaches a plateau at higher levels of feed intake (Whittemore, 1993). As the maintenance costs occur daily, utilising feed, no matter whether the pig grows fast or whether it grows slowly, the absolute rate of growth is a prime determinant of the efficiency of conversion into pork because of saving in feed used for maintenance (Whittemore, 1993).

Rapid growth may be limited by the appetite of the pig and differences in intake between sexes are notable, the greatest being castrated males, compared to boars and gilts (Cole & Chadd, 1989). Apart from the ability to withstand

invasion by pathogenic organisms, the ability of the animal to regulate its metabolic activities in response to changes in the nutritional environment seems to be the most crucial form of adaptation that it is called upon to perform. However, the nutritional regulation of the metabolic activity of tissue growth involves three factors, namely: the consumption of the diet itself; the influence of the quality of the diet, that represents the degree to which the nutritional value of a specific diet can sustain the anabolic responses induced by its consumption; and the presence of metabolic regulators such as hormones (Reeds *et al.* 1993). But as quoted by Cole and Chadd (1989), any regulation of feed intake should not be considered in a restricted sense, but as part of the integrated process of growth.

2.1.3 Health

It is long known that sick animals have retarded growth, mostly due to the failure of sick animals to eat. Fowler and Gill (1989) postulated that any factors which undermine health will have a damaging effect on feed intake. Bacterial and viral infections can alter the normal function of the gastrointestinal tract, producing maldigestion/malabsorption diseases and diarrhoea. Young animals are more susceptible than older animals and take longer to recover after gastrointestinal infections (Buddle & Bolton, 1992).

Parasites, defined by Holness (1991) as organisms which live on and obtain food from the body of another (host), may live on the exterior of the pig (external parasites) or within the internal tissues and organs (internal parasites). Liveweight loss and even high mortality rates due to morbidity, parasitism and crippling afflictions is enormous, particularly in pig production in the tropics, where the humid climates favour the presence of destructive protozoan, helminthic and arthropod parasites (Eusebio, 1980). Among the external parasites, mange is the most common, which stunts the growth of the infested pigs and in severe cases can cause death. Internal parasites are responsible for erratic appetite, weakness, diarrhoea and damage of visceras, which in turn affect the feed intake, abnormal digestion and absorption of nutrients. These lead to failure of gaining weight or later rejection of meat/organs in the slaughterhouses (Eusebio, 1980, McNitt, 1983).

The reason why sick animals do not grow has postulated an explanation from Kelly *et al.* (1993), in which toxins and other pathogenic agents induce the immune system to produce and release pro-inflamatory molecules called cytokines. These alter the normal function of certain aspects of the neuroendocrine system, and thus the secretion of important growth-promoting hormones such as somatotropin and insulin-like growth factor. A direct effect of the cytokines on the intermediary metabolism through the liver, muscle and adipose tissue is further suggested to play a critical role, reducing the feed intake or leading to a complete anorexia.

2.1.4 Management

Management is the skill and expertise of the farmer or producer in their day-to-day work of running the pig unit (Goodwin, 1973). Modern pig management includes feeding, housing, care and other well-planned practices that are essential for increasing efficiency in pig production (Eusebio, 1980). In all types of pig production there is a relationship between management and health. The level of disease found in the herd can be largely determined by the quality of management (Thornton, 1988). So, many of the management procedures are aimed at disease prevention or at mitigating the effects of those diseases that cannot be prevented (Holness, 1991). To manage the pig unity successfully, proper record-keeping is essential (Botha, 1993). Under commercial conditions, priorities may be given to factors such as feed conversion efficiency and feed cost per pig, growth rate and carcass grading (Holness, 1991).

2.1.5 Environment

The environmental complex is known to affect a pig's growth (Curtis, 1993). Stress factors such as extreme temperature, direct sunlight, fear, pain and interference with the pig's natural behavioural patterns, will quickly lead to reduced performance and productivity. Thus production systems must be designed to minimise these effects (Holness, 1991). Effects of the stressors when present simultaneously are additive, decreasing bodyweight gain (Curtis, 1993).

The environmental components considered in pig farming include air temperature, air movement, relative humidity, group size, stocking density and atmospheric concentrations of various gasses and dust (Close, 1989). The environmental temperature is a factor often neglected but through its direct effect on the animals' heat exchange, influences metabolism, voluntary feed intake and growth (Lindsay, 1983; Close, 1989; Curtis, 1993). It influences the extent to which energy intake is utilised within the body for maintenance and growth (Close, 1989). The energy required for maintenance increases when temperatures are below the zone of thermal neutrality, and the voluntary feed intake decreases when the environmental temperature rises above that zone (Lindsay, 1983; Close, 1989; Holness, 1991).

2.2 Manipulation of growth

Many people are predicting a second green revolution in agriculture, following the introduction of growth stimulants into the livestock industries. Increases in the amount of lean meat per pig for example, may actually lead to a reduction in the total number of pigs slaughtered (Peterson *et al.* 1992). According to Lamming (1986), there are still areas of the world where food storage and human and animal starvation occur, and this has stimulated the sympathy of affluent societies. It is an important and opportune time to draw the many different factors of animal growth into quantitative physiological terms.

Considering the exciting possibilities for its manipulation with attention to genetics, growth-promoting agents and potential use of anabolic agents.

Recently there has been increasing interest in improving the nutritional quality of agricultural products, with particular emphasis on increasing awareness of the need to reduce the dietary fat consumption of the human population. As a consequence of this, research efforts into the mechanisms controlling fat and lean tissue deposition in animals has been enhanced. Several potential methods of safely and humanely treating animals to increase their rate of muscle deposition and reduce their rate of fat deposition have been developed (Buttery & Sweet, 1993). According to Whittemore (1993), pig growth can be manipulated at three levels, namely: genetics, the environment and the endocrine system.

2.2.1 Genetic make-up

The pig, with an average generation interval of 2 - 2.5 years has a great advantage over other domestic meat-producing species, such as sheep and cattle to increased genetic progress. Porcine performance can be increased by changing the genetic make-up in order to improve the genetic potential. As various characteristics of a pig are genetically controlled and inherited through genes, these genes can be manipulated to achieve genetic improvement by either increasing the frequency of favourable genes, or the combination of genes by selection, or by introducing new genes into the herd by crossbreeding (Holness, 1991). The aim of the applied geneticist is to design the most efficient programme to select for genetic improvement of a desirable trait. To do this, the first problem is his lack of knowledge of the precise mechanism whereby a gene operates through biochemical and physiological pathways to control the trait. The second problem is that the character he is forced to measure and select is often removed from the actual character he wishes to improve and, furthermore, is controlled by many genes. These problems are dominant obstacles in the desire to breed genetically superior meat-producing animals (Bulfield, 1980). Therefore, and according to Whittemore (1993), the fewer the selection objectives, the faster the rate of improvement in those selected. For growth rate, a rate of improvement of 20g/day liveweight annually, appears to be readily acceptable by pig breeders.

Pig breeding programmes have selected for increased growth rate, feed conversion rate and carcass quality to improve the efficiency of lean meat production (Cameron, 1993). These programmes are a long term and disciplined effort, which can only be achieved if days to target liveweight and fat thickness are carefully recorded for each individual animal in the different breeding lines (Whittemore, 1980). According to Leat and Cox, (1980), selection against fat thickness has resulted in back fat thickness of commercial animals being reduced over the last couple of years. Hovenier *et al.* (1992), considering the heritabilities and genetic correlations of production traits and meat quality, concluded that pork quality can be improved if less emphasis is put on lowering backfat thickness or increasing lean tissue growth. It was also concluded (Jones *et al.* 1994), that while selection for reduced fat and increased

growth rate in pigs has increased carcass lean content, carcass length and viscera weights, there have been relatively minor changes in meat quality.

2.2.2 Nutrition

Quantitatively, skeletal muscle is the most important lean tissue of the body. Its composition changes after birth more than that of any other soft tissue, except perhaps, the skin. Muscle atrophy occurs as a result of undernutrition more than any other tissue, except fat. The number of muscle fibres is determined genetically, but the size of the fibres depend on the nutrition and the size of the individual, and on how much the muscle is exercised (Widdowson, 1980). The high rate of fat deposition in domestic pigs is well-known and it can be deduced that any feed-back mechanisms from fat to the controlling centres of nutrition are not as sensitive as those in other species. This insensitivity presumably arose during selection for rapid weight gain at a time when fat was readily accepted as part of the human diet. Now that fat is not recommended for human consumption, the pig's propensity to fatten can be counteracted in farming practice by restricting feeding (Forbes, 1983). Earlier, McMeekan (1959) said that the growth curve, shape and internal structure (composition) of pigs can be controlled by the method of feeding at different levels of nutrition (high level and low level), according to the animal's age. The amount of feed is related to most of the controlable factors in pig production management, and not least with slaughter weight. Thus the grade classification and the grade premium offered per kg of carcass weight depends on an appropriate feeding scale (Whittemore, 1980). Because fat can downgrade the meat, and the animal is less efficient in feed conversion for fattening than for lean tissue deposition, the farmer must select an appropriate feeding system to produce baconers or porkers, depending on consumer demands (Gordijn, 1993). According to Mersmann *et al.* (1989), modest restriction of feed intake to 90% of *ad libitum* intake in finishing pigs causes a reduction in fat deposition with no change in gain of lean weight.

Animals may experience periods during which, through nutritional limitation, they either grow at a suboptimal rate relative to their genetic capacity, or their gain is of abnormal composition, or both (Stamataris *et al.* 1991). However, as stated by Mersmann *et al.* (1989), compensatory growth following feed restriction, yields fatter pigs. The manipulation of nutrition in growth is considered by Forbes (1983) as expensive in terms of the pig producer's time.

2.2.3 Growth promoting agents

Growth-promoting agents, mainly used as feed additives, are products to be used to enhance the rate of growth, or at least to prevent a depressed growth rate or feed conversion efficiency in conditions where intensive systems of husbandry may limit maximum growth. These agents can be classified in three main groups: antibiotics, antibacterial and antiparasitic drugs (Lamming, 1986). According to Armstrong (1986), growth promoters are those products that fulfil the role of enhancement of animal performance in terms of increased

growth rate and/or feed conversion rate in clinically healthy and nutritionally normal animals, fed a balanced diet adequate in all known nutrients. In this concept, feed additives that act prophylactically as disease suppressants are not considered as growth promoters.

It has been clearly established that germ-free animals grow faster than conventionally reared animals. Thus, it is suggested that antimicrobial substances have a growth promotional effect through their action on enteric bacteria. This is by reduction of harmful bacterial metabolites, suppression of potentially pathogenic organisms, suppression of competition for nutrients, alteration in metabolic activity and enhanced intestinal absorptive capacity (Smith, 1993). Considerable loss in growth can occur during subclinical infections. One explanation for the growth-promoting properties of antibiotics when added to the diet on farm animals, is that the incidence of sub-clinical infections is reduced (Halliday, 1980). Despite new hybrids with greater potential for growth and better feed conversion efficiency being introduced, little attention has been given to the development of genotypes resistant to disease. At the same time new diseases have continued to appear. Because of their growth-promoting effects, antibiotics have been incorporated in animal feed for almost four decades. The public however, have become increasingly concerned about food safety, and fears relating to the use of antibiotics in animal foodstuffs and the use of these animal proteins in human consumption have been widely expressed (Smith, 1993). The possible development of resistant organisms which create a decreased response or avert disease, and the potentially serious hazard of these resistant strains of organisms is a possibility. The question of transferred resistance to organisms which are pathogenic to man, makes the use of these compounds not entirely problem free (Lamming, 1986). Detectable residues in tissue resulting from the use of ionosphere antibiotics such as coccidiostats have been attributed to the absorption from the digestive tract of farm livestock. These products can be of sufficiently small molecular weight to permit absorption, although very limited. A longer withdrawal time before slaughter is, therefore, recommended (Armstrong, 1986). A group of growth promoting agents, termed the probiotics, are the selected strains of *Lactobacillus* (*L.acidophilus*, *L.bifidus*, *L.bulgaricus* and *L.casei*), or *Streptococcus* (*S.faecium*), which are given orally to alter the intestinal flora with resultant improvement in liveweight gains and feed conversion efficiency. Besides these, some enzymes, usually a mixture of amylase, lipase and protease, acting on foodstuffs to liberate nutrients not normally released by the digestive processes, can also promote growth in non-ruminants (O'Connor, 1980).

2.2.4 Anabolic agents/Growth stimulants

The sex of the animal is an important determinant of growth rate, feed conversion efficiency and carcass composition, due to the secretion of the sex steroid hormones from the gonads (Roche & Quirke, 1986). The tendency of gilts and barrows to deposit large quantities of fatty tissue, makes them especially susceptible to treatment with pharmacological substances to manipulate growth and reduce the deposition of fat (Sheridan *et al.* 1990;

Martinez *et al.* 1992). Anabolic agents, which are generally hormonal in action, achieve their effect by causing a nett increase in nitrogen retention in the form of muscle protein. They can either be classified according to whether they are oestrogen, androgen or progestagenic in action, or to whether they are steroids which are endogenous to farm animals, or are exogenous steroid or non-steroid compounds (Patterson & Salter, 1985). Generally the anabolic effect can be achieved by increasing the concentration of anabolic hormones in the system, by increasing the sensitivity of target organs to existing hormone concentrations or by diminishing the effects of the feedback control mechanisms. This latter method may be achieved either by compromising the feedback system or by desensitizing the primary system to the effects of the feedback by immunisation (Whittemore, 1993). Anabolic steroids tend to increase the lean to fat ratio in the carcass (Sheridan *et al.* 1990). Although its administration has been shown to improve growth rate and carcass quality in cattle and sheep, very little information is available about its use in pigs (Martinez *et al.* 1992).

Beta-adrenergic agonists are another type of agent used to manipulate animal growth, through beta-adrenergic receptors of the tissues. Generally they act as repartitioning agents that markedly favour lean (muscle) deposition in all major livestock species when included in the diet (Murray & Oberbauer, 1992). Lindsay *et al.* (1993) considered β-adrenergic agonists as probably the most interesting class of growth stimulants, due to their activity in increasing protein deposition and decreasing fat deposition.

Marked improvements in composition and growth performance of market pigs may be realised by commercial producers through exogenous administration of anabolic agents or other metabolic modifiers (Beermann, 1993). The current difficulty in the use of anabolic agents is not the mode of action, but the question of public acceptance, concerning drug residues and toxicological safety of these products. This problem has resulted in delays in their widespread use (Lamming, 1986).

2.2.4.1 Anabolic steroids

The steroids include such biologically important compounds as the sex hormones, the adrenal hormones, the bile acids and the sterols. They have a common basic structural unit of a phenanthrene nucleus linked to a cyclopentane ring. The individual compounds differ in the number and position of their double bonds and in the nature of the side chain of carbon atom 17 (McDonald *et al.* 1973). Sex steroids are used as conventional growth promoters in farm animals by administration in the form of ear implants or injectables. Androgens are the male sex hormones which are steroid compounds produced by the interstitial cells (Leydig cells) of the testis. The main androgen is testosterone, but there are some modified forms of testosterone, such as androsterone and dehydroandrosterone, which are due to the metabolism of testosterone in the kidneys. Testosterone and related androgens are responsible for the male secondary sex characteristics, body conformation, muscular development and *libido* or sex drive (Bone, 1979).

They are derivatives of cholesterol and chemically built around the steroid nucleus. The androgens are also produced in the cortex of the adrenal gland, exerting some effects on the metabolism of carbohydrates and proteins, but with minor effects on reproductive functions (Svendsen, 1974). Androgens will increase growth rate by binding to specific muscle receptors and increasing protein deposition (Roche & Quirke, 1986). These hormones circulate in the blood, bound to plasma proteins and are either rapidly utilised or are degraded by the liver and/or kidneys. They are then excreted through the bile duct into the intestine or are excreted as part of the urine (Bone, 1979). The major androgens commercially available as growth stimulants are testosterone, trenbolone acetate and nandrolone (Roche & Quirke, 1986).

The female gonads or ovaries produce two female sex hormones, oestrogens and progesterone. The oestradiol is produced mostly in the ovarian follicle (Graafian follicle), by the cells of the *theca interna*, while the hormone progesterone is produced by the *corpus luteum*, an endocrine structure into which the Graaffan follicle is transformed after ovulation. Oestrogens are responsible for the development of the female secondary sex characteristics and body conformation. In addition, they enter into the complex hormonal interrelationships of the oestrous cycle (Bone, 1979). The mechanism of action of oestrogens is not clear, but increased growth hormone secretion, increased thyroid activity, a direct effect at muscle level through special oestradiol receptors in muscle tissue have been postulated as a possible mode of action (Roche & Quirke, 1986). According to Leat and Cox (1980), the appreciable uptake of oestrogens by fat cells confirms the influence of the female sex hormones in the growth of adipose tissue. The major oestrogens available are oestradiol - 17β and zeranol (Roche & Quirke, 1986).

Zeranol, a weak synthetic chemically available oestrogen, (Van der Merwe & Pieterse, 1994), is identified as a non-steroid substance used as growth promoter in cattle and sheep. Its application in pigs has been envisaged too. A subcutaneous implantation in the pig produces the appearance of zeranol, taleranol and zearalanone in the plasma as free and conjugated metabolites. In the urine, bile and faeces, however, it is identified as the metabolite zearalanone, resulting from the oxidation of zeranol (Bories *et al.* 1992).

Nandrolone (17β-19-Nortestosterone), is an injectable androgenic steroid used as anabolic agent. Its major metabolite is 17α-epimer, which is found in a very low concentration in the urine of treated animals, owing its extensive metabolism. Nandrolone has been frequently used in the fattening of veal calves and cattle (Van der Merwe & Pieterse, 1994).

2.2.4.2 Beta-adrenergic agonists

Beta-adrenergic agonists are phenethanolamine compounds, analogues of the naturally occuring catecholamines norepnephrine (noradrenalin) and epinephrine (adrenalin), with whom they share pharmacological properties (Beermann, 1993). β-adrenergic agonists mediate their effects on various organs, including muscle and fat tissue, modifying the activity of endocrine

systems such as insulin. However, the effects of β-agonists are variable and depend on the structure of the β-agonist, duration of application, anatomical location, age, species, breed, sex and also nutritional factors (Bracher-Jakob & Blum, 1990). Beta-adrenergic agonists enhance lean (muscle) content and reduce the fat content of animals. They exert control over fat metabolism by stimulating lipolysis through beta receptors in the adipose tissue and also cause a rapid increase in nitrogen retention essentially in the skeletal muscle. Muscle hypertrophy by β-agonists has been suggested to be associated with an increase in the diameter of muscular fibres (Buttery & Sweet, 1993), and a decreased protein degradation (Bracher-Jakob & Blum, 1990; Wheeler & Koohmarale, 1993). After an application of β-adrenergic agonists, metabolic, endocrine, cardiovascular, respiratory and skeletal muscle activities are initially markedly altered. Such changes rapidly disappear during continued exposure to β-adrenergic agonists. This indicates that chronic metabolic effects, leading to changes in body composition and growth performance, are due to intracellular changes (Bracher-Jakob & Blum, 1990).

Mammals tend to be much more responsive to β-agonists than are birds. The reason probably being related to a fundamental difference between mammals and birds in the relative importance of adrenalin in the control of their metabolism. Although there is evidence that β-agonists demonstrate marked sexual dimorphism, there is some suggestion that females may be more responsive than males (Buttery & Sweet, 1993).

The beta-adrenergic agonists are compounds usually available and termed clenbuterol, cimaterol, ractopamine and L-644, 969. Clenbuterol, the compound originally developed for the treatment of asthma in humans and horses, was shown to increase protein deposition and decrease fat deposition. It is pharmacologically classified as a β_2-agonist (Lindsay *et al.* 1993), and is weak when compared with epinephrine (Spurlock *et al.* 1993). Some literature have recommended that athletes use clenbuterol, due to its significant improvement of strength inspiratory and expiratory power (Meyer, 1993). According to Mersmann *et al.* (1989), the reduction of fat accretion by the use of clenbuterol is not the result of clenbuterol interaction with the β-adrenergic receptor on the porcine adipocite. It is rather indirect, through changing blood flow to adipose tissue, which might effect adipose metabolism. For anabolic purposes dosage needs to be ten times higher than during therapy. After application, clenbuterol is found in blood plasma, and high levels are accumulated in the eye and the liver. The elimination occurs via the urine (Meyer, 1993).

2.3 Carcass characteristics and meat quality

It is generally acknowledged that animal proteins are more savory and more detectable to the palate than any other source of protein (Aykroyd, 1964). According to Whittemore (1993), pork accounts for more than 40% of the world's meat consumption. There is a broad range of preferences in its consumption patterns, which causes pig to be finished and consumed at a wide

variety of weights, between the suckling and heavier weight stages. This variation occurs because as an animal grows, its physical and chemical composition changes and these changes play a major role in determining the economics of production and acceptability of the carcass, besides the general implications of meat quality. Carcasses are sold to butchers on the basis of weight, shape and some estimate of the proportion of lean and fat they contain. Butchers, on the other hand sell joints and cuts of meat on their appearance, gauged in terms of proportion of fat, colour and freedom from drip or exudate. Finally, consumers judge the quality of meat by its tenderness and taste (Lister, 1980).

While the long term objective of the swine industry had been to produce lean tissue more efficiently through a reduction in carcass fat, it is becoming increasingly clear that the quality criteria will become more complex. In the future it could include factors such as pork colour, drip loss, marbling and protein content, tenderness, flavour and freedom from chemical residues (Jones *et al.* 1994).

2.3.1 Carcass characteristics

The pig has been bred both for supreme fatness (lard) and supreme leanness (pork), and the popularity of its meat differs widely throughout the world (Whittemore, 1993). A consistent trend towards leaner pig carcasses has been apparent over recent years, because lean pigs convert feed into meat more efficiently than fat pigs and lean carcasses usually realize higher price per kilogram (Wood *et al.* 1981). The relative amount of muscle, bone and fat tissue in the carcass of livestock is often referred to as carcass composition. Livestock industries and consumers are greatly concerned about carcass composition, because it relates to the cost of production and product quality (Laymaster, 1989). Visually assessing carcasses has been a commonly used technique to estimate the composition of pork. Unfortunately, visual predictions of composition are difficult to describe and standardize so they can be consistently applied over time (Kauffman & Warner, 1993). Dressing percentage, a parameter expressed as the dressed weight of the cold carcass as a ratio of the liveweight of the animal, is a ratio affected mainly by the weight of the animal at slaughter. It becomes an important parameter as some market outlets specify a certain carcass weight range (Thornton, 1981). Usually payment for pig carcasses is on the basis of their weight, adjusted for some assessment in carcass quality such as backfat thickness (P_1, P_2 and P_3), carcass length, ham length and eye muscle area (Whittemore, 1993). Carcasses with good conformation are preferred in some markets because they are associated with a higher dressing percentage, higher ham percentage and also a higher carcass lean content (Blasco *et al.* 1994).

2.3.2 Meat quality

No one has been able to predict the ultimate eating quality of meat from knowledge only of the composition of the carcass from which it came. In reality, quality, whether of the carcass or of meat, is a function of the animal

and its responses to the pre- and post-slaughter environments. Just as the system of husbandry during an animal's life can influence its growth and composition, so the post-slaughter handling of the carcass can radically alter the quality of the meat it yields (Lister, 1980). The increasing importance of product quality has focussed attention on factors that may affect quality at all stages of the pork production chain, from the farm through to the consumer. Although it is generally accepted that the factors which have the biggest influence on eating quality exert their effect after the animal has left the farm, there is increasing interest in the influence of pig management, or on-farm factors, on the organoleptic properties of pigmeat (Ellis & McKeith, 1993). The meat industry wishes to maintain or improve the eating quality, although it is often believed that this will necessarily decrease as meat becomes leaner (Wood *et al.* 1981).

In lean meat, proteins always predominate, being about 65% of the muscle dry matter. Although there is quite a large spectrum within which the lipid content of a carcass may vary, the intramuscular lipids are not greater than 4 - 5% of the weight of fresh meat. The proportion of phospholipids is relatively constant, at about half of the lipids. Finally there are the carbohydrates and the minerals, each about 1% (Alais & Linden, 1991). According to Lister (1980), there is a variation in meat quality, depending on the animal's age at slaughter. So, the meat from young animals tends to be pale in appearance and almost devoid of fat. Although it is apt to lack flavour, it is very tender to eat. Older animals produce a darker meat, due to the increased concentration of myoglobin in the muscle, and there is more intra and inter muscular fat, while the tenderness tends to decrease. Swatland (1984) quotes that the most important physical property of meat is its degree of tenderness when eaten, usually after some degree of cooking. Maximum tenderness is reached when the meat reaches a certain temperature, and then it becomes tougher at higher temperatures.

Palatability of meat products encompasses a broad spectrum of factors, including appearance, tenderness, juiciness and flavour. The appearance of meat products is very important in the purchase decision of the consumer. Consumers expect meat products to have an attractive colour and a desirable fat to lean ratio. However, cooked meat appearance seem to be less important than the tenderness, juiciness and flavour (Ellis & McKeith, 1993). Off-odours and flavours in pigmeat can be caused by lipid rancidity during storage, or by the presence of the male pheromone 5α-androstenone, which produces the so-called boar taint (Willeke, 1993).

One particular area that has received considerable attention over recent times from both geneticists and meat scientists is the halothane gene. This is so-called because animals homozygous for the recessive form of this gene show a distinctive response when exposed to the anaesthesic gas, halothane. The halothane gene is of interest because it influences all aspects of the pig production and marketing chain with both beneficial and deterious effects. Halothane reactors have enhanced carcass lean content, compared to homozygous non-reactors, but are stress susceptible and produce a high

incidence of pale, soft and exudative (PSE) meat (Ellis & McKeith, 1993). It has been suggested that stress susceptibility in pigs is the result of selection for, and is associated with heavily muscled pigs that have a high growth rate, improved feed efficiency, and lean carcasses (Heinze & Mitchell, 1987). According to Jones *et al.* (1994), there is a suggestion that PSE is one of the major problems in the pig industry, which is produced by ante-mortem environments and procedures, such as poor pre-slaughter handling, poor stunning and chilling of carcasses or a combination of all these factors.

Meat quality is generally assessed using muscle pH, muscle colour, drip loss, muscle texture, muscle tenderness, muscle flavour; fat colour, fat firmness, fat wetness, fat flavour, fat texture and muscle and fat taint (Cameron, 1993; Whittemore, 1993; Shaw *et al.* 1995).

2.3.3 Anabolic agents and carcass quality

Economic objectives and consumer preferences have prompted scientists to seek methods for restricting fat deposition in meat animals (Spurlock, 1993). Anabolic agents appear to have some effect on the carcass composition of animals, and depending upon the type used, they can cause an increase in the ratio of muscle to fat, or a decrease in this ratio. However, the basic eating quality attributes to the consumer are largely unaffected by hormonal treatment (Patterson & Salter, 1985). According to Heitzman (1986), during the period of growth manipulation, the animals may exhibit unwanted side effects which adversely affect their health, welfare and productivity. On the other hand, there is an also increasing concern about the safety of anabolic agents to public health.

Anabolic steroids

Martinez *et al.* (1992), using trenbolone acetate implants in pigs, found some important changes in carcass composition that may be useful in reducing the fatness, while no significant differences were observed in carcass weight and length. Treatment of gilts with a combined androgen and oestrogen preparation nearly always resulted in some reduction in backfat thickness, accompanied by small increases in eye muscle area and the percentage lean cuts (Patterson & Salter, 1985). Sheridan *et al.* (1990) reported that average daily gain and carcass weights were not significantly improved following treatment of gilts with androgens and oestrogens, decreased backfat thickness being the only significant effect in the carcass characteristics. Higher uterine weights and lower ovarian weights were found in gilts treated with oestrogens, and lower weights of both the uterus and ovaries were observed in those treated with androgens. Most of the endocrinological side effects of anabolic agents in breeding animals are undesirable and it is recommended that anabolic agents should not be used in animals intended for breeding (Heitzman, 1986).

β-Agonists

The major overall effects of the β-agonists are to alter the normal allometric patterns of growth in animals fed normal diets. To increase the weight of the carcass relative to liveweight, to increase feed utilization efficiency, and in some cases, to increase growth rate (Beermann, 1993). Wallace *et al.* (1988), found that weight gain was higher when β-agonists were administered to pigs. Feed convertion efficiency as well as the lean meat percentage and the area of *longuissimus dorsi* muscle was significantly higher while backfat thickness was lower. Stecchini *et al.* (1991) administered clenbuterol orally to heavy pigs and this treatment, however, did not improve lean meat yield, but increased the potential to produce dark, firm and dry meat. Meanwhile, Warris *et al.* (1990) treated pigs with β-agonists and found no effect on growth rate, higher dressing percentage, reduced fat content and larger *longuissimus dorsi*. The livers of treated animals were smaller than the untreated animals. A tougher muscle was experienced in treated animals. Side effects of β-agonist treatment include endocarditis (Schaefer *et al.* 1992) and hoot lesions (Wallace *et al.* 1988).

