Institute of Animal Husbandry and Animal Breeding
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# Effects of immunological castration on the regulation of metabolism in boars

DISSERTATION

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**Abbreviations**

| | |
|---|---|
| **DHT** | 5α-dihydrotestosterone |
| **FSH** | Follicle stimulating hormone |
| **GCR** | Glucocorticoid receptor |
| **GH** | Growth hormone |
| **GH-RH** | Growth hormone releasing hormone |
| **GnRH** | Gonadotropin releasing hormone |
| **HSD** | 3β-hydroxysteroid dehydrogenase |
| **IGF-I** | Insulin-like growth factor I |
| **LH** | Luteinizing hormone |

# 1 Introduction

## 1.1 Tradition of castration in pig production

Surgical castration of male animals is one of the oldest biotechnical measures applied in animal production and has been known to almost all nations and cultures. The primary reason was the abolishment of testicular hormones and thus their stimulating effect on aggressive behaviour. In consequence, the castrated males were easier to handle.
The tradition to castrate male pigs goes back to medieval times and was additionally performed because the castrated male pigs (barrows) deposit much more fat instead of protein than entire male pigs (boars). Fat accumulation was preferred by the market until a couple of decades ago because its high energy content was required by a hard working human population (EFSA, 2004). When muscle work was substituted by machines and automation in industry started about 60 years ago, this process was paralleled by a rapid change of market requirements toward carcasses with less fat and more muscle protein. Today, carcasses which contain maximal amounts of lean meat are definitely preferred. In consequence, discussions arose to leave boars uncastrated because their carcasses are characterized by high deposition of protein and accordingly decreased accumulation of fat. But the main cause of still castrating male pigs is the occurrence of the so-called boar taint in the carcass of entire male pigs. This undesirable urine-like odour is not acceptable to many consumers (Matthews et al., 2000). Nevertheless, fattening of entire male pigs is still under discussion, hoping that the formation of anabolic hormones and boar taint, which are both synthesized in the testes, may be separated some day.

## 1.2 Advantages and disadvantages of fattening entire male pigs

Many studies confirmed that differences in fattening performance exist between castrates (barrows), intact males (boars) and females (gilts) (Witt and Schröder, 1969; Walstra, 1974; Campbell et al., 1989; Neupert et al., 1995) which are explained by the availability of the anabolic gonadal hormones. However, intensive breeding for protein deposition led to a remarkable shift in the meat-fat-ratio so that barrows today have the same fattening performance as reported for boars 25 years ago (Claus, 1993). Nevertheless, the influence of gonadal steroids still exists and thus the differences between the sexes.
Economic advantages may result when leaving boars uncastrated and useing them routinely for meat production. These advantages include lower production costs, e.g. no labour costs resulting from castration, the absence of animal losses, and a decrease in growth

performance following castration. In addition, entire boars grow more rapidly and exhibit an improved feed conversion rate (Table 1a-c). In a study by Spring et al. (2009; Table 1c), barrows had a higher daily weight gain and a higher daily feed consumption. But this supposed better performance resulted in fat deposition instead of lean meat, which is underlined by the thickness of back fat and the less lean meat content. Additionally, barrows in this study had a worse feed conversion rate compared with boars. Thus, boars produce carcasses with higher retail yields due to the fact that they synthesize more lean meat and less fat than their castrated counterparts (Martin, 1969; Ellis et al., 1983; Dobrowolski et al., 1993). Gilts usually are intermediate in these criteria while barrows are generally inferior in all these traits (Walstra and Kroeske, 1968; Kay and Houseman, 1975).

Further boars have the ability of a higher protein retention which is caused by the anticatabolic effect of gonadal steroids. Therefore, the nitrogen excretion is reduced. In areas with a high production density, the fattening of boars was considered as a possible alternative to avoid higher pollution of the soil and drinking water with nitrogen, but to maintain or even increase the number of animals per production unit (Claus, 1993). In studies where barrows were treated with a combination of androgens and oestrogens, the nitrogen retention was increased by 25%. (Van der Wal et al., 1975; Van Weerden and Grandadam, 1976; Berende et al., 1980). Furthermore, the treatment with androgens and oestrogens resulted in an average increase in protein deposition of 40%, and fat deposition was decreased by 15-20%. These results confirm that gonadal steroids affect the balance between protein synthesis and breakdown in favour of a higher protein deposition (Van Weerden and Grandadam, 1976). The anticatabolic effect of androgens, and thus the improved N-retention again, is explained at least partly by the fact that androgens antagonize glucocorticoids and their catabolic functions (Mayer and Rosen, 1977; Snochowski et al., 1981).

Table 1a: Differences between the sexes during fattening from 40-110 kg body weight (Witt and Schröder, 1969)

| | boars | barrows | gilts |
|---|---|---|---|
| feed consumption (kg/d) | 2.45 | 2.54 | 2.48 |
| weight gain (g/d) | 808 | 727 | 724 |
| feed conversion rate | 3.05 | 3.51 | 3.45 |
| back fat thickness (cm) | 3.12 | 3.53 | 3.32 |

Table1b: Differences between the sexes during fattening from 60-100 kg body weight (Campbell et al., 1989)

| | boars | barrows | gilts |
|---|---|---|---|
| feed consumption (kg/d) | 3.21 | 3.67 | 3.38 |
| weight gain (g/d) | 1186 | 1057 | 1011 |
| feed conversion rate | 2.72 | 3.46 | 3.34 |
| back fat thickness (cm) | 1.48 | 2.50 | 2.12 |

Table 1c: Differences between the sexes during fattening from 28-207 kg body weight (Spring et al., 2009)

| | boars | barrows |
|---|---|---|
| feed consumption (kg/d) | 2.06 | 2.35 |
| weight gain (g/d) | 885 | 927 |
| feed conversion rate | 2.33 | 2.54 |
| back fat thickness (cm) | 1.29 | 1.95 |
| lean meat content (%) | 56.6 | 53.2 |

An argument against routine fattening of boars is an inferior meat quality. Meat of boars can be too lean and thus is often found to be less tender, and meat with a deficit in intramuscular fat is inferior in flavour and juiciness (Branscheid, 1993). Similarly, the processing industry needs a minimum of fat content, but the fat of entire males is softer and less resistant to oxidation due to a higher quantity of unsaturated fatty acids (Malmfors and Nilsson, 1978; Barton-Gade, 1987). In addition to these criteria, the most eminent limitation to use entire male pigs is the occurrence of boar taint.

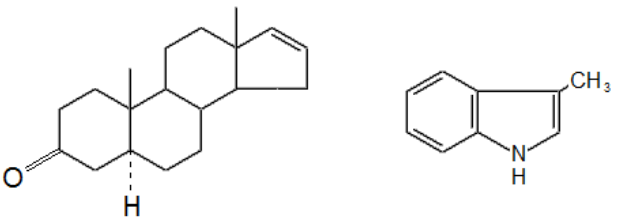
## 1.3 Chemical aspects of boar taint

Consumer acceptance of pork from boars is hindered by a strong, objectionable odour of the heated fat and meat of many boars (Malmfors and Lundström, 1983; Matthews et al., 2000). Table 2 shows the percentage of pigs separated in androstenone classes. 87% of the boars had androstenone concentrations higher than 0.3 µg/g fat, whereas all barrows were below this value. This “perspiration-like” or “urine-like” odour has become known as “boar odour”, “boar taint” or “male sex odour” in pork and is associated with the Δ16-steroid 5α-androst-16-en-3-one (androstenone; Patterson, 1968). More recently, 3-methylindole (skatole, Void 1970) with a faecal odour was suspected as another reason for “boar taint”, which stimulated discussions on the relative contribution to the unpleasant odour (Bonneau, 1982).

Table 2: Percentage of animals in androstenone classes influenced by sex (Stamer et al., 1993)

| androstenone level (µg/g fat) | barrows (%) | boars (%) |
|---|---|---|
| < 0.04 | 86.5 | 10.3 |
| 0.04-0.3 | 13.5 | 2.6 |
| 0.3-1 | 0 | 59.0 |
| > 1 | 0 | 28.1 |

Figure 1 compares typical characteristics of the two compounds. Androstenone is a steroid which has a chemical structure similar to the testicular sex hormones, but it has no hormonal activity (Claus, 1979).The characteristic double bond for the Δ16-steroids at the C-16 results in smell properties of androstenone instead of hormonal activity. In contrast, skatole is derived from the amino acid trypthophan and is not directly linked to testicular function.




| | androstenone | skatole |
|---|---|---|
| chemistry | steroid | indole |
| site of synthesis | testes | colon |
| type of smell | urine-like odour | faecal-like odour |
| possibilities to inhibit the formation | not selectively influencable | influencable by nutrition |

Figure 1: Compounds contributing to boar taint (Claus, 2005)

### 1.3.1 Androstenone

Androstenone is synthesized in the Leydig cells of the testes in parallel to the synthesis of anabolic sex hormones (Gower and Ahmad, 1967) which in the porcine species comprise androgens and oestrogens (Claus and Hoffmann, 1980). Synthesis of androstenone and of testicular hormones is stimulated by the luteinizing hormone (LH), which in turn is stimulated by gonadotropin releasing hormone (GnRH) (Andresen, 1975; Carlström et al., 1975). Thus, the degree of boar taint is strictly coupled to the degree of formation of anabolic hormones.
The occurrence of a group of steroids with smell properties (“16-unsaturated steroids” or Δ16-steroids) in testicular tissue from boars was first described by Prelog and Ruzicka (1944) who also reported on the urine-like taint of androstenone due to the presence of a ketogroup at the C-3 position. This substance was later found to be present in high concentrations in adipose tissue and to be the reason for the characteristic urine-like boar taint (Patterson, 1968; Claus et al., 1970; Claus et al., 1971; Beery and Sink, 1971; Fuchs, 1971; Thompson et al., 1972).

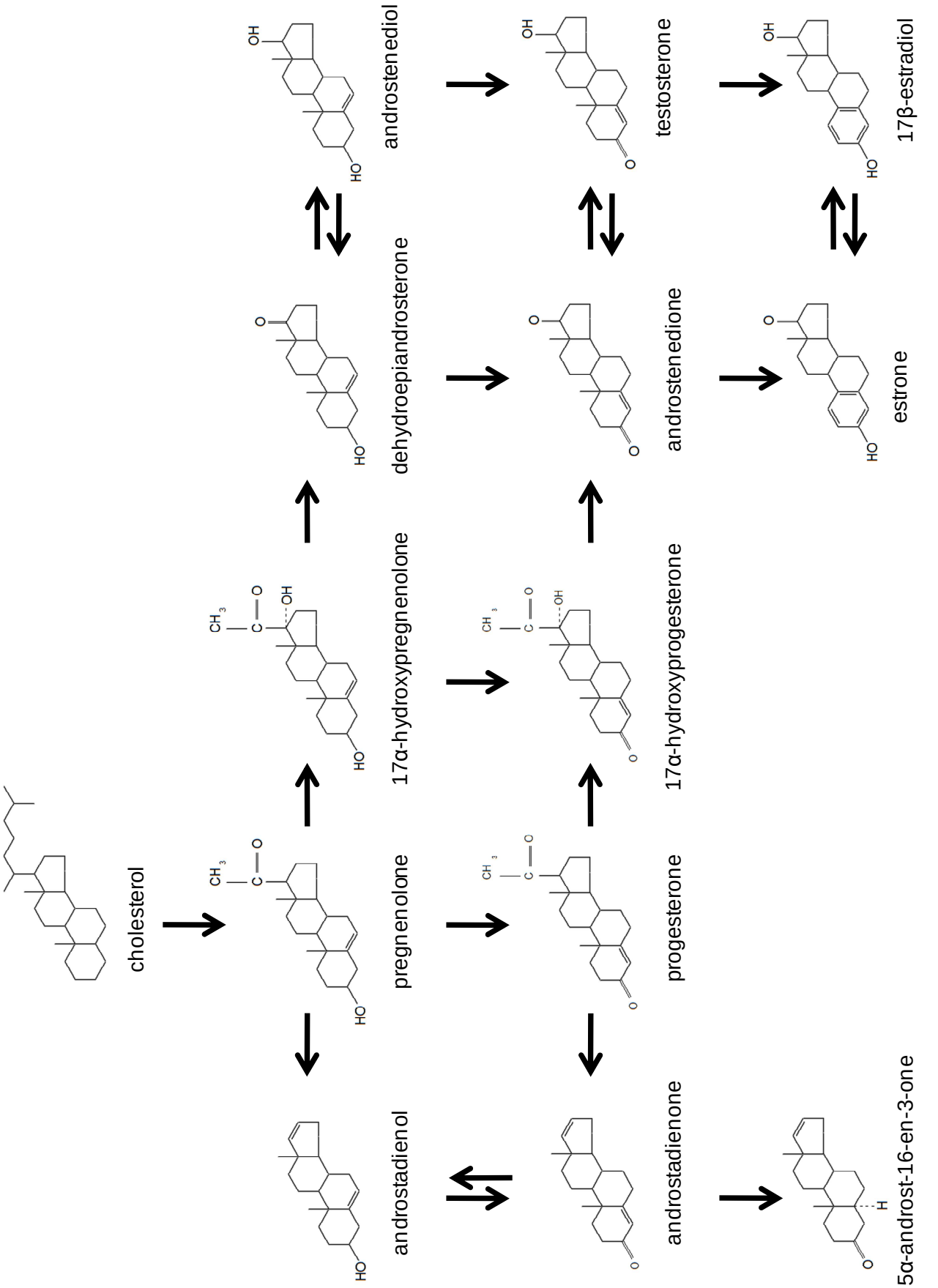



Figure 2: Biosynthesis of gonadal steroids (Bonneau, 1982)

The principle of biosynthesis of androstenone together with the other testicular steroids is summarized in Figure 2. LH stimulates the synthesis of the precursor steroid pregnenolone which then is delivered by the enzyme 3β-hydroxysteroiddehydrogenase (HSD) both for further synthesis of the gonadal hormones (androgens and oestrogens) and the Δ16-steroids (androstenone). Thus a close relationship exists between the secretion of androstenone (boar taint) and the sexual status of the male pig. Many attempts to suppress androstenone selectively but to maintain the anabolic potential of boars have been unsuccessful due to the common biosynthesis (see 1.4.2).