2.3.3.1 Anabolic agents and consumer safety

The potential hazards of growth manipulation using chemicals are present both on and off the farm. Of major concern is the fact that meat from animals reared under these modern conditions will not only be reduced in quality, but also adversely affect the health of the consumer. In the case of anabolic agents, the concern is their potential to adversely affect the human hormonal or endocrine status or to produce cancer (Heitzman, 1986). Residues following administration of anabolic steroids can be present in tissues, especially the liver and kidneys. The urine, bile and faeces being the major way of excretion following treatment (Heitzman *et al.* 1984). The β-agonists are possible toxic substances which affect the cardiovascular system, but there is no information of this in field trials (Heitzman, 1986). β-agonists are excreted to a considerable extent unmetabolized in the urine, at a range of 34 to 43% of the administered dose. It seems as if the liver is the target area where edible tissue are to be monitored (Sauer *et al.* 1993).

According to Heitzman (1986), in many countries legislation regulating the use of drugs has been instituted with the primary aim of avoiding residues in food and thus, a guarantee of consumer safety. The primary goal of such safety is to assess the pharmacological and toxicological properties of residues in edible animal tissues at the time of slaughter following treatment of the animal. This evaluation also requires adequate information on the metabolism and pharmacokinetic profile of the drug (Hoffmann, 1984).

The presence or absence of residues following the administration of any animal drug depends on the sensitivity of the analytical method used (Heitzman, 1986). Techniques for measurement of residues of anabolic agents in farm animals, their meat and meat products have improved dramatically during the last couple

of years, due to the introduction of new methodology (Heitzman *et al*, 1984). The highest concentration of residues are seen after administration of the drug, but the concentration falls rapidly after withdrawal. The rate at which residue concentrations fall, depends on the formulation of the agent and may be very rapid after oral administration, or very slow with implants. The liver and kidney are often the site of metabolism and excretion of a drug. Thus, higher concentrations of metabolites are found in these organs (Heitzman, 1986). A constant supply of the minimum amount of anabolic agent necessary for the maximum anabolic response is desirable. This reduces the risk of unwanted residues and the wasteful use of agent (Heitzman *et al*, 1984).

Residues in edible tissues following treatment with anabolic agents have been reported. Residual activity of β-agonists have been found (Meyer, 1993) after consumption of livers from animals treated with clenbuterol. These organs contained amounts above human therapeutic level, probably almost up to an anabolic level. Sterility in male foxes fed chickens following the administration of anabolic steroid implants, has also been reported (Elton, 1964). According to Hoffmann (1984), following recommended treatments and based on the tissue hormone levels measurable, no differentiation can be made at the time of slaughter between treated and untreated animals. Thus, a residue problem does not exist. A further safety margin is obtained by the low oral bio-availability of these agents. The fact that in respect to production of sex steroids in the human itself, the amount consumed with food of animal origin seem to be negligible.

Exciting as the responses of pigs to exogenous hormones might be, it still remains to be ascertained whether the consumer will accept pork products as being of the highest quality in the broadest sense, even after pigs concerned have been treated with exogenous hormone preparations. Whether the same benefits of increased growth rate, efficiency and leanness cannot be achieved more economically and more simply through conventional genetic selection for lean tissue growth rate are factors to be borne in mind (Whittemore, 1993).

CHAPTER 3

MATERIAL AND METHODS

Location:

Crossbred gilts (Large White x Landrace) purchased as weaners (6 weeks of age: 15.5 ± 0.9kg liveweight) at a commercial piggery were randomly allocated to four treatment groups and submitted for an observation period of three phases. The trial was carried out in an indoor system, at an experimental unit of the Faculty of Agriculture (UOFS), from September 1994 to February 1995. The pigs were fed a pig growth diet (16% crude protein) *ad libitum* with free access to water. Blood and urine samples were analysed in two different laboratories at the Medical Faculty (UOFS). The pigs were slaughtered at a commercial abattoir at a mean liveweight of 85 kg, and carcass and meat measurements and parameters were taken at the Department of Animal Science, Meat Science Section, Faculty of Agriculture, UOFS.

3.1 Animal health programme and environment control

The experimental unit was cleaned daily and the internal temperature controlled by closing/opening windows and/or switching on/off an electrical air ventilation unit. Flies and other insects were controlled by spraying an insecticide every 28 days, inside and outside the unit. All animals were preventively injected with an antibiotic before the trial and an anti-mange/internal parasite remedy every 35 days. Other occasional treatments were applied using antibiotics, sulphamides and wound spray preparations.

3.2 Material

24 Large White x Landrace gilts of approximately 15.5 ± 0.9 kg liveweight and 6 weeks of age were randomly allocated to four groups (n = 6/group) and individually housed in pens (0.5 x 1.5 x 0.7m) (Figure 3.1). Four animals out of each group were identified to serve for blood sampling in the weekly determination of hematocrit, blood creatinine, blood glucose, blood urea and blood estradiol levels.

3.3 Maintenance of the experimental animals

Following an adaptation period of 12 days all pigs were maintained on a commercial pig growth diet (Table 3.1), in which the nutrient requirements of swine (NRC, 1988) were met. The animals were fed *ad libitum* and provided with fresh water throughout the observation period until a target liveweight of 85 kg was reached. At this point all animals were slaughtered.

3.4 Observation period

Excluding the adaptation period of 12 days, the observation period was divided into three phases:

a) Phase 1: Treatment Phase, in which the animals were treated with the anabolic agents. This period lasted for a fixed time of 9 weeks;

b) Phase 2: This phase could be seen as the clearance period of the anabolic agents. Started after the 9th week, up to the final liveweight of 85 kg;

c) Phase 3: This phase was the post slaughter period, in which certain carcass evaluations were done.

3.5 Treatments

The 24 gilts were allocated to four treatment groups. Each group (n = 6) received the following treatment:

Treatment 1: Gilts (n = 6) were implanted (subcutaneously, in the ear) with zeranol implants (Ralgro-Hoechst Ag-vet Ltd) (36mg), every 3 weeks for a total period of 9 weeks (four treatments of 36mg/animal);

Treatment 2: Gilts (n = 6) received a daily oral dose of 0.5 mg clenbuterol (Sigma) in a water solution, mixed in the feed, for a period of 9 weeks;

Treatment 3: Gilts (n = 6) were administered an intramuscular injection of 1.0 ml (50 mg) nandrolone (Laurabolin-Intervet) every 3 weeks, for a period of 9 weeks (4 treatments of 50 mg/animal);

Treatment 4: Gilts (n = 6) received no anabolic agents and acted as the control.

Table 3.1 Feed composition

Component	Minimum Amount (g/kg)	Maximum Amount (g/kg)
Moisture	-	120.0
Crude Protein	160.0	-
Crude Fibre	-	80.0
Calcium	8.0	10.0
Phosphorus	6.0	-
Total Lysine	9.0	-

3.6 Sampling and measurements

3.6.1 Phase 1

During Phase 1 (treatment period of 9 weeks), the following parameters were measured:

i) Body weight of all animals were recorded every 48 hours to monitor the average daily gain (ADG) (Radnóczi & Fésus, 1993) for the individual animals and the mean of the groups for the observation period.

ii) Weekly feed intake was monitored and the feed convertion rate (FCR) (Cole & Chadd, 1989) determined for the individual animals and the groups up to a target liveweight of 85 kg.

iii) Backfat thickness measurements (P_2) and eye muscle diameter was measured weekly in all the animals, with the aid of a sonar apparatus (Sonolayer-L SAL-32B-Toshiba) to monitor the deposition of backfat and eye muscle thickness (Zhang *et al*, 1993). These measurements were taken at the level of the last rib, 65mm away from the dorsal mid-line for the observation period (see Figure 3.2).

iv) Blood was sampled weekly during the observation period from 4 specific animals per group, for the determination of hematocrit, blood creatinine, blood glucose, blood urea and blood estradiol levels.

3.6.2 Phase 2

During this phase (metabolic clearance period), the following parameters were measured:

i) Body weight of all animals was recorded every 48 hours to determine the average daily gain (ADG) (Radnóczi & Fésus, 1993) for the individual animals and the mean of the groups, up to the target liveweight of 85 kg.

ii) Weekly feed intake was noted and the feed convertion rate (FCR) (Cole & Chadd, 1989) determined for the individual animals and the groups, up to the target liveweight of 85 kg.

iii) Backfat thickness measurements (P_2) and eye muscle diameter were measured weekly in all animals with the aid of a sonar aparatus (Sonolayer-L SAL-32B-Toshiba) to monitor the deposition of fat and muscle (Zhang *et al.* 1993). These measurements were taken at the level of the last rib, 65mm away from the dorsal mid-line for the observation period.

iv) Blood was sampled weekly during the observation period from 4 specific animals per group, for the determination of hematocrit, blood creatinine, blood glucose, blood urea and blood estradiol levels.

v) Urine was sampled every second day from all the animals treated with anabolic agents. This was used to determine the metabolic clearance rate of the anabolic agents, from the cessation of treatment to the attainment of the target slaughter liveweight of 85 kg.

3.6.3 Phase 3

This phase started at slaughter (85 kg final liveweight).

- The days to slaughter were observed in all animals, to determine the time to the final liveweight for the individual animals and the mean of the groups (Gordijn, 1993);

- The chest diameter (mm) was measured in live animals, at the level of the *scapula* and below the elbow joint just prior to slaughter.

After slaughter, the following carcass evaluations were performed:

3.6.3.1 Carcass measurements

i) The carcasses were weighed (kg), to determine the dressing percentage (Heinze & Mitchell, 1987):

   a) 60 minutes after slaughter (warm carcass weight)
   b) 24 hours after slaughter (cold carcass weight);

ii) Carcass length (mm) was measured from the anterior edge of the aitch bone to the anterior edge of the first rib (Martinez *et al.* 1992);

iii) Thorax depth (mm) was measured at the deepest part of the chest (see Figure 3.3);

iv) Chest depth (mm) was measured, from the shoulder to the sternum cartilage;

v) Carcass backfat thickness was physically measured (mm) (Thornton, 1981) with the aid of a caliper as follows(see Figure 3.4):

   a) 45 mm away from the mid-line, at the level of the 10th rib (P_1)
   b) 65 mm away from the mid-line, at the level of the 10th rib (P_2)
   c) 80 mm away fro the mid-line, at the level of the 10th rib (P_3)

vi) The area (cm^2) of the eye muscle (*longissimus dorsi*), at the level of the 10th rib, from the left side of the carcass, was determined (Cordray *et*

al. 1978) using a digital planimeter (Placom KP-90 Sokkisha Co. Ltd - Japan);

vii) The left hindquarter length (mm) was measured, from the *symphisis pubis* to the distal end (*cochlea*) of the tibia (Wood *et al.* 1981);

viii) The circumference of the first third of the hindleg was measured (mm);

ix) Carcass composition (bone, muscle, fat and skin) was determined using the 13th rib dissection technique (Naudé, 1974).

3.6.3.2 Meat quality parameters

A meat sample (100g) taken from the muscle *longissimus dorsi* was weighed uncooked, and then cooked in water at 70°C, for 60 minutes (Heinze & Mitchell, 1987). The cooked meat sample was then dried on filter paper and processed as follows:

i) weighed to determine the percentage water loss;

ii) prepared and cut with a dinamometer scale (Chatillon-USA), to determine the cutting resistance (Warner-Bratzler shear force) of the muscle;

iii) A small sample (± 0.5g) was weighed, put on filter paper and pressed at 600 kg in a carver laboratory press (Freds Carver INC-Model B-USA), for 60 seconds and weighed again to determine the percentage of free water.

iv) Muscle pH was measured with the aid of a microprocessor pH-meter electrode (Hanna Instruments HI 851-Singapore), from the muscle *Semimembranosus* and from the muscle *Longissimus dorsi*, at the level of the last rib. These measurements were taken 45 minutes after slaughter (initial muscle pH), and 24 hours after slaughter (ultimate muscle pH) (Oliver *et al.* 1994).

3.6.3.3 Viscera measurements

i) The gastro-intertinal tract (including stomach, small intestine, large intestine and the *mesenterium*) were weighed (kg) (Yen *et al.* 1989). These parameters were measured in order to determine and serve as an indicator of the percentage that these organs make out of the total liveweight and also what percentage digesta are present in the digestive tract:

a) full
b) empty

ii) The empty stomach was then separated and weighed (g);

iii) The uterus (*corpus uteri* and the horns), free from the *ligamentum latum*, was weighed (kg);

iv) The right and left ovaries, free from the ovarian bursa, were weighed (g) separately;

v) The right and left kidneys were weighed (kg) separately without the capsule and the perirenal fat;

vi) The lungs, separated from the trachea, were weighed (kg);

vii) The liver was weighed (kg);

viii) The spleen was weighed (kg);

ix) The heart was separated from the *epicardium* and weighed (kg).

3.7 Methodology

3.7.1 Blood sampling

Blood samples were taken weekly from four specific gilts in each group. A modification of the method described by Duran and Walton (1994) were used, in which the gilts under the effect of a tranquilizer sedative (Stressnil, 1ml/20kg liveweight - Janssen Pharmaceutica Ltd) were restrained lying laterally with the fore legs pulled back (see Figure 3.5). The *vena jugularis* was then punctured with a 18 gauge needle attached to a syringe to draw the blood (Figure 3.6). Blood (except for blood glucose determination) was allowed to coagulate and later centrifuged for 15 minutes. The serum was aspirated and stored at -20°C until assayed for the various serum hormones and metabolites. Blood was sampled for the determination of blood glucose levels, blood urea, creatinine and serum oestrogen concentrations. Capillary tubes were used to take a sample of whole blood for the determination of the hematocrit.

3.7.2 Urine sampling

During Phase 2 (metabolic clearance rate of the anabolic agents), urine samples were taken from all the animals treated with anabolic agents. At intervals of 24 hours, the total volume of urine was collected and weighed (kg). Alliquots of approximately 18 ml from each sample was frozen at - 20°C for determination of the anabolic metabolites in the urine (Van der Merwe & Pieterse, 1994).

3.7.3 Laboratory analysis

3.7.3.1 Blood creatinine

For the determination of blood creatinine, a creatinine reagent kit (p/n 668306 - Beckman Astra System - Ireland 1987) was used. Its methodology is based on the reaction of the creatinine contained in the sample (serum) with an alkaline picrate reagent contained in the reaction tube, which combines to produce a red colour complex. An increased absorbance monitored by a detector is directly proportional to the sample creatinine concentration. An accuracy at medical decision level has been demonstrated in blood samples, for this assay. The aim of the creatinine determinations was to serve as an indicator of kidney function or malfunction.

3.7.3.2 Blood glucose

Blood glucose levels were determined using the glucose reagent kit assay (p/n 668300 - Beckman Astra System - Ireland, 1986). The technique is based on an oxygen reaction, in which a precise volume of sample is introduced into the enzyme reagent in a reaction tube containing an electrode that monitors oxygen concentration and the rate of it consumption. This rate is directly proportional to the concentration of glucose in the sample. The accuracy of this method is assessed by linear regression and was demonstrated to be high, even at critical concentration levels of glucose in blood. Blood glucose concentrations were used to possibly serve as an indicator of the more efficient feed convertors.

3.7.3.3 Blood urea

The concentration of urea in the blood was determined using the reagent kit (BUN reagent kit, p/n 667530 - Beckman Astra System - Ireland 1987). Its methodology is based on enzymatic conductivity rate, in which a precise volume of sample is injected into the urease reagent in a reaction tube containing an electrode that monitors the solution's conductivity. Urease catalyses the hydrolysis of urea to ammonium carbonate. The increase of this conductivity is directly proportional to the concentration of urea in the sample. This assay demonstrated accuracy even at critical concentration levels of urea in the blood. The parameter once again was aimed at indicating the more efficient feed convertors.

3.7.3.4 Serum oestrogen (Estradiol)

The determination of oestrogen levels was done according to the method of coat-a-count estradiol (DPC - Los Angeles). The methodology is based on antibody-coated tubes, in which labelled estradiol competes with estradiol in the sample for antibody sites. After incubation and separation of bound and free oestradiol, the tube is counted in a gamma counter. The counts are inversely related to the amount of estradiol present in the sample. The assay is accurate over a wide range of estradiol values and offers clinically appropriate

reproducibility. The aim of the serum estradiol determinations was to monitor the effect of the anabolic agents on the endogenous serum oestradiol levels.

3.7.3.5 Urine: Anabolic agents excretion

The anabolic agent metabolites was determined in the urine, to serve as an indicator of the clearance rate. Samples from zeranol and clenbuterol treated gilts were analysed by selective ion monitoring with the aid of gas chromatography - mass selective detector. A three point calibration curve was obtained by spiking clenbuterol in known concentrations to normal pig urine. From these values the concentration of clenbuterol in the unknown samples could be calculated (Van der Merwe & Pieterse, 1994). As the metabolites to which nandrolone is broken down in porcine is still unknown and there are differences amongst species, it was not possible to validate the assay for pigs (Van der Merwe, 1995 - personal communication).

Although the urine samples were taken every second day, for practical reasons and reasons beyond the laboratory's control, the laboratory could not process the total number of samples obtained. Thus, for each of the two treatments (zeranol and clenbuterol), some critical times (in terms of the anabolic agent's concentrations in the body) were selected and the corresponding samples processed.

Figure 3.1 Partial image of the individual pens used for housing the 24 Large White x Landrace gilts during the experiment

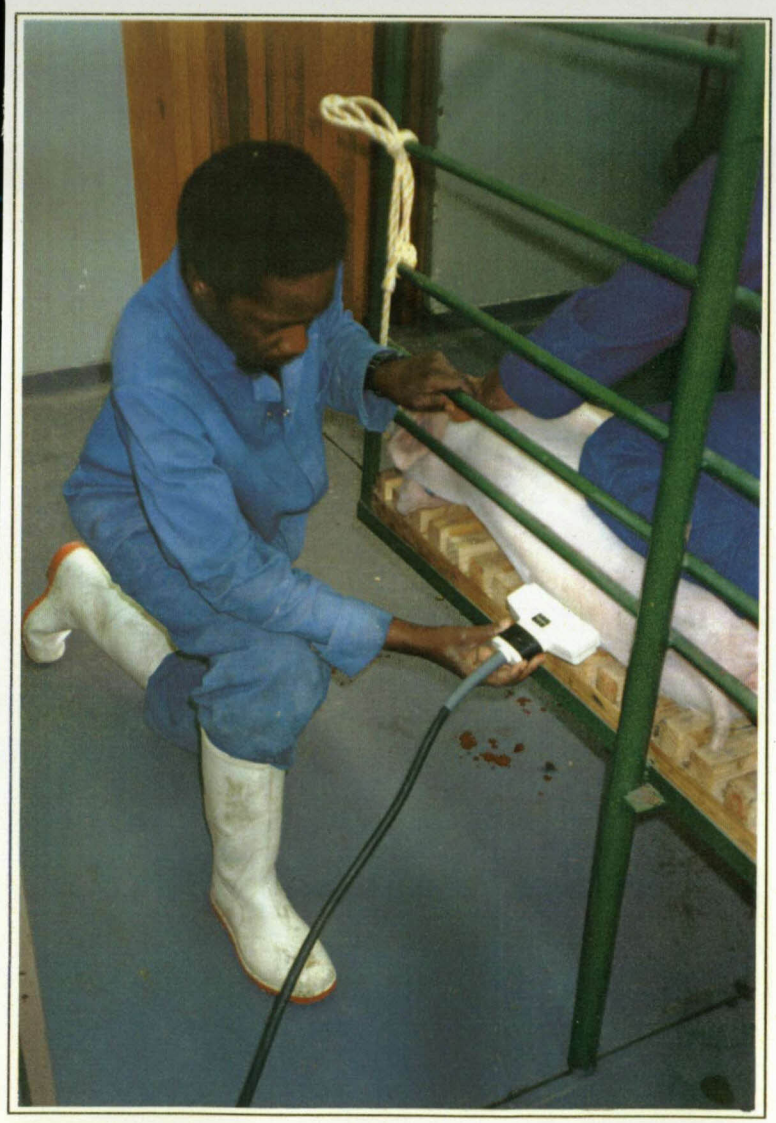

Figure 3.2 Backfat thickness and eye muscle diameter measurements taken at the level of the last rib,with the aid of a sonar apparatus

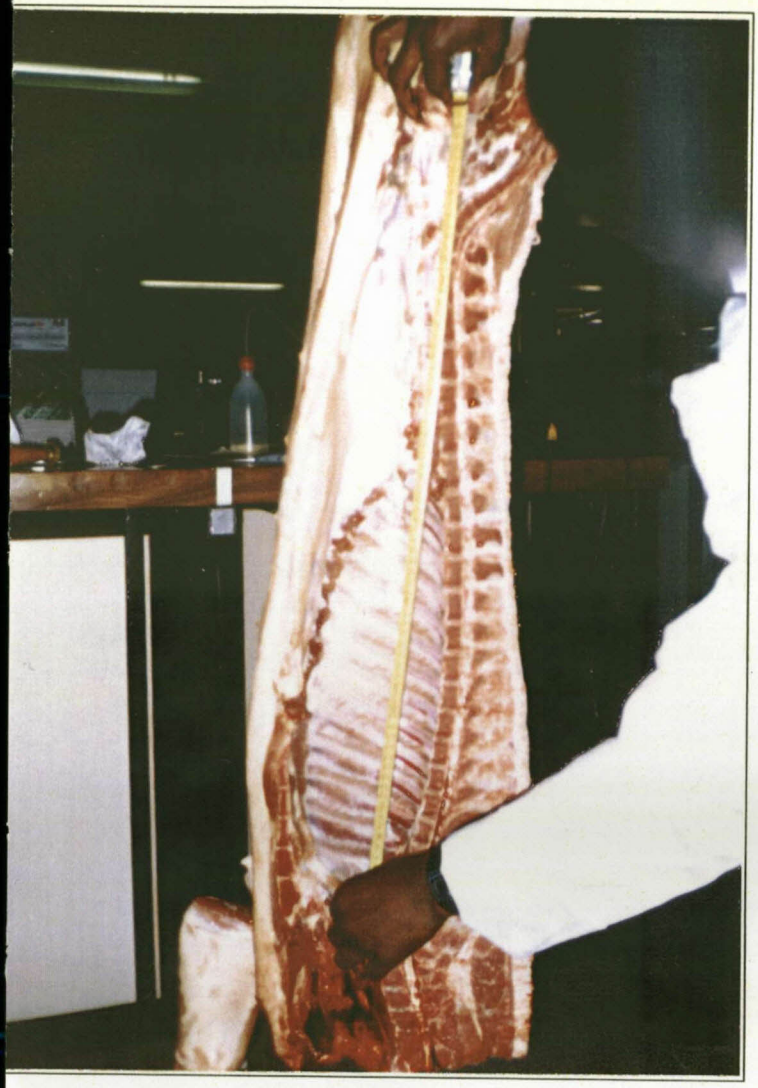

Figure 3.3 Carcass conformation indigators: Measurent of carcass length

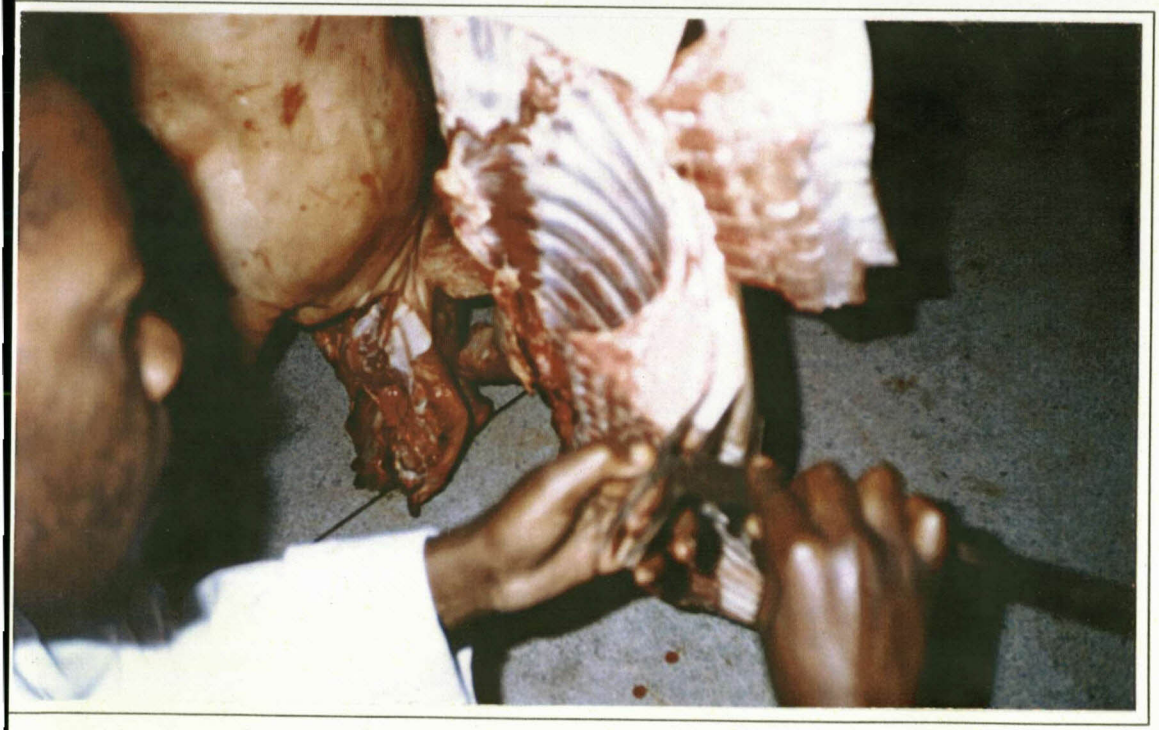

Figure 3.4 Carcass parameters and characteristics: Physical measurements of backfat thickness in the carcass, using a caliper

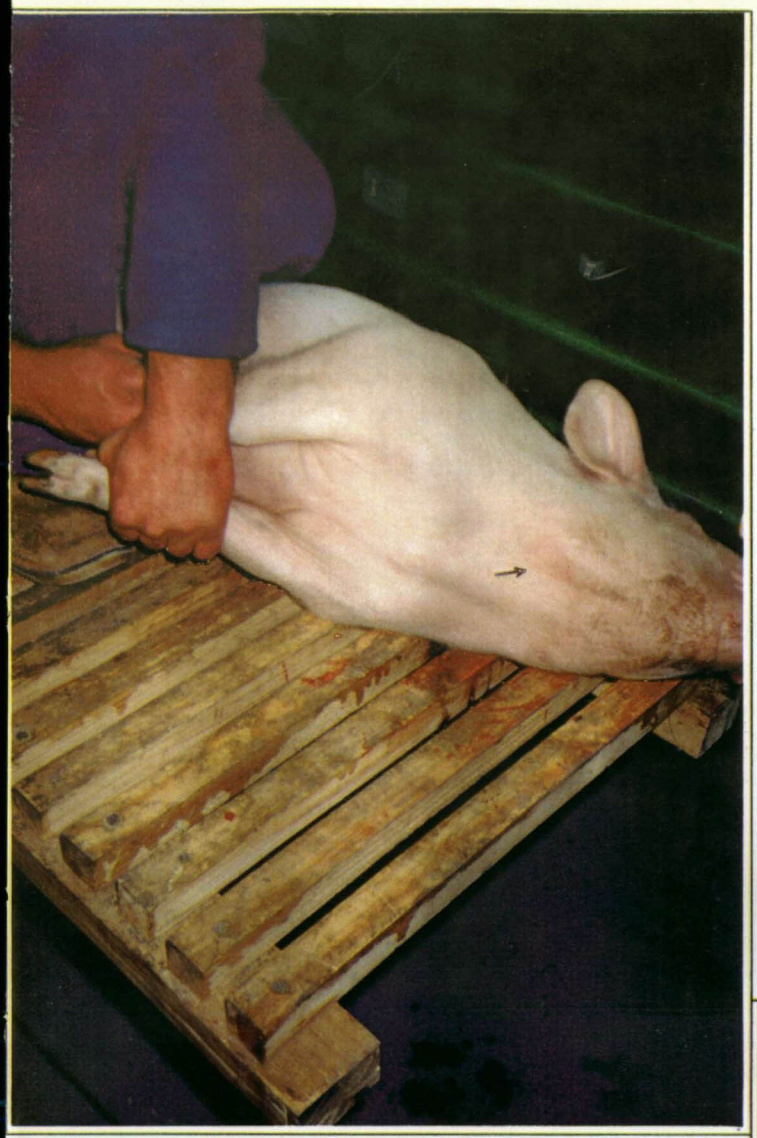

Figure 3.5 Restrainment of a pig showing the site (→) for blood sampling

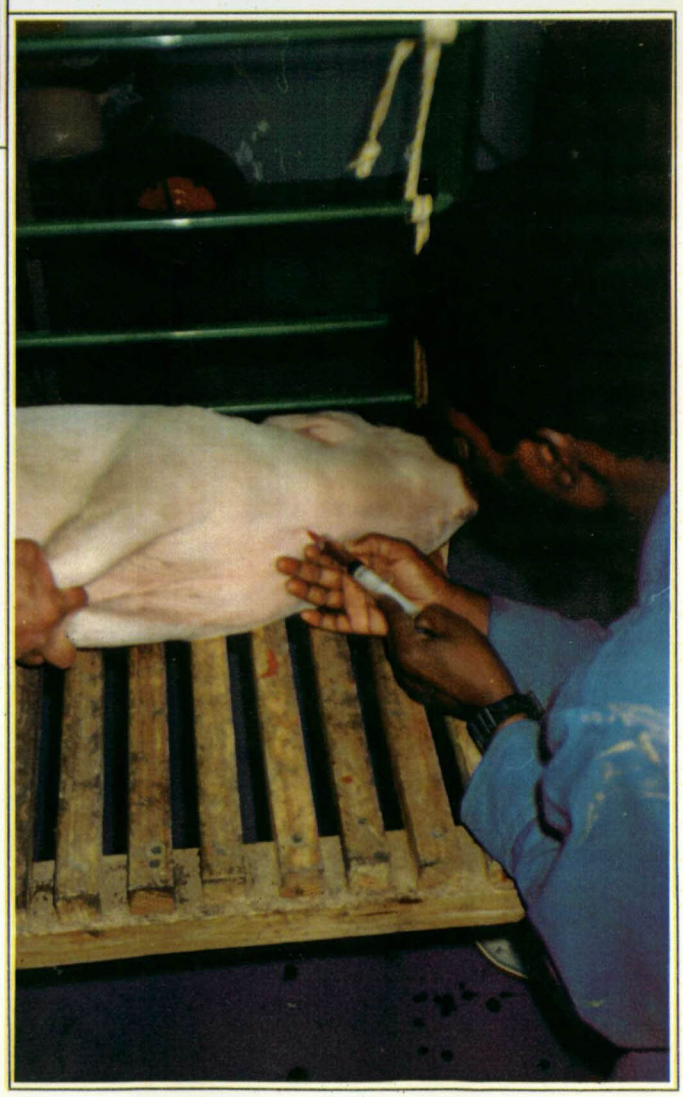

Figure 3.6 Blood sampling from the v*ena jugularis*

CHAPTER 4

RESULTS

4.1 Growth traits and feed conversion rate

Overall growth parameters are given in Table 4.1. In Table 4.2 the growth parameters are set out separately for Phase 1 (treatment period) and Phase 2 (post-treatment period).