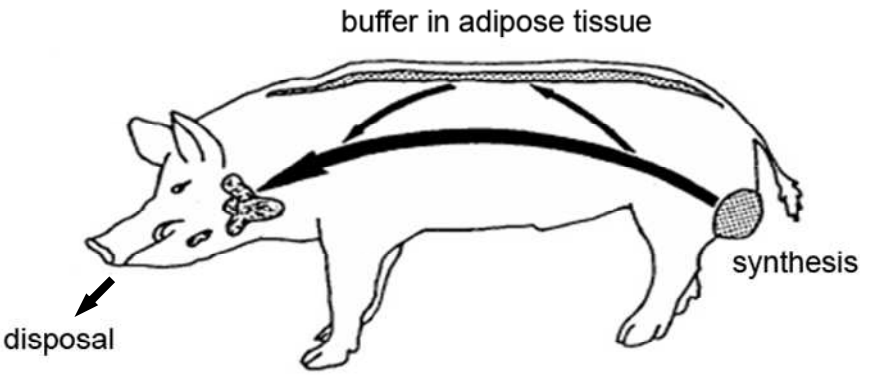



Figure 3: Synthesis of androstenone in the testes and its release via the salivary gland (Claus, 1991)

Androstenone is transferred from the testes into the blood circulation via the vena spermatica (concentrations of 20 ng/mL plasma; Andresen, 1974; Claus and Hoffmann, 1980). Due to its lipophilic character, androstenone is accumulated in adipose tissue. The adipose tissue acts like a reservoir and androstenone can be released from fat when de novo synthesis is decreased in the testes (Figure 3). Claus (1979) found androstenone concentrations of 0.2-4 µg/g fat in carcasses with 80 kg of weight. Neupert et al. (1995) analysed carcasses with 85 kg of weight and determined mean androstenone values of 1.21 µg/g fat. The high variation of androstenone concentrations is due to differences in the stage of pubertal development which is reached during the final period of fattening and depends e.g. on breed. The final pubertal spurt is expected at an age of 15-17 weeks (Figure 4) (Booth, 1975; Allrich et al., 1982; Bonneau, 1987; Schwarzenberger et al., 1993). In addition, the variability of androstenone levels is influenced by housing conditions (e.g. groups of boars and thus social interactions) (Giersing et al., 1997, 2000).

Physiological androstenone acts as a pheromone during mating behaviour and induces the standing reflex of the sow before copulation (Signoret et al., 1960; Melrose et al., 1971;

Willems, 1972; Reed et al., 1974). Additionally, it may lead to the release of oxytocin as a stimulant of sperm transport during mating (Claus and Schams, 1990).

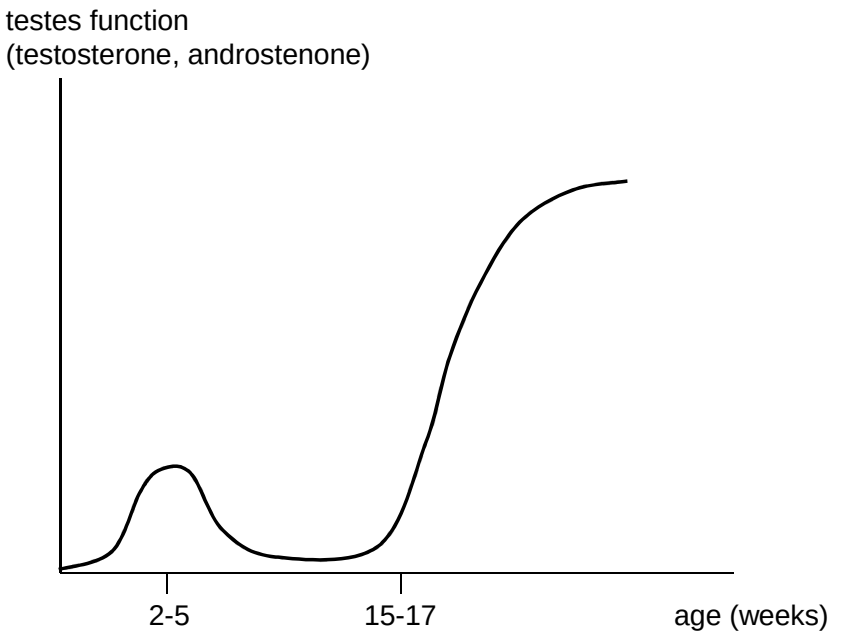



Figure 4: Time course of pubertal development of boars (Claus et al., 1994)

The sensibility of consumers for androstenone differs individually. Some persons have a specific anosmia for androstenone, i.e. they are not able to smell androstenone although these persons have a normal sense of smell for other substances (Table 3). Among the persons who are able to smell androstenone, some are highly sensitive even to concentrations below the threshold of 0.5 µg/g fat. Another limitation for the organoleptic evaluation of androstenone is that even persons with the ability to smell this substance can judge negative samples as positive ones because after smelling androstenone, the receptors are occupied for a certain time (“reverberation effect”).

Table 3: Ability to smell androstenone

| persons (n) | none (%) | less (%) | medium (%) | strong (%) | |
|---|---|---|---|---|---|
| 286 | 25.0 | 23.5 | 33.5 | 18.0 | Elsley, 1968 |
| 301 | 27.5 | - | - | 17.5 | Griffith and Patterson, 1970 |
| 520 | 28.5 | 22.0 | 29.0 | 20.5 | Claus unpublished |

### 1.3.2 Skatole

Skatole is produced by microbial degradation of the amino acid tryptophan in the colon (Figure 5). A part of skatole is excreted with faeces but another part is resorbed from the gut and then accumulated in fat tissue. Neupert et al. (1995) found mean skatole concentrations of 131 ng/g fat but also a considerable variation between individuals. The faecal odour of skatole, which is smelled by almost all persons, can be found in barrows as well as in gilts, but the frequency of high concentrations above a cut-off value of 200 ng/g fat is clearly higher in entire boars. The presence of high levels in pig meat certainly is an olfactoric problem, but this off odour can be controlled with feeding strategies. Feeding e.g. potatoe starch during the last week before slaughter decreases skatole concentrations far below the cut-off value (Claus et al. 1994, 2003; Lösel and Claus, 2005; Lösel et al., 2006; Spring et al., 2009).

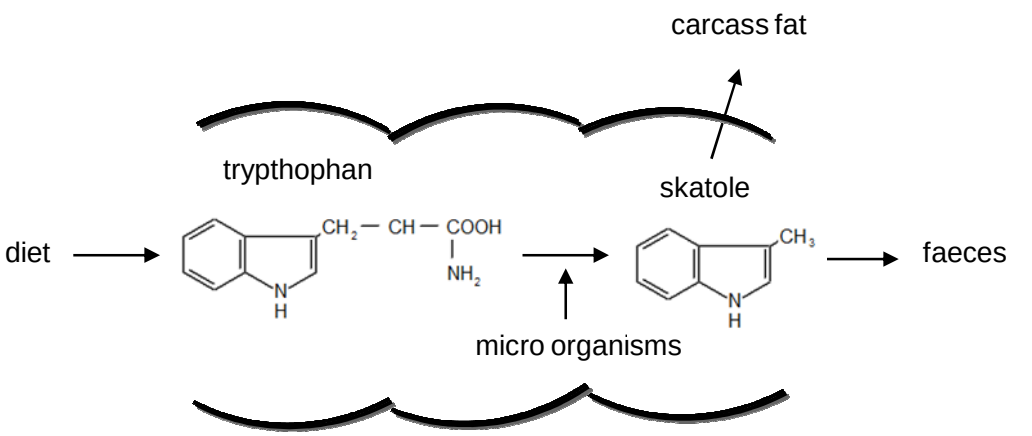



Figure 5: Schematic view of skatole formation in the colon (Weiler et al., 1992)

## 1.4 Anabolic effects of testicular hormones

### 1.4.1 Physiological principles

In boars, testosterone and 5α-dihydrotestosterone (DHT) are the most important androgens produced in testes. DHT was measured with maximum concentrations of 2.75 ng/mL in blood plasma (average concentrations of 0.75 ng/mL). It stimulates specifically reproductive phenomena such as the development and function of accessory glands (Parrot and Booth, 1984). Testosterone occurs in average plasma concentrations of 2.15 ng/mL (maximum of 11.50 ng/mL; Claus et al., 1983). The variation in androgen concentration depends on the time of day (Claus and Gimenez, 1977; Claus and Hoffmann, 1980), on age (Lapwood and Florcruz, 1978; Allrich et al., 1982; Schwarzenberger et al., 1993) and on the seasonal effect of the photoperiod (Hoagland and Diekmann,1982; Claus et al., 1983; Andersson et al., 1997).
In addition to its role for reproduction, testosterone is involved in the stimulation of male behaviour including aggression, and it exerts various effects on metabolism. The latter effects are mediated by androgen receptors in muscle tissue (Snochowski et al., 1981) and include both a stimulation of protein synthesis and deposition (“anabolic effect”; Claus and Weiler, 1994) and an inhibition of catabolic effects provided by glucocorticoids (“anticatabolic effect”; Mayer and Rosen, 1977; Sharpe et al., 1986; Chen et al., 1997). This anticatabolic effect is mainly responsible for the improved protein deposition found in entire boars compared to barrows and, less pronounced, compared to gilts (Metz, 2003).

Boars additionally synthesize high amounts of oestrogens such as 17β-oestradiol. Its concentrations in boar blood plasma may even exceed levels found in oestrus sows (Claus and Hoffmann 1980; Claus et al., 1983, 1987). Oestrogens have synergistic effects to androgens, e.g. for male behaviour and libido (Parrot and Booth, 1984), and also for spermatogenesis (Wagner et al., 2006). Oestrogens also have an effect on growth because they stimulate protein deposition indirectly by stimulating the GH-IGF-I system (GH growth hormone, IGF-I insulin-like growth factor I) (Claus and Weiler, 1994). For this role, oestrogens lead to a rise of IGF-I by increasing the expression of GH receptors in the liver (Gabrielsson et al., 1995). In consequence, normalization of a boar-specific growth potential by steroid application in barrows requires a combination of an androgen and an oestrogen (Van Weerden and Grandadam, 1976; De Wild and Lauwers, 1984) as outlined in Figure 6.

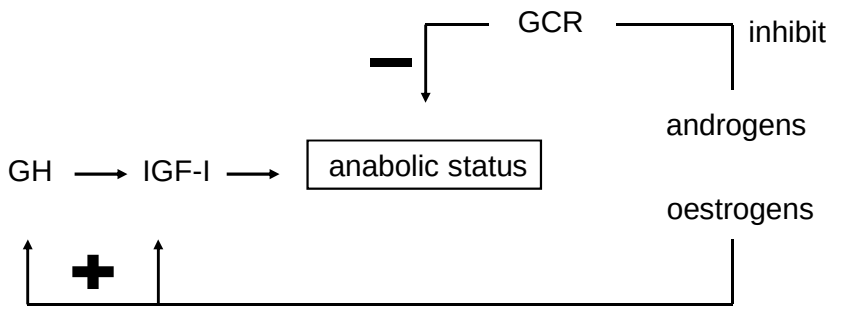



Figure 6: Principles of endocrine regulation of protein deposition (Claus et al., 1994) (GCR glucocorticoid receptor)

Producers should take advantage of the anabolic hormones produced by the testes. But the close relationship between synthesis of anabolic steroids and of androstenone makes the success of pork production with intact boars doubtful. Attempts to separate the secretory capabilities of the testes into anabolic hormones and pheromones failed so far.

### 1.4.2 Attempts to abolish androstenone but to maintain the anabolic potential

Many attempts were made to use the metabolic advantages of boars but to abolish boar taint selectively. They were all directed towards an elimination of androstenone from the carcass because it is the main substance responsible for the urine taint in almost every boar slaughtered beyond puberty.

One possibility was the “hormonal castration” and thus the control of androstenone synthesis by exogenous application of hormones (Echternkamp et al., 1969; Ertl et al., 1972). This treatment influenced the secretion of LH causing an atrophy of the Leydig cells and decreased testicular activity (Sheridan et al., 1990). In consequence, androstenone as well as testosterone were reduced, i.e. the anabolic potential was also lost in treated boars (Daxenberger et al., 2001). The implantation of anabolic preparations into barrows increased nitrogen retention and decreased fat deposition (Van Weerden and Grandadam, 1976; De Wild and Lauwers, 1984). Therefore, surgical castration together with application of anabolic hormones can improve the fattening performance of barrows. But these attempts are theoretical possibilities because they have no practical relevance due to residue considerations and consumer protection.

Genetic selection for pigs with low levels of tissue androstenone concentrations at the time of slaughter is possible (Willeke et al., 1980; 1987) and a high heritability was confirmed. But

these attempts also resulted in a decreased performance due to joint testicular biosynthesis of anabolic steroids and androstenone which could not be decoupled. The genetic selection also led to a delay of puberty in boars as well as in gilts. Therefore, genetic selection for low androstenone concentrations has a negative influence on reproductive traits which is not desirable for breeding.

Another approach was the active immunization against androstenone which was basically successful (Claus, 1975; Williamson et al., 1985; Brooks et al., 1986). However, this possibility was not investigated further because titre development differed considerably between individuals and repeated immunization injections were required.

An alternative could be to sort X from Y carrying sperm so that only X-sperm are used for artificial insemination to produce only gilts for fattening. But this method is currently only in a state of research, i.e. this strategy is far from practical application and is not paralleled with the higher anabolic potential of boars. One problem is the huge number of sperm needed for a satisfactory insemination with the ordinary insemination technique (Johnson et al., 2005; Martinez et al., 2005). In addition, sorting of sperm would considerably rise the costs of pig production.

Slaughter at a young age before the final pubertal spurt and thus less probability of occurrence of androstenone is an alternative which has traditionally been performed in England and explains the acceptance of boar meat by part of the consumers. Today, economic considerations require slaughter of pigs with much higher and still increasing final weights. Therefore, the number of tainted carcasses from entire boars will inevitably rise.

Processing of meat by procedures which remove androstenone from the product or destroy the chemical structure of the substance could be a possible solution to use pork from entire males. Claus et al. (1985) found that almost all of the traditional products still contain the levels of androstenone as measured in raw material before processing. Only products which were subject to prolonged heating may have partially reduced androstenone concentrations due to evaporation. Another study investigated the possibility to overlay boar taint with flavours from fermentation or smoking (Stolzenbach et al., 2009). Mixing of processed meat with spices revealed no satisfactory results (Lunde et al., 2008).

As there are no alternatives to inhibit boar taint, surgical castration is performed in most countries up to now. It is performed usually without anaesthesia early after birth. Since a couple of years, however, surgical castration without anaesthesia became an animal welfare issue so that alternatives are urgently required.

## 1.5 Animal welfare and castration

Every year approximately 100 million male piglets are castrated without anaesthesia in the European Union (Thun et al., 2006; Heinritzi et al., 2008). Due to economical priorities and lack of suitable alternatives, the animal welfare legislation in most European countries allows castration up to seven days without anaesthesia. If castration is practiced after seven days of age, it shall only be performed under anaesthesia and additional prolonged analgesia by a veterinarian. This practice was also confirmed by the EU Commission Directive (2001/93/EC).

Castration without anaesthesia is meanwhile regarded to induce acute pain and stress and will therefore not be tolerated any longer by animal welfare organizations. Piglets that were not anaesthetized during castration produced a higher proportion of screaming sounds, which probably indicates acute pain provoked by castration (Puppe et al., 2005). Llamas Moya et al. (2008) confirmed that the behaviour of piglets is modified after castration. During the initial hours following castration, pigs showed less locomotion, more trembling and spasms, they huddled-up more and scratched their rump. Three days later, castrated piglets were more isolated, showed less social interactions and increased dog sitting behaviour. Thus, welfare organizations demand a method that is more in accordance with the animal welfare issue. In Norway, surgical castration without anaesthesia has not been allowed since 2003. Until another solution is found, only veterinarians may perform castration under anaesthesia. In Switzerland, since 2010 castration has been permitted under anaesthesia only. The Netherlands are considering to stop surgical castration at the latest by 2015, on the basis of increasing reactions from consumers in relation to the daily castration routine. Sweden will prohibit castration in 2012.

Apart from the boar taint problem, raising uncastrated boars is not an optimal alternative because of fighting between boars and mounting behaviour resulting in leg injuries, lameness and skin lesions especially towards the end of the fattening period as the boars become sexually mature (EFSA, 2004). Some studies indicate that aggression in entire males may be a welfare problem as well (Rydhmer et al., 2006; Salmon and Edwards, 2006; Boyle and Björklund, 2007; Fredriksen and Hexeberg, 2008; Tuyttens et al., 2008). This problem will be intensified by split marketing. Fredriksen et al. (2008) found reduced levels of aggression lesions shortly before slaughter if groups stayed together from birth to slaughter without any mixing. Some countries as the United Kingdom, Ireland and Spain, which raise entire males but slaughter at a lower age, gradually increase the age for slaughter on the grounds that producers earn more when slaughtering heavier pigs. Therefore, the problem with fighting and boar taint will continuously become more evident in these countries.

One possible alternative is the castration under general or local anaesthesia followed by analgesia. The narcotic treatment permits to perform the castration of the piglets without feeling pain and therefore the welfare aspect is guaranteed. Problems with anaesthesia are that at the moment only veterinarians are allowed to administer these narcotics, introducing unacceptable costs for the farmers. The anaesthetic gas isoflurane is currently under discussion but it additionally requires an analgesic drug to suppress pain the days following castration (Schulz, 2007; Schulz et al., 2007a, 2007b). In Switzerland, routine castration is practised under isoflurane inhalation anaesthesia with a specially designed application system, combined with additional pain treatment before castration. Kupper et al. (2008) found that 92% of the castrated piglets showed no defence movement.

Another anaesthetic gas used for general anaesthesia is $CO_2$. A mixture of 30% oxygen and 70% $CO_2$ has been found to be the most effective combination (Kluivers-Poodt et al., 2007; Gerritzen et al., 2008). This anaesthesia is applied in the Netherlands. But $CO_2$ exposure has been reported to cause distress until loss of consciousness (EFSA, 2004; Rodrigez at al., 2008), and the anaesthetic and the lethal dose are close together.

Lahrmann et al. (2006) investigated general anaesthesia by an injection method. They found that anaesthetics are easily given with an injection pistol and that analgesia is satisfactory. But they reported losses of 3-5%. Other studies showed that the same anaesthetic was insufficient to induce adequate depth of anaesthesia in a significant number of piglets (Leeb et al., 2008; Schmidt and von Borell, 2008). It was demonstrated that pigs showed pedal reflex, vocalisation and withdrawal movements.

In Germany, it was recommended to perform castration with an analgesic agent (“Düsseldorfer Erklärung”, 2008). An injection of lidocaine into the testis or the spermatic cord was considered effective in reducing pain during and after the castration (Heinritzi et al., 2006; Zöls et al., 2006a, 2006b). In a study of Fredriksen and Nafsted (2006), 54% of the veterinarians and 19% of the producers evaluated the effect of predominantly subcutaneous and then intratesticular injection of lidocaine with adrenaline to be satisfying. The most common complications with local anaesthesia reported in this study were abscesses. A general problem of castration with anaesthesia is its need for a waiting time between application and the onset of its pain-suppressing effect, as well as the problem that the use of an anaesthetic can hardly be controlled under routine practice by the farmers. Therefore, the question arises if surgical castration under anaesthesia is a practical method for the daily routine in pork production.

Another approach which will not compromise animal welfare is the active immunization against GnRH. Immunization is a well established technique in animal production, and a promising and realistic alternative to surgical castration.

## 1.6 Principles of immunization and its effects on metabolism

Active immunization against substances produced naturally in the body has been known since the 1930s (Scaramuzzi et al., 1993). It is an effective method which can abolish individual endocrine signals so that their role for regulation of physiological phenomena can be studied in detail. Specifically abolishment of GnRH by active immunization was an early and promising approach because GnRH is on top of a complex system which influences the complete reproductive system, i.e. the gonadotropins and the gonadal steroids (Hage-van Noort et al., 1992; D'Occhio, 1993). Moreover, GnRH is synthesized only in tiny amounts in the hypothalamus, so that these low amounts can easily be inactivated by antibodies. In consequence, immunization against GnRH has proven to be a powerful tool for practical, on-farm applications as well as for studying basic aspects of reproduction in the field of reproduction research (Thompson, 2000). As GnRH is a hapten, which for its own is too small to be immunogenic, it is chemically linked to a carrier protein. This complex is mixed with an adjuvant to form a dose for vaccination (Talwar, 1985; Oonk et al., 1998).

GnRH is produced in cell bodies in the hypothalamic neurons and is transported with blood flow to its target cells in the anterior pituitary. There, GnRH binds to the gonatotrope cells and LH and follicle stimulating hormone (FSH) are synthesized and released (Figure 7). During the short transport from the hypothalamus to the pituitary, the hormone can be attacked by antibodies. Binding to antibodies neutralizes GnRH either by preventing it from diffusion through the capillary walls (due to the size of the complex) or by masking the receptor binding site on the GnRH molecule itself (Thompson, 2000). The immunoneutralization results in a blockade of the GnRH-LH axis and of the synthesis of anabolic steroids and androstenone in the testicular Leydig cells. Consequently, the taint levels are decreased but the anabolic potential is lost as well. Today, the on-farm application of immunization to avoid surgical castration is of primary interest as a method to inhibit sexual development and boar taint (Caraty and Bonneau, 1986; Bonneau et al., 1994).

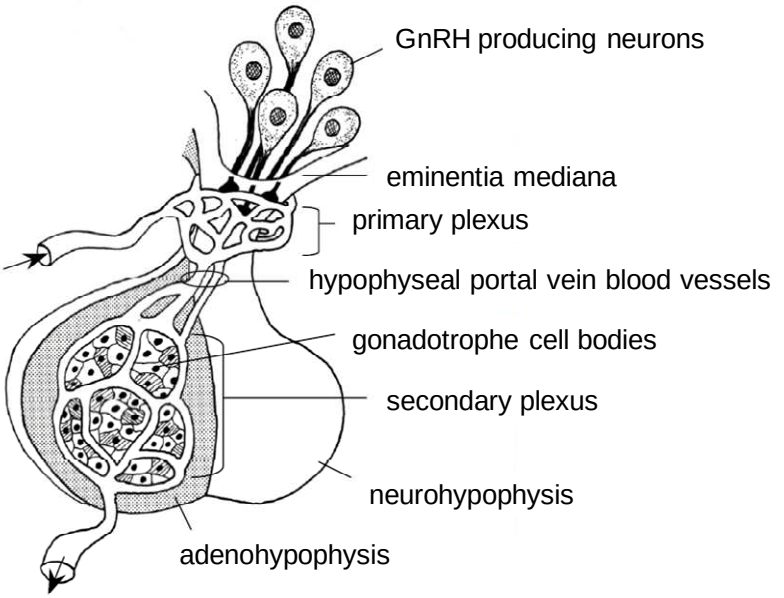



Figure 7: Neuro-endocrine structures relevant for “immunological castration” (Rosenzweig et al., 1996)

Falvo et al. (1986) vaccinated boars twice with GnRH conjugated to human serum globulin in two different adjuvants. The second (so-called anamnestic) vaccination resulted in high antibody titres and thus had a castration effect. LH and testosterone concentrations were significantly reduced and weights of testes and accessory glands were lower compared to control boars. Further, the incidence of boar taint in immunized male pigs was lower than in intact boars. Other studies, which compared immunized boars with surgically castrated and intact ones, confirmed that two injections are required to achieve high and thus effective antibody titres (Bonneau et al, 1994; Manns and Robbins, 1997; Hennessy et al., 1997; Dunshea et al., 2001). In addition, these studies found that immunized boars had less feed intake and better feed efficiency rates compared to surgical castrates. The thickness of backfat was significantly lower in immunized boars: Boars had 18.3 mm of backfat, immunized boars 24.5 mm, and barrows 28.0 mm (Manns and Robbins, 1997).

One commercially available vaccine is Improvac® (Pfizer Animal Health). Improvac® contains a modified form of GnRH in an aqueous adjuvant that causes little tissue aggravation (Dunshea et al., 2001). All pigs in this study that were treated with two doses of Improvac® showed an immune response, and the majority of immunized boars had no tissue reactions following the vaccination. Testosterone levels were reduced and testicular growth had ceased within two weeks after the second dose. 24% of the control boars had

androstenone concentrations of 0.5-1.0 µg/g fat, and another 49% had levels higher than 1.0 µg/g fat. The immunized male pigs did not differ from surgical castrates. Only 3% of the Improvac® treated boars had androstenone levels of 0.5-1.0 µg/g fat, the remaining immunized boars were below 0.5 µg/g fat which is the threshold value for acceptance by consumers. Thus, immunization with Improvac® is an effective method to inhibit the testicular development and boar taint. A relevant factor regarding animal welfare is the problem of fighting between boars. 30 pigs in this study exhibited fighting lesions, 26 were untreated boars whereas only 4 were immunized boars. All other animals showed no fighting scars. Surprisingly, the immunized boars showed greater daily gain and grew 7-32% faster in the last weeks of the fattening time (Dunshea et al., 2001). It was concluded that this effect was due to increased feed intake rather than a change in feed conversion rate. Nevertheless, feed conversion rate and backfat in immunized boars and entire boars were similar and less than in barrows. Turkstra et al. (2002) reported that the growth performance (i.e. average daily gain and feed efficiency) in entire boars and in immunized boars which responded late to the immunization was better than in barrows and in immunized, early responding boars. These results indicate that the time schedule of the immunization and subsequent reactions are of great importance.

Thus, immunized male pigs had a better fattening performance than surgical castrates even though the anabolic hormones were very low. Therefore, other compensatory mechanisms have to be considered. Metz and Claus (2003) found high GH concentrations in immunized boars (3.88 ng/mL) similar to control boars (4.19 ng/mL). This maintenance of GH in immunized boars was confirmed in another study (see 2.1), but it is not known why GH is elevated. The possibility that immunized boars retain at least part of their anabolic potential and thus an improved fattening performance and carcass quality is of high practical relevance. It might help to compensate the costs of the vaccinations. Additionally, an improved protein deposition at the expense of fat might be used to elevate the slaughter weight and thus to compensate the costs for the piglet. It is necessary, however, to confirm remaining fattening advantages by detailed studies that also include physiological parameters to characterize metabolic reactions. Such studies should also characterize endocrine reactions which are not directly related to testicular functions. Based on published data, three different hypotheses were raised. They are given below.

## 1.7 Theories underlying the own experimental studies

### 1.7.1 Interaction between the releasing hormones GnRH and GH-RH in the hypothalamus or the pituitary

This hypothesis is based on the finding that the GH concentrations in immunized boars are as high as in entire males (Table 4), whereas surgically castrated barrows exhibited much lower GH concentrations. Thus GH levels in barrows were 31% lower than in immunized boars although gonadal steroids were absent in both groups.
The mechanisms which maintain GH at a high male level are still unclear and cannot be put down to the abolishment of testicular steroids and their possible negative feedback on GH secretion (Breier et al., 1989). It rather may depend on an interaction between GnRH and growth hormone releasing hormone (GH-RH) either in the hypothalamus or in the pituitary. Infusion of GnRH increased LH and decreased GH, whereas infusion of GH-RH led to high GH concentrations and low LH values (Claus and Weiler, 1994; Weiler, 1995).