4.1.1 Total weight gain

No significant differences in the overall weight gain was found between the four groups. The mean total weight gain per group was 67.5 kg; 66.1 kg; 67.5 kg and 67.8 kg respectively for the control, clenbuterol, nandrolone and zeranol treated groups. A significantly ($P<0.05$) faster growth rate was obtained (Table 4.1) by the animals treated with zeranol implants (mean age of 147.3 days at slaughter), with the clenbuterol and the nandrolone treated groups having a slower rate (163.8 and 164.0 days respectively) than the control (160.2 days). The growth curves obtained for the different groups for the entire period of the experiment are represented in Figure 4.1.

4.1.2 Average daily gain (ADG)

Gilts implanted with zeranol had a significant ($P<0.05$) improved overall growth rate (ADG 728 g/d), compared to the other treated groups (Table 4.1). The treatment with clenbuterol and nandrolone resulted in a lower growth rate (mean ADG of 612 g/d and 618 g/d respectively), although not significantly different when compared to the control (640 g/d). The control group did not differ statistically from the zeranol group.

It can be seen (Table 4.2) that for the zeranol treated group, the ADG was higher (735 g/d) during Phase 1 than in Phase 2 (662 g/d). The contrary happened in the control (662 and 682 g/d), the clenbuterol group (577 and 623 g/d) and the nandrolone group (579 and 648 g/d), where Phase 2 showed a faster growth rate than Phase 1.

4.1.3 Feed conversion rate (FCR)

Although not statistically significant (Table 4.1 and 4.2) the control group showed a better overall FCR (2.76 kg feed/kg gain), when compared to the treated groups (a mean of 2.79, 2.78 and 2.87 kg feed/kg gain for zeranol, clenbuterol and nandrolone groups respectively). A better feed conversion rate was generally observed during the treatment period than during the post-treatment period. The most dramatic change was found in the zeranol treated group (from 2.55 to 3.32 kg feed/kg gain), with little difference in the control group (from 2.69 to 2.85 kg feed/kg gain).

Table 4.1 Growth parameters and characteristics of Large White x Landrace gilts throughout the treatment and post-treatment period with different anabolic agents up to the target final liveweight of 85 kg.

PARAMETERS	TREATMENTS							
	ZERANOL		CLENBUTEROL		NANDROLONE		CONTROL	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Initial liveweight (kg)	18.40	1.60	17.90	0.80	17.90	1.10	18.10	0.80
Final liveweight (kg)	86.20	1.40	84.00	2.00	85.24	0.50	85.60	0.60
Total weight gain (kg)	67.80	1.70	66.10	2.70	67.50	1.10	67.50	1.00
Total feed intake (kg)	189.20	7.10	184.00	12.10	194.10	9.10	186.20	7.90
Average daily gain(g/day)	727.00^{b}	34.00	602.00^{a}	68.30	615.00^{a}	44.00	636.00^{ab}	51.00
Average daily feed intake(kg/day)	2.03^{b}	0.11	1.68^{a}	0.15	1.76^{a}	0.08	1.75^{a}	0.14
Feed Conversion Rate(kg feed/kg gain)	2.79	0.15	2.78	0.10	2.88	0.11	2.76	0.12
Fat deposition increase (mm/week)	2.40	0.51	1.07	0.24	1.09	0.19	1.26	0.25
Eye muscle diameter increase (mm/week)	4.12^{ab}	0.52	3.97^{b}	0.50	4.16^{ab}	0.55	4.43^{a}	0.70
Age at slaughter (days)	147.30^{b}	3.70	163.80^{a}	14.80	164.00^{a}	8.90	160.20^{a}	8.10
Total observation period (days)[1]	93.30	3.70	109.80	14.80	110.00	8.90	106.20	8.10

^{a,b} Figures within a row having the same/without superscripts are not significantly different

[1] Duration of observation period = age at slaughter - (age at onset of trial + adaptation period)

Table 4.2 Growth parameters and characteristics of Large White x Landrace gilts during (Phase 1) and after (Phase 2) treatment with different anabolic agents up to the final liveweight of 85 kg.

PARAMETERS	TREATMENTS							
	ZERANOL		CLENBUTEROL		NANDROLONE		CONTROL	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Initial liveweight (kg)								
Phase 1	18.40	1.60	17.90	0.80	17.90	1.10	18.10	0.80
Phase 2	64.70	1.50	54.20	5.90	54.40	3.80	56.70	2.20
Total weight gain (kg)								
Phase 1	46.30	2.10	36.40	5.50	36.50	2.90	38.55	2.30
Phase 2	21.50	1.40	29.70	7.30	31.00	3.60	29.00	2.00
Total feed intake (kg)								
Phase 1	117.70	4.80	97.60	11.30	101.90	5.60	103.70	8.00
Phase 2	71.50	8.00	86.40	21.20	92.20	11.90	82.50	9.40
Average daily gain (g)								
Phase 1	734.90	33.00	577.10	87.40	579.10	46.70	612.20	36.10
Phase 2	722.10	112.10	655.60	66.00	672.70	77.40	690.10	111.80
Average daily feed intake (kg)								
Phase 1	1.87	0.08	1.55	0.18	1.62	0.09	1.65	0.13
Phase 2	2.38	0.25	1.90	0.20	1.98	0.12	1.94	0.20
Feed conversion rate (kg feed/kg gain)								
Phase 1	2.55	0.17	2.70	0.12	2.80	0.17	2.69	0.18
Phase 2	3.32	0.23	2.90	0.16	2.97	0.21	2.85	0.23

No significant differences between groups.

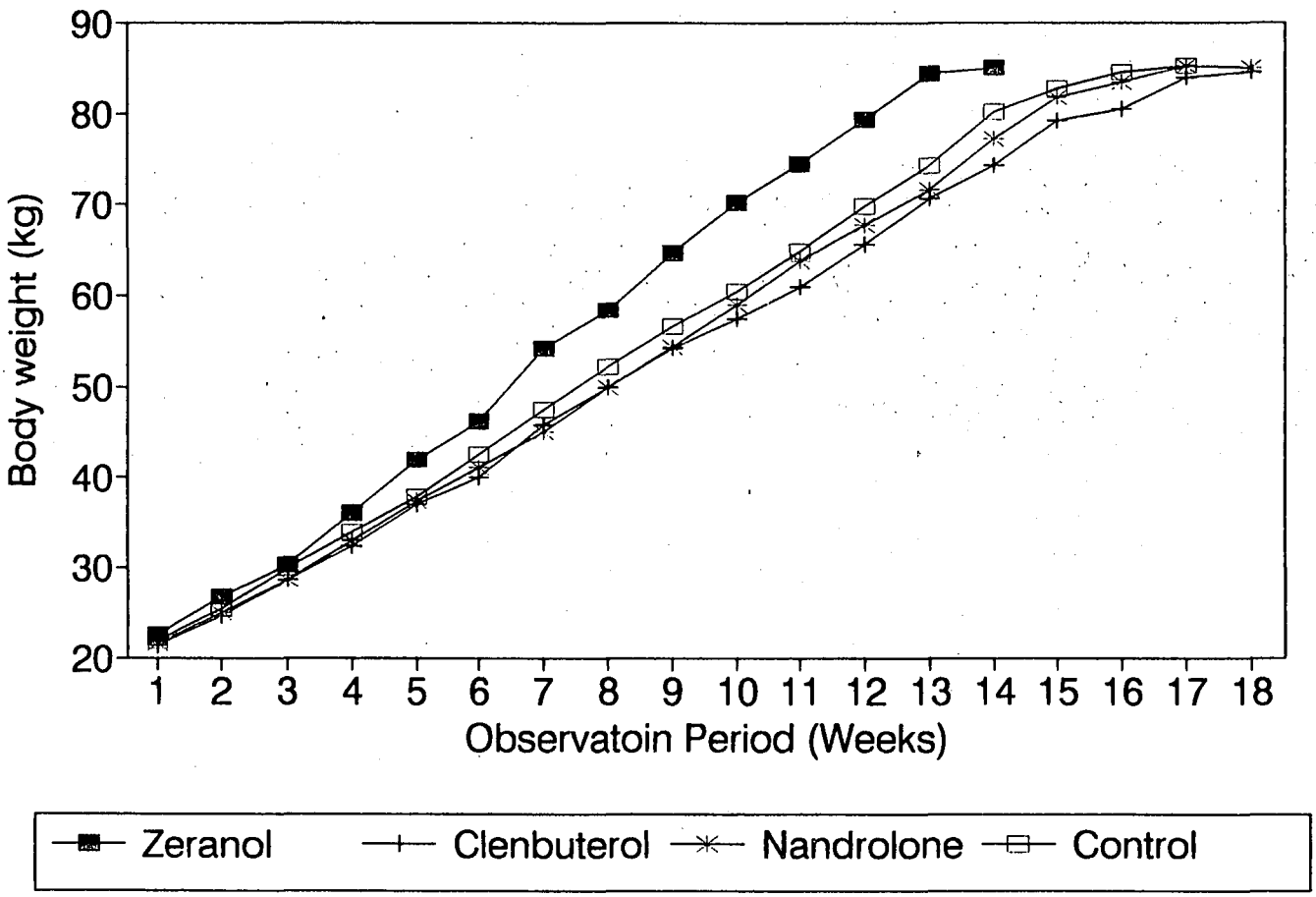

Figure 4.1 Effect of anabolic agent treatments on body weight in Large White x Landrace gilts, up to the target liveweight of 85 kg

4.1.3.1 Feed intake

Total feed intake throughout the experiment showed no significant difference between groups (Table 4.1 and 4.2). It was observed that more than 50% of all the feed was consumed during Phase 1. Zeranol treatment resulted in a significantly ($P<0.05$) higher overall average daily intake, compared to the control, clenbuterol and nandrolone treated groups. During Phase 1 and Phase 2, the average daily intake increased in all the four groups, but more notably in the zeranol group (from 1.87 to 2.38 kg feed/day), and less in the control (from 1.65 to 1.94 kg feed/day).

4.2 Ultrasonic measurements of P_2 and eye muscle diameter

The results of ultrasonic measurements of backfat (P_2) deposition and eye muscle diameter are presented in Table 4.1, Table 4.3, Table 4.4 and Table 4.5. A graphic illustration of weekly increase in these two parameters is given in Figure 4.2 and Figure 4.3.

4.2.1 Backfat (P_2) deposition

Ultrasonic measurements of backfat (P_2) deposition showed no significant differences between the different groups. However, abnormal high overall values (Table 4.4) for fat deposition were found in the zeranol group (2.4 mm/week), and clenbuterol treatment yelded the lowest overall values (1.07 mm/week).

4.2.2 Eye muscle diameter

Eye muscle diameter measurements indicated higher protein (muscle) deposition for the control group (4.43 mm/week), which was significantly ($P<0.05$) more than the clenbuterol treated group (3.97), but not significantly different from the zeranol (4.12 mm/week) and nandrolone (4.16 mm/week) treated groups (Table 4.1).

4.3 Carcass characteristics and parameters

The results of carcass characteristics and parameters are presented in Table 4.6. Correlation coefficients between carcass characteristics and several other parameters are shown in Table 4.22 and Table 4.23. There was a general tendency (although not always significant) for the zeranol treated pigs to differ from the control and the nandrolone and clenbuterol treated animals in carcass weight, dressing percentage and backfat thickness.

4.3.1 Carcass weight and dressing percentage

Significant ($P<0.05$) differences between the zeranol treated gilts and the clenbuterol treated group were found in respect to warm and cold carcass weight and dressing percentage (warm and cold weight: 70.8 and 68.8 kg, and

Table 4.3 Means of ultrasonic measurements of weekly backfat (P_2) deposition and eye muscle diameter in Large White x Landrace gilts during (Phase 1) and after (Phase 2) treatment with different anabolic agents.

PARAMETERS	TREATMENTS							
	ZERANOL		CLENBUTEROL		NANDROLONE		CONTROL	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Weekly fat deposition increase (mm)								
Phase 1	1.00	0.30	0.86	0.26	0.88	0.23	0.92	0.28
Phase 2	3.79	0.71	1.27	0.22	1.29	0.14	1.60	0.22
Total	2.40	0.51	1.07	0.24	1.09	0.19	1.26	0.25
Weekly eye muscle diameter (mm)								
Phase 1	3.52	0.86	3.28	0.74	3.41	0.86	3.50	1.00
Phase 2	4.72	0.17	4.65	0.36	4.91	0.23	5.35	0.39
Total	4.12	0.52	3.97	0.50	4.16	0.55	4.43	0.70

No significant differences between groups.

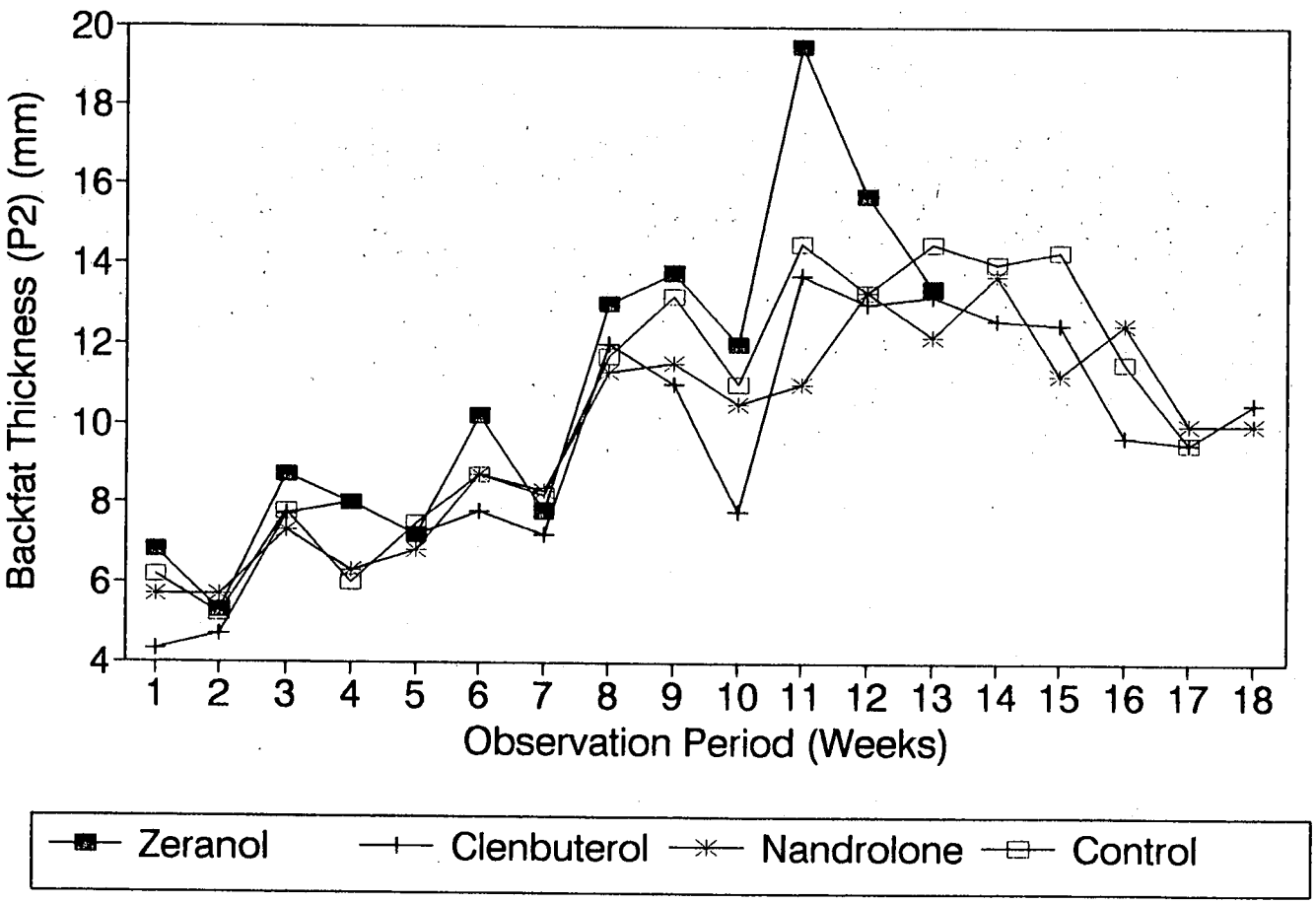

Figure 4.2 Effect of anabolic agent treatments on backfat (P_2) thickness in Large White x Landrace gilts.

Table 4.4 Mean (±SD) of ultrasonic measurements of backfat (P_2) diameter in Large White x Landrace gilts during Phase 1 (treatment phase) and Phase 2 (clearance phase) with different anabolic agents.

PHASE	WEEK	TREATMENTS							
		ZERANOL		CLENBUTEROL		NANDROLONE		CONTROL	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
1	1	6.8	2.1	4.3	0.9	5.7	0.7	6.2	0.7
	2	5.3	0.9	4.7	0.7	5.7	0.9	5.2	0.7
	3	8.7	0.7	7.7	0.7	7.3	1.2	7.8	1.1
	4	8.0	1.3	8.0	1.2	6.3	1.1	6.0	1.5
	5	7.2	1.5	7.2	0.4	6.8	0.9	7.5	2.0
	6	10.2	2.1	7.8	0.7	8.7	1.7	8.7	1.7
	7	7.8	1.9	7.2	0.9	8.3	1.2	8.2	0.9
	8	13.0	1.2	12.0	2.2	11.3	1.4	11.7	1.2
	9	13.8	2.9	11.0	1.0	11.5	0.8	13.2	2.3
2	10	12.0	1.2	7.8	0.9	10.5	1.3	11.0	1.8
	11	19.5	3.6	13.7	1.2	11.0	1.0	14.5	4.1
	12	15.7	3.4	13.0	1.0	13.3	1.6	13.3	0.7
	13	13.4	1.0	13.2	0.7	12.2	0.7	14.5	2.7
	14	*	*	12.6	1.4	13.7	1.2	14.0	2.2
	15	*	*	12.5	3.0	11.2	1.9	14.3	0.9
	16	*	*	9.7	0.5	12.5	0.5	11.5	1.5
	17	*	*	9.5	1.5	10.0	0.0	9.5	1.5
	18	*	*	10.5	1.5	10.0	0.0		
	Total	10.9	3.9	9.5	2.8	9.8	2.5	10.4	3.2

No significant differences between groups for the respective phases.

* Missing data due to attainment of target liveweight.

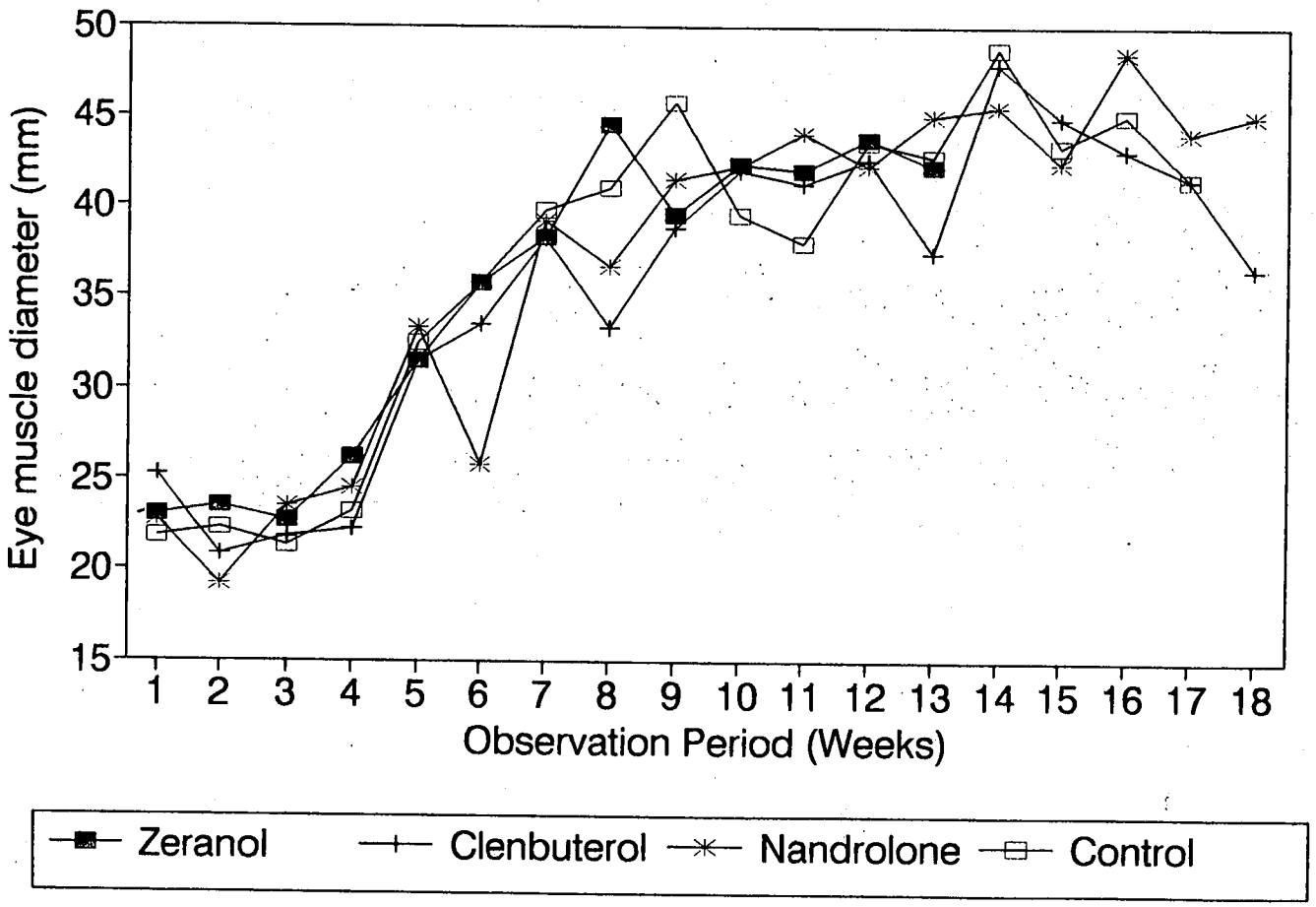

Figure 4.3 Effect of anabolic agent treatment on eye muscle diameter in Large White x Landrace gilts.

Table 4.5 Mean (±SD) of ultrasonic measurements of eye muscle diameter in Large White x Landrace gilts during Phase 1 (treatment phase) and Phase 2 (clearance phase) with diferent anabolic agents.

PHASE	WEEK	TREATMENTS							
		ZERANOL		CLENBUTEROL		NANDROLONE		CONTROL	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
1	1	23.0	3.6	25.2	2.0	22.8	2.0	21.8	2.4
	2	23.5	2.2	20.8	0.9	19.2	2.0	22.3	3.9
	3	22.7	2.1	21.8	1.3	23.5	0.5	21.3	3.5
	4	26.2	3.6	22.2	3.3	24.5	3.6	23.2	3.8
	5	31.5	2.4	31.5	3.2	33.3	5.6	32.5	3.6
	6	35.8	3.3	33.5	3.4	35.8	2.6	35.8	7.6
	7	38.3	2.2	38.2	1.7	39.2	4.7	39.8	5.4
	8	44.5	3.6	33.3	2.9	36.7	4.1	41.0	4.1
	9	39.5	7.9	38.8	4.0	41.5	4.3	45.7	5.1
2	10	42.3	2.3	42.0	2.9	41.2	3.4	39.5	2.6
	11	42.0	3.5	41.2	3.5	44.0	3.4	38.0	5.5
	12	43.7	4.5	42.5	6.3	42.2	1.6	43.5	2.4
	13	42.2	7.4	37.4	2.3	45.0	3.4	42.7	4.5
	14	*	*	47.8	3.0	45.5	1.5	48.7	4.0
	15	*	*	44.8	0.8	42.4	4.2	43.3	8.4
	16	*	*	43.0	5.7	48.5	1.5	45.0	4.0
	17	*	*	41.5	1.5	44.0	0.0	41.5	1.5
	18	*	*	36.5	1.5	45.0	0.0		
	Total	35.0	8.2	35.7	8.1	37.5	8.8	36.8	8.9

No significant differences between groups for the respective phases.

* Missing data due to attainment of target liveweight.

Table 4.6 Carcass characteristics of Large White x Landrace gilts slaugthered at 85 kg liveweight, following a treatment period with different anabolic agents.

PARAMETERS	TREATMENTS							
	ZERANOL		CLENBUTEROL		NANDROLONE		CONTROL	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Carcass weight: Warm (kg)	70.8[a]	1.9	66.0[b]	2.6	68.9[ab]	1.9	65.7[b]	3.1
Cold (kg)	68.8[a]	1.9	63.9[b]	3.5	66.9[ab]	2.5	63.9[b]	2.7
Dressing percentage: Warm (%)	82.1[a]	2.6	78.6[ab]	2.4	80.7[ab]	2.4	76.8[b]	3.6
Cold (%)	79.8	2.3	75.9	2.9	78.3	3.2	74.7	3.0
Backfat thickness: (mm)								
Last lumbar vertebra	21.1[a]	5.7	9.9[b]	2.9	11.7[b]	1.5	14.2[ab]	5.9
Last rib	17.2	5.3	9.6	3.8	12.8	3.4	13.6	4.7
Tenth rib	21.6[a]	4.9	11.3[b]	4.3	14.6[b]	3.3	16.1[ab]	2.7
P_1	13.5[a]	4.3	6.7[b]	2.7	8.2[b]	1.0	8.7[ab]	2.1
P_2	14.8[a]	4.2	7.4[b]	3.3	9.2[b]	1.1	9.7[ab]	2.9
P_3	16.6[a]	4.8	8.6[b]	3.9	10.1[b]	1.3	10.9[ab]	3.4
Eye muscle area: (cm^2)	30.6	2.4	31.1	3.1	34.3	1.9	32.1	5.9

[a,b] Figures within a row having the same/without superscripts are not significantly different.