Table 4: GH concentrations (ng/mL) of entire boars, immunized boars and barrows (Metz, 2003)

| age (weeks) | boars | immunized boars | barrows |
|---|---|---|---|
| 18 | 4.69 | 4.18 | 2.94 |
| 23 | 4.09 | 4.09 | 2.88 |
| 25 | 3.81 | 3.36 | 2.83 |
| mean | 4.19 | 3.88 | 2.88 |

Barrows are a good model to test this hypothesis of a possible interaction between these releasing hormones. It is known that barrows have high concentrations of GnRH and LH due to the surgical castration causing the lack of negative feedback of testosterone from the testes. If there is an interaction between GnRH and GH-RH, the immunization of barrows against GnRH (see 2.2) should lead to a drop of GnRH which in turn should result in higher GH-RH values and consequently in higher GH concentrations and a better growth performance (Figure 8).

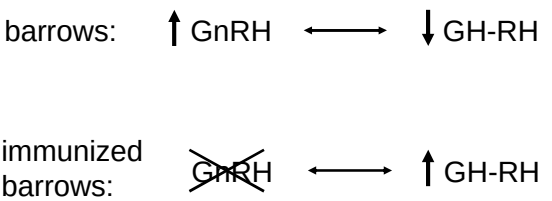



Figure 8: Hypothetical interaction between GnRH and GH-RH in barrows and immunized barrows

### 1.7.2 Influence of different feed intake on GH and IGF-I levels

Growth and thus protein synthesis is regulated by GH and mainly by its growth factor IGF-I. GH binds to its receptors in the liver and then stimulates the synthesis and release of IGF-I into blood. But in addition, the GH-IGF-I axis is influenced by oestrogens and by carbohydrates with a high glycaemic index such as glucose or starch (Figure 9).

In the pig, oestrogens lead to an additional rise in IGF-I concentrations (Claus et al., 1992; Claus and Weiler, 1994) by increasing the expression of the GH receptors in the liver (Gabrielsson et al., 1995). Similar to oestrogens, carbohydrates stimulate the secretion of insulin which in turn also increases the expression of GH receptors in the liver (Brameld et al., 1999).

The immunization against GnRH leads to a drop of testicular steroids. In consequence, after the second vaccination oestrogens are absent, and their influence on IGF-I is no longer prominent. All boars in our study (see 2.3) were immunized, and the two groups were fed with different amounts of feed (2 kg vs. 3 kg). After the second vaccination the only difference between groups was the energy supply through the diet. For this reason, we could investigate the influence of energy on IGF-I concentrations.

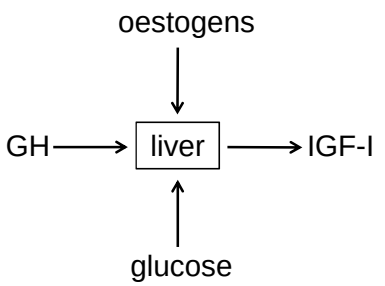



Figure 9: GH-IGF-I system and regulative parameters (Claus, 2005)

### 1.7.3 Imprinting of a male specific GH secretion pattern

The theory for this study (see 2.4) is based on studies in rats where perinatal presence of testicular steroids imprints a male pattern of GH later in life (Jansson et al., 1985). This typical male secretion was characterized by a high pulse frequency and a low baseline. In mice and rats, sexual differentiation is not completely finished during the short gestation period, but differentiation is extended to some short time after birth. Male pigs produce testosterone just like rodents about 4 weeks after birth (Schwarzenberger et al., 1993). This rise in testosterone concentration may influence GH secretion. But this rise in testosterone cannot occur in surgical castrates because the testes are removed within the first week of life (early castration, Figure 10). In immunized boars, in contrast, this neonatal steroid production can take place due to the fact that the testes are still there and the immunization effect is not relevant before the second vacciantion.

We hypothesize that the difference in GH concentrations between immunized boars and barrows is caused by an imprinting mechanism of the neonatal steroid synthesis. Therefore, we compared barrows which were either surgically castrated before (“early”) or after (“late”) the neonatal rise of testosterone (Figure10).

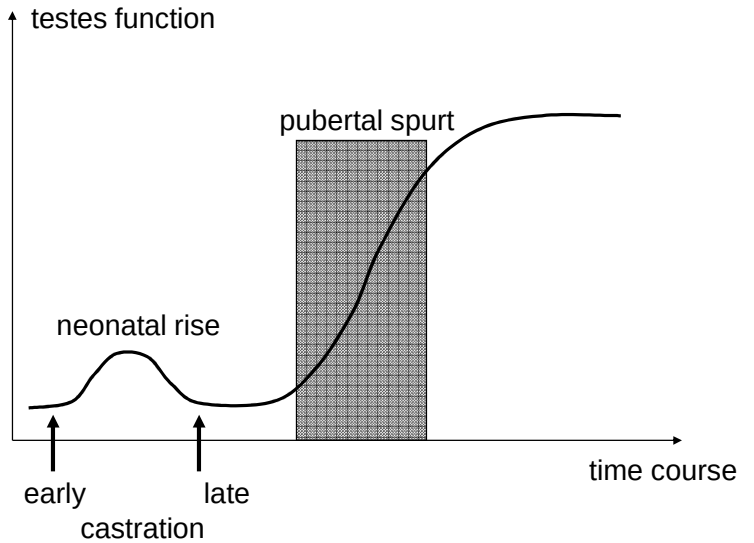



Figure 10: Early and late castration in the time course of pubertal development of boar testes function

# 2 Own Experimental Studies

## 2.1 *Short-term endocrine and metabolic reactions before and after second immunization against GnRH in boars*

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# Short-term endocrine and metabolic reactions before and after second immunization against GnRH in boars

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**Abstract**

Immunization of boars against GnRH inhibits synthesis of testicular steroids including androstenone (sex odour). Timing of the second vaccination (anamnestic reaction) should occur as late as possible to maintain anabolic effects of testicular hormones, but early enough to remove androstenone from body fat. Five catheterized boars received the second dose (Improvac®) at age 22 weeks. Titre, hormones and parameters reflecting protein turnover were determined in blood. An increased antibody titre and drop of LH and steroids occurred within 5 days. Metabolism adapted after 7 days. Results from this study in conjunction with previous work suggest that after two doses of Improvac given 4 weeks apart, clearance of androstenone from body fat may be achieved as early as 3 weeks after the second vaccination. Thus, it might be possible to extend the duration of anabolic effect in male pig production.


*Keywords:* Immunological castration; Boar sex odour; Anamnestic vaccination

## 1. Introduction

Due to the anabolic function of their testicular hormones, boars have superior fattening performance and carcass composition compared with barrows, while gilts are intermediate in these traits [e.g. 1–4]. This hierarchy is explained by the availability of gonadal hormones. Gilts secrete estrogens from the ovary, whereas testicular steroid synthesis in boars includes androgens and high amounts of estrogens [5] which differ in their anabolic mechanisms. Androgens improve protein synthesis via muscular receptors [6] and decrease protein turnover, mainly by interacting with the glucocorticoid receptor [7]. In contrast, estrogens act indirectly by stimulating growth hormone (GH) secretion from the pituitary gland and, even more pronounced, the GH-dependent secretion of the insulin-like growth factor 1 (IGF-1) from the liver by increasing the hepatic GH-receptors [8].

In addition to sex hormones, boars also synthesize androstenone in the testes. This steroid has no hormonal activity [9] but functions as a pheromone [10]. Androstenone accumulates in adipose tissue and its urine-like smell is regarded as a major cause of boar taint [11] which may result in rejection of meat from uncastrated pigs by a considerable proportion of consumers [12]. Manifold attempts to suppress androstenone but maintain anabolic status have been unsuccessful due to the common biosynthesis of anabolic steroids and androstenone in the testes. Implantation of anabolic sex hormones was effective to decrease steroid synthesis due to their negative feedback on gonadotropins. The anabolic effect cannot be used, however, due to residue considerations [13]. Surgical castration without anaesthesia has usually been used to control boar taint but is an important animal welfare issue which led to legal intervention e.g. in Norway. Immunological castration by immunization against gonadotropin releasing hormone (GnRH) [e.g. 14–16] is increasingly discussed as an alternative to surgical castration. However, immunization blocks the GnRH-luteinizing hormone (LH)-axis and thus also synthesis of anabolic steroids

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in the Leydig cells. In consequence, immunization leads not only to inhibition of boar taint but also anabolic effects. So far, the only commercially available vaccine for immunological castration is Improvac® [17] but it is not registered in all countries. The manufacturer recommends that two doses are given at least 4 weeks apart with the second dose given 4–6 weeks prior to slaughter. An anamnestic immunological response occurs resulting in high levels of antibody against GnRH 10–14 days after the second dose, as described by the manufacturer. Such a scheme not only suppresses boar taint, but also retains anabolic effects for a large part of the fattening period which probably explains the excellent performance data in immunized boars found in a field study [18]. In a previous study, we used an early onset of immunization: the first dose was given at age 10 weeks followed by two additional doses at age 16 and 23 weeks to abolish anabolic steroids over most of the fattening period. We found not only inhibition of androstenone but also a loss of anabolic function. Nitrogen retention in this study did not differ between immunocastrates and barrows but was about 24% higher in boars [19]. Therefore, the timing of vaccination in the fattening period is of high practical relevance.

To optimize the vaccination schedule, the following need to be considered: time till sufficient antibody formation after the second dose of vaccine; time to cessation of Leydig cell function and the resulting drop in peripheral steroid concentrations; time required for clearance of androstenone stored in adipose tissue. Only the latter question had been answered by earlier published studies: androstenone was completely eliminated from slaughterweight boars approximately 2 weeks after castration [20]. However, clearance from adipose tissue is age dependent, and it may take more than 6 weeks in older boars [20].

Detailed data on the immunological and metabolic responses to immunological castration are still missing. The aim of this study was to examine immunological, hormonal, and metabolic responses following the second dose of Improvac® in a two-dose immunocastration schedule.

## 2. Materials and methods

### 2.1. Animals

Five German Landrace boars were obtained from the University of Hohenheim swine herd and kept in individual pens (3 × 2.5 m). For blood collection, each boar was fitted with a cephalic vein catheter as described in detail earlier [21]. Catheters were surgically implanted at an age of 17 weeks (body weight range: 52–61 kg) under general anesthesia. Average duration of anesthesia did not exceed 30 min and pigs were fully recovered from surgery 2 days later. Animals were then immunized at weeks 18 and 22 with 2 mL doses of Improvac® subcutaneously in accordance to the manufacturer's recommendations (CSL Ltd., Parkville, Australia). Deuterium oxide administrations for later determination of total body fat (see below) were performed in ages 20 and 25 weeks. At age 28 weeks, the boars were slaughtered. The pigs were fed twice daily at 8:00 and 15:00 h with portions of 1.5 kg of a ration (Table 1) containing 16.7% crude protein and 13.5 MJ ME/kg.

Table 1
Feed composition

| Component | Wet weight (g/kg) |
|---|---|
| Barley | 373 |
| Triticale | 200 |
| Wheat | 170 |
| Pea | 70 |
| Soybean meal | 150 |
| Vitamin and mineral premix | 25 |
| Calcium carbonate | 2 |
| Soybean oil | 10 |

Blood was sampled daily before feeding at 8:00 h into heparinized vials and plasma was stored at −20 °C until assayed. Catheters were rinsed using sterile heparinized saline after each sampling. The whole experiment including the cannulation had been approved by the local animal welfare committee of the state of Baden-Württemberg.

### 2.2. Analytical methods

#### 2.2.1. GnRH-antibody titre

For titre determination, GnRH (Bachem, Weil am Rhein, Germany) was dissolved in PBS and diluted in 0.1 M carbonate buffer (pH 9.6) at a concentration of 10 ng/mL. One hundred μL/well were pipetted into Immobilizer Amino microtiterplates (Nunc, Wiesbaden, Germany) and allowed to react for 1 h at 37 °C under shaking. Plates were emptied and blocked with testing buffer (0.12 M NaCl, 0.02 M $Na_2HPO_4$, 0.01 M EDTA, 0.1% hydrolyzed gelatine, 0.05% Tween 20, 0.002% phenol red, 0.005% chlorhexidine gluconate; pH 7.2) overnight at 4 °C. All subsequent washing steps were performed using 10% PBS containing 0.05% Tween 20. Plasma samples from boars were diluted either 1:1600 or 1:3200 with testing buffer and 100 μL of each dilution per well was incubated for 2 h at 37 °C. For the next step, sheep-anti-pig IgG was biotinylated using EZ-link-NHS-LC-Biotin according to the manufacturer's instruction (Pierce Biotechnology, Rockford, IL, USA) and added at 3.2 ng/well. StreptABComplex (Dako, Hamburg, Germany) was added at a dilution of 1:5000 in testing buffer and incubated for 30 min at 37 °C in the dark. For colour reaction tetramethylbenzidine/$H_2O_2$ in acetate buffer was used. Reaction was stopped after 40 min at 37 °C using 2 M $H_2SO_4$. Optical density (OD) was determined at 450/690 nm and used to characterize the titre quantitatively.

#### 2.2.2. LH

Radio-immunological determination of LH was performed as described earlier [22]. Because there was no need to characterize LH pulsatility, only daily samples were used for

determination of LH. The intra- and interassay coefficients of variation were 10% and 8.3%, respectively, at a concentration of 0.6 ng/mL. The lower limit of detection was 0.02 ng/mL.

*2.2.3. Testosterone*

The radio-immunoassay used for measurement was described earlier [23] and revealed a coefficient of variation between days of 6.8% (2.95 ng/mL). The lower limit of detection was 0.05 ng/mL and average recovery was above 95% for concentrations ranging from 0.5 to 10 ng/mL.