66.0 and 63.9 kg respectively for zeranol and clenbuterol; dressing percentage; 82.1% and 78.6% respectively for zeranol and clenbuterol groups) (Table 4.6). The nandrolone treated gilts did not differ significantly from any of the other groups in respect to warm and cold carcass weight and dressing percentage. The dressing percentage of the cold carcasses showed no significant difference between the four groups. In all four groups, there was a high positive correlation ($P<0.01$) between warm and cold carcass weights ($r = 0.95$) and a positive correlation ($P<0.05$) between carcass weight and the final liveweight ($r = 0.58$) (Table 4.22 and 4.23).

4.3.2 Backfat thickness

The backfat thickness (Table 4.6) physically measured on the carcass at the level of the last rib showed no significant differences between groups. However, measurements of backfat at the level of the last lumbar vertebra and tenth rib, and P_1, P_2 and P_3, were significantly ($P<0.05$) higher in the zeranol treated group (mean values of 21.1, 21.6, 13.5, 14.8 and 16.6 mm respectively) - compared to the clenbuterol treated group (mean values of 9.9, 11.3, 6.7, 7.4 and 8.6 mm respectively) and the nandrolone treated animals (mean values of 11.7, 14.6, 8.2, 9.2 and 10.1 mm respectively). The control group, with mean values of 14.2, 16.1, 8.7, 9.7 and 10.9 mm for backfat thickness at the above corresponding levels, did not differ significantly from any of the other three treatment groups. Backfat thickness at the level of the last rib was not significantly correlated to growth traits and other carcass characteristics (Table 4.23).

4.3.3 Eye muscle area

The eye muscle area (Table 4.6) measurements resulted in non-significant differences being recorded between the groups, although the nandrolone treatment yielded the largest area (34.3 cm^2), and the zeranol treatment yielded smallest area (30.6 cm^2). The mean area obtained from the clenbuterol treated group and the control was 31.1 cm^2 and 32.1 cm^2 respectively. A high correlation ($P<0.05$; $r = 0.56$) between eye muscle and carcass mass existed, for all the four groups (Table 4.23).

4.4 Carcass composition

The results obtained from carcass composition determinations are presented in Table 4.7. An illustration of carcass composition is showed in Figure 4.4. In Table 4.22 and Table 4.24 the correlation coefficients between carcass composition and several other variables are demonstrated for all the groups. A significant ($P<0.05$) high lean muscle content was observed in the clenbuterol treated group (60.5%), when compared to the zeranol treated animals (54.1%), which yielded the lowest lean muscle ratio of all four groups. This however was not significantly different from those of the control (57.5%) and the nandrolone treated groups (59.2%). The ratio of carcass fat was higher in the zeranol group (28.3%) and lowest in the nandrolone treated animals (19.8%), while the clenbuterol treatment and the control produced 20.1% and 22.2% fat

content in the carcass respectively. These differences in fat content were however not significantly different between the different treatment groups. Moisture loss during the carcass composition determinations showed a relative constant mean value in the control and the zeranol and nandrolone treated groups (0.5% in each). The clenbuterol treatment tended to have a higher (0.7%) moisture loss, although these differences were not statistically significant. Fat content in the carcass was highly ($P<0.01$) correlated ($r = 0.73$) with total feed intake, feed conversion rate ($r = 0.73$) and the age ($r = 0.63$) of the animal at slaughter (Table 4.22).

4.5 Carcass conformation

The results obtained from the measurement of carcass conformation indicators are shown in Table 4.8, and correlation coefficients concerning carcass conformation indicators, meat quality parameters and organ weights are set out in Table 4.23. Statistical differences were found in cold carcass weight, in which zeranol treated animals were significantly ($P<0.05$) heavier (68.8kg) than the control (63.9kg) and the group treated with clenbuterol (63.9), but not significantly different from those animals treated with nandrolone (66.9kg). No statistical differences were observed in the four treatment groups regarding carcass length, chest diameter, chest depth and thorax depth. From Table 4.23, it can be seen that carcass conformation indicators such as ham circumference ($P<0.05$; $r = 0.52$), chest depth ($P<0.01$; $r = 0.64$)and chest diameter ($P<0.05$; $r = 0.56$) had a high correlation with cold carcass weight.

4.6 Meat quality parameters

Table 4.9 represents the results of meat quality parameters. In Table 4.22 and Table 4.23 the correlation coefficients between carcass, meat quality and growth traits are shown.

4.6.1 Muscle pH

Means of the initial and the final pH measurements from the *M. Longissimus dorsi* and from *M. Semi-membranosus* showed no significant difference between the groups (Table 4.9). For all the groups treated with anabolic agents, the mean pH values were generally slightly higher in the *M. Semi-membranosus* than in the *M. Longissimus dorsi*. The contrary tendency was observed in the control group. From the initial (45 minutes) to the final (24 h) pH, the mean values of *M. Semi-membranosus* measurements dropped lower than those measured from the *M. Longissimus dorsi*. The control group kept the initial difference in pH of the two sites, which was not different from the other groups. The lower mean values of final pH (*M. Semi-membranosus*) were obtained from the Zeranol treated group ($pH = 5.52$) and the higher values were observed in both the Clenbuterol and the Nandrolone ($pH = 5.68$), while an intermediate value of 5.71 were found in the Control group. A significantly high negative correlation ($P<0.05$; $r = -0.53$) was observed between muscle pH and fat content in the carcass (Table 4.22).

Table 4.7 Carcass composition of Large White x Landrace gilts slaughtered at 85.0 kg liveweight, following treatment with different anabolic agents.

PARAMETERS		TREATMENTS							
		ZERANOL		CLENBUTEROL		NANDROLONE		CONTROL	
		Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	SD
Muscle	(%)	54.1[b]	2.5	60.5[a]	4.0	59.2[ab]	2.9	57.5[ab]	4.5
Fat	(%)	28.3	4.9	20.1	6.5	19.8	3.1	22.2	4.7
Bone	(%)	12.7	2.6	14.3	3.0	15.6	2.2	14.3	1.6
Skin	(%)	4.4	0.6	4.4	0.7	4.9	0.6	5.4	1.1
Moisture loss	(%)	0.5	0.1	0.7	0.4	0.5	0.2	0.5	0.2

[a,b] Figures within a row having the same/without superscripts are not significantly different.

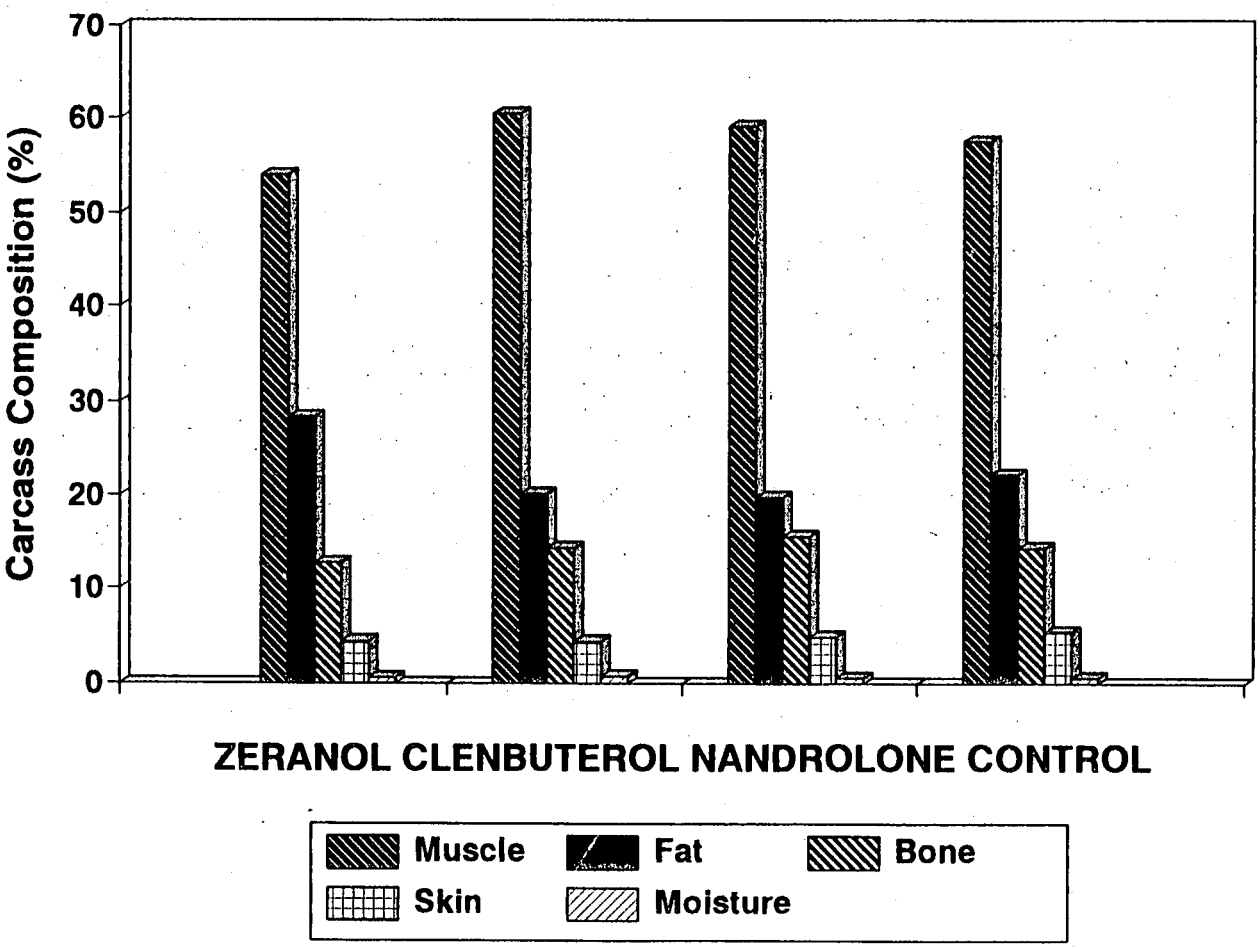

Figure 4.4 Carcass composition of Large White x Landrace gilts slaughtered at 85 kg liveweight, following treatment with different anabolic agents.

Table 4.8 Carcass conformation indicators of Large White x Landrace gilts slaughtered at 85 kg liveweight, following treatment with different anabolic agents.

PARAMETERS	TREATMENTS							
	ZERANOL		CLENBUTEROL		NANDROLONE		CONTROL	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Cold carcass weight (kg)	68.8[a]	1.9	63.9[b]	3.5	66.9[ab]	2.5	63.9[b]	2.7
Carcass length (mm)	828.0	15.0	815.0	13.0	831.0	21.0	832.0	22.0
Chest diameter (mm)	981.0	16.0	964.0	44.0	983.0	27.0	960.0	24.0
Chest depth (mm)	320.0	11.0	327.0	25.0	329.0	7.0	320.0	10.0
Thorax depth (mm)	92.0	8.0	89.0	9.0	89.0	5.0	86.0	4.0
Ham length (mm)	363.0	9.0	366.0	11.0	380.0	10.0	367.0	10.0
1/3 Hindleg circumference (mm)	596.0	12.0	596.0	15.0	595.0	14.0	584.0	22.0

[a,b] Figures within a row having the same/without superscripts are not significantly different.

Table 4.9 Meat quality parameters obtained from Large White x Landrace gilts slaughtered at 85 kg liveweight, following a treatment period with different anabolic agents.

PARAMETERS	TREATMENTS							
	ZERANOL		CLENBUTEROL		NANDROLONE		CONTROL	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Initial (45 minutes) muscle pH:								
M. Longissimus dorsi	5.56	0.17	5.99	0.21	5.81	0.82	6.24	1.03
M. Semimembranosus	5.77	0.17	6.23	0.15	5.97	0.31	6.08	0.18
Final (24 hours) muscle pH:								
M. Longissimus dorsi	5.54	0.05	5.76	0.21	5.71	0.08	5.76	0.23
M. Semimembranosus	5.52	0.03	5.68	0.13	5.68	0.04	5.71	0.26
Cooking loss (%)	28.70	1.10	30.30	3.40	30.00	2.80	30.00	5.20
Cutting resistance (kg)	3.74[b]	0.61	4.99[ab]	0.85	5.69[a]	0.65	5.39[a]	0.85
Water holding capacity (%)	46.50	3.70	37.20	4.50	43.00	7.50	43.50	4.30

[a,b] Figures within a row having the same/without superscripts are not significantly different.

4.6.2 Cooking loss

No statistical differences were observed between the four groups (Table 4.9) concerning cooking loss. A relative uniform mean value was obtained in the groups treated with clenbuterol, nandrolone and the control group (30.3%, 30% and 30% respectively), with the mean for the zeranol treated group being slightly lower (28.7%). From Table 4.22, it can be seen that no significant correlation was obtained between cooking loss and the other parameters measured.

4.6.3 Cutting resistance

Of all the meat quality measurements taken a significant ($P<0.05$) difference in cutting resistance (Table 4.9) was observed in the group treated with zeranol compared to the control and the nandrolone treated animals. The clenbuterol group (with a mean value of 4.99 kg shear force) was not significantly different from either the zeranol group which had the lower mean (3.74 kg) shear force or the control (5.39 kg) and the nandrolone (with the higher mean of 5.69 kg shear force) groups.

4.6.4 Water holding capacity

Water holding capacity values (Table 4.9) showed no statistical differences between the four groups. Zeranol treated animals had the highest mean value (46.5%), and in the clenbuterol group the lowest (37.2%). The control and the nandrolone groups (43.5 and 43% respectively) were very similar. No relevant correlation coefficients could be found between water holding capacity and other carcass and meat quality parameters (Table 4.23).

4.7 Organ weights

4.7.1 Digestive tract and digesta weights

In Table 4.10 and Table 4.11 the weights and the percentage of the digestive tract and digesta are set out. No significant differences between groups were found in all the measurements taken (Table 4.10) and the ratios (%) calculated (Table 4.11). Correlation coefficients between digestive tract weights and several other measurements and parameters are shown in Table 4.25. Zeranol treated animals showed slightly higher weights of the full gastrointestinal tract (11.1 kg) and empty stomach (667.0 g); than the other three groups (8.9 kg and 622.0 g; 9.8 kg and 612.0 g; 9.9 kg and 563.0 g for the full gastro-intestine and the empty stomach weights for the clenbuterol, nandrolone and the control groups, respectively). The weights of the empty intestines were less variable between the groups with the highest weights being 4.9 kg (nandrolone group and the control) and the lowest being 4.4 kg (clenbuterol group). The zeranol treated group had a mean of 4.8 kg. On the other hand, the ratio of the full gastrointestine tract as a percentage of the final liveweight were also higher (not significant) in the zeranol treated gilts (12.8%) compared to those of the control (11.5%), clenbuterol (11.2%) and nandrolone (11.5%) treated animals. The

Table 4.10 Digestive tract measurements of Large White x Landrace gilts slaughtered at 85 kg liveweight following treatment with different anabolic agents.

PARAMETERS		TREATMENTS							
		ZERANOL		CLENBUTEROL		NANDROLONE		CONTROL	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
Full gastro-intestine	(kg)	11.1	2.5	8.9	1.5	9.8	0.9	9.9	0.6
Empty intestine	(kg)	4.8	0.6	4.4	0.4	4.9	0.5	4.9	0.3
Empty stomach	(g)	667.0	110.1	622.0	58.0	612.0	145.9	563.0	48.9

No significant differences between groups.

Table 4.11 Digesta and gastro-intestinal tract (GIT) weights as a percentage of the final liveweight in Large White x Landrace gilts following treatment with anabolic agents.

PARAMETERS	TREATMENTS							
	ZERANOL		CLENBUTEROL		NANDROLONE		CONTROL	
	Mean %	SD	Mean %	SD	Mean %	SD	Mean %	SD
Full GIT	12.8	2.9	11.2	1.1	11.5	0.9	11.5	0.8
Empty GIT	6.3	0.7	5.9	0.6	6.5	0.5	6.3	0.4
Digesta	6.5	3.1	5.3	0.6	5.0	0.5	5.2	0.7

No significant differences between groups.

Table 4.12 Reproductive organ weights of Large White x Landrace gilts slaughtered at 85 kg liveweight following a period of treatment with different anabolic agents.

PARAMETERS			TREATMENTS							
			ZERANOL		CLENBUTEROL		NANDROLONE		CONTROL	
			Mean	SD	Mean	SD	Mean	SD	Mean	SD
Uterus	(kg):		0.134[a]	0.011	0.064[b]	0.016	0.058[b]	0.026	0.091[b]	0.042
Ovaries	(g):	Left:	0.522[b]	0.132	3.411[a]	0.427	3.591[a]	0.911	4.244[a]	1.033
		Right:	0.593[b]	0.244	3.240[a]	0.491	3.022[a]	0.770	3.754[a]	1.222

[a,b] Figures within a row having the same/without superscripts are not significantly different.

mean percentage of the digesta as a ratio of the final liveweight was higher, although not significant, in the zeranol treated group (6.5%) compared to that obtained in the clenbuterol (5.3%) nandrolone (5.0%) and the control (5.2%) groups. There was a significant ($P<0.05$) positive correlations between empty intestine weights and average daily intake ($r = 0.43$), average daily gain ($r = 0.45$) and between empty stomach weight ($r = 0.44$) and feed conversion rate (Table 4.25).

4.7.2 Reproductive organ weights

The uterus and ovary weights are set out in Table 4.12. Generally, the treatment of gilts with zeranol resulted in a significantly ($P<0.05$) reduced growth rate of the reproductive tract and ovaries, when compared to the other groups. The uterus weights of the zeranol treated gilts (0.134 kg) were significantly ($P<0.05$) more than the control (0.091 kg) and the clenbuterol (0.064 kg) and nandrolone (0.058 kg) treated animals. No significant differences were observed between the control and the clenbuterol and nandrolone treated gilts. Zeranol application resulted in significantly ($P<0.01$) smaller ovary weights (0.522 g and 0.593 g for the left and right ovaries respectively), compared to the other three groups. Between the control (4.244 g and 3.754 g left and right ovaries respectively), the clenbuterol (3.411 g and 3.240 g) and the nandrolone (3.591 g and 3.022 g) treated gilts, no significant differences were found concerning ovary weight. In these three groups, slight (although not significant) differences could be seen between the left and the right ovary weights. The left ovaries (3.75 ± 0.36g) being heavier than the right ovaries (3.34 ± 0.31 g). The opposite effect was observed in the zeranol treated animals.

4.7.3 Visceral organ weights

The weights of the liver, kidneys, heart and spleen are set out in Table 4.13 and expressed as percentages of the final liveweight in Table 4.14. In Tables 4.22, 4.23 and 4.24 correlation coefficients between these organs and other parameters are shown. No statistical differences between the four groups were found, whether as absolute weights of all the organs considered, or as calculated percentages of the final liveweight.

4.7.3.1 Liver

Liver weights were heavier in the zeranol treated group (a mean of 1.51 kg) and lighter in both the control and the clenbuterol groups (a mean of 1.38 kg in each group). The mean liver weight in the nandrolone treated gilts was 1.45 kg. The ratio of the livers as a percentage of the final liveweight tended to be higher in the treated groups than in the control (1.62%), although not significant. The highest weight was recorded in the zeranol treated group (1.75%), followed by the nandrolone (1.69%) and the clenbuterol (1.64%) treated animals.

Table 4.13 Organ weights of Large White x Landrace gilts slaughtered at 85 kg liveweight following a period of treatment with different anabolic agents.

PARAMETERS			TREATMENTS							
			ZERANOL		CLENBUTEROL		NANDROLONE		CONTROL	
			Mean	SD	Mean	SD	Mean	SD	Mean	SD
Liver	(kg)		1.51	0.16	1.38	0.14	1.45	0.10	1.38	0.09
Kidneys	(kg):	Left	0.15	0.05	0.12	0.01	0.11	0.01	0.13	0.02
		Right	0.14	0.03	0.13	0.03	0.11	0.01	0.13	0.01
Heart	(kg)		0.31	0.04	0.32	0.04	0.34	0.03	0.31	0.03
Lungs	(kg)		0.57	0.14	0.54	0.23	0.58	0.06	0.65	0.15
Spleen	(kg)		0.12	0.02	0.13	0.03	0.13	0.02	0.12	0.02

* No significant differences between groups.

Table 4.14 Liver, heart, lungs and spleen weights as a percentage of the final liveweight in Large White x Landrace gilts following treatment with anabolic agents.

PARAMETERS	TREATMENTS							
	ZERANOL		CLENBUTEROL		NANDROLONE		CONTROL	
	Mean %	SD	Mean %	SD	Mean %	SD	Mean %	SD
Liver	1.75	0.16	1.64	0.19	1.69	0.11	1.62	0.11
Heart	0.36	0.05	0.38	0.05	0.39	0.04	0.36	0.04
Lungs	0.67	0.17	0.75	0.08	0.68	0.07	0.76	0.17
Spleen	0.14	0.02	0.15	0.03	0.15	0.02	0.14	0.02

* No significant differences between groups.

4.7.3.2 Kidneys

Heavier kidneys, although not significantly different from the other groups, were observed in the zeranol treated group (a mean of 0.15 and 0.14 kg for the left and the right kidneys respectively). In nandrolone treated gilts these organs were lighter and of similar weight (0.11 kg each). The control also had similar same mean weight (0.13 kg) for left and right kidneys respectively, while in the clenbuterol group the mean of the right (0.13 kg) was heavier than that of the left (0.12 kg). A frequency of 50% in kidney systs was observed in those gilts treated with zeranol, although no clinical signs of disturbances relating to this abnormality was noted throughout the experiment.

4.7.3.3 Lungs

No significant differences were found in the weight and ratios of the lungs (Table 4.13). Higher weights were obtained from the control (0.65 kg), and a small difference was found between the zeranol (0.57 kg) and the nandrolone (0.58 kg) groups. The smallest lungs were observed in the clenbuterol treated group (0.54 kg).

4.7.3.4 Heart

The mean weights of the heart were higher in the nandrolone treated gilts (0.34 kg) compared to the other three groups. The lightest mean heart weight was obtained in the zeranol group and the control (0.31 kg), while the clenbuterol group had a mean of 0.32 kg. The same sequence of differences were observed for the ratio of the heart as a percentage of the final liveweight. However, all these differences were not statistically significant.

4.7.3.5 Spleen

Of all the visceral organ measurements, the most uniform was that from the weights of the spleen (Table 4.13). Fluctuations (standard deviations) of no more than 0.03 kg and the means and respective ratios of 0.12 kg and 0.14% (zeranol treated group and the control), and 0.13 kg and 0.15% (clenbuterol and nandrolone treated animals), were obtained from these respective groups. Spleen weight tended to be correlated some growth traits (Table 4.24).

4.8 Serum urea concentrations

Means (and standard deviations) obtained from the determination of serum urea levels for the treatment and recovery periods are presented in Table 4.15 and graphically illustrated for the treatment (Phase 1) and post-treatment or recovery (Phase 2) periods in Figure 4.5. Correlation coefficients between serum urea levels and the main growth characteristics are shown in Table 4.22.

Mean serum urea levels of Phase 2 were generally higher (4.3, 3.9 and 3.7 mmol/l for the zeranol, clenbuterol and control groups respectively) than the levels for the

Table 4.15 Means and standard deviation of serum urea concentrations (mmol/l) during (Phase 1) and after (Phase 2) treatment of Large White x Landrace gilts with different anabolic agents.

PHASE	WEEK	TREATMENTS							
		ZERANOL		CLENBUTEROL		NANDROLONE		CONTROL	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
1	1	3.2	0.8	3.6	0.5	4.6	0.8	4.1	0.7
	2	4.1	1.7	3.8	0.3	5.1	0.3	4.9	0.8
	3	3.3	0.5	3.3	0.2	4.3	0.7	3.7	0.5
	4	3.7	0.5	3.6	0.4	4.2	0.5	3.5	0.5
	5	3.3	0.4	3.3	0.5	3.8	0.3	3.2	0.5
	6	3.1	0.7	3.5	0.6	4.3	0.3	4.0	0.2
	7	3.5	1.0	3.6	0.3	3.6	0.2	2.5	0.6
	8	4.3	0.8	3.6	0.3	4.1	0.1	3.4	0.5
	9	4.3	1.2	3.3	0.6	3.8	0.7	3.4	0.8
2	10	4.4	0.9	4.0	0.7	4.1	0.3	3.3	0.4
	11	3.3	0.4	5.1	1.1	3.5	0.8	4.0	0.1
	12	3.0	1.3	5.7	1.2	3.6	1.3	3.9	0.3
	13	5.6	1.1	4.0	0.8	4.3	0.5	4.0	1.0
	14	5.2	1.1	3.7	0.5	4.1	0.5	3.4	0.6
	15	*	*	3.6	0.4	4.2	0.8	3.4	0.4
	16	*	*	4.0	0.5	2.9	0.5	3.9	0.0
	17	*	*	2.6	0.0	3.8	0.0	3.4	0.0
	18	*	*	2.7	0.0	3.2	0.0	*	*
	Phase 1	3.6	0.4	3.5	0.2	4.2	0.4	3.6	0.6
	Phase 2	4.3	1.0	3.9	0.9	3.7	0.5	3.7	0.3
	Total	3.9	0.8	3.7	0.7	4.0	0.5	3.6	0.5

* Missing data due to attainment of target liveweight.
No significant differences between groups for the respective phases

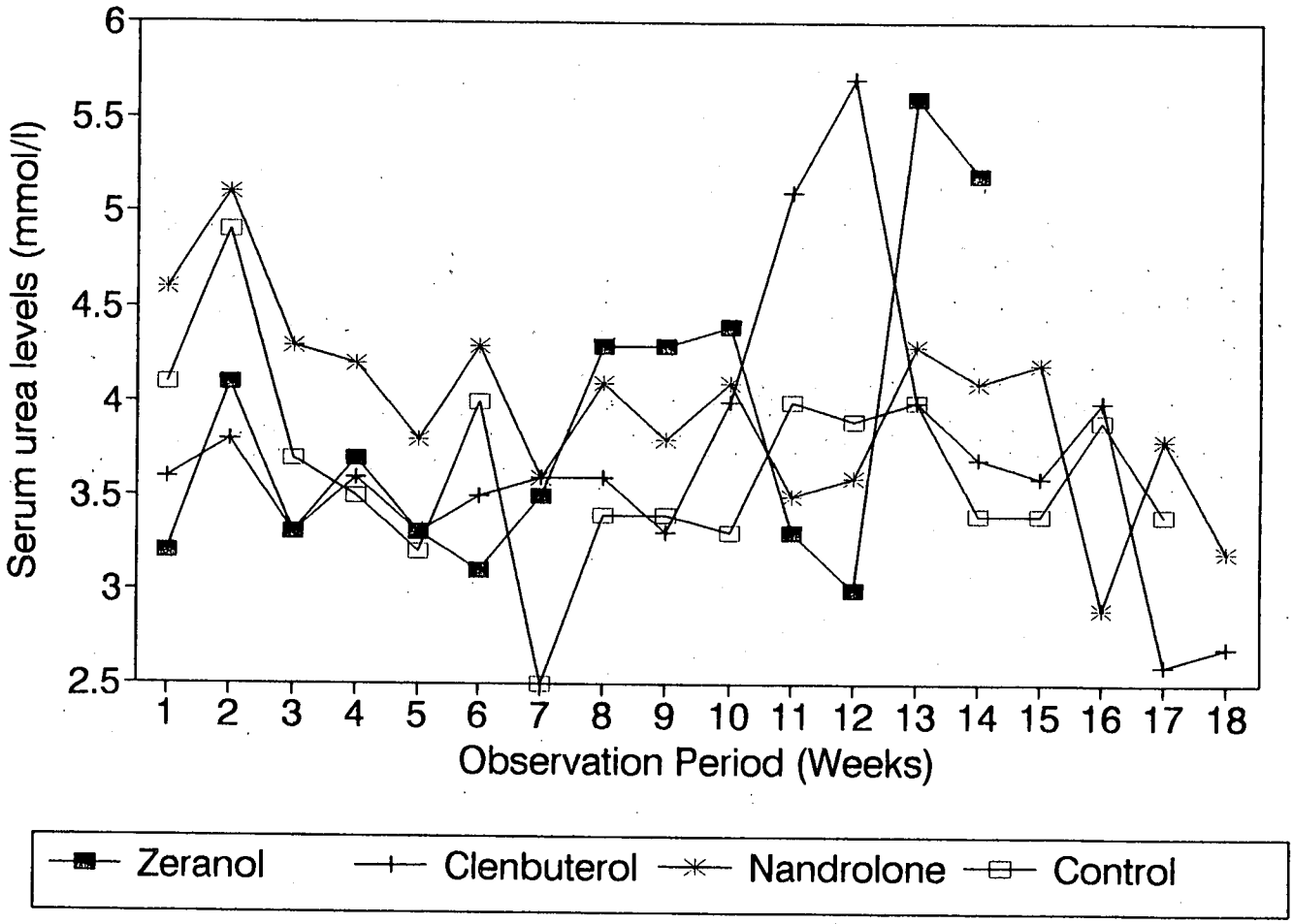

Figure 4.5 Effect of anabolic agent treatments on the serum urea levels in Large White x Landrace gilts.

treatment period (3.6, 3.5 and 3.6 mmol/l) for the same respective groups. In the nandrolone treated group, the contrary was obtained (4.2 mmol/l during Phase 1 and 3.7 mmol/l during Phase 2). A tendency for an increased urea level (not significant) over the entire period was observed in the zeranol treated animals, while in the other three groups, no clear trend was noted. From Table 4.22 it can be seen that there were no significant correlations between serum urea and growth parameters.

4.9 Serum glucose concentrations

The mean (and standard deviation) obtained by assay for serum glucose levels are presented in Table 4.16. A graphic illustration of serum glucose concentrations over the entire treatment (Phase 1) and post-treatment (Phase 2) period is given in Figure 4.6. Correlation coefficients between serum glucose concentrations and the main growth characteristics are shown in Table 4.22. Serum glucose levels decreased on average, from Phase 1 to Phase 2 in all the groups. The greatest decrease was observed in the zeranol treated group (from 5.7 to 4.9 mmol/l) and in the control group (from 5.8 to 5.0 mmol/l). In the clenbuterol and the nandrolone treated animals the decrease was much less (from 5.3 to 5.2 mmol/l and 5.4 to 5.1 mmol/l respectively). The group with the most uniform decrease of serum glucose concentration over time, was the control. The correlation coefficients between glucose levels in the serum and several other parameters (Table 4.22) showed a negative tendency, although none of these were significant.

4.10 Serum creatinine concentrations

The mean serum creatinine concentrations are set out in Table 4.17 and graphically illustrated over the entire period in Figure 4.7. Correlation coefficients between serum creatinine levels and the main growth parameters are set out in Table 4.22. A clear tendency of increased levels over the entire observation period can be seen in all the groups. Greater fluctuations of 53.1 mmol/l (from 99.6 to 152.7 mmol/l) and 51.0 mmol/l (from 96.9 to 147.9 mmol/l) in the mean values for Phase 1 and Phase 2, were obtained in the nandrolone and clenbuterol treated groups. The control group showed a fluctuation of 43.1 mmol/l (from 83.5 to 126.6 mmol/l), and the zeranol a fluctuation of 47.7 mmol/l (from 86.1 to 130.8 mmol/l). No significant correlations were found between creatinine levels in the serum and the parameters shown in the Table 4.22.

4.11 Serum oestradiol concentration

The mean serum oestradiol concentration are presented in Table 4.18. These are graphically illustrated over the treatment and post-treatment period in Figure 4.8. Correlation coefficients between serum oestradiol levels and the main growth characteristics are shown in Table 4.22. Serum oestradiol concentrations over the observation period showed a tendency to increase in all the groups. A dramatic decrease in the mean concentration was observed in weeks 4 and 5 (Phase 1) in all four the groups. In the nandrolone treated gilts, however, a higher average concentration during Phase 1 (6.8 pmol/l) and Phase 2 (8.3 pmol/l), resulted in a bigger difference, (Phase 1 and Phase 2) of 1.5 pmol/l, when compared to that of the zeranol treated gilts

Table 4.16 Mean and standard deviation of serum glucose concentrations (mmol/l), during (Phase 1) and after (Phase 2) treatment of Large White x Landrace gilts with different anabolic agents.

PHASE	WEEK	TREATMENTS							
		ZERANOL		CLENBUTEROL		NANDROLONE		CONTROL	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
1	1	6.7	0.7	5.9	0.7	5.9	0.6	6.5	0.6
	2	6.0	0.3	5.6	0.3	5.4	0.3	5.9	0.9
	3	6.3	0.4	5.8	0.3	5.8	0.5	6.0	0.5
	4	5.4	0.3	5.3	0.3	5.3	0.2	5.6	0.2
	5	5.5	0.3	4.5	0.3	5.2	0.2	5.3	0.2
	6	5.7	0.6	5.9	0.7	5.4	0.4	5.9	0.5
	7	5.5	0.3	4.7	0.2	5.6	0.5	5.6	0.8
	8	5.2	0.5	5.0	0.3	4.8	0.4	5.6	0.2
	9	4.9	0.4	4.9	0.6	5.0	0.3	5.4	0.5
2	10	4.9	0.3	4.9	0.32	5.1	0.6	5.3	0.3
	11	4.9	0.4	4.7	0.3	5.3	0.1	5.0	0.3
	12	4.5	0.2	4.6	0.5	5.1	0.2	4.9	0.5
	13	5.1	0.3	5.1	0.3	5.1	0.3	5.2	0.3
	14	5.2	0.6	5.4	0.5	5.3	0.5	4.8	0.4
	15	*	*	5.7	0.4	5.4	0.3	5.5	0.1
	16	*	*	5.2	0.2	4.5	0.0	4.6	0.1
	17	*	*	4.6	0.0	4.7	0.0	5.0	0.0
	18	*	*	6.6	0.0	5.5	0.0	*	*
	Phase 1	5.7	0.5	5.3	0.5	5.4	0.3	5.8	0.3
	Phase 2	4.9	0.3	5.2	0.6	5.1	0.3	5.0	0.3
	Total	**5.4**	**0.5**	**5.2**	**0.6**	**5.2**	**0.3**	**5.4**	**0.5**

* Missing data due to attainment of target liveweight

No significant differences between groups for the respective phases

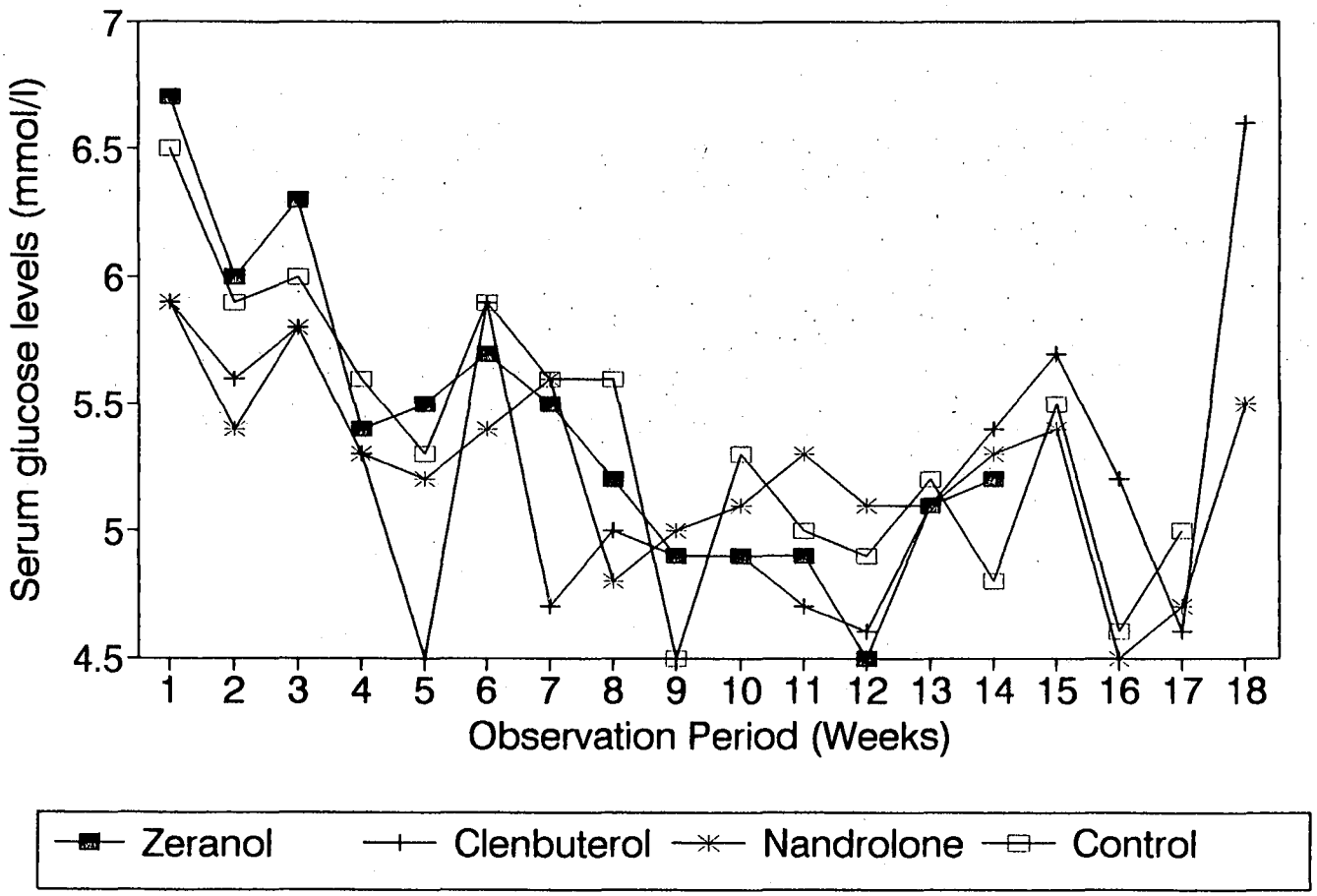

Figure 4.6 Effect of anabolic agent treatments on the serum glucose concentrations in Large White x Landrace gilts

Table 4.17 Mean and standard deviation of serum creatinine concentrations (mmol/l) during (Phase 1) and after (Phase 2) treatment of Large White x Landrace gilts with different anabolic agents.

PHASE	WEEK	TREATMENTS							
		ZERANOL		CLENBUTEROL		NANDROLONE		CONTROL	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
1	1	70.0	6.4	76.5	5.9	89.0	12.6	74.8	14.9
	2	80.5	11.7	85.0	8.1	86.5	2.9	69.8	13.8
	3	72.8	6.1	72.5	16.0	89.5	10.6	66.5	7.4
	4	82.3	7.4	80.8	20.3	93.5	7.8	83.0	4.2
	5	83.5	4.3	92.5	16.9	90.0	10.6	80.5	6.6
	6	91.0	6.1	114.5	14.4	102.3	23.5	89.8	15.8
	7	86.5	4.3	105.8	8.8	105.5	6.9	85.5	14.0
	8	104.3	8.3	119.0	8.6	116.3	7.3	102.3	14.8
	9	103.8	5.1	125.5	9.4	123.5	6.7	99.5	18.5
2	10	120.0	8.3	135.3	16.9	116.0	30.7	111.5	10.7
	11	122.5	7.4	135.8	18.8	135.8	5.0	112.8	14.0
	12	135.0	16.8	134.0	13.1	142.0	5.6	127.0	3.7
	13	129.0	9.1	125.3	12.6	145.0	10.6	121.5	7.9
	14	147.7	13.3	136.7	6.1	149.0	8.8	128.8	16.8
	15	*	*	144.3	8.2	158.5	8.0	129.3	7.5
	16	*	*	157.0	9.0	149.7	12.8	135.5	8.5
	17	*	*	178.0	0.0	195.0	0.0	146.0	0.0
	18	*	*	185.0	0.0	183.0	0.0	*	*
	Phase 1	86.1	11.3	96.9	18.6	99.6	12.5	83.5	11.6
	Phase 2	130.8	9.9	147.9	19.8	152.7	22.5	126.6	10.7
	Total	**102.1**	**24.0**	**122.4**	**31.9**	**126.1**	**32.2**	**103.8**	**24.2**

* Missing data due to attainment of target liveweight

No significant differences between groups for the respective phases

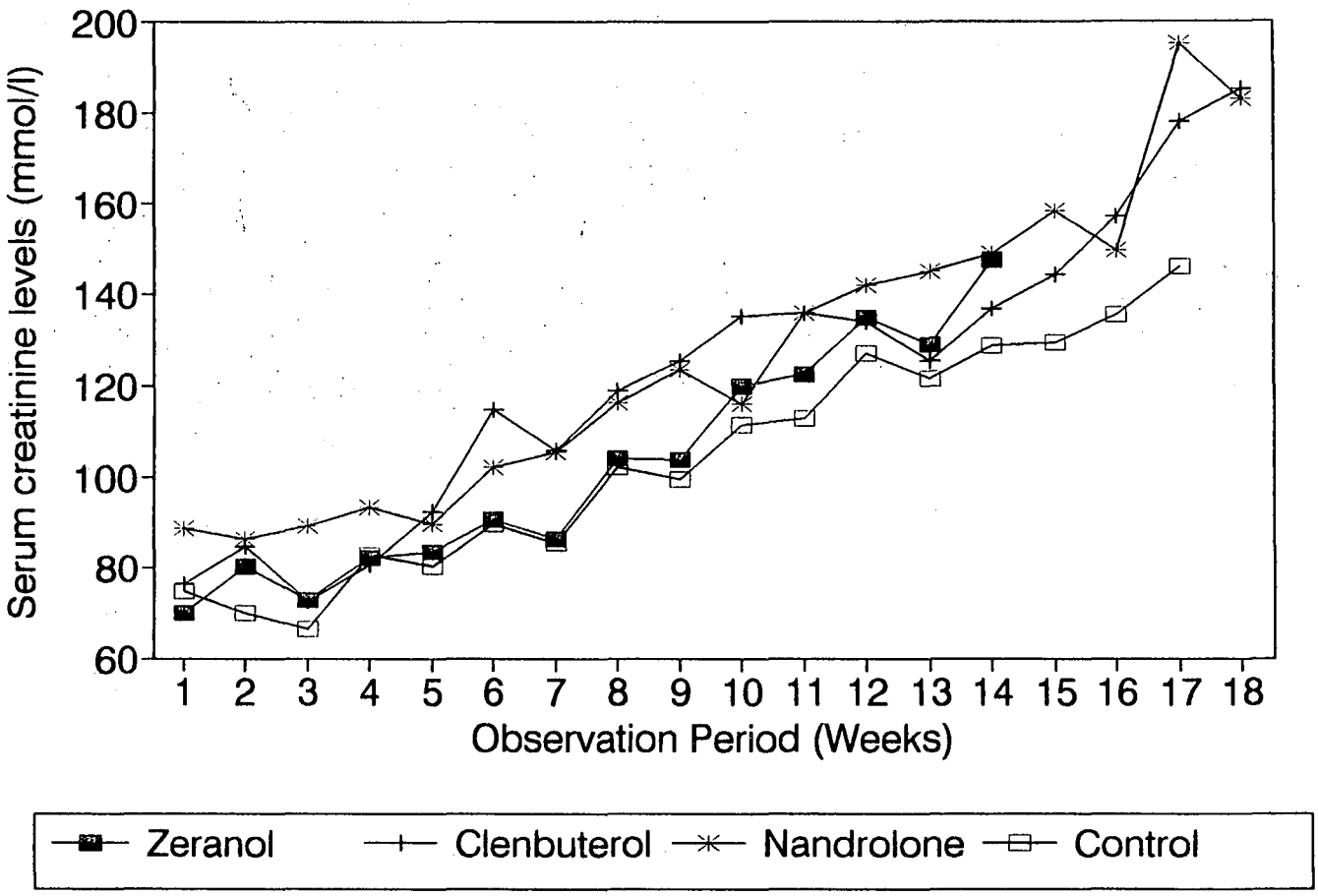

Figure 4.7 Effect of anabolic agent treatments on the serum creatinine concentrations in Large White x Landrace gilts

(0.1 pmol/l; 4.3 and 4.4 pmol/l in Phase 1 and Phase 2 respectively). Relatively moderate increases were seen in the clenbuterol treated animals (0.7 pmol/l; 5.1 and 5.8 pmol/l in Phase 1 and Phase 2 respectively) and the control group (0.4 pmol/l; 5.1 and 5.5 pmol/l in Phase 1 and Phase 2 respectively). No significant correlations were observed between serum oestradiol concentration and the growth parameters as set out in Table 4.22.

4.12 Hematocrit

The mean over the entire observation period for hematocrit values are set out in Table 4.19 and graphically illustrated over the treatment and post-treatment period in Figure 4.9. The readings of the first week (Phase 1) were shown to be lower than any other means in all the four groups (Figure 4.9). An increase in the hematocrit from Phase 1 to Phase 2 were observed in all the groups (not significant). The mean values obtained during the same period were relatively similar (34.6%, 33.5%, 34.3% and 35.7% for zeranol, clenbuterol, nandrolone and control groups respectively, during Phase 1; and 37.8% 37.8%, 36.4% and 39.2% during Phase 2). Within the same phase, the differences between groups were negligible. No significant correlation was observed between the hematocrit and any of the other parameters presented in Table 4.22.

4.13 Anabolic agent excretion rate

4.13.1 Zeranol

Table 4.20 sets out the concentrations of zeranol and it metabolites in urine samples collected from zeranol implanted gilts, and determined during three critical times. Mean peak concentrations (32.8 ng/ml) were obtained at day 6 after implantation, followed by a slow excretion rate from day 12 (12.7 ng/ml) to day 19 (11.7 ng/ml). An illustration indicating the excretion rate is set out in Figure 4.10.

4.13.2 Clenbuterol

Clenbuterol and it metabolite concentrations in urine samples taken from clenbuterol treated gilts are presented in Table 4.21. The mean and standard deviation were determined from the known concentrations of the individual animals, and non-detectable levels were ignored. A mean peak concentration (58.0 ng/ml) was obtained at day 2 after the last oral dose, followed by a relatively rapid reduction in concentration observed by day 6 (44.3 ng/ml) and day 10 (9.5 ng/ml).

Table 4.18 Mean and standard deviation of serum oestradiol concentrations (pmol/l) during (Phase 1) and after (Phase 2) treatment of Large White x Landrace gilts with different anabolic agents.

PHASE	WEEK	TREATMENTS							
		ZERANOL		CLENBUTEROL		NANDROLONE		CONTROL	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
1	1	5.8	1.4	7.4	1.0	7.8	3.3	5.5	1.8
	2	4.6	1.2	6.2	0.8	10.7	1.3	6.4	1.7
	3	3.0	2.9	1.5	2.4	5.7	3.7	3.9	3.8
	4	0.2	0.1	0.2	0.1	0.4	0.2	0.2	0.1
	5	0.4	0.2	0.3	0.1	0.9	0.2	0.5	0.1
	6	7.3	2.6	12.4	9.0	12.2	4.0	8.8	1.2
	7	7.7	2.8	7.4	3.3	9.3	3.7	9.6	2.3
	8	5.6	2.8	5.3	2.4	8.7	1.7	6.4	3.1
	9	4.3	1.2	5.1	1.9	5.8	1.3	4.4	0.9
2	10	4.4	1.3	6.2	5.4	7.0	1.4	4.1	1.1
	11	4.8	0.9	6.2	2.4	6.9	1.9	5.7	0.7
	12	4.3	1.0	4.3	1.5	5.7	1.7	4.1	1.0
	13	4.1	1.6	5.1	2.1	5.9	1.7	4.4	0.3
	14	4.5	0.8	5.9	2.5	6.0	0.8	5.2	1.6
	15	*	*	6.5	0.9	7.0	1.3	5.9	1.3
	16	*	*	6.1	1.1	7.6	1.9	6.8	0.1
	17	*	*	6.0	0.0	13.5	0.0	7.6	0.0
	18	*	*	5.9	0.0	12.5	0.0	*	*
	Phase 1	4.3	2.5	5.1	3.7	6.8	3.8	5.1	3.1
	Phase 2	4.4	0.2	5.8	0.6	8.3	2.8	5.5	1.2
	Total	**4.4**	**2.0**	**5.4**	**2.7**	**7.4**	**3.4**	**5.3**	**2.4**

* Missing data due to attainment of target liveweight

No significant differences for the groups in the respective phases

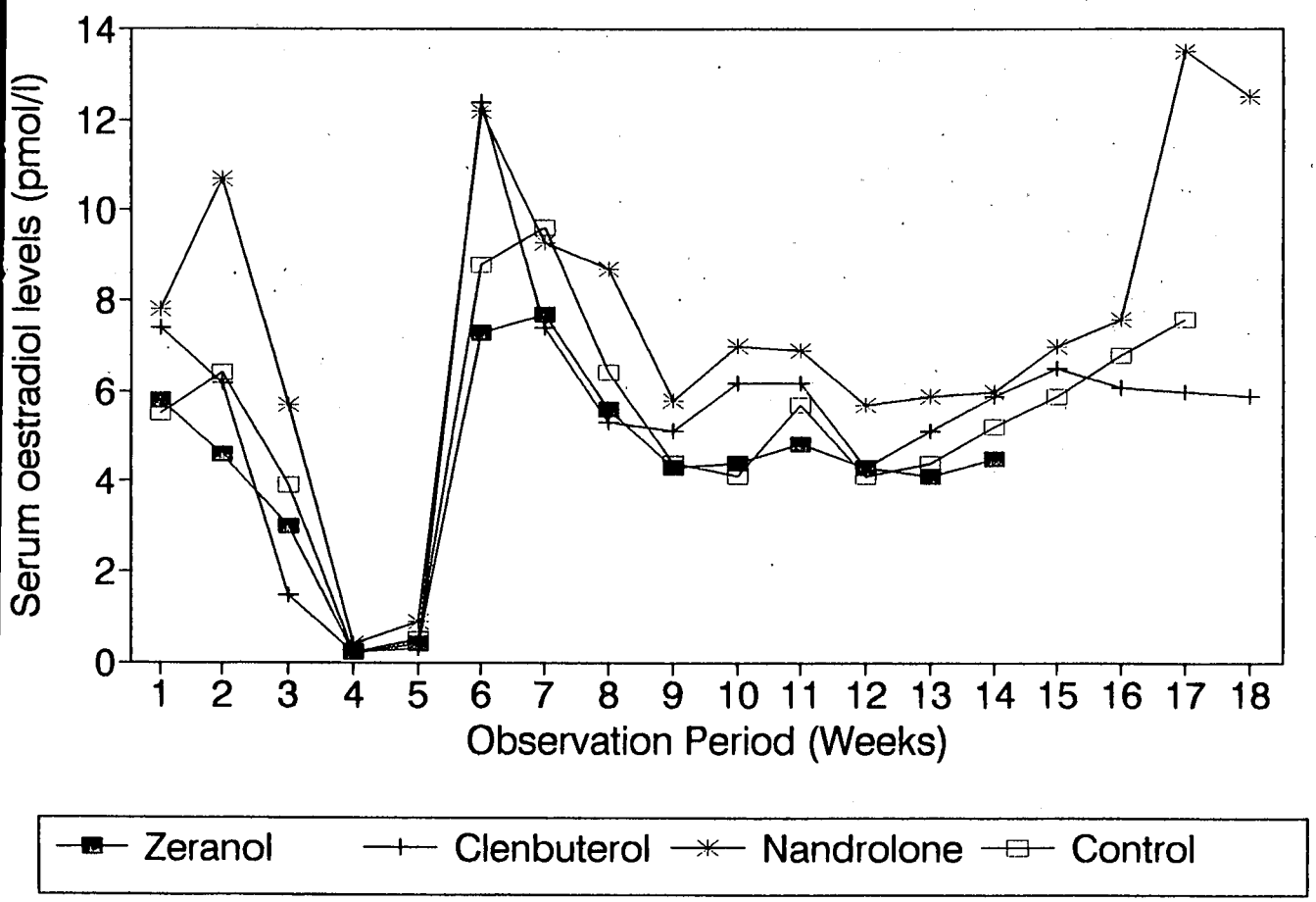

Figure 4.8 Effect of anabolic agent treatments on the serum oestradiol levels in Large White x Landrace gilts

Table 4.19 Mean and standard deviation of hematocrit (%) of Large White x Landrace gilts during (Phase 1) and after (Phase 2) treatment with different anabolic agents.

PHASE	WEEK	TREATMENTS							
		ZERANOL		CLENBUTEROL		NANDROLONE		CONTROL	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
1	1	23.0	2.3	23.0	4.9	27.5	10.1	26.8	9.1
	2	35.6	1.9	33.3	3.4	32.5	3.6	31.0	5.2
	3	35.0	0.5	32.4	3.3	36.3	1.1	36.1	1.3
	4	34.5	1.5	33.3	1.5	35.0	2.0	36.9	1.9
	5	36.9	0.9	34.6	1.8	34.1	1.3	36.6	1.6
	6	36.1	1.4	36.5	1.8	34.4	4.0	37.4	2.3
	7	34.4	1.9	36.9	2.1	35.1	3.9	36.6	2.9
	8	37.6	4.2	33.6	2.6	36.5	1.8	39.3	1.3
	9	38.4	1.8	37.6	2.7	36.9	0.9	40.6	1.3
2	10	37.3	3.9	36.4	1.7	32.6	1.8	38.4	0.7
	11	37.6	2.6	36.1	5.0	36.5	2.1	39.3	1.8
	12	38.9	2.9	37.8	2.9	38.0	1.5	40.1	1.3
	13	38.4	2.0	38.0	1.3	36.9	1.7	38.8	1.1
	14	36.7	1.6	38.5	0.8	36.4	2.0	40.5	1.8
	15	*	*	38.8	1.0	36.3	1.4	39.4	1.3
	16	*	*	41.5	2.0	39.2	0.8	41.3	0.3
	17	*	*	35.5	0.0	36.5	0.0	36.0	0.0
	18	*	*	37.5	0.0	35.5	0.0	*	*
	Phase 1	34.6	4.3	33.5	4.1	34.3	2.7	35.7	4.0
	Phase 2	37.8	0.8	37.8	1.7	36.4	1.7	39.2	1.5
	Total	**35.7**	**3.8**	**35.6**	**3.8**	**35.3**	**2.5**	**37.4**	**3.6**

* Missing data due to attainment of target liveweight.
No significant differences for the groups during the respective phases

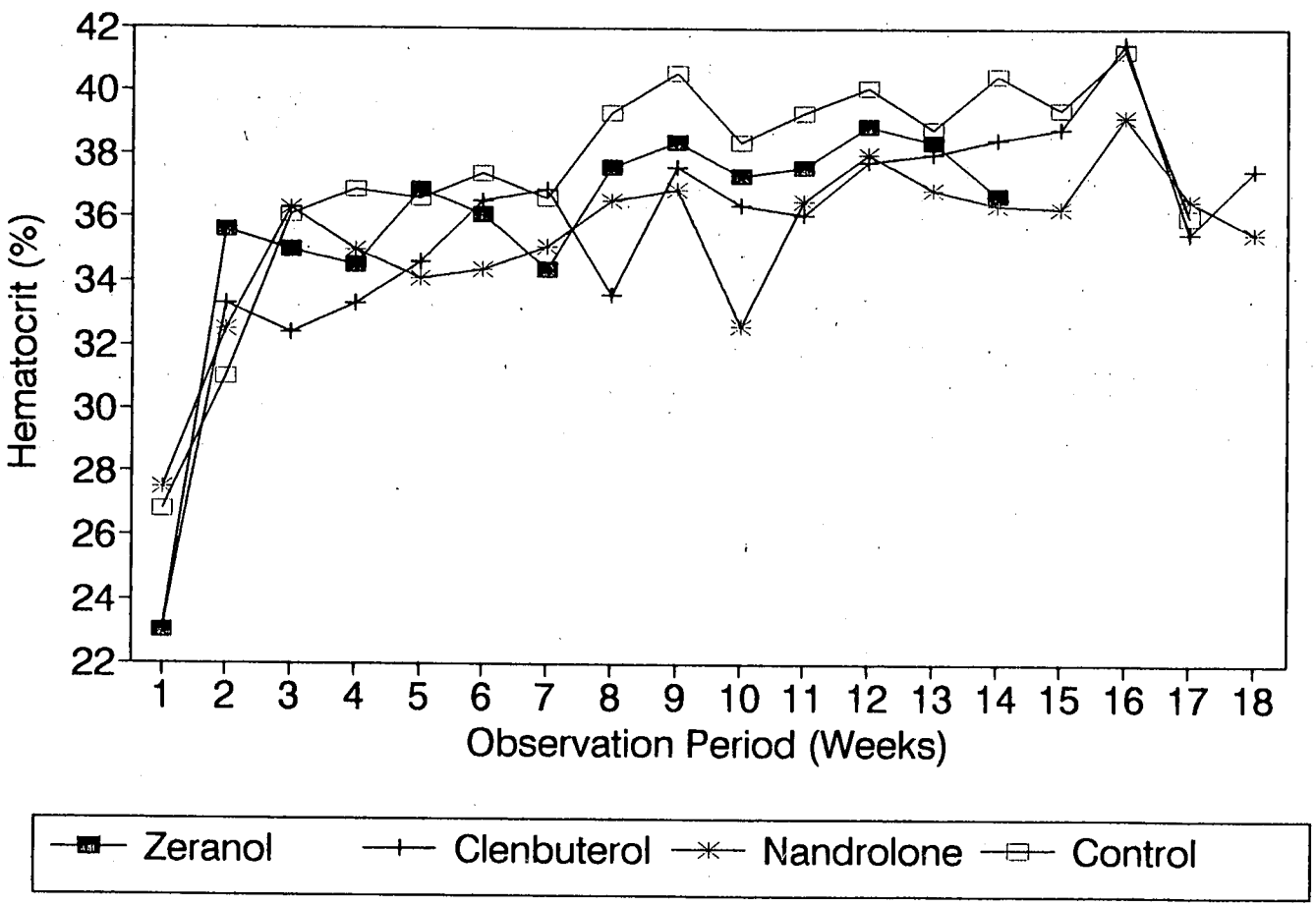

Figure 4.9 Effect of anabolic agent treatments on the hematocrit in Large White x Landrace gilts