*2.2.4. Androstenone*

For determination of 5α-androst-16-en-3-one (androstenone) 1 mL plasma was extracted with 3 mL *n*-hexane by shaking in a sealed glass vial for 30 min. After freezing at −30 °C for 30 min, the liquid hexane phase was decanted in a glass vial and evaporated in a rotary evaporator for 10 min until only a small volume of hexane was left. The vial was vortexed and evaporated to complete dryness within 5 min in the evaporator. We found earlier that such a short period does not lead to losses of androstenone. Dried residues were resolved in 100 μL methanol containing 5 ng of 5α-androstan-3-one as an internal standard for the gas chromatographic-mass spectrometric (GC-MS) determination. The system comprised a GC-17A coupled to a QP 5050 mass spectrometer (Shimadzu, Duisburg, Germany). GC-MS conditions were as follows: column, 10 m × 0.25 mm Zebron Amino Acid (Phenomenex, Aschaffenburg, Germany); injection volume, 3 μL; injection port temperature program, 110 °C (1 min), 300 °C (250 °C/min); carrier gas was Helium, 3 mL/min (splitless); column temperature program, 80 °C (2 min), 300 °C (30 °C/min), 300 °C (3 min); transfer nozzle temperature, 280 °C; multiplier voltage, 1.75 kV; single ion monitoring at *m/z* 272 and 274 (*m/z* 257 as another characteristic ion for androstenone could not be used because of co eluting substances). For calibration androstenone-free plasma of a barrow was spiked with 0.5, 1.0, 2.5, 5, and 10 ng/mL. Repeatability was determined by measurement of a boar plasma sample either on the same day ($n = 5$, intraassay coefficient of variation of 4.9%) or on consecutive days (interassay coefficient of variation of 8.2%). Sensitivity (threefold signal-to-noise ratio) was evaluated by spiking plasma of a barrow with increasing amounts of androstenone and revealed a detection limit of 0.3 ng/mL.

To save limited plasma, only samples of every fifth day during the whole experiment were measured for androstenone, except samples taken 2–8 days after the second dose of Improvac® to display short term daily changes.

*2.2.5. IGF-1*

Concentrations of IGF-1 in plasma were determined as described before [24]. In brief, samples were extracted with HCl/ethanol and measured using a specific double-antibody RIA. Coefficients of variation within and between assays were 8.4% (160 ng/mL) and 5.7% (183 ng/mL), respectively.

*2.2.6. Urea*

Nitrogen excretion is mainly represented by urea but transfer of pigs into metabolic crates to obtain urine and faeces is stressful, resulting in metabolic artefacts. Instead, urea was measured in plasma using microtiterplates because it had been shown previously, that urea determination is an excellent analytical parameter to characterize N-retention [25]. In brief, plasma was diluted four-fold and pre-incubated with glutamic-dehydrogenase to remove ammonia by reaction with oxoglutarate, ADP and NADH. After addition of urease released ammonium was quantified at 340 nm using urea standards. Intraassay and interassay coefficients of variation were 8.9% ($n = 10$; 332 μg/mL) and 9.7% ($n = 10$; 332 μg/mL), respectively.

*2.2.7. Total body fat*

Composition of the fat-free body mass is strongly correlated with the total body mass and not influenced by the body fat content [26]. Fat-free body mass is calculated from total body water which is determined by application of a known dose of $D_2O$ and measurement of $D_2O$ content in body water after equilibrium. The difference between total body mass and fat-free body mass is the fat content. For determination, animals were weighed and injected intravenously through the catheter with $D_2O$ (70%; Chemotrade, Leipzig, Germany) at 0.21 g 70% $D_2O$/kg body weight. The exact amount given to each pig was determined by weighing the filled syringe before and after injection. Following administration of $D_2O$ the catheter was rinsed with 40 mL sterile heparinized saline. After equilibration with body water, three 20 mL blood samples were drawn at 4, 4.5, and 5 h after $D_2O$ application into heparinized vials and stored at −20 °C. For clean-up, the method described by Tissier et al. [27] was used. Five millilitres of the blood were freeze-dried and the water phase sublimated in a cryo trap using liquid nitrogen. $D_2O$-content was determined at 3960 nm in an infrared spectrophotometer using $D_2O$ calibrators (0, 252, 311, 505 mg/kg). Intraassay and interassay coefficients of variation were 0.2% ($n = 5$) and 2.2% ($n = 10$), respectively. Total body fat content was calculated as described by Susenbeth [26].

*2.2.8. Hydroxyproline (OH-Pro)*

Hydroxyproline in blood plasma and urine reflects collagen breakdown. This breakdown is lowered when protein synthesis and deposition is reduced as well [25,28]. Lopes et al. [25] also described the method used in this study. In brief, 400 μL 7.5 M HCl was placed in a screw-capped vial and 100 μL plasma added to the acid. Hydrolysis was performed for 12 h at 110 °C. The hydrolysates were evaporated in a heating chamber and redissolved in 5 mL bidistilled water. Derivatization and HPLC-analysis were modified according to Einarsson [29]. In brief, *cis*-hydroxyproline was added before derivatization as an internal standard and primary amino acids are derivatized using 9-fluorenylchloroformiate. After extraction with diethyl ether, secondary amino acids were derivatized with *o*-phthaldialdehyde and diluted 20-

fold. Chromatographic conditions were as follows: column, Lichrospher 100 RP 18 125 mm × 4.6 mm 5 μm (pre-column of the same material); injection volume, 10 μL; eluent A: 0.1% aqueous trifluoroacetic acid/acetonitrile (70:30, v:v), eluent B: acetonitrile; gradient elution (minutes/% eluent B): 0/0, 2/0, 8/40, 8.5/100, 11.5/100, 12/0, 15/0, flow, 1.5 mL/min; fluorescence detector, ex/em: 255 nm/325 nm. Calibration was performed with OH-Pro standards. Coefficients of variation within and between assays were 2.7% (22.6 μg/mL) and 8.5% (21.8 μg/mL), respectively. To save limited plasma, only samples of every fifth day during the whole experiment were measured.

### 2.3. Statistics

Data are presented as daily means ± S.E.M. Day 0 is the day of the first immunization and day 28 the day for second immunization. For statistical evaluation the mean for period I (days 0–28) and period II (day 39 until slaughter on day 72) were calculated for every animal and tested for differences between these periods using paired *t*-test. The period between days 29 and 38 are omitted because of increasing or decreasing values. To determine the onset of antibody titre development and rise of urea in each pig, the mean value and the standard deviation (S.D.) were calculated from day 0 until day 28 (second immunization). The first value above the mean value plus the threefold S.D. ($p < 0.05$) was defined as the onset. Similarly, LH, testosterone, androstenone, IGF-1, and OH-Pro were checked for a decrease. In this case, the mean value and the standard deviation (S.D.) were calculated from the last 4 weeks of the experiment (days 44–72). For analysis of significant trends in titre development between days 37 and 72 Neumann's test was used [30]. Correlations between parameters were determined by Spearman's rank correlation coefficient because LH determination led to concentrations below the detection limit. The Statistical Package for the Social Sciences (SPSS Version 12.0; Chicago, IL, USA) was used.

## 3. Results

Developments of antibody titres against GnRH are shown in Fig. 1. It was found that all pigs had a significant increase in antibodies against GnRH from the period before the second immunization and thereafter, but the extent of antibody formation differed between individuals. While pigs 1, 4, and 5 revealed high antibody titres with an extinction of more than 2, pigs 2 and 3 exhibited a weak titre development which had been measured at a dilution of 1:1600 to obtain extinctions above 1. Titres raised within 3–5 days after second dose in all pigs, reaching the highest levels between days 4 and 6. Thereafter titres decreased ($p < 0.05$, Neumann Test), except in one pig which had a second peak 4 weeks after the booster. At the end of the sampling period all pigs still had elevated titres but they were about half the peak values.

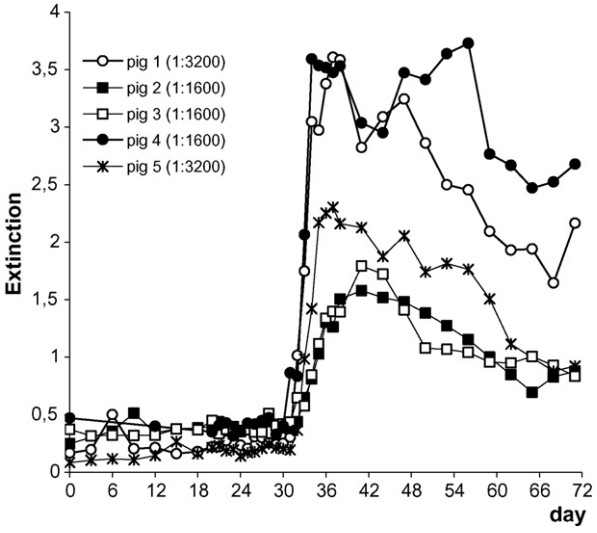


Fig. 1. Development of GnRH-antibody titres for each pig (individual antiserum dilution given in brackets). First Improvac immunization was on day 0, whereas the second immunization was on day 28.

Changes in the concentrations of LH are shown in Fig. 2. They decreased from mean values of 0.158 ng/mL to concentrations of 0.03 ng/mL which is close to the detection limit. This significant decrease ($p < 0.01$) was found within 4–8 days after second dose. Therefore, there was no apparent time lag between antibody development and LH decrease. None of the pigs showed an increase in LH during the whole period after the second vaccination, so that the low antibody titres found in three of the pigs at the end of the sampling period were still sufficient to suppress GnRH and LH in consequence.

Similar to LH, testosterone (Fig. 3) remained at high mean concentrations of about 2.75 ng/mL after the first immunization and decreased to values of about 0.25 ng/mL ($p < 0.001$)

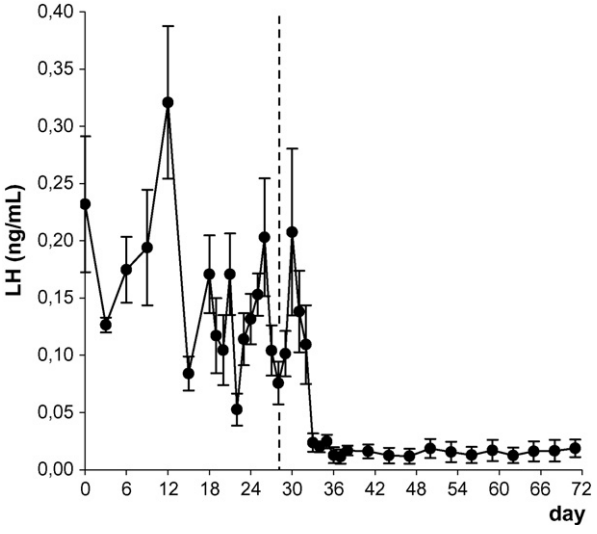


Fig. 2. LH in plasma ($n = 5$; daily means ± S.E.M.). First immunization was on day 0, whereas second immunization was on day 28 (dotted line).

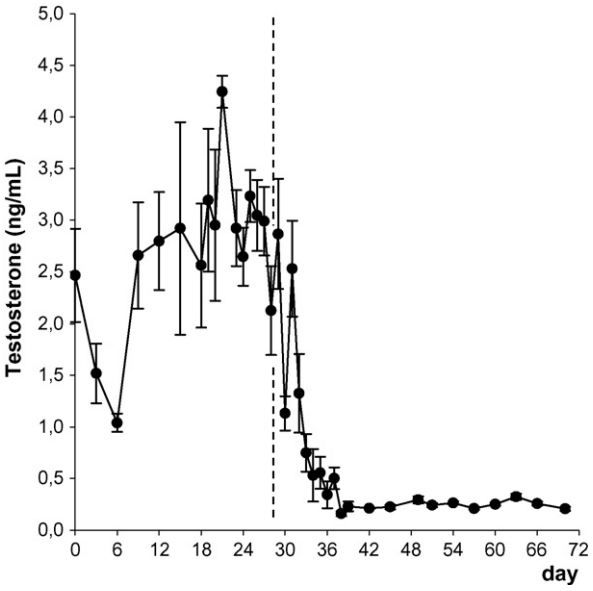


Fig. 3. Testosterone in plasma ($n = 5$; daily means ± S.E.M.). First immunization was on day 0, whereas second immunization was on day 28 (dotted line).

5–10 days after second dose. No rise of testosterone was found before the end of sampling.

Before second dose the androstenone concentrations differed between individuals (range of mean values from days 0 to 27: 0.62–2.67 ng/mL). The development of plasma androstenone, concentrations is shown in Fig. 4. Mean values decreased significantly ($p < 0.05$) from 1.48 to 0.34 ng/mL after the second dose. Basal concentrations in an order of 0.35 ng/mL were reached 4–8 days after second dose. As for testosterone, there was no rise of concentrations until the end of the experiment.

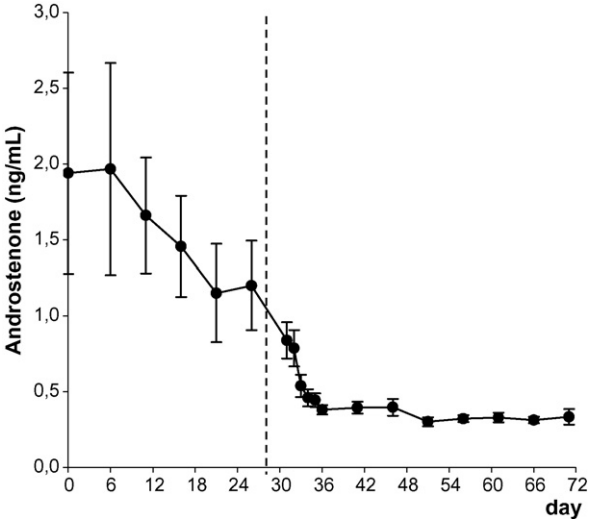


Fig. 4. Androstenone in plasma ($n = 5$; daily means ± S.E.M.). First immunization was on day 0, whereas second immunization was on day 28 (dotted line).