Table 4.20 Zeranol and it metabolites concentrations in urine samples collected from Large White x Landrace gilts after subcutaneous implantation with 36 mg zeranol

ANIMAL NUMBER	ZERANOL CONCENTRATIONS (ng/ml) OVER TIME (days))			
	DAY 0	DAY 6	DAY 12	DAY 19
9	20.0	27.0	24.0	19.0
10	21.0	22.0	6.0	7.0
13	20.0	53.0	9.0	8.0
15	13.0	46.0	16.0	1.0
25	18.0	13.0	7.0	12.0
27	30.0	36.0	14.0	23.0
Mean	20.3	32.8	12.7	11.7
SD	5.1	13.8	6.2	7.4

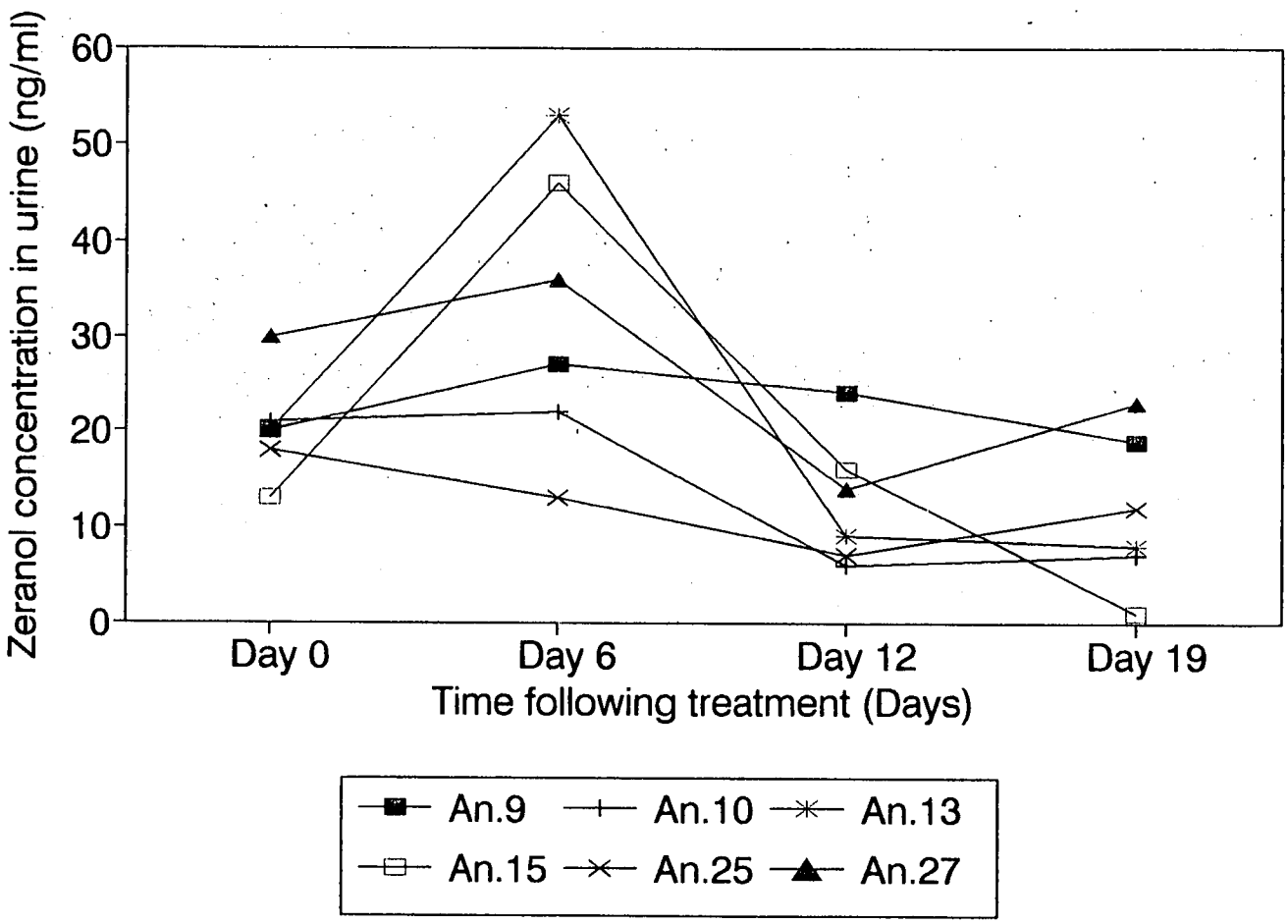

Figure 4.10 Zeranol and it metabolites excretion rate for individual gilts following zeranol implantation

Table 4.21 Clenbuterol and it metabolites concentrations in urine samples collected from Large White x Landrace gilts after an oral administration of 0.5mg clenbuterol

ANIMAL NUMBER	CLENBUTEROL CONCENTRATION (ng/ml) OVER TIME (days)		
	DAY 2	DAY 6	DAY 10
3	37.0	70.0	8.0
16	ND	ND	ND
17	ND	ND	ND
18	29.0	ND	ND
21	133.0	54.0	11.0
22	33.0	9.0	ND
Mean	58.0	44.3	9.5
SD	43.4	25.8	1.5

ND = Not detectable.

Table 4.22 Correlation coefficients between growth traits, carcass and meat characteristics, organ weights and serum, glucose, urea, creatinine, oestradiol concentrations and the hematocrit for gilts.

	VARIABLE	VARIABLE NUMBER																
	VARIABLE	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1	Hematocrit																	
2	Serum glucose	-0.28																
3	Serum urea	0.27	* -0.53															
4	Serum creatinine	0.09	0.24	-0.28														
5	Serum oestradiol	-0.02	-0.10	-0.19	0.28													
6	Weight gain	0.30	-0.46	0.13	-0.18	-0.05												
7	Total feed intake	0.21	-0.46	* 0.62	-0.43	-0.22	0.25											
8	Feed conversion rate	0.11	-0.33	* 0.60	-0.40	-0.22	-0.07	** 0.95										
9	Age at slaughter	-0.28	0.51	** -0.68	** 0.62	0.40	-0.21	** -0.85	** -0.82									
10	Average daily feed intake	0.20	-0.45	** 0.68	* -0.54	-0.31	0.17	** 0.96	** 0.94	** -0.94								
11	Average daily gain	0.30	* -0.56	** 0.67	** 0.63	-0.36	0.44	** 0.86	** 0.75	** -0.96	** 0.92							
12	% of Muscle in carcass	0.07	-0.20	0.29	0.48	-0.11	-0.21	** -0.74	** -0.69	* 0.58	** -0.70	** -0.62						
13	% of Fat in carcass	-0.07	0.36	-0.24	-0.43	-0.09	0.02	** 0.73	** 0.73	** -0.61	** 0.74	* 0.61	** -0.89					
14	Muscle pH (24h)	-0.30	0.10	0.09	0.49	0.24	0.33	** -0.55	** -0.68	* 0.59	** -0.68	* -0.52	0.35	* -0.53				
15	Ovary weight	-0.19	0.17	** -0.62	-0.14	0.38	-0.16	** -0.74	** -0.72	* 0.58	** -0.69	* -0.59	0.38	-0.38	0.28			
16	Uterus weight	-0.08	-0.08	0.50	0.18	0.10	-0.03	0.16	0.16	-0.06	0.15	0.08	-0.41	0.41	0.02	-0.27		
17	Kidney weight	0.25	-0.15	0.36	-0.23	-0.13	0.33	0.30	0.16	* -0.61	0.44	* 0.56	-0.23	0.25	-0.28	-0.37	0.30	

* P<0.05
** P<0.01

Table 4.23 Correlation coefficients between carcass parameters, carcass conformation indicators, meat quality parameters and organ weights, for gilts in all four groups.

		VARIABLE NUMBER														
	VARIABLE	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	Final liveweight															
2	Carcass warm weight	0.44														
3	Carcass cold weight	0.58*	0.95**													
4	Ham length	0.16	-0.45	-0.32												
5	Ham circumference	0.05	0.56*	0.52*	-0.62*											
6	Carcass length	0.25	-0.23	-0.20	0.45	-0.60*										
7	Chest depth	0.59*	0.64**	0.64**	-0.22	0.32	0.03									
8	Chest diameter	0.62*	0.55*	0.56*	-0.22	0.34	-0.18	0.75**								
9	Tharax depth	-0.38	-0.12	-0.18	-0.05	-0.15	-0.24	-0.40	-0.30							
10	Backfat thickness	0.46	0.15	0.18	-0.12	0.94	0.30	0.03	0.34	0.24						
11	Eye Muscle area	0.08	0.56*	0.49	-0.30	0.74**	-0.03	-0.03	0.40	-0.14	0.00					
12	Cooking loss	-0.09	0.14	0.09	-0.53*	0.11	-0.12	0.24	0.02	-0.47	-0.15	0.04				
13	Water holding capacity	0.00	-0.09	-0.02	0.18	-0.13	-0.17	-0.41	-0.34	0.38	0.43	-0.11	-0.40			
14	Liver weight	0.01	-0.17	-0.24	0.17	0.30	0.58*	-0.32	-0.16	0.21	0.14	-0.53*	-0.29	-0.04		
15	Heart weight	0.12	0.02	-0.05	0.20	-0.21	0.61*	0.19	-0.07	-0.39	-0.44	0.06	0.08	-0.17	0.09	

* $P<0.05$

** $P<0.01$

Table 4.24 Correlation coefficients between growth traits, organ weights and carcass composition, for gilts in all four groups.

	VARIABLE	VARIABLE NUMBER									
		1	2	3	4	5	6	7	8	9	10
1	Average daily gain										
2	Age at slaughter	-0.96**									
3	Average daily feed intake	0.92**	-0.94**								
4	Feed conversion rate	0.75**	-0.82**	0.75**							
5	Spleen weight	-0.64**	0.66**	-0.51*	-0.39						
6	Kidney weight	0.56*	-0.61*	0.44	0.31	-0.58*					
7	Lung weight	0.09	-0.03	-0.10	-0.21	-0.27	-0.03				
8	% of Fat in carcass	0.61*	-0.61*	0.74**	0.73**	-0.36	0.25	-0.35			
9	% of Skin in carcass	-0.19	0.29	-0.34	-0.40	0.01	-0.37	0.57*	-0.35		
10	% of Bone in carcass	-0.35	0.39	-0.45	-0.45	0.16	-0.14	0.22	-0.77**	0.28	

* $P<0.05$
** $P<0.01$

Table 4.25 Correlation coefficients between gastrointestinal tract and other visceral organ weights, and growth traits and carcass characteristics, for gilts in all four groups.

		VARIABLE NUMBER													
	VARIABLE	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	Empty intestine weight														
2	Empty stomach weight	-0.06													
3	Liver weight	0.29	0.16												
4	Heart weight	-0.06	-0.09	-0.15											
5	Lungs weight	-0.07	-0.10	-0.07	-0.24										
6	Spleen weight	-0.11	0.10	0.00	-0.02	-0.15									
7	Kidneys weight	-0.09	-0.21	0.32	0.14	-0.17	-0.45*								
8	Final liveweight	0.23	0.08	0.10	0.04	0.02	0.17	-0.42*							
9	Average daily feed intake	0.43*	0.18	0.44*	-0.09	-0.06	-0.24	0.19	0.29						
10	Age at slaughter	-0.29	0.15	-0.63**	0.20	-0.03	0.66*	-0.60**	0.04	-0.94**					
11	Feed conversion rate	-0.19	0.44*	-0.10	0.02	-0.27	0.32	-0.02	-0.20	0.19	0.10				
12	Average daily gain	0.45*	-0.21	0.40	-0.05	0.15	-0.42*	0.18	0.39	0.63**	-0.96**	-0.64**			
13	% of Lean muscle in carcass	-0.36	0.01	-0.43*	0.03	0.33	0.26	-0.31	-0.17	-0.77**	-0.72**	0.00	-0.59**		
14	% of Fat in carcass	0.31	0.05	0.29	-0.09	-0.29	-0.23	0.34	0.10	0.82**	0.67**	0.08	0.57**	-0.90**	

* $P<0.05$

** $P<0.01$

CHAPTER 5

DISCUSSION

5.1 Growth traits and feed conversion rates

There was no significant difference in the overall weight gain between the four groups (Table 4.1 and 4.2) because an uniform initial and final liveweight was targeted in the experimental design. The possible differences in growth rates should be noted in the age at which the gilts reached this target liveweight of 85 kg. Indeed, a faster growth rate was achieved by the group treated with zeranol implants (147.3 days to the final liveweight of 85 kg) compared to the control (160 days), and nandrolone and clenbuterol groups (164.0 and 163.8 days respectively). This growth rate was slower, when compared to those reported by Siebrits (1993) (130 and 133 days in Large White and Landrace entire boars, respectively) achieved with no anabolic agent treatment.

A linear comparison may be misleading in the analysis of these results, as there are at least three variable factors (crossbreeding, sex and treatment with anabolic agents) that play a role in determining growth rate. It is known that gilts generally grow slower than boars (Lindsay, 1983; Whittemore, 1993). A significantly improved overall growth rate (Tables 4.1 and 4.2) was observed after the administration of zeranol implants (727 g/d), but the treatment with clenbuterol and nandrolone resulted in a lower ADG than the control (636 g/d). A lower ADG has been published by Sheridan *et al* (1990) (588 to 682 g/d) after the application of different combinations and dosages of anabolic agents in gilts. Bracher-Jakob and Blum (1990), working with the β-agonist Ro 16-8714, obtained an increase in ADG (927 g/d) which is far higher than the results (602 g/d) obtained in this trail, using the β-agonist clenbuterol. On the other hand, Brennan and Joyce (1979) who used antibiotics as growth promoters, obtained an ADG of 577 g/d to 592 g/d.

The ADG of the zeranol treated group was higher during Phase 1, period in which the animals attained about 76% of the target final liveweight (Table 4.2). This effect is in agreement with Whittemore (1993) and is typical of fast growing animals. In the clenbuterol and nandrolone treated groups, the opposite phenomenon tended to occur, Phase 2 having a higher ADG than Phase 1. In both these groups, about 37% of the final liveweight was realised during Phase 2. There was no significant difference between the groups regarding the FCR, although the control group had a better average (2.76 kg feed/kg gain), when compared to the nandrolone treated group (2.88 kg feed/kg gain) which had the highest mean FCR of all the groups. This indicates that the faster growth rate observed in the zeranol treated group was not the result of a higher efficiency in the feed utilisation. Sheridan *et al* (1990) found an improved FCR (2.53 kg feed/kg gain) in trenbolone acetate implanted gilts, while Mitchell *et al* (1990), using the β-agonist ractopamine added in the feed, obtained a FCR of 3.58 kg feed/kg gain. Similar results

(3.52 kg feed/kg gain) were reported by Brennan and Joyce (1979) following the use of antibiotics at a growth promoting level in pigs.

In all four treatment groups, as the animals were growing older (from Phase 1 to Phase 2) and as could be expected, their FCR became higher. This observation is in agreement with older animals being less efficient in converting feed into liveweight gain (McMeekan, 1959; Whittemore, 1993). As no significant differences were observed between the groups in the feed conversion rate (FCR), and considering the fixed targeted final liveweight not being significantly different between the groups, no significant difference in the overall amount of feed consumed throughout the experiment was expected (Tables 4.1 and 4.2). The only possible difference was as to how much the animals could eat on average per day. There was in fact a significant difference ($P<0.05$) in the average daily intake (ADI) between the different treatment groups. The highest mean was observed in the treatment with zeranol (2.03 kg/d), with a corresponding better ADG (727 g/d), and thus, a faster growth (147.3 days to the mean final liveweight of 85 kg). Possible side effects observed in the clenbuterol group were hypersensitivity (signs of nervousness), the danger of rectal prolapses, the tendency to develop arthritis (signs of limping/crippling) and an occasional loss of appetite. This could have affected the ADI and therefore the growth rate (163.8 days to the mean final liveweight) in this group. The nandrolone group, having a slightly lower ADI (1.76 kg/d) and ADG (615 g/d), compared to the control group (1.75 kg/d ADI; 640 g/d ADG), took 160 days to achieve the final liveweight of 85 kg.

Dunshea *et al.* (1993) reported no marked changes in behaviour of gilts treated orally with ractopamine, while Lindsay *et al.* (1993) reported a decrease in ADI of animals treated with β-agonists. According to Cole and Chadd (1989), a rapid growth in pigs may be limited by the lack of appetite. Generally for all four groups the ADI and the FCR were higher during Phase 2 (post-treatment period), which could be an interaction of the cessation of anabolic agent treatment and the fact that a higher body weight demands a higher feed intake for maintenance and growth. This phenomenon is confirmed that as an animal grows, its efficiency in feed utilisation decreases, (Lindsay *et al.* 1993; Whittemore, 1993). An ADI of 2.5 kg/d to 3.5 kg/d, and ADG of 590 g/d was reported by Yen *et al.* (1991), after the addition of ractopamine (β-agonist) to pig diets, while Hanrahan (1989), using somatotropine as a growth promoter for boars, obtained 2.5 kg/d ADI and a corresponding ADG of 801 g/d. He *et al* (1994) reported a decrease in ADI with no altered ADG following the use of porcine somatotropine in gilts.

5.2 Ultrasonic measurements of P_2 and eye muscle diameter

No significant differences between the four groups were found in the mean values obtained from weekly measurements of the backfat thickness (P_2) using ultra-sonography (Tables 4.1, 4.3 and 4.4). Generally the values obtained for weekly fat deposition at the level of the P_2 site (2.4 mm, 1.07 mm, 1.09 mm and 1.26 mm for zeranol, clenbuterol, nandrolone and the control groups respectively), were higher than those obtained by Zhang *et al* (1993) from serial

ultrasonic measurements, where a mean of 0.64 mm/week was recorded. According to Kallweit (1991), the difficulty in taking ultrasonic measurements successfully from the same anatomical place as the animal grows could possibly be a limitation for this technique in the accurate measuring for backfat thickness.

Eye muscle diameter, measured weekly (Tables 4.1, 4.3 and 4.5) using an ultrasonic apparatus at the site of the P_2 measurement, indicated significantly higher eye muscle values for average weekly protein deposition in the control (4.43 mm/week), than in the clenbuterol treated group. These control group measurements were not significantly higher than those in the zeranol and the nandrolone treated groups. The values were not consistent throughout the time and showed much variations. No similar measurements over time were found in the literature. Martinez *et al* (1992) reported a mean eye muscle diameter of 66 mm by physical measurements on the carcasses of gilts slaughtered at 95 kg liveweight, following an implantation with trenbolone acetate. These results are in agreement to the considered limits described by Fisher (1992). Fisher (1992) reported that subjective interpretation of scans are necessary to quantify the reflection information and the fact that the determination of the diameter of the *M.Longissimus dorsi* is less successful than that of backfat thickness.

5.3 Carcass characteristics and parameters

A positive effect in carcass weight (68.8 kg cold carcass weight) and dressing percentage (79.8%) was obtained after the application of zeranol implants in gilts (Table 4.6). Brennan and Joyce (1979), who used antibiotics as growth promoters, reported a 74% dressing percentage in gilts. The use of trenbolone acetate in gilts slaughtered at weights over 100 kg was reported by Martinez *et al* (1992) as yielding a dressing percentage of 78% for the cold carcass weight. Bonneau (1991) reported a general decrease in dressing percentage following the use of porcine somatotropine, the same as that reported by Hanrahan (1989), who obtained a dressing percentage of 76%.

From the physical measurements of backfat thickness at several sites, a significant ($P<0.05$) increase in backfat thickness in the group treated with zeranol implants was recorded. The higher value being that measured (Table 4.6) at the site of the tenth rib (21.6 mm) and the lowest at the P_1 site (13.5 mm). Lower backfat thickness were obtained by treating gilts with the β-agonist, clenbuterol. P_2 values, the most commonly referred to in the literature, was also observed to be high in the zeranol group (14.8 mm) and lower in the clenbuterol group (7.4 mm), which indicates that this latter treatment yielded leaner carcasses. Backfat thickness (P_2 = 19.5 mm) was not reduced in carcasses from pigs treated with antibiotics as a stimulant at growth promoting levels (Brennan & Joyce, 1979). Martinez *et al.* (1992) reported that trenbolone acetate implants did not decrease the P_2 values in gilts (19.0 mm), but did reduce it in barrows (17 mm). Dunshea *et al.* (1993) reported a decrease in these P_2 values, as a direct effect of an increase in dietary protein following treatment with the β-agonist ractopamine.

No significant differences in this trial were found in the mean values of measurements of eye muscle area (as a possible indicator of protein deposition) between the groups. The mean area of the nandrolone treated animals was higher (34.3 cm^2), compared to the zeranol group (30.6 cm^2), where the smallest eye muscle area was obtained. Yen *et al.* (1990) reported increases in the eye muscle area (33.4 cm^2) in pigs treated with the β-agonist cimaterol. Average values of 34 cm^2 in the eye muscle area in gilts slaughtered at the mean weight of 80 kg were reported by Sheridan *et al.* (1990) following treatment with trenbolone acetate. The eye muscle values obtained in this trial thus compare favourably with those found in the literature. Interesting is the relatively high positive correlation ($P<0.05$; $r = 0.56$) found between eye muscle area and carcass mass.

5.4 Carcass composition

Before further consideration is given to the results obtained regarding carcass composition (Table 4.7 and Figure 4.4), it should be noted that there are a number of techniques available to predict or physically determine carcass composition. Their common limitations are usually referred to as being either time consuming, or having a limited repeatability, or lacking accuracy in predicting composition, or a combination of all these aspects. Estimation of lean muscle and fat content in carcasses have also been made by regression equations (Edwards *et al.* 1981; Fahey *et al.* 1977); by non-invasive methods such as X-ray, ultrasound B-scan (Kallweit, 1991; Fisher, 1992), as well as dissection and cutting methods (Kauffman & Warner, 1993; Naudé, 1974). It seems that an ideal method of determining carcass composition does not exist as such. Depending on the aim of the carcass composition determination, the method to be selected should consider aspects such as economics, time, repeatability, the level of accuracy and consumers' safety. The method of three rib cuts described by Naudé (1974) was repeatable to enable comparisons to be carried out and to show possible differences in carcass composition between the groups.

Lean muscle (or protein) content in the carcass showed a significant ($P<0.05$) difference between the zeranol treated animals (54.1%) and the clenbuterol group (60.5%). The nandrolone group and the control did not differ from the other two groups. No significant differences between the groups were found in respect to bone, fat and skin percentages of the carcasses although the zeranol treated group deposited more fat (mean 28.3%).

The yield of a higher lean content observed after the treatment of gilts with clenbuterol is in a agreement with the enhanced efficiency of nitrogen retention brought about by the application of β-agonists reported by Bracher-Jakob and Blum (1990). An increased protein deposition in the carcass was also reported by Mitchell *et al.* (1991), and Lindsay *et al.* (1993) following the addition of β-agonists in pig diets.. Yen *et al.* (1991) reported obtaining 66.7% lean muscle and 21.2% fat and 12.1% bone in carcasses from pigs treated with the β-agonist, ractopamine which is slightly less than that obtained with clenbuterol.

Ratios of 6%; 58.7%; 11.9% and 10.1% for skin, lean muscle, fat and bone respectively, in pigs treated with recombinant porcine somatotropine were reported by Hanrahan (1989). A ratio of 62.9% for lean muscle was shown to be achievable in lean carcasses (Wood *et al.* 1981) From the results in this trial the mean of percentage bone and skin in the carcass were stable between the groups and no significant differences were obtained between the four groups. Some interesting (although not statistically significant) differences in lean content and fat content were observed, especially between the zeranol (28.3% fat in the carcass) and the clenbuterol (20.1% fat in the carcass) treated groups. In fact, zeranol treated animals grew faster and deposited more fat in the body.

The earlier noted higher average daily feed intake (ADI) and feed conversion rate (FCR) could explain the lower efficiency of fat deposition in the swine. These results confirm the statement that zeranol, as an oestrogen hormone, influences the deposition of adipose tissue (Leat & Cox, 1980). Notably during Phase 2, in the zeranol treated group, when fat deposition was presumably highest, the ADI (2.3 kg/d) and FCR (3.52 kg feed/kg gain) had the highest recorded values in the experiment. For both Phase 1 and Phase 2, the lowest values of ADI (1.55 kg/d and 1.88 kg/d respectively) were observed in the clenbuterol treated group, which could implicate that lean meat production (growth) in pigs demands less feed or energy. In the statistical analysis, high ($P<0.01$) correlations were noted between fat content in the carcass and feed conversion rate ($r = 0.73$). Thus the higher the feed conversion rate, the better the chances of fat deposition.

5.5 Carcass conformation

The anabolic agents zeranol, clenbuterol and nandrolone given to gilts did not significantly alter carcass conformation indicators (Table 4.8) with the exception of cold carcass weight, which was significantly ($P<0.05$) higher in the zeranol treated group compared to the other groups. The rest of carcass measurements did not differ significantly between the four groups.

It would seem that carcass conformation indicators depend more on the age and weight at slaughter, the individual genetic pattern of growth and nutrition to a certain extent. Henry (1993) observed that a substantial change in pig carcass conformation is achieved due to selection for later maturing animals. Carcass length is probably the most commonly measured indicator, which in this trial did not show any difference between the groups (means of 828 mm; 815 mm; 831 mm and 832 mm for the zeranol, clenbuterol, nandrolone and the control group respectively). Martinez *et al.* (1992) found similar measurements (varying between 827 and 835 mm) and reported no significant differences in carcass length of carcasses weighing 76 to 79 kg, following trenbolone acetate treatment in pigs. Yen *et al.* (1990), however, reported shorter (784 mm) carcasses following the administration of the β-agonist cimaterol to pigs. In further work (Yen *et al.* 1991), using ractopamine, obtained an increased carcass length (825 mm). Ham length varied between 363 mm (zeranol group) and 380 mm (nandrolone group), and these lengths are

less than those reported by Wood *et al.* (1981), who obtained values from 534 mm to 546 mm in carcasses of 56 kg. Ham circumference, thorax and chest depth and chest diameter showed no variation for the different anabolic agent treatments. From the results obtained, it is of interest to note that carcass cold weight was significantly ($P<0.05$) and positively correlated to ham circumference ($r = 0.52$) chest depth ($r = 0.64$) and chest diameter ($r = 0.56$). This could indicate that the wider and deeper the chest, and the bigger the ham, the heavier the carcass. In the literature reviewed, there was no reference to these parameters.

5.6 Meat quality parameters

The initial (45 minutes) and final (24 hours) pH values did not differ significantly between carcasses in gilts treated with zeranol, clenbuterol, nandrolone, and the control (Table 4.9). In all three treated groups, the pH measurements taken from the *M. Semimembranosus* were generally higher (although not significant) than those taken from the *M. Longissimus dorsi*. This phenomenon was not repeated in the control group. Differences however, in muscle pH from the same carcass are likely, due to different energetic metabolic rates between the muscles concerned (Whittemore, 1993). Apart from possible poor pre-slaughter animal handling practices that can influence the post-mortem muscle pH (Whittemore, 1993; Jones *et al.* 1994), these parameters are of great importance in identifying stress susceptible pigs, where the frequency of occurrence of pale, soft and oxidative (PSE) meat is high. In agreement with the results obtained (final pH taken from the *M. Semimembranosus*, with mean values of 5.52; 5.68; 5.68 and 5.71 for zeranol, clenbuterol, nandrolone and control groups, respectively), Oliver *et al.* (1994) obtained a mean value in final pH of 5.72 for Large White x Landrace pigs, and reported no incidence of PSE meat. Stecchini *et al.* (1991) reported the occurrence of dark, firm and dry (DFD) meat in pigs, following an oral administration of clenbuterol.

Treatment of gilts with the anabolic agents zeranol, clenbuterol and nandrolone did not alter the percentage cooking loss or moisture loss. No significant differences were found in this parameter between the groups, where almost uniform values (28.7% to 30.3%) were obtained. Heinze and Mitchell (1987) reported similar values of 30.2% to 31.4% in cooking loss, from meat of stress resistant pigs.

Cutting resistance measurements (Table 4.9) as an indicator of possible meat tenderness, showed significant ($P<0.05$) differences between groups. The more tender meat was produced by the zeranol treatment (mean value of 3.74 kg shear force) compared to the control. Relatively tougher meat (mean value of 5.69 kg shear force) was obtained following treatment with nandrolone. Yen *et al.* (1991) reported no significant differences in cutting resistance of pigmeat from animals treated with the β-agonist ractopamine. In an earlier study (Yen *et al.* 1990) in which the β-agonist cimaterol was used, shear force values were higher, indicating the obtaining of tougher meat. The use of

porcine somatotropine was reported by Wander *et al.* (1993) to increase the mean value of cutting resistance in pig meat.

No statistical differences were found between the groups regarding water holding capacity. Mean values varying between 37.2% (clenbuterol group) and 46.5% (zeranol group) were obtained. These values were not significantly different from those reported by Heinze and Mitchell (1987) (from 36.7% to 48.2%) in Large White x Landrace stress resistant pigs.

5.7 Organ weights

The treatment of gilts with zeranol implants, injectable nandrolone and oral dose of clenbuterol did not produce a significant difference in the weights of the gastrointestinal tract (Tables 4.10 and 4.11). Mean values varying between 8.9 kg and 11.1 kg; 4.4 kg and 4.8 kg; and 563 g and 667 g for full gastro-intestines, empty intestines and empty stomach weights were obtained respectively. Contradictory to these results, lower gastro-intestine weights were reported by Yen *et al.* (1989), following the use of neomycin and carbadox as growth promoters in pigs. The possible reason for this difference could be that the anabolic agents used in this experiment act in a different way from the antibiotics and other gut active promoters, whose action is mainly within the gastro-intestinal tract (Smith 1993). However, Bracher-Jakob (1990) also reported reduced weights of the empty stomach (500 g) and empty intestines (2.75 kg) as result of β-agonist treatment in pigs. Full gastro-intestine tract weights were found to be about 11.2% to 12.8% of the final liveweight, while the mean percentage of digesta to the final liveweight was 5.0% to 6.5% (not significant). In the literature reviewed, no reference was made to these ratios. Mersmann (1989) reported a decreased gut weight in slower growing pigs. Stamataris *et al.* (1991) however, reported similar effect in pigs given a restricted diet, compared to those fed *ad libitum*.

Implantation of gilts with zeranol resulted in the suppression of ovary growth (mean weight 0.6 g), while the uterus was recorded as significantly ($P<0.05$) enlarged (mean weight 134 g), when compared to non treated animals and those treated with the androgen, nandrolone and the β-agonist, clenbuterol (Table 4.12). The age at which the implantation was made, the dose and the duration of treatment of zeranol could have had a definite influence on the level of the ovary growth. An observation of interest in the zeranol treated gilts was the clearly increased size of the vulvas, the tendency to develop uterine prolapse and traumatic vulvo-vaginitis. Decreased ovarian weights (2.9 g) and increased uterine weights (mean 113 g) were reported by Sheridan *et al.* (1990), after an application of oestradiol 17-β in gilts. Etienne and Dourmad (1994) reported the oestrogenic properties of zerealenone to produce changes in the morphology of uterine tissue in sows by decreasing LH and progesterone secretion. Beltranena *et al.* (1993) reported that porcine somatotropine administered to gilts did not affect the development of the reproductive organs. Ovaries from gilts treated with clenbuterol and nandrolone showed signs of activity, as they had follicles of different sizes, *corpora lutea* and *corpora albicans*, similar to those in the control gilts. Cardenas and Pope (1994)

reported that the administration of small doses of testosterone during the follicular phase increased the number of *corpora lutea* and blastocysts in swine.

The means of the liver, kidneys, heart and spleen expressed as absolute weights or as a percentage of the final liveweight showed no statistical differences between the four groups. These parameters (Tables 4.13 and 4.14) were monitored to assess possible detrimental effects of the growth promoters on organ development. Means of liver weights varied from 1.38 kg (control and clenbuterol group) to 1.51 kg (zeranol group), and its ratio to final liveweight varied from 1.62% to 1.75%. These results are in agreement to those reported by Bracher-Jakob and Blum (1990), who treated pigs with the β-agonist Ro 16-8714 and obtained no significant differences (mean values between 1.48 kg and 1.59 kg). Decreased liver weights and a ratio of 1.11% to the slaughter weight was reported by Yen *et al.* (1990), after the administration of cimaterol in pigs. In a further study with the β-agonist ractopamine, Mitchell *et al.* (1991) reported a decreased liver weight (1.4 kg). It would thus also seem that in this study, the liver was not significantly affected by the administration of growth promoters.

Zeranol treated animals showed a frequency of 50% in kidney cysts, and presumably this was the reason why the mean weight of these organs (0.29 kg) were higher (although not statistically) than those of the control (0.26 kg) and the other groups. No explanations could be given for this abnormality, and no clinical signs of disturbances relating to kidney function were noted throughout the trial. Increased kidney weights were reported in pigs treated with cimaterol (0.28 kg) (Yen *et al.* 1990) and with porcine somatotropine (Hanrahan, 1989).

No significant differences were found between the four groups with respect to lungs weights (Table 4.13). Clenbuterol treatment resulted in lighter (0.54 kg) lungs, and heavier lungs were recorded in nandrolone treated animals (0.58 kg). Apart from their growth promoting activity, the β-agonists are also shown to act on the rate of the respiratory system (Lindsay *et al.* 1993). The significant improvement in the inspiratory and expiratory power mentioned by Meyer (1993) as an effect of oral administration of clenbuterol, could have lead to the heavier lungs. Bracher-Jakob and Blum (1990) however, reported no effect on lungs weight, following the application of β-agonists in pigs. It should be noted that whenever lung weights are taken post mortem, it could be of interest (for the reliability of the results obtained and inspection of the organs), that probable blood inspiration by the animal during the dissanguination during slaughter could influence the weights of the lungs significantly.

Mersmann (1987) reported an increased heart rate as result of oral dosages of clenbuterol in pigs, and this could suggest an increased heart weight (hypertrophy) in those animals submitted to a prolonged treatment. Results obtained from the clenbuterol treated animals did however not indicate such a trend, as there was no significant difference between this group (0.32 kg) and the control (0.31 kg). Heavier heart weights (not significant) were observed in the nandrolone group (0.34 kg) and lighter weights were found in the zeranol

treated group (0.31 kg). It seems that the physiological way to which clenbuterol treated pigs react to high heart rate, is not necessarily an increased growth of the organ concerned. Mitchell *et al.* (1991) reported a decreased heart weight after treating pigs with ractopamine (β-agonist), the same conclusion was reported by Yen *et al.* (1990) following the administration of cimaterol in pigs.

Spleen weights resulted in mean values of 0.12 kg and 0.13 kg, and percentages of 0.14% and 0.15% of the final liveweights. No significant differences were found between the four groups with respect to these measurements. A decreased weight of spleen following treatment of pigs with β-agonists was reported by Bracher-Jakob and Blum (1990), while increased weights (0.15 kg) of the same organ, in response to treatment with the β-agonist cimaterol, were obtained by Yen *et al.* (1990). Yen *et al.* (1991) reported a ratio of 0.12% of spleen weight as a percentage of the slaughter weight in pigs treated with ractopamine - which is similar to the 0.15% obtained with clenbuterol in this trial.

5.8 Serum urea concentrations

The determination of serum urea levels as an indicator of nitrogen utilisation, showed no definite trends of increase or decrease over the observation period. So, for example, if any abnormal renal function occurred in the treated animals, especially in the zeranol treated group, where 50% of the gilts showed kidney cysts, a rise in the mean values of blood urea levels should be recorded, according to Dukes (1970). Higher mean serum urea levels were generally observed in the nandrolone group during the treatment period (Phase 1), compared to the other three groups. During the recovery period (Phase 2), higher concentrations were measured in the clenbuterol and zeranol treated groups (see Table 4.15). Relatively stable (but not constant) concentrations over the entire observation period were monitored in the control group. Yen *et al.* (1990) reported increases in serum urea levels after treating pigs with cimaterol (β-agonist). Veum *et al.* (1986) reported increases in serum urea as a result of different levels and sources of crude protein in diets given to pigs. According to Wilson *et al.* (1972), the levels of serum urea in swine does not remain constant and is influenced by the amount and quality of feed consumed, especially the level of protein in the diet. From the graphic illustration of serum urea concentrations (Figure 4.5) it is difficult to determine any specific trend. A significantly high ($P<0.01$) positive correlation existed between this parameter and the average daily intake ($r = 0.68$) and average daily gain ($r = 0.67$). More available urea (N) in the blood, could thus indicate a higher growth rate potential. The fact that these correlations are positive is contrary to Wilson *et al.* (1972), who found that serum urea values are inversely proportional to gain in body weight and the efficiency of feed conversion.

5.9 Serum glucose concentrations

A tendency for lower glucose concentrations (Table 4.16 and Figure 4.6) was observed in all four groups (control group included) during the anabolic agent

treatment period. Due to increased metabolic rates following the use of anabolic agents, a higher blood glucose concentration was expected in the treated groups - to be available for a higher metabolic rate and increased protein deposition. Results seem to indicate that the expected higher metabolic rate at cellular level, had a decreasing effect on the blood glucose concentrations. During the recovery period (when stimulation ceased), no clear trend was observed. For all the groups the concentrations rose during the second half of this period and decreased again two weeks later. As could be expected, a tendency for a negative correlation between serum glucose levels and some growth parameters was observed. For average daily gain this negative correlation was significant ($P<0.05$; $r = -0.56$). This could indicate that a higher ADG is associated with a higher rate of glucose utilisation at tissue level, and less glucose is found in the blood. It would thus seem as if serum glucose levels give some indications of growth performance and metabolic activity in a broad sense. This parameter alone could not point out marked differences in serum concentration between zeranol, clenbuterol, nandrolone treatments and the control. Rule *et al.* (1989) stated that porcine adipose tissue is refractory to stimulation by glucose oxidation and incorporation into lipids by insulin. No inhibition of anabolic function in porcine adipose tissue was found after treatment with β-agonists. Dubreuil *et al,* (1990), reported no changes in serum glucose levels after the application of growth hormone releasing factors to Large White x Landrace pigs, similar to results found in this study. Maybe the restriction in feed intake (lower blood glucose levels) would show more dramatic responses following treatment by anabolic agents.

5.10 Serum creatinine concentrations

A tendency of an increased level over the entire observation period (Table 4.17) was observed in all the treatment groups, regarding serum creatinine concentrations. The control group had a lower mean concentration (not significant) throughout the trial, and the nandrolone group exhibited the highest level. As renal dysfunction can be diagnosed through the determination of blood creatinine concentrations (Guyton, 1971), these determinations could be expected to show possible abnormal increases in serum creatinine in the treated animals. This being due to the disturbed catabolism (especially in the zeranol group where kidney cysts were found in 50% of the animals). In these animals and the clenbuterol and nandrolone treated animals, no dramatic differences in creatinine levels were observed, compared to the control.

Serum creatinine levels were negatively correlated with average daily intake ($r = -0.54$) and average daily gain ($r = -0.63$). These results are contrary to those reported by Dubreuil *et al.* (1990), who found a decrease in serum creatinine concentrations following the administration of growth hormone releasing factor in pigs. Similar results were reported by Haydon *et al.* (1988) after giving an amino acid deficient diet to growing pigs. Riond and Riviere (1988) reported no differences in serum creatinine levels in pigs treated with the antibiotic gentamicin. The gradual and continuous increase in serum creatinine concentration observed in this trial (Figure 4.7) (that was slightly higher in the

treated groups than in the control), give an indication of proportional and continuous accretion of lean meat (protein) in the body, as steady creatinine production is also proportional to the muscle tissue growth (Dukes, 1970). These results seem to indicate no detrimental effect on kidney function by the treatment of gilts with these specific anabolic agents.

5.11 Serum oestradiol concentrations

No definite pattern in the serum oestradiol concentrations was observed in all four groups throughout the trial (Table 4.18). However, all the groups showed a sudden drastic decrease during weeks 3 and 4 (see Figure 4.8) followed by a sudden increase. The reasons for this phenomenon is unclear. The nandrolone group maintained the highest level of serum oestradiol in all the groups, and the zeranol group was generally the lowest. This is in agreement with the ovary weights obtained in Table 4.12. Suppression of ovarian activity (and size) was, however, expected in the nandrolone (testosterone) treated group, with a resultant lower oestradiol level. The sensitivity of the assay used for porcine oestradiol concentration determination is a factor to be considered. Maybe the nandrolone dose injected was also not sufficient to induce suppression of ovarian growth. Cardenas and Pope (1994) reported that small doses of testosterone during follicular phase increase the number of corpora lutea. Thus, it would seem that nandrolone treatment can induce an anabolic response, rather than a hormonal (androgenic) side-effect. Oestradiol levels were not significantly correlated to any growth parameters. These results are not in agreement with those reported by Iyayi and Tewe (1991), who found significant increases in serum oestradiol levels accompanying the increase in liveweight in Large White x Landrace female pigs fed normal diets. Beltranena *et al.* (1993) reported that serum oestradiol levels were unaffected by the implantation or injection of porcine somatotropine in gilts.

5.12 Hematocrit

The mean of hematocrit readings show no abnormal values over the entire observation period for all the four groups (Table 4.19). Slightly higher values were observed in the control (mean 39.2 ± 1.5%), and the nandrolone treated group showed the lowest values (mean 36.4 ± 1.7%). These variations, however, were all within normal limits. Apparently, the treatment of gilts with the anabolic agents zeranol, nandrolone and clenbuterol did not affect the hematocrit and thus packed blood cell volume. It was surmised expected a high percentage of red cells (hematocrit) in the treated animals could be possible. This could be due to relative more oxygen at tissue level being required because of a higher rate of metabolite oxidation, due to an increased metabolic rate induced by the anabolic agents. Water deprivation and diarrhoea (Balsbaugh *et al.* 1986) were shown to increase the hematocrit in pigs. Harun (personal communication, 1994) reported that the application of the growth promoter olaquindox to calves decreased the hematocrit. Increased blood volume in pigs slaughtered at 90 kg to 100 kg liveweight following treatment with the β-agonist Ro 16-7814 was reported by Bracher-

Jakob and Blum (1990). The use of anabolic agents did not affect the percentage of red blood cells and thus possibly the metabolic rate.

5.13 Anabolic agent excretion rate

Zeranol and its metabolite concentrations in urine from zeranol treated gilts were found to maintain a relatively high level (Table 4.20) during the post implantation period. Peak urine values of 32.8 ng/ml were obtained, followed by a slow reduction or rate of excretion (11.7 ng/ml by day 19 after implantation). This could indicate that for zeranol implanted animals, a clearance period of at least three weeks could be suggested to give acceptable residual concentrations of the anabolic agent in the animal. Bories *et al.* (1992) stated that urine is the major excretion pathway of zeranol and its metabolites. Concentrations of about 40 ng/ml in bovine urine, 10 days after implantation, have been reported by Van der Merwe and Pieterse (1994).

Clenbuterol and its metabolite concentrations in urine samples from clenbuterol orally administered gilts decreased rapidly after the last dose (Table 4.21). From the results obtained, it would seem as if the clearance period for this β-agonist is relatively short because of the high clearance rate and short half-life. Sauer *et al.* (1993), reported that there were important differences between the concentration of clenbuterol excreted in the urine and those found in the blood plasma and body tissues. According to Sauer *et al.* (1993) the liver should possibly be used for the target sample, rather than the urine.

5.14 An economic and practical point of view

Feed represents 70 to 80% of the total production costs in producing pork (Krider & Carroll, 1971; Gordijn, 1993). Table 5.1 sets out the basic cost feed framework of feed for the production of 1 kg of pork from gilts treated with zeranol, clenbuterol and nandrolone. Some management implications in the use of these anabolic agents are further indicated as useful advice for on-farm conditions.

5.14.1 Zeranol treatment

Zeranol treated gilts grew faster than those of the control (91.95% of time needed for growth), demanded more feed and yielded more fat carcasses. The apparent advantage of saving time in pork production by shortening the growth period is counteracted by the lower feed efficiency utilisation and the possible penalties obtained in down-grading of the carcasses at the market. Feed costs for the production of 1 kg of lean zeranol treated meat is R0.02 above the cost to produce 1 kg of lean meat in untreated or control animals. From the point of view that pigs treated with zeranol are more feed consuming and less efficient in converting feed into lean meat, and given the actual market demands for lean meat, the application of zeranol for baconer carcass production is showed to be uneconomical. Irrespective of the above mentioned factors regarding zeranol implantation in growing gilts, this anabolic agent has the advantage of being administered as an implant. This makes the treatment very

practical and the general management of treated animals was shown to be easy. No side-effects were observed regarding the general health of the animals. It could be of interest, however, in further trials to investigate a lower final target liveweight so that the better feed conversion rate observed in the earlier stages of growth could be predominant and the high rate of fat deposition that occurs at heavier live weights and ages could be avoided.

5.14.2 Clenbuterol treatment

The production of baconers is faced with a high fat content in the carcass due to the fact that from a certain weight, the pig grows with the increased rate of fat deposition rather than lean muscle deposition. Anabolic agents like the β-agonist clenbuterol could be used as growth agents or manipulators. What could be expected from this growth manipulation is probably a better carcass composition with a high lean muscle content and minimum fat content, and thus, more orientated to the consumer's demand for lean meat. By using clenbuterol, there was no saving of the farmers time (102.25% of time needed for growth), but clenbuterol's potential is in costing less for lean meat production (R4.95/kg), compared to the control (R5.27/kg). With the use of this anabolic agent the obtainment of a more acceptable carcass for the market is a distinct possibility. The application of clenbuterol to gilts, as a daily oral treatment has the first disadvantage of ensuring that the exact dose, per day, per animal is given. When given in the feed, the consideration of feed wastage becomes crucial, not ignoring the decision of feeding *ad lib.* or limiting the feed. Secondly, the side-effects observed with clenbuterol included rectal prolapses and arthritis, which can be difficult to control if a large number of animals are treated.

Table 5.1 Feed costs for pork production from Large White x Landrace gilts treated with zeranol, clenbuterol and nandrolone

	TREATMENTS			
Item	**Zeranol**	**Clenbuterol**	**Nandrolone**	**Control**
Total feed intake/animal (kg)	189.20	184.00	194.10	186.20
Total liveweight gain (kg)	67.80	66.20	67.60	67.60
Cold carcass mass/animal (kg)	68.80	63.90	66.90	63.90
Lean meat content/carcass (kg)	37.22	38.66	39.60	36.74
Lean meat & fat content/carcass (kg)	56.69	51.50	52.85	50.93
Age at slaughter (days)	147.30	163.80	164.00	160.20
Total feed cost/animal (R)	196.77	191.36	201.86	193.65
Feed cost/kg liveweight gain (R)	2.90	2.89	2.99	2.86
Feed cost/kg carcass (R)	2.86	2.99	3.02	3.03
Feed cost/kg lean meat (R)	5.29	4.95	5.10	5.27
Feed cost/kg lean & fat (R)	3.47	3.72	3.82	3.80
Age as % of the Control	91.95	102.25	102.37	100.00

Given values: R1.04/kg feed (September, 1994)

5.14.3 Nandrolone treatment

An intramuscular injection of the anabolic steroid nandrolone to gilts gives results similar to those obtained by the clenbuterol treatment. No faster growth rates were observed (102.37% of time needed for growth), and lean meat costs in feed were R0.17 lower (R5.10/kg lean meat) than for the control. In the nandrolone treated animals however, the cost per kg of liveweight gain during the growing period was higher than for all the other treated groups (R2.99/kg of liveweight gain). It would seem that nandrolone can be used economically for the production of leaner baconer carcasses from gilts. Due to the fact that nandrolone is injectable and the treatments are done every three weeks, and no visible side-effects are observed after the application, the general management is problem free. In an on-farm unit this agent can be easily applied. However, a vast lack of knowledge concerning the use of nandrolone in pigs is predominant, especially in the ideal dose and treatment period for the best growth performance and carcass traits.

CHAPTER 6

CONCLUSIONS

No significant improvements in the growth parameters were achieved by the use of the anabolic agents clenbuterol and nandrolone. Zeranol treated gilts did, however, respond. In this group the overall growth rate and the overall average daily gain was significantly higher ($P<0.05$), when compared to the control. Clenbuterol treatment tended to suppress the growth rate. The best growth performance in terms of total weight gain, average daily gain and feed conversion rate, in all treated gilts, was recorded during the treatment period. After this it slowed down and a higher average daily intake and feed conversion rate was recorded.

In this study, the measurement of backfat thickness (P_2) and eye muscle diameter using the sonar apparatus did not serve as an accurate and reliable predictor of fat thickness or leanness of the carcass. The means obtained did not generally agree when compared to the results obtained with physical measurements made on the carcass and when compared to the results from the determination of carcass composition. One of the main problems encountered in this facet, was taking the measurement on exactly the same site every week. Noticeable differences between zeranol treated gilts and the other two treatment groups concerning some carcass characteristics were found. The main findings were the higher warm and cold carcass weights and dressing percentage, and the backfat thickness physically measured from the different sites (last rib, tenth rib, P_1, P_2 and P_3) obtained in the zeranol treated group. Leaner carcasses were obtained following the use of the β-agonist clenbuterol, together with a higher lean muscle content in the carcass. Carcass conformation characteristics are clearly unaffected by the administration of zeranol, clenbuterol and nandrolone. Besides a more tender meat being produced by zeranol treatment and a tougher meat found in the clenbuterol treated animals, meat quality parameters generally were not affected by the use of anabolic agents in pigs. Implantation of gilts with zeranol at a young age suppresses the development of the gonads, while the reproductive tract is seemingly over-developed. Moreover, the dysfunction of the ovaries and the general trend of these treated animals towards a uterus prolapse and vulvo-vaginitis, makes this treatment unacceptable for gilts intended for breeding. The macroscopical development of the reproductive organs are seemingly not affected by the administration of clenbuterol and nandrolone.

The anabolic agents used in this study did not affect the growth of the digestive tract and other organs such as liver, heart, kidneys, spleen and lungs. These agents also did not affect the ratio of these organs to the final liveweight. The anabolic effect observed in the zeranol treatment concerning growth characteristics was not mediated through an increased gastrointestinal capacity. The gastrointestinal tract and its capacity was not increased.

The laboratory methods used (and considering the difficult handling consequences of blood sampling), to monitor growth performance and feed efficiency utilisation through blood levels of glucose and urea seem to be time consuming and not always practical. These results were also less dramatic and difficult to interpret, compared to

the physical and routine measurements of animal growth parameters easily obtained for an on-farm evaluation. Further investigations in the field of blood biochemistry is recommended to monitor the effect of blood glucose, creatinine, oestradiol and urea in the anabolic process, or to evaluate the influence of zeranol, clenbuterol and nandrolone on these blood parameters. More frequent blood sampling periods may have given a clearer picture of nitrogen and glucose utilisation.

A longer withdrawal period is necessary after an implantation of pigs with zeranol, compared to the rapid clearance realised after an oral dose of clenbuterol. The precise clearance period is still unclear but at this stage lack of finances and expertise in the determination of anabolic agent metabolites is a major stumbling block. The determination of the excreted anabolic agents or metabolites in the urine does not seem to be the solution to the consumer's concern regarding residues. The answer would be to seek for possible residues in edible meat. As there is still little information available regarding the biochemistry of zeranol and even less regarding nandrolone and clenbuterol metabolites in swine, further investigation in the rate of clearance seem to be of crucial importance for its future legal and safe use in the pig industry.

From an economical point of view, it would seem that although the feed cost per kg carcass was the least in the zeranol group and it achieved its target weight 12 days earlier than the control animals, the higher rate of fat deposition gives it an undesirable carcass and zeranol's long half-life forces a long withdrawal period prior to slaughter. By the implementation of smaller doses of zeranol and a lighter target weight (porker), the detrimental or disadvantageous factors could possibly be overcome or minimised. From the results obtained in this trial and taking into consideration the cost of the agents and other impracticalities it would thus seem that the use of these anabolic agents at these doses and for the respective treatment periods in gilts, are not justified.

CHAPTER 7

SUMMARY

24 Crossbred gilts (Large White x Landrace) purchased as weaners were randomly allocated to four treatment groups (n = 6) and submitted for an observation period of three phases: Phase 1, in which the animals were treated with anabolic agents (nandrolone, clenbuterol, zeranol); Phase 2, could be seen as the anabolic agent clearance period; and Phase 3, in which certain carcass characteristics and meat quality parameters were measured. The trial was aimed to compare the effect of the different anabolic agents zeranol (implants of 36 mg/pig, every three weeks, for 9 weeks), clenbuterol (daily oral dose of 0.5 mg/pig, for 9 weeks) and nandrolone (intramuscularly injected, 50 mg/pig, every 3 weeks, for a period of 9 weeks), on growth rate parameters, carcass and meat characteristics, visceral organ growth and blood concentrations of urea, glucose, creatinine, oestradiol and the hematocrit. The gilts were individually housed and fed a pig growth diet (16% crude protein) *ad libitum*, with free access to water. Body weight of all animals were recorded every 48 hours to monitor the average daily gain (ADG) and the growth rate up to the target liveweight of 85 kg. Weekly feed intake was monitored and the feed conversion rate (FCR) determined for the individual animals and the mean of the groups. Backfat thickness (P_2) and eye muscle diameter were measured weekly with the aid of a sonar apparatus in all animals, to monitor the deposition of fat and lean muscle. Blood was sampled weekly from 4 specific animals per group for the determination of hematocrit, blood urea, blood glucose, blood creatinine and blood oestradiol concentrations. The clearance rate of the anabolic agents was monitored in the urine sampled every second day from all anabolic agent treated animals following cessation of treatment. At slaughter (85 kg liveweight), several carcass measurements were done. Visceral organ weights were noted and meat quality parameters (water loss, cutting resistance, pH) were determined.

Zeranol treatment revealed an improved growth rate (ADG of 727 g/d and 147.3 days to attainment of 85 kg) compared to the control and the other treatment groups. None of the three anabolic agents improved the FCR significantly, although the control showed the lowest mean value (2.76 kg feed/kg liveweight gain). A tendency for an increase in this parameter was observed over time, in all the groups, the highest mean value being encountered in the group treated with zeranol (3.32 kg feed/kg liveweight gain). Overall average daily feed intake was significantly ($P<0.05$) greatest in the zeranol treated animals (2.03 kg/d). Backfat thickness (P_2) deposition assessed through ultrasonic measurements, showed no significant differences between the treatments and the control. The diameter of the eye muscle, weekly monitored by the same method, from the P_2 site, showed significant ($P<0.05$) differences - the control having the highest value (4.43 mm/week). The clearance rate of the anabolic agents was faster in clenbuterol treated animals than in the zeranol group, while for nandrolone group this could not be assessed, because its metabolites in swine are still unknown. Zeranol treated animals had a significantly ($P<0.05$) improved cold carcass weight and dressing percentage (68.8 kg and 79.8% respectively). Mean values for backfat thickness were generally high in carcasses from zeranol treated animals (P_1 = 13.5 mm; P_2 = 14.8 mm; P_3 = 16.6 mm) which leaner carcasses were obtained in the

clenbuterol group (P_1 = 6.7 m; P_2 = 7.4 mm; P_3 = 8.6 mm). The eye muscle area (physically measured) was significantly ($P<0.05$) higher in the nandrolone group (34.3 cm^2) compared to the Zeranol group (30.6 cm^2), but not statistically different from the control and the nandrolone group. Zeranol, clenbuterol and nandrolone treatments did not significantly alter carcass conformation indicators. However, carcass weight was recorded to be positively ($P< 0.05$) correlated to ham circumference ($r = 0.52$); chest depth ($r = 0.64$) and chest diameter ($r = 0.56$).

With the exception of cutting resistance values, in which the zeranol treatment group produced more tender meat (3.74 kg shear force) than the control and the other two treatments, the rest of meat quality parameters measured (muscle pH; cooking loss of water holding capacity) were not affected by the treatment with anabolic agents. No significant differences in the weights of digesta, digestive tract and the visceral organs (liver, kidneys, lungs, heart and spleen) were found following anabolic agent treatment. Suppressed ovary growth (0.6 g of weight) and over-growth of the reproductive tract (134 g uterus weight) and increased size of the vulvas were observed following zeranol implantation of gilts. The reproductive organs from clenbuterol and nandrolone groups were functional and apparently unaffected.

The determination of blood urea, blood glucose, blood creatinine and blood oestradiol levels using specific kits to assess the concentrations of the metabolites and hormones generally did not result in definite trends of increases or decreases over time. These determinations could thus not be accurately used as possible indicators of the metabolic status following the use of zeranol, clenbuterol and nandrolone in gilts.