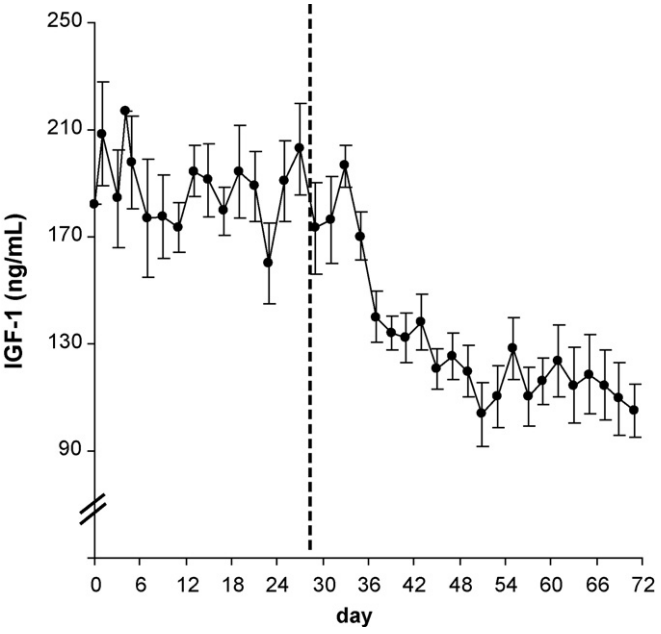


Fig. 5. IGF-1 in plasma ($n = 5$; daily means ± S.E.M.). First immunization was on day 0 whereas second immunization was on day 28 (dotted line).

Measurement of IGF-1 in plasma (Fig. 5) revealed a decrease of about 36% from mean values of 186 to 119 ng/mL ($p < 0.01$). The decrease started 5 days after second vaccination and reached basal values, 6–10 days after the booster.

Decreased N-retention due to the drop of anabolic hormones is reflected by the concentrations of urea in plasma (Fig. 6). Urea also showed an immediate reaction after second dose (203 μg/mL) which was significantly higher ($p < 0.001$) compared to the level before the booster (126 μg/mL). In contrast to urea, measurement of OH-Pro (Fig. 7) revealed a decrease of about 24% from mean values of 26.9–20.4 μg/mL ($p < 0.01$). Basal values were reached 6–10 days after sec-

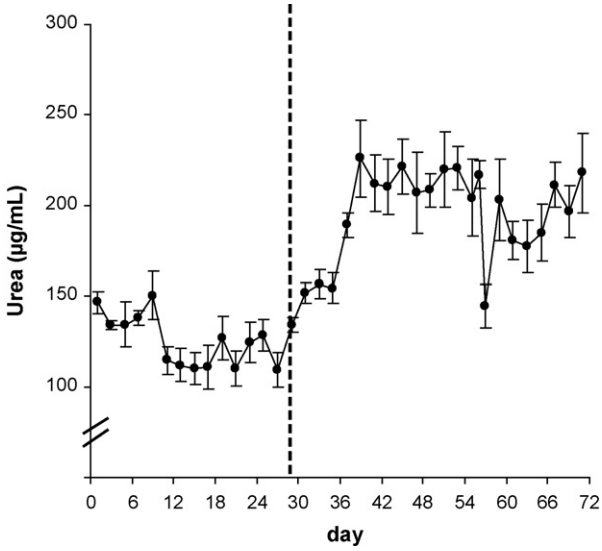


Fig. 6. Urea in plasma ($n = 5$; daily means ± S.E.M.). First immunization was on day 0 whereas second immunization was on day 28 (dotted line).

Table 2
Correlation between parameters investigated ($^{**}p < 0.001$)

| | IGF-1 | Urea | LH | Testosterone | GnRH-Titre | Androstenone | OH-Pro |
|---|---|---|---|---|---|---|---|
| IGF-1 | – | −0.455** | 0.398** | 0.455** | −0.371** | 0.466** | 0.485** |
| Urea | | – | −0.563** | −0.634** | 0.603** | −0.489** | −0.575** |
| LH | | | – | 0.526** | −0.745** | 0.580** | 0.534** |
| Testosterone | | | | – | −0.501** | 0.669** | 0.470** |
| GnRH-Titre | | | | | – | −0.744** | −0.525** |
| Androstenone | | | | | | – | 0.367 |

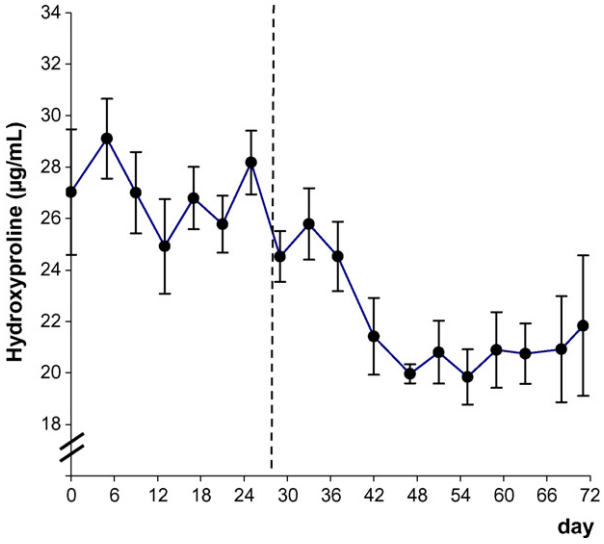


Fig. 7. Hydroxyproline in plasma ($n = 5$; daily means ± S.E.M.). First immunization was on day 0, whereas second immunization was on day 28 (dotted line).

ond immunization. The total body fat content after the first and second immunization revealed slightly increasing values from 19.5% (week 20) to 21.8% (week 25), which was not significant.

Correlations between the parameters determined are summarized in Table 2. Plasma androstenone and OH-Pro were not correlated significantly. All other parameters, either representing an anabolic metabolism or being members of the hormonal regulators of anabolism, were significantly correlated ($p < 0.001$).

## 4. Discussion

Many field studies have demonstrated the efficacy of GnRH-immunization using different antigens, including Improvac® [15–18,31]. A vaccination scheme with two doses is generally recommended. The first vaccination is followed by a primary immune response in which a limited amount of antibody is produced with a relatively high proportion of non-specific IgM antibodies, and immunological memory is established. Following the second dose, there is a rapid, large and persistent increase in titres of circulating antibodies dominated by high affinity IgG [32]. In contrast to our study in which the first immunization had no obvious effect, other studies detected biological effects after a single injection, probably due to the use of more aggressive adjuvants [33–35].

To date detailed temporal relationships between the second dose and endocrine and metabolic reaction have not been examined in detail. Our data from cannulated pigs revealed a very rapid increase of antibody formation after the second dose. Quantitative aspects, however, were highly variable depending on the individual. Nevertheless, it was fully sufficient to release the castration effects. The continuous decrease after the maximal titre might allow recovery of testicular function before slaughter if the second dose of a vaccine is given too early. Timing of the second dose of Improvac® should be optimised to achieve a high enough titre of antibodies to ensure the castration effect in all boars and, secondly, make optimal use of the anabolic effect of gonadal hormones when they have reached levels typical in the mature boar. Currently, the manufacturer recommends giving the dose 4–6 weeks before slaughter. One study using a limited number of boars reported that in slaughter weight boars, 3 weeks are sufficient for androstenone to be eliminated from adipose tissue after surgical castration [20]. Further research is needed to determine whether the currently recommended 4–6 weeks window before slaughter can be shortened and still achieve reliable boar taint suppression but an extended period of anabolic function.

Age-dependent testicular function is well investigated in the boar. Apart from a transient rise of testicular endocrine function 2–4 weeks postnatally [36], the pubertal spurt starts at around 16–17 weeks leading to peak levels of steroids in blood plasma at an age of about 19 weeks [37]. Thereafter, they decrease slightly and stabilize at a high level [37–39].

As shown in the present study, LH and testosterone concentrations decreased immediately with the rise of titre. The rapid decrease of testosterone is explained by the effect of LH on cholesterol side chain cleavage which is the rate limiting step in testicular steroidogenesis [40,41]. Maintenance of the titre of antibodies against GnRH and thus withdrawal of LH for prolonged periods led to Leydig cell involution characterized by an almost complete loss of cytoplasm [22]. The gradual decrease of testosterone is not a reflection of low levels of ongoing synthesis, but can be explained by the clearance of testosterone which was stored in adipose tissues of the boar [42] and becomes measurable in blood plasma. The same kinetic can be assumed for androstenone, but androstenone is more lipophilic so it achieves much higher tissue concen-

trations in adipose tissue and release from the tissue is more protracted [43].

In contrast to gonadal steroids, the decrease of IGF-1 was much slower. IGF-1 is increased by estrogens [44]. Estrogens were not measured in this study due to limited availability of blood plasma but have been shown earlier to be highly correlated to androgens [43] and to decrease because of GnRH-immunization [19]. The slow decrease of IGF-1 after second dose is explained by its long half life, which is due to binding to specific binding proteins [45].

The loss of anticatabolic androgens and the decrease of the estrogen/IGF-1-dependent proliferative functions are also reflected by the metabolic parameters. The increase of urea signals enhanced protein degradation and N-excretion. In contrast to urea, OH-Pro decreased after second vaccination. Synthesis and breakdown of collagen reveal a sex-dependent equilibrium and it was shown that androgens primarily stimulate collagen synthesis [46,47]. The reduced OH-Pro concentrations thus reflect a decrease of collagen breakdown to compensate for the drop in collagen de novo synthesis. Apart from the rapid drop of protein anabolism after the second vaccination, it is also known that the voluntary feed intake increases remarkably due to the absence of both estrogens and androgens [48]. It is advisable therefore to adapt amount of daily feed supply and feed composition around the time of second vaccination to finishing rations for barrows, to avoid excessive fat accumulation in the carcass.

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## 2.2 *Effects of immunization against GnRH on gonadotropins, the GH-IGF-I-axis and metabolic parameters in barrows*

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# Effects of immunization against GnRH on gonadotropins, the GH-IGF-I-axis and metabolic parameters in barrows

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*Surgically castrated male piglets (barrows) reveal an increase in LH and a decrease in GH compared to untreated boars. Boars that were castrated by immunization against gonadotropin releasing hormone (GnRH) have decreased LH but maintain GH. The difference in GH levels between barrows and immunological castrated boars cannot be explained by testicular steroids because they are low in surgical and immunocastrated boars as well. Therefore, differences in GH concentrations might be due to an interaction between GnRH and growth hormone releasing hormone (GRH) in the hypothalamus or the pituitary. This hypothesis was tested with twelve male piglets that had been castrated within 1 week postnatally and fitted with indwelling cephalic vein catheters at 17 weeks of age. They were split into a control group and an immunized group (each* n = *6). Vaccination with Improvac® was performed at 18 and 22 weeks of age. Specific radioimmunoassays were used for hormone determinations (GH, LH, FSH, testosterone and IGF-I). Additionally, metabolic responses were evaluated by measuring analytical parameters that characterize protein synthesis and breakdown, and body fat content. The second vaccination led to a rapid decrease of LH below the limit of detection whereas FSH decreased more slowly, over a period of 5 weeks, from 2.2 to 0.5 ng/ml. This level of FSH, which corresponds to boar-specific concentrations, was maintained thereafter. GH decreased with increasing age but was not influenced by vaccination and remained at a low concentration typical for barrows. Similarly, IGF-I was not altered by vaccination. Consequently, metabolic status was not changed by immunization. It is concluded that the difference in GH levels between surgical and immunocastrated boars is not explained by an interaction between GnRH and GRH.*

**Keywords:** pig, GnRH immunization, metabolism, GH, FSH

## Introduction

Boars synthesize high amounts of androgens, oestrogens and the pheromone 5α-androst-16-en-3-one (androstenone) in testicular Leydig cells (Claus and Hoffmann, 1980). Apart from their regulatory role in reproductive functions, androgens and oestrogens have a high anabolic potential. Androstenone has no hormonal activity but stimulates female reproductive functions as a pheromone (Reed *et al*., 1974) and is mainly responsible for the unpleasant 'boar taint' (Patterson, 1968). To prevent this taint, male piglets are usually castrated surgically without anaesthesia shortly after birth, even though fattening performance, nitrogen retention and the ratio of lean to fat are inferior in barrows compared with boars. In recent years, castration without anaesthesia has become an important animal-welfare issue. Norway expects to ban surgical castration completely in 2009.

Currently, there are two approaches under discussion for the control of boar taint, which will not compromise animal welfare. The first is surgical castration with anaesthesia (Prunier and Bonneau, 2006; Thun *et al*., 2006; Zöls *et al*., 2006). The second is the use of vaccines (immunological castration) to inactivate gonadotropin releasing hormone (GnRH), causing a drop of LH, a reduction of steroid synthesis and, consequently, decreased taint levels. FSH is reported to remain unchanged after immunization, probably due to a different regulation via the activin system (Li *et al*., 1998). Metz and Claus (2003) found that GH concentrations in immunocastrated boars remained at the same high level typically found in intact males. Normal barrows, however, have GH levels that are 31% lower than immunocastrates (Metz and Claus, 2003) although steroid concentrations are low as in barrows. Thus, the mechanism that maintains GH in immunocastrates at a level typical for intact boars cannot be due to the feedback effect of steroids on GH secretion (Breier *et al*., 1989). Rather, it may depend on an interaction between GnRH and growth hormone releasing hormone

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(GRH) either in the hypothalamus or in the pituitary. In the pituitary, such an interaction may occur by cells that are thought to be able to synthesize GH and gonadotropins (Childs, 2000; Childs *et al*., 2000). The hypothesis is substantiated by the infusion of GnRH in pigs, which led to an increase of LH by 86% but to a concomitant decrease of GH by 18%. Infusion of GRH in turn elevated GH by 78% and decreased LH by 16% (Claus and Weiler, 1994). Since immunization against GnRH leads to an inactivation of GnRH, a rise of GH might be expected. Barrows are an appropriate model to clarify this hypothesis because they have higher levels of GnRH and gonadotropins due to the absence of negative feedback by gonadal steroids compared to boars. Immunization against GnRH should then lead to a rise of GRH and GH. Because GH is known as the anabolic 'master-hormone', such a rise should also be reflected by corresponding changes of parameters that characterize an anabolic metabolism. Such a mechanism might also be of practical relevance to improve the growth performance of barrows.

## Materials and methods

*Study design*

Experimental pigs were German Landrace barrows that had been surgically castrated at 1 week of age. The aim of the study was to compare groups of six immunized and six non-immunized barrows. Because the maintenance of catheters and animal welfare over prolonged periods require extensive care, it is not possible to keep 12 cannulated pigs at the same time. Consequently, the study was performed in two replicates where each replicate comprised three non-immunized barrows as controls and three immunized barrows. Care was taken to provide the same environment in the two replicates. For that reason, animals were kept under a constant light regime (12 h light : 12 h dark). Each pig was kept separately in an individual pen measuring $3 \times 2.5$ m, and fed twice daily at 0800 and 1500 h with 1.5 kg of a diet containing 16.7% crude protein and 13.5 MJ ME/kg (Table 1). The barrows were slaughtered at 28 weeks of age.

*Immunization*

Immunization was performed with GnRH, which had been linked to a glycoprotein carrier. This antigen is commercially available (Improvac®, Pfizer Animal Health, Parkville, Australia) and was administered in accordance with the manufacturer' recommendations. The first 2 ml dose was given when pigs were 18 weeks old; a second 2 ml dose was given 4 weeks later at 22 weeks of age.

The control group was not injected because the adjuvant alone is not available. Furthermore, it is known that it does not lead to tissue reactions (Dunshea *et al*., 2001).

*Blood sampling*

For blood sampling, each barrow was fitted with a cephalic vein catheter under general anaesthesia at 17 weeks of age (BW range: 50 to 57 kg in replicate 1; 53 to 58 kg in replicate 2) as described earlier (Claus *et al*., 1990). Blood samples were drawn daily at 0900 h in heparinized vials and stored at −20°C after centrifugation.

**Table 1** *Feed composition*

| Component | Wet weight (g/kg) |
|---|---|
| Barley | 373 |
| Triticale | 200 |
| Wheat | 170 |
| Pea | 70 |
| Soybean meal | 150 |
| Vitamin and mineral premix | 25 |
| Calcium carbonate | 2 |
| Soybean oil | 10 |

*Metabolic measurements*

To determine body fat content, the deuterium oxide method was performed at an age of 20 and 25 weeks as described earlier (Claus *et al*., 2007). Protein synthesis was measured by $^{13}$C-leucine infusion at the day of slaughter as described below. For GH determination, additional samples (window sampling) were drawn for 24 h every 20 min at 19 and 24 weeks of age. Catheters were rinsed using sterile heparinized saline after each sampling. The whole experiment including the cannulation had been approved by the animal welfare committee of the state of Baden-Württemberg.

*Analytical methods*

*GnRH–antibody titre*. GnRH (Bachem, Weil am Rhein, Germany; 15.6 ng/well in 0.05 M carbonate buffer, pH 9.6) was coated on MaxiSorp microtitre plates (Nunc, Wiesbaden, Germany) for 1 h at 37°C under shaking. Blocking with testing buffer (overnight at 4°C) and washing steps have been described before (Claus *et al*., 2007). Plasma samples were diluted 1 : 1600 with testing buffer and 100 μl of each dilution per well was incubated for 2 h at 37°C. Thereafter, rabbit anti-swine IgG-horseradish peroxidase (P0164, Dako, Hamburg, Germany) was added at a dilution of 1 : 10 000 in testing buffer and incubated for one hour at 37°C in the dark. Colour reaction and optical density determination were measured as described previously (Claus *et al*., 2007). Repeatability was determined using three different plasma samples from immunized boars with different known titres ($n = 7$). Interassay coefficients of variation were 24%, 9.4% and 3.6% for extinctions of 0.486, 2.754 and 3.903, respectively.

*LH*. Earlier determinations of LH in barrows were based on window sampling at 20-min intervals and led to an erratic pattern of LH concentrations (Metz, 2003). Because the diurnal pattern of LH was not relevant for this publication, we preferred to determine LH in samples taken at a standardized time over prolonged periods. Such a sampling regime was shown earlier to reflect major changes over prolonged periods without short-term fluctuation (Claus *et al*., 2007). LH in samples taken every third day during the

whole experiment was measured by radioimmunoassay (RIA) (Wagner and Claus, 2004). The specific antiserum (AFP15103194) was kindly provided by Dr Albert Parlow (NIDDK, Torrance, CA, USA) and a highly purified pLH standard (AFP11043B) was used for radio-iodination and for calibration. The coefficients of variation within one day and between days were 6.8% (mean: 0.4 ng/ml) and 6.7% (mean: 0.37 ng/ml), respectively. The lower limit of sensitivity was 0.024 ng/ml. For determination of recovery, spiked samples were prepared, which reflect mean physiological concentrations. Thus the recovery for 0.5 ng/ml was 105% ($n = 6$).

*FSH*. FSH was measured in samples from every third day by RIA (Wagner and Claus, 2004). A specific antiserum (AFP2062096) was provided by Dr Parlow (NIDDK, Torrance, CA, USA) and a highly purified pFSH standard (AFP10640B) was used for radio-iodination and for calibration. The coefficients of variation within one day and between days were 3.3% (mean: 1.92 ng/ml; $n = 10$) and 9.2% (mean: 1.7 ng/ml; $n = 5$), respectively. The lower limit of sensitivity was 0.03 ng/ml and the recovery for spiked samples with 2.5 ng/ml was 105% ($n = 6$).

*Testosterone*. A specific RIA including a solvent extraction step was used as described earlier (Wagner and Claus, 2004) to measure plasma testosterone every third day. Antiserum against testosterone-3-carboxymethyloxime-BSA was raised in rabbits. Cross-reactivities with other steroids were 30% for dihydrotestosterone and 0.5% for dehydroepiandrosterone, whereas 17ß-estradiol, progesterone and epitestosterone showed cross-reactivities less than 0.1%. Repeatability was determined by measurement of spiked samples on consecutive days ($n = 12$) and the interassay coefficient of variation was found to be 8% for concentrations between 0.5 and 2.5 ng/ml. The lower limit of detection was 0.05 ng/ml. The average recovery after extraction was above 95%.

*GH*. Due to the pulsatile secretion pattern of GH, which is more important than the mean level, this hormone was determined in plasma obtained by window sampling. Determination was performed as described before (Claus *et al*., 1990). A specific antiserum (AFP422801) was kindly provided by Dr Parlow (NIDDK, Torrance, CA, USA) and a highly purified pGH standard (AFP10864P) was used for radio-iodination and calibration. The intraassay coefficient of variation was 9.3% at a concentration of 4.2 ng/ml ($n = 10$) while the interassay coefficient of variation was 10% (mean: 3 ng/ml, $n = 6$). Recovery was above 95% for spiked samples (1.5 to 6 ng/ml). Each GH profile was evaluated for pulses. The mean for all 74 samples per window for each animal was determined. A pulse was assumed when at least two consecutive GH concentrations exceeded this mean value by at least 50%. The maximal level was the mean of the maximal concentrations of these pulses. The base levels were calculated by the mean of the 14 lowest values. The frequency was defined as the number of pulses per 24 h.

*IGF-1*. Concentrations of IGF-1 were determined by a double-antibody RIA in daily plasma samples after HCl/ethanol-extraction using a specific antiserum raised in rabbits (Claus *et al*., 1992). The highly purified IGF-I standard was obtained from Gro Pep (CU020, Adelaide, Australia) and was used for radio-iodination and for calibration. Extraction yield was between 61% and 70% for samples spiked from 75 to 400 ng/ml ($n = 9$). The coefficients of variation within and between assays were 6.9% and 5.7% for concentrations of 186 ng/ml ($n = 10$) and 183 ng/ml ($n = 9$), respectively.

*Urea*. Urea in plasma was measured to characterize protein degradation and therefore nitrogen excretion. Determination was performed on microtitre plates as reported earlier (Claus *et al*., 2007). Intra- and interassay coefficients of variation were 8.9% ($n = 10$) and 9.7% ($n = 14$) at concentrations of 322 and 347 μg/ml, respectively.

*Hydroxyproline*. Total hydroxyproline (OH-Pro) in blood reflects collagen breakdown and thus catabolic mechanisms. It was measured using an HPLC method published before (Claus *et al*., 2007). Coefficients of variation were 2.7% ($n = 10$) for intraassay and 9.1% ($n = 14$) for interassay variations in samples containing 22.6 and 20.9 μg/ml, respectively.

*Protein synthesis by infusion of $^{13}C$-leucine*. Determination of protein synthesis by infusion of $^{13}C$-leucine is an established method (Waterlow *et al*., 1978). It is based on a constant infusion of leucine up to a constant equilibrium so that the maintenance of a plateau during ongoing infusion represents leucine used for protein synthesis.

To obtain background values for $^{13}C/^{12}C$-enrichment, a sample of heparinized blood was taken before giving the bolus of leucine described below. A bolus of 30 mg L-[1-$^{13}C$] leucine (Euroisotope, Saarbrücken, Germany) dissolved in 5 ml saline was administered intravenously, followed by continuous infusion at the rate of 30 mg/h for 6 h (150 ml/h) via the implanted catheter using a peristaltic pump. Blood samples were taken at 30 min intervals during the 6 h infusion period. To measure the exact concentration of leucine solution infused to each pig, an aliquot of 10 ml was taken from the residual solution at the end of the experiment, and all animals were weighed before slaughter. Leucine was isolated from plasma and derivatization was performed using the test kit EZ:faast (Phenomenex, Aschaffenburg, Germany). In brief, plasma samples were acidified by 1 M HCl (40 μl/100 μl plasma), amino acids were extracted by a cation exchanger, eluted and derivatized using propyl chloroformate. For quantification, norvaline was added as internal standard. GC-MS (GC-17A coupled to a QP 5050, Shimadzu, Duisburg, Germany) was conducted in the single ion mode (*m*/*z* 158 for internal

standard, *m/z* 200 and 201 for leucine) under conditions described by the manufacturer of the test kit. For quantification of $^{13}$C-leucine in infusion solutions, an external standard containing 200 nmol/ml was processed as described above and the leucine content calculated using the internal standard, after which the infusion rate was estimated. To determine $^{13}$C-enrichment, peak areas for *m/z* 201 were related to *m/z* 200 and plotted against time of sampling. Intraassay coefficient of variation was 2.75%. Constant enrichment was obtained from 3 h to 6 h after onset of infusion and was used to calculate the total leucine flux together with the infusion rate (see above). For calculation, a percentage of 7.6% leucine in total protein was assumed (Waterlow *et al.*, 1978; Eggum, 1989).

*Total body fat content*. Because GH primarily changes the lean/fat ratio, anabolic parameters are insufficient and the additional determination of body fat with the $D_2O$ method was included. Application of $D_2O$, measurement of isotopic enrichment by infrared spectroscopy and calculations including coefficients of variations were published recently (Claus *et al.*, 2007).

*Statistics*

Data were analysed using Statistical Package for the Social Sciences (SPSS v.13.0; Chicago, IL, USA). Data in figures are presented as means ± s.e. of six animals per group, with day 0 being the day of the first immunization and day 28 the day of the second immunization. In the tables, data for IGF-1, urea, OH-Pro and fat content are presented for two phases. Phase 1 comprised values from day 0 to day 27; phase 2 comprised values from day 39 to day 72 as similarly reported earlier (Claus *et al.*, 2007). Days 28 to 38 were not included because of transient increasing or decreasing values due to the onset of immunization effects. Trend analysis was performed as described by Neumann (1941) and Hart (1942). Changes in titre were tested as described previously (Claus *et al.*, 2007). A linear mixed model with repeated measures was used to compare barrows and immunized barrows. Fixed effects (group and phase) and their interaction were tested for statistical significance. Replicate was included as a random effect. For none of the parameters investigated, a significant replicate effect was found (estimate of covariance parameter by the Wald test). Interactions were significant for FSH and antibody titres.

## Results

Changes in titre of antibodies against GnRH are shown in Figure 1a and b. In immunized barrows (Figure 1a), antibody titres rose rapidly and peaked seven days after administration of the second dose of Improvac. Titres then declined until the end of the study on day 72 after initial vaccination ($P < 0.05$; trend analysis). Nevertheless, on day 72, titres were still higher than they had been before the second immunization ($P < 0.001$). Differences between both groups and phases were highly significant. Control

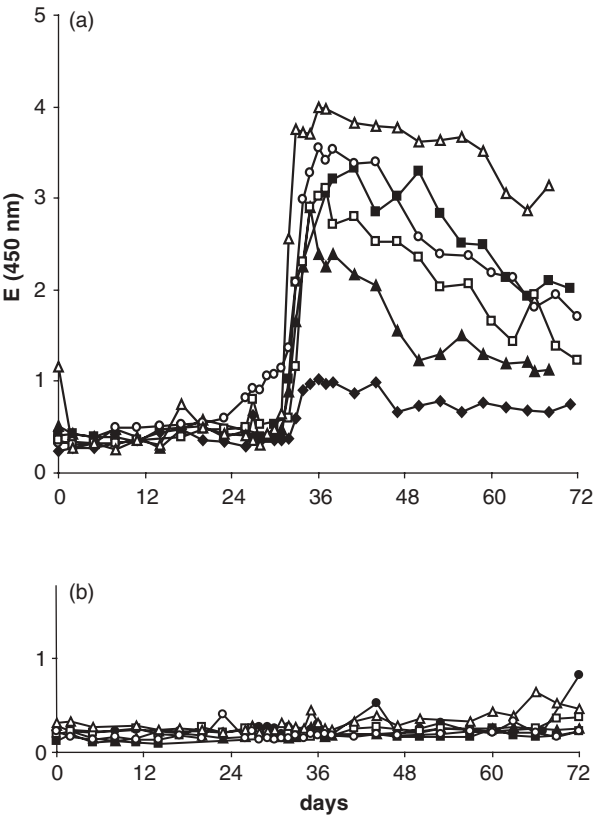


**Figure 1** Antibody titre in immunized barrows (a) and control barrows (b). First immunization was on day 0 (18 weeks of age). Second immunization was on day 28 (22 weeks of age). Closed circles represent animals from replicate 2, open circles animals from replicate 1.

barrows (Figure 1b) had low extinctions around 0.3 throughout the experimental period, comparable to values in immunized animals before they were given a second dose of Improvac.

A decrease in LH to values near the detection limit within 5 days was observed after the second immunization (Figure 2a; $P < 0.01$). The decrease in LH between the first and second immunization was significant in four of the six pigs as shown by trend analysis ($P < 0.05$). Plasma LH in control barrows (Figure 2b) remained at about 0.5 ng/ml throughout the experiment.

FSH determination (Figure 3a) revealed decreasing values already before the second immunization in five of the six immunized barrows ($P < 0.05$; trend analysis). After the second dose of the vaccine, FSH decreased from 1.3 to 0.5 ng/ml ($P < 0.001$) and remained at this level until the end of the study. Control barrows (Figure 3b) began with levels of about 2 ng/ml and these did not decrease significantly.

Testosterone values in all animals remained below the detection limit of 0.05 ng/ml throughout the experiment (data not shown). There was no significant difference between vaccinated and unvaccinated barrows.

The GH parameters are given in Table 2. Mean GH concentrations decreased significantly ($P = 0.01$) from the first window at age 19 weeks (1 week after first vaccination)

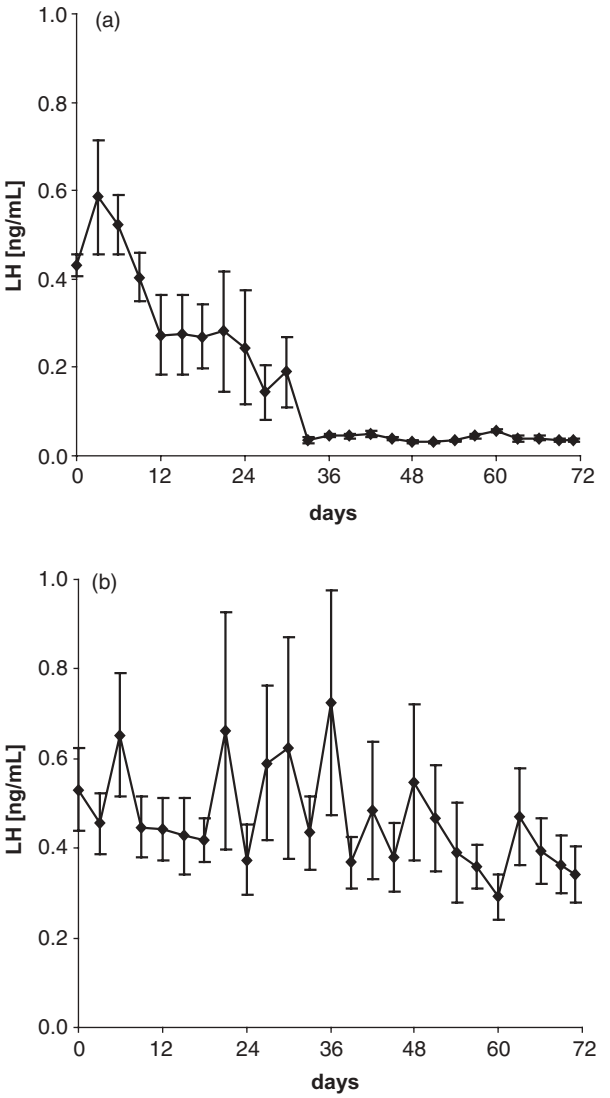


**Figure 2** LH concentrations (mean ± s.e.) in immunized barrows (a) and control barrows (b). First immunization was on day 0 (18 weeks of age). Second immunization was on day 28 (22 weeks of age).

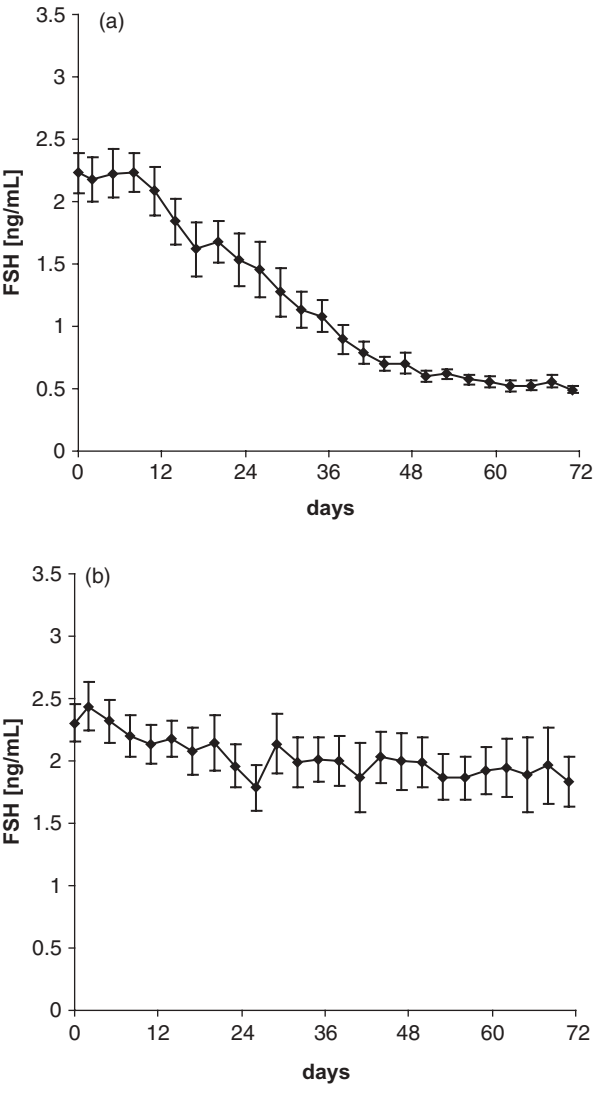


**Figure 3** FSH concentrations (mean ± s.e.) in immunized barrows (a) and control barrows (b). First immunization was on day 0 (18 weeks of age). Second immunization was on day 28 (22 weeks of age).

to the second window at age 24 weeks (2 weeks after the second vaccination) both in control barrows and in immunized barrows. The decrease was 18% in the control barrows and 22% in the immunized barrows. While maxima decreased in the same way ($P < 0.015$) the base levels remained unchanged. The pulse frequency was lower in the second window compared with the first one ($P = 0.048$). Nevertheless, there were no significant differences between the two treatment groups in any of the GH parameters evaluated, in either the first or second sampling windows.

IGF-1 (Table 3) tended to decrease due to immunization but was not significantly different from the controls ($P = 0.067$).

Data for metabolic parameters are also presented in Table 3. Compared to phase 1, urea concentrations in phase 2 were increased by 15 μg/ml in both groups (not significant) while differences between groups exist ($P = 0.002$). There was a significant decrease in OH-Pro by about 10% after the second immunization ($P = 0.001$). Protein synthesis values were 5.16 and 5.55 g/kg BW per day for barrows and immunized barrows, respectively, and were not significantly different. Comparison of deuterium oxide applications in weeks 20 and 25 demonstrated that fat content tended to increase by 15% in this period ($P = 0.073$) and there was no statistical difference between the groups.

## Discussion

In an earlier study (Metz and Claus, 2003), using immunocastrated entire male pigs at ages of 10, 16 and 23 weeks, we found that GH concentrations in blood plasma at 23 weeks were identical to entire control boars (both 4.09 ng/ml). In contrast, GH concentrations in blood plasma of surgical castrates only reached 2.88 ng/ml, and thus were 42% lower than the other two groups. The maintenance of

**Table 2** *Characterization of GH concentrations in blood plasma of barrows and immunized barrows in 24-h windows at an age of 19 and 24 weeks*

| Group | Window week | Mean level (ng/ml) | Basal level (ng/ml) | Maximum (ng/ml) | Pulses (*n*/24 h) |
|---|---|---|---|---|---|
| Barrows | 19 | 2.53 ± 0.12 | 1.66 ± 0.09 | 5.36 ± 0.48 | 4.50 ± 0.31 |
| | 24 | 2.06 ± 0.07 | 1.43 ± 0.19 | 4.27 ± 0.71 | 3.50 ± 0.39 |
| Immunized barrows | 19 | 2.21 ± 0.17 | 1.43 ± 0.16 | 4.95 ± 0.26 | 4.33 ± 0.19 |
| | 24 | 1.72 ± 0.12 | 1.16 ± 0.10 | 3.95 ± 0.25 | 3.17 ± 0.50 |
| | Group | n.s. | n.s. | n.s. | n.s. |
| | Window | 0.01 | n.s. | 0.015 | 0.048 |

Values are given as mean ± s.e.

**Table 3** *Metabolic parameters*

| Group | | IGF-1 (ng/ml) | Urea (μg/ml) | Hydroxyproline (μg/ml) | Protein synthesis (g/kg per day) | Fat content (%) |
|---|---|---|---|---|---|---|
| Barrows | Phase 1 | 145.1 ± 17.6 | 328 ± 8 | 23.2 ± 0.3 | – | 17.7 ± 2.9 |
| | Phase 2 | 122.1 ± 14.1 | 343 ± 6 | 20.4 ± 0.5 | 5.16 ± 0.31 | 22.1 ± 1.1 |
| Immunized barrows | Phase 1 | 139.5 ± 9.0 | 378 ± 9 | 23.6 ± 0.4 | – | 19.0 ± 1.7 |
| | Phase 2 | 133.8 ± 10.0 | 393 ± 5 | 21.7 ± 0.4 | 5.55 ± 0.81 | 21.2 ± 0.7 |
| | Group | n.s. | 0.002 | n.s. | n.s. | n.s. |
| | Phase | 0.067 | n.s. | 0.001 | – | 0.073 |

Phase 1 refers to day 0 (day of first vaccination at age 18 weeks) to day 27 while phase 2 comprise day 39 to day 72. Protein synthesis was determined on the last day of phase 2, while fat content was measured on day 2 and day 49.
Values are given as mean ± s.e.

GH concentration after immunocastration was recently confirmed in another study (paper in preparation; boars: 3.57 ng/ml; immunized boars: 3.24 ng/ml; not significant). These results indicated a systematic difference in GH concentrations between surgically castrated and immunocastrated pigs. It is well known that GnRH and thus gonadotropins are elevated in barrows due to the absence of negative feedback by steroids. Since surgically castrated and immunocastrates have low steroid values, we hypothesized that there may be an interaction between elevated GnRH and GRH, which might be abolished by GnRH vaccination of barrows.

In contrast to our earlier study with boars, gonadotropin concentrations in barrows already decreased in response to the first vaccination, which is known to lead to low levels of immunoglobulins primarily represented by IgM. Such an effect was also found previously (Oonk *et al.*, 1998; Turkstra *et al.*, 2001 and 2002). It is likely that our assay system did not detect low levels of IgG and did not detect IgM at all because it was raised against IgG.

The difference in gonadotropin reaction between boars and barrows might be due to the absence of negative steroid feedback in the barrows, which thus have higher gonadotropin concentrations in the beginning and are probably more sensitive to low levels of immunoglobulins. Detailed mechanisms of a possible interaction of the immune system and hormonal feedback regulation have not been investigated. Such an assumption, however, is supported by the maintenance of an intermediate LH level of about 0.2 ng/ml until the second vaccination, which corresponds to the physiological LH concentration in boars (Wagner and Claus, 2004; Claus *et al.*, 2007). The second vaccination then led to a rise of antibody titre and to a further decrease of LH down to base levels. Interestingly, FSH also decreased continuously after a latency period of about 10 days after the first immunization. In earlier studies we found no change in FSH concentrations following immunization of boars: levels remained the same (0.5 ng/ml) in immunized and control boars (Wagner and Claus, 2004). In the present study, barrows of both groups had higher FSH concentrations due to the absence of negative steroid feedback. Immunization clearly decreased FSH but values were again stabilized at 0.5 ng/ml. With regard to FSH regulation, differences exist with other species. Both the function of activin from the anterior pituitary and the existence of a hypothalamic releasing hormone, other than GnRH, were assumed (Kauffold *et al.*, 2005). This does not exclude the fact that GnRH plays a role in stimulating the high FSH concentrations due to the absence of negative feedback in barrows. From other species it is known that GnRH and activin interact (Liu *et al.*, 1996; Shupnik and Weck, 1998; Gregory *et al.*, 2005) so that high GnRH concentrations might be paralleled by elevated activin. Because activin in turn inhibits the synthesis and release of GH (Childs and Unabia, 1997), these effects might also explain lower GH levels in surgical castrates. The comparison of the two windows in the immunized barrows, one during high and one during low FSH, however, did not lead to different GH concentrations so that lower levels of GH in surgical castrates do not support the assumption of activin effects even if this substance was not measured in this

study. Furthermore, GH secretion was not influenced by immunization of barrows, so the hypothesis of an interaction between the releasing hormones GnRH and GRH in the hypothalamus or pituitary also could not be substantiated.

As many of the metabolic effects of GH are mediated by IGF-I, levels of this hormone were also determined and again revealed no differences between barrows and immunized barrows. As expected, metabolic parameters confirm the absence of an effect of immunization on GH in barrows. In both groups, however, the age-dependent influence is obvious such as the increase of urea concentrations and the decrease of OH-Pro levels in blood plasma as it is known from other studies (Miller *et al.*, 1990). The fat content also increased with age, as expected. It is concluded that interactions between GnRH and GRH in the hypothalamus or the pituitary do not explain why GH concentrations in surgically castrated males are lower than in GnRH immunized boars. It might be that boars, which remain intact until GnRH immunization, e.g. at 18 weeks, react differently compared to those that were castrated during the first week of life. Indeed, it is known from rodents that a later 'male pattern' of GH secretion is imprinted by gonadal steroids early postnatally (Jansson *et al.*, 1985). Such a transient increase of gonadal steroids in pigs is known to occur about 3 to 4 weeks after birth (Schwarzenberger *et al.*, 1993).

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## 2.3 *Effects of two levels of feed allocation on IGF-I concentrations and metabolic parameters in GnRH-immunized boars*

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ORIGINAL ARTICLE

# Effects of two levels of feed allocation on IGF-I concentrations and metabolic parameters in GnRH-immunized boars

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**Keywords