It was concluded that the use of clenbuterol and nandrolone in gilts yielded no improvements in the growth parameters. In gilts treated with zeranol, overall growth rate was higher. Ultrasonic measurements of backfat thickness and eye muscle diameter proved to be an inaccurate and unreliable predictor of fat thickness or leanness of the carcass. A longer withdrawal period is necessary after an implantation of pigs with zeranol compared to the rapid clearance realised after an oral dose of clenbuterol. The faster growth rate obtained following the use of zeranol implants is counteracted by higher feed costs of lean meat production and the yield of poorer ratio of lean-to-fat content in the carcass when compared to the clenbuterol and nandrolone treatments. The growth of the digestive tract, liver, heart, kidneys, spleen and lungs were not affected by anabolic agent treatment, and thus the anabolic effect of zeranol concerning growth characteristics is not through an increased gastrointestinal capacity. The assessment of growth performance and feed utilisation efficiency through blood levels of glucose and urea appear to be time consuming and not always practical. Further investigations regarding blood biochemistry, ideal doses of the anabolic agents, their metabolism and clearance rate in swine, as well as the margin of consumer's safety, is still of crucial importance for the future legal and safe use of anabolic agents in the pig industry. From the results obtained, it would seem that the use of these anabolic agents for the respective treatment periods and doses in gilts are not justified.

REFERENCES

ALAIS, C. & LINDEN, G., 1991. Meat and Blood products. In: *Food Biochemistry* p. 174 (Ed. I. Morton) - Ellis Horword - New York.

ARMSTRONG, D.G., 1986. Gut-active growth promoters. In: *Control and Manipulation of Animal Growth* p. 21 (Ed. P.J. Buttery, D.B. Lindsay, N.B. Haynes). Butterworths - London.

AYKROYD, W.R., 1964. Veterinary Medicine and Human Nutrition. In: *Veterinary Medicine and Human Health* p. 301 (Ed. Calvin W. Schwabe). The Williams & Wilkins Company - Baltimore.

BAKER, D.H., HANN, J.D., CHUNG, T.K. & HAN, Y., 1993. Nutrition and Growth: The concept and application of an ideal protein for swine growth. In: *Growth of the Pig*, pp. 133 (Ed. G.R. Hollis) Cab International.

BALSBAUGH, R.K., CURTIS, S.E. & MEYER, R.C., 1986. Body weight, total body water and hematocrit in diarrheic piglets. *J. Anim. Sci.* 62: 2, 307 - 314

BARBER, R.S., BRAUDE, R., MITCHELL, K.G. & PITTMAN, R.J., 1972. Effect of level of feed intake on the performance and carcass composition of growing pigs. *Anim. Prod.* 14: 199 - 208

BEERMANN, D.H., 1993. Use of exogenous agents to regulate growth composition. In: *Growth of the pig* p. 185 (Ed. G.R Hollis) Cab International.

BELTRANENA, E., SCHAEFER, A.L., AHERNE, F.X. FOXCROFT, G.R., 1993. Recombinant porcine somatotropin effects on sexual development and metabolic status of gilts. *Can. J. Anim. Sci.* 74: 265 - 271

BLASCO, A., GOU, P., GISPERT, M., ESTANY, J., SOLER, Q., DIESTRE, A. & TIBAU, J., 1994. Comparison of five types of pig crosses. I Growth and carcass traits. *Livestock Production Science* 40 171 - 178

BOATFIELD, G., 1983. Pigs. In: *Farm Livestock*, p. 47 (Ed. Farming Book Series) Farming Press Limited - England.

BONE, J.F., 1979. The Endocrine System. In: *Animal Anatomy and Physiology* p. 333 Reston Publishing Company, Inc - Virginia.

BONNEAU, M., 1991. Regulation of pig growth by somatotropic hormones: II. The effect of exogenous GRF or pST administration on performance and meat quality. *Pig news and information* 12: 1 39-45

BORIES, G.F., SUTRA, J-F.P., & TULLIEZ, J.E., 1992. Metabolism and disposition of [^{3}H] Zeranol Implanted in the pig. *J. Agric. Food Chem.* 40, 284 - 288

BOTHA, L., 1993. Management of the pig herd. In: *Pig production in South Afric2a* p. 5 (Ed. E.H. Kemm) - The Agricultural Research Council - Pretoria.

BRACHER-JAKOB, A. & BLUM, J.W., 1990. Effects of a β-adrenergic agonist on growth performance, body composition and nutrient retention in finishing pigs fed normal or low amounts of protein. *Anim. Prod.* 51: 601 - 611

BRENNAN, P.J. & JOYCE, E.J., 1979. An on-farm evaluation of three growth promotants for pigs. *Aust. J. Exp. Agric. Husb.*, 19: 32 - 35

BUDDLE, J.R. & BOLTON, J.R., 1992. The pathophysiology of Diarrhoea in pigs. *Pig News and Information* Vol. 13 no. 1 pp. 41N - 45N

BULFIELD, G., 1980. The biochemical and genetical determinants of selection for growth. In: *Growth in Animals*, p. 11 (Ed. T.L.J. Lawrence) Butterworths - London - Boston.

BUTTERY, P.J. & SWEET A., 1993. Manipulation of lean deposition in animals. *Animals feed science and technology,* 45: 97 - 115

CAMERON, N.D., 1993. Selection for meat quality: objectives and criteria. *Pig news and information* Vol. 14 no. 4: 161N - 168N.

CARDENAS, H. & POPE, W.F., 1994. Administration of testosterone during the follicular phase increased the number of corpora lutea in gilts. *J. Anim. Sci.* 72: 2930 - 2935

CLOSE, W.H., 1989. The influence of the thermal environment on the voluntary food intake. *Occasional publication* No. 13, 87 - 96

COLE, D.J.A., & CHADD, S.A., 1989. Voluntary food intake of growing pigs. *Occasional publication* No. 13, 61 - 70

CORDRAY, J.C., HUFFMAN, D.L. & McGUIRE, J.A., 1978. Predictive equations for estimating protein and fat in the pork carcass. *J. Anim. Sci.* 43. 3. 666 - 672

CURTIS, S.E., 1993. The physical environment and swine growth. In: *Growth of the pig* p. 93 (Ed. G.R. Hollis) Cab International.

DEATHERAGE, F.E., 1975. World population growth and future food supplies. In: *Food for life*, p. 351 (Ed. United Kingdom Edition). Plenum Press - London.

DOBSINSKA, E., HARUN, M. & NKATA, P., 1982. Verificaciòn de los efectos del estimulante de crecimiento olaquindox en terneros, en las condiciones tropicales de Mozambique. Personal communication.

DUBREUIL, P., COUTURE, Y., PELLETIER, G., PETITCLERC, D., LAPIERRE, H., POMMIER, S., GAUDREAU, P., MORISSET, J. & BRAZEAU, P., 1990. Effect of porcine growth hormone - releasing factor (1-29) NH_2 and thyrotropin - releasing factor on pig growth performance. *Can. J. Anim. Sci.* 70: 2, 459-467

DUBREUIL, P., PELLETIER, G., PETITCLERC, D., LAPIERRE, H., COUTURE, Y., GAUDREAU, P., MORISSET, J. & BRAZEAU, P., 1990. Influence of growth hormone - releasing factor and (or) thyrotropin - releasing factor on sow blood components, milk composition and piglet performance. *Can. J. Anim. Sci.* 70: 3, 821-832

DUKES, H.H., 1970. Protein metabolism. In: *Dukes' Physiology of Domestic Animals* p. 583 (Ed. Melvin J. Swenson) Comstock Publishing Company New York.

DUNSHEA, F.R., KING, R.H. & CAMPBELL, R.G., 1993. Interrelationships between dietary protein and ractopamine on protein and lipid deposition in finishing gilts. *J. Anim. Sci.* 71: 2931 - 2941

DURAN, C.O. & WALTON, J.R., 1994. Care of potbellied pigs in companion animal practice. In: *The Veterinary Annual 34* p. 81 - 88 (Ed. Mary-Elizabeth Raw & T.J. Parkinson) Blackwell scientific publications.

EDWARDS, R.L., SMITH, G.C., CROSS, H.R. & CARPENTER, Z.L., 1981. Estimating lean in pigs carcasses differing in backfat thickness. *J. Anim. Sci.* 52: 4 704-709

ELLIS, M. & McKEITH, F.K., 1993. Factors affecting the eating quality of pork. In: *Growth of the pig* p. 215 (Ed. G.R. Hollis). Cab International.

ELTON, C., 1964. The ecological study of desease. In: *Veterinary medicine and human health* p. 186 (Ed. Calvin W. Schwabe). The Williams & Wilkins Company - Baltimore.

ETIENNE, M. & DOURMAD, J. -Y., 1994. Effects of zearalenone on glucosinolates in the diet on reproduction in sows: A review. *Livestock production science* 40: 99 - 113

EUSEBIO, J.A., 1980. Pig breeding. In: *Pig production in the tropics* p. 7 (Ed. W.J.A. Payne). Longman Group Ltd.

FAHEY, H.J., SCHAEFER, D.M., KAUFFMAN, R.G., EPLEY, R.J., GOULD, P.F., ROMANS, J.R., SMITH, G.C. & TOPEL, D.G., 1977. A comparison of practical methods to estimate pork carcass composition. *J. Anim. Sci.* 44, 1: 8-17

FAO - Quarterly bulletin of statistics, 1992, 5:3, 62 - 103 Food balance sheets.

FAO - Quarterly bulletin of statistics, 1992, 5: 3, 62 - 103 Per caput food supply

FAO - Quarterly bulletin of statistics, 1991 4: 2, iii - xvii - The world food balance cheet.

FISHER, A.V., 1992. Estimation of body composition using the velocity of ultrasound. *Pig news and information Vol.* 13 no. 4 149N - 154N

FORBES, M., 1983. Physiology of regulation of food intake. In: *Nutritional physiology of farm animals* p. 177 (Ed. J.A.F. Rook and P.C. Thomas). Longman group Ltd - London.

FOWLER, V.R. & GILL, B.P., 1989. Voluntary food intake in the young pig. *Occasional publication* no. 13, 51 - 60

GILLIN, E.D. & KRANE, J., 1989. Where does the increase in crop production come from? FAO - *Quarterly bulletin of statistics*, 2: 4, iii - viii

GOODWIN, D.H., 1973. Pig products and market requirements. In: *Pig management and production*, p. 19 (Ed. Derek H. Goodwin) Hutchinson Educational.

GORDIJN, R.J., 1993. Economics of pig production. In: *Pig production in South Africa*, p. 129 (Ed. E.H. Kemm). The Agricultural Research Council - Pretoria.

GUYTON, A.C., 1971. Micturition, renal disease, and diuresis. In: *Textbook of Medical Physiology* p. 452 (Ed. Arthur C. Guyton) W.B. Saunders Co. - Philadelphia.

HALLIDAY, R., 1980. Interrelationships between immunity and growth. In: *Growth in animals*, p. 65 (Ed. T.L.J. Lawrence). Butterworths - London - Boston.

HANRAHAN, T.J., 1989. Use of somatotropin in livestock production: Growth in pigs. In: *Use of somatropin in livestock production* (Ed. K. Sejrsen, M. Vestergaard, A. Neimann-Sorensen). Elsevier applied science - London.

HAYDON, K.D., HALE, O.M. & NEWTON, G.L., 1988. Effect of essential amino acid balance on performance and selected serum components in growing swine. *Nutrition reports - International* 37: 1, 33-39

HEINZE, P.H. & MITCHELL, G., 1987. Growth, carcass and meat characteristics of stress susceptible and stress resistant South African Landrace gilts. *S. Afr. J. Anim. Sci.* 1988, 18(1)

HEITZMAN, R.J., 1986. Safety aspects of growth manipulation in animals. In: *Control and manipulation of animal growth.* (Ed. P.J. Buttery, D.B. Lindsay, N.B. Haynes). Butterworths - London.

HEITZMAN, R.J., CARTER, A., DIXON, S.N., HARWOOD, D.J. & PHILLIPS, M., 1984. Recent studies on pharmacokinetics and residues of anabolic agents in beef cattle and other farm animals. In: *Manipulation of growth in farm animals*, p.1 (Ed. J.F. Roche & D. O'Callaghan). Martinus Nijhoff Publishers; Boston.

HENRY, Y., 1993. Recent developments in pig production systems: Effect on carcass and meat quality. *Pig news and information* Vol. 14, no. 4 149N - 156N

HE, P., AHERNE, F.X., SCHAEFER, A.L., THOMPSON, J.R., NAKANO, T. & JONES, S.D.M., 1994. Differentiation of the effects of somatotropin and enhanced growth rate on the occurrence of osteochondrosis in pigs. *Can. J. Anim. Sci.* 74: 251 - 255

HOFFMANN, 1984. Aspects on tolerance levels of anabolic agents with sexhormone like activities in edible animal tissues. In: *Manipulation of growth in farm animal*, p. 17 (Ed. J.F. Roche & D. O'Callaghan). Martinus Nijhoff Publishers; Boston.

HOLNESS, D.H., 1991. Distribution, potentials and constraints. In: *The tropical agriculturalist - pigs*, p. 1 (Ed. René Coste and Anthony J. Smith). MacMillan Education Ltd. - London.

HOVENIER, R., KANIS, E., ASSESDONK, T. & WESTERINK, N.G., 1992. Genetic parameters of pig meat quality traits in a halothane negative population. *Livestock production science*, 32: 309 - 321

IYAYI, E.A & TEWE, O.O., 1991. Effect of cassava peel-based diets on serum estradiol of growing female pigs. *Tropical agriculture* 68 (3) 239-242

JONES, S.D.M., CLIPLEF, R.L., FORTIN, A.F., McKAY, R.M., MURRAY, A.C., POMMER, S.A., SATHER, A.P. & SCHAEFER, A.L., 1994. Production and ante-morten factors influencing pork quality. *Pig news and information* Vol. 15 no. 1 15N - 18N

KALLWEIT, E., 1991. Measurement of body, carcass and tissue composition in meat animals by non-invasive methods. *Pig news and information* vol. 13 no. 4: 147N-148N

KAUFFMAN, R.G. & WARNER, R.D., 1993. Evaluating pork carcass for composition and quality. In: *Growth of the pig.* (Ed. G.R. Hollis). Cab international.

KELLY, K.W., KENTS, S. & DANTZER, R., 1993. Why sick animals don't grow: an immunological explanation. In: *Growth of the pig* p. 119 (Ed. G.R. Hollis). Cab International.

KRIDER, J.L. & CARROLL, W.E., 1971. Organization, a factor in the cost of production. In: *Swine production.* (Ed. James L. Smith, Joseph F. Murphy). McGraw - Hill Book Company.

LAMMING, G.E., 1986. Introductory comments. In: *Control and manipulation of animal growth* (Ed. P.J. Buttery, D.B. Lindsay, N.B. Haynes). Butterworths - London.

LAYMASTER, K.A., 1989. Estimation of carcass leanness by use of X-ray computed tomography. *Swine research. Progress report* no. 3: 7 - 9. (Ed. Roman L. Hruska). The agricultural research service - U.S. Dept. of Agriculture).

LEAT, W.M.F. & COX. R.W., 1980. Fundamental aspects of adipose tissue growth. In: *Growth in animals*, p. 137 (Ed. T.L.J. Lawrence). Butterworths - London - Boston.

LINDSAY, D.B., 1983. Growth and fattening. In: *Nutritional physiology of farm animals*, p. 261 (Ed. J.A.F. Rook & P.C. Thomas). Longman Group Ltd. - London.

LINDSAY, D.B., HUNTER, R.A., GAZZOLA, C., SPIERS, W.G. & SILLENCE, N.M., 1993. Energy and growth. *Aust. J. Agric. Res.* 44, 875 - 99

LISTER, D., 1980. Growth and meat quality in animals. In: *Growth in animals*, p. 287 (Ed. T.J. Lawrence). Butterworths - London - Boston.

MARTINEZ, M., LOPEZ-BOT, C., SANCHO, G. & VENTANAS, J., 1992. Effects of trenbolone acetate on swine carcass characteristics and backfat composition. *Can. J. Anim. Sci.* 72: 969 - 972

McDONALD, P., EDWARDS, R.A. & GREENHALGH, J.F.D., 1973. Lipids. In: *Animal nutrition* p. 25 (Ed.Oliver & Boyd). T. & A. Constable Ltd. - Edinburg.

McMEEKAN, C.P., 1959. Growth and development of pigs. In: *Principles of animal production* p. 116 (Ed. C.P. McMeekan) Whitcombe and Tombs Ltd.

McNITT, J.I., 1983. Pigs. In: *Livestock husbandry techniques*, p. 182 (Ed. Richard Clay) Granada Publishing Limited.

MERSMANN, H.J., 1987. Acute metabolic effects of adrenergic agonists in swine. *Am. J. Physiol.* 252: E85

MERSMANN, H.J., 1989. Acute changes in blood flow in pigs infused with β-adrenergic agonist. *J. Anim. Sci.* 67: 2913 - 2920

MERSMANN, H.J., MacNEIL, M.D., SEIDEMAN, S.C. & POND, W.G., 1989. Compensatory growth in finishing pigs after feed restriction. In: *Swine research. Progress report* no. 3: 34 - 36 (Ed. Roman L. Hruska). The Agricultural Research Service - U.S. Dept. of Agriculture.

MEYER, H.H.H., 1993. Anabolic β-agonists - biochemistry of action, pharmacokinetics, adverse effects in man and control in urine or blood. In: *Blood samples in doping control - proceedings of the second international symposium on drugs in sports - Lillehammer, Norway, August* 29 - 31, 1993 Ed: Peter Hammersbach & Käre I. Birkeland.

MITCHELL, A.D., SOLOMON, M.B. & STEELE, N.C., 1990. Response of low and high protein select lines of pigs to the feeding of the beta-adrenergic agonist ractopamine (Phenethanolamine). *J. Anim. Sci.* 68: 3226 - 3232

MITCHELL, A.D., SOLOMON, M.B. & STEELE, N.C., 1991. Influence of level of dietary protein or energy on effects of ractopamine in finishing swine. *J. Anim. Sci.* 69: 4487 - 4495

MUÑOZ, C., ARÉVALO, M.S.M., LóPEZ, M.T.M. & SCHLESINGER, M.D.L., 1994. Impaired interleuken - 1 and tumor necrosis factor

production in protein-calorie malnutrition. *Nutrition research*, vol. 14, no. 3, 347 - 352

MURRAY, J.D. & OBERBAUER, A.M., 1992. Growth hormone manipulation and growth promotants in sheep. In: *Progress in sheep and goat research* (Ed. A.W. Speedy) C.A.B. International.

NATIONAL RESEARCH COUNCIL (Ninth revised edition, 1988). Nutrient requirements of swine. *National academy press - Washington, D.C.*

NAUDÉ, R.T., 1974. Intensiewe vleisproduksie uit melkrasbeeste. *D.Sc. (Agric) - Proefskrif, Universiteit van Pretoria.*

O'CONNOR, J.J., 1980. Mechanisms of growth promoters in single-stomach animals. In: *Growth in Animals*, p. 207 (Ed. T.L.J. Lawrence) Butterworths - London - Boston.

OLIVER, M.A., GOU, P., GISPERT, M., DIESTRE, A., ARNAU, J., NOGUERA, J.L. & BLASCO, A., 1994. Comparison of five types of pig crosses. II. Fresh meat quality and sensory characteristics of dry cured ham. *Livestock production science* 40: 179 - 185

PATTERSON, R.L.S. & SALTER, L.J., 1985. Anabolic agents and meat quality: A review. *Meat science* 14: 191 - 220

PETERSON, E.B., PRACKEL, P.V., HERTEL, T.W. & McGUIRK, A.M., 1992. Impacts of growth stimulants in the domestic livestock sector. *Agribusiness - New York* 8: 4, 287 - 307

POND, W.G. & MANER, J.H., 1974. Pork as human food. In: *Swine production in temperate and tropical environments*, p. 31 (Ed. G.W. Salisbury & E.W. Crampton) H. Freeman and Company - San Francisco.

RADNóCZI, L. & FÉSÜS, L., 1993. Pig production in Hungary. *Pig news and information*, Vol. 14 no. 3 113N - 117N

REEDS, P.J., BURRIN, D.G. & DAVIS, T.A., 1993. Growth regulation with particular reference to the pig. In: *Growth of the pig*, p. 1 (Ed. G.R. Hollis) Cab. International.

RIOND, J.L. & RIVIERE, J.E., 1988. Multiple intravenous dose pharmacokinetics and residue profile of gentamicin in pigs. *J. Vet. Pharmacology and therapeutics* 11: 2, 210-214

ROCHE, J.F. & QUIRKE, J.F., 1986. The effect of steroid hormones and xenobiotics on growth of farm animals. In: *Control and manipulation*

of animal growth, p. 39 (Ed. P.J. Buttery, D.B. Lindsay & N.B. Haynes) Butterworths - London.

RULE, D.C., SMITH, S.B. & MERSMANN, H.J., 1989. Effects of adrenergic agonists and insulin on porcine adipose tissue lipid metabolism in vitro. In: *Swine research progress report* no. 3: 28-29 (Ed. Roman L. Hruska) The agricultural research service U.S. Dpt. of Agriculture.

SAUER, M.J., PICKETT, R.J.H., DIXON, S.N. & JACKMAN, R., 1993. Distribution and elimination of clenbuterol in calves following prolonged oral administration at a growth-promoting dose. In: *Residues of veterinary drugs in food* (Ed. N. Haagsma, A. Ruiter, P.B. Czedik - Eyenberg). Proceedings of the euro residue II conference - Veldhoven, The Netherlands 3 - 5 May, 1993.

SCHAEFFER, A.L., JONES, S.D.M., TON, A.K.W., De PASSILLE, A.M.B., RUSHEN, J. & MERRILL, J., 1992. The effect of feeding the beta-adrenergic agonist ractopamine on the behaviour of market-weight pigs. *Can. J. Anim. Sci.* 72: 15 - 21

SHAW, F.D., TROUT, G.R. & McPHEE, C.P., 1995. Plasma and muscle cortisol measurements as indicators of meat quality and stress in pigs.

SHERIDAN, P.J., AUSTIN, F.H., BOURKE, S. & ROCHE, J.F., 1990. The effect of anabolic agents on growth rate and reproductive organs of pigs. *Livestock - Production - Science*, 26: 4, 263 - 275

SIEBRITS, F.K., 1993. The pig industry in South Africa. In: *Pig production in South Africa*, p.1 (Ed. E.H. Kemm). The Agricultural Research Council - Pretoria.

SMITH, W.J., 1993. Antibiotics in feed, with special reference to pigs: a veterinary viewpoint. *Animal feed science and technology*, 45: 57 - 64

SMITH, W.W. & HUTCHINGS, L.M., 1952. General view. In: *Pork production*, p. 1 - 8 (Ed. The MacMillan Company) London.

SPURLOCK, M.E., CUSUMANO, J.C. & MILLS, S.E., 1993. [^{3}H]-Dihydroalprenolol binding to β-adrenergic receptors in porcine adipose tissue and skeletal muscle membrane preparations. *J. Anim. Sci.* 71: 1778 - 1785

STAMATARIS, C., KYRIAZAKIS, I. & EMMANS, G.C., 1991. The performance and body composition of young pigs following a period of growth retardation by food restriction. *Anim. Prod.* 53: 373 - 381

STECCHINI, M.L., GIOMO, A., CORINO, C. & POLIDORI, F., 1991. Effetti del clenbuterolo sol suino pesante: aspetti della qualita delle carcasse e delle carni. *Selezione-Veterinaria* 32: Supplement 1, 251 - 257

SVENDSEN, P., 1974. Food of domestic animals (Chapter 6). In: *"An introduction to animal physiology"* p. 49 - 67 (Ed. Per Svendson) medical and technical publishing Co. Ltd.

SWATLAND, H.J., 1984. The structure and properties of meat. In: *Structure and development of meat animals* (Ed. Library of congress cataloging in publication data) Prentice - Hall. Inc. New Jersey.

THORNTON, K., 1981. Feeding. In: *Practical pig production*, p. 124 (Ed. Farming Press Ltd - Suffolk).

THORNTON, K., 1988. Some health considerations. In: *Outdoor pig production*, p. 102 (Ed. Farming Press Books) British Library Cataloguing - U.K.

VAN DER MERWE, P.J., & PIETERSE, J.W., 1994. Stability of Zeranol, Nandrolone and Trenbolone in bovine urine. *Analyst*, vol. 119, 2651 - 2653

VEUM, T.L., ZAMORA, R.G. & SHERRY, M.P., 1986. Utilization of soybean and milk proteins by neonatal pigs reared artificially. In: *Swine in biomedical research* Vol. 2 p. 1113-1124 (Ed. Tumbleson, M.E.) Plenum press - New York.

WALLACE, D.H., HEDRICK, H.B., SEWARD, R.L., DAURIO, C.P. & CONVEY, E.M., 1988. Growth and efficiency of feed utilization of swine fed a Beta-adrenergic agonist. In: *Beta-agonist and their effects on animal growth and carcass quality*, p. 143 - 151 (Ed. U.K. Barking) Elsevier Applied Science Publishers Ltd.

WANDER, R.C., CLARK, S.L., HU, C.Y., HOLMES, Z.E. & SCHRUMPF, E., 1993. Interaction of porcine somatotropin administration to growing pigs and frozen storage of carcass on lipids and quality characteristics of roasts. *Journal of Food Composition and Analysis* 6, 62-74

WARRISS, P.D., KETIN, S.C., ROLPH, T.P. & BROWN, S.N., 1990. Effects of the Beta-adrenergic agonist salbutamol on meat quality in pigs. *J. Anim. Sci.* 68: 1, 128 - 136

WARRISS, P.D., NUTE, G.R., ROLPH, T.P., BROWN, S.N. & KESTIN, S.C., 1991. Eating quality of meat from pigs given the Beta-Adrenergic Agonist Salbutamol. *Meat-Science*, 30: 1, 75 - 80

WATANABE, T., 1976. Vegetable protein as a human food - background and present situation. In: *"Meat animals - growth and production"* (Ed. D. Lister, D.N. Rhodes, V.R. Fowler & M.F. Fuller) Plenum Press - New York and London.

WHEELER, T.L. & KOOHMARALE, M., 1993. Effects of a β-Agonist on muscle protein degradation, enzyme activity, and meat tenderness in steers. In: *Beef research - progress report* no. 4: 128 - 130 (Ed: Roman L. Hruska) The agricultural research service - U.S. Meat Animal Research Center.

WHITTEMORE, C.T., 1980. Records and recording. In: *Pig production - The scientific and practical principles.* (Ed. C.T. Whittemore, R.J. Thomas, J.H.D. Prescott) Longman Handbooks in Agriculture - London.

WHITTEMORE, C.T., 1993. Growth and body composition changes in pigs. In: *The science and practice of pig production*, p. 48 (Ed. Longman Group UK Limited). British Library Cataloguing in Publication Data.

WIDDOWSON, E.M., 1980. Definitions of growth. In: *Growth in animals*, p. 1 (Ed. T.L.J. Lawrence) Butterworths - London - Boston.

WILLEKE, H., 1993. Possibilities of breeding for low 5α-androstenone content in pigs. *Pignews and Information*, Vol. 14 No. 1 31N - 33N

WILSON, G.D.A., HARVEY, D.G. & SNOOK, C.R., 1972. A review of factors affecting blood biochemistry in the pig. *Br. Vet. J.*, 128, 596

WOOD, J.D., MOTTRAM, D.S. & BROWN, A.J., 1981. A note on the eating quality of pork from lean pigs. *Anim. Prod.* 32: 117 - 120

YEN, J.T., MERSMANN, H.J., NIENABER, J.A., HILL, D.A & POND, W.G., 1990. Responses to cimaterol in genetically obese and lean pigs. *J. Anim. Sci.* 68: 2698 - 2706

YEN, T.J., NIENABER, J.A., KLINDT, J. & CROUSE, J.D., 1991. Effect of ractopamine on growth, carcass traits and fasting heat production of U.S. contemporary crossbred and chinese meishan pure- and crosbred pigs. *J. Anim. Sci.* 69: 4810 - 4822

YEN, J.T., NIENABAR, J.A. & POND, W.G., 1989. Effect of neomycin and carbadox on growth, fasting metabolism and gastrointestinal tract of young pigs. In: *Swine research progress report* no. 3: 60 - 61 (Ed. Roman L. Hruska) The Agricultural Research Service - US. Dept. of Agriculture.

ZHANG, W., HUISKES, J.H. & RAMAKERS, P.J.L., 1993. Serial ultrasonic measurements of backfat thichness in growing - finishing pigs II. Relationship with carcass traits. *Pig News and information* Vol 14 No. 4 177N - 180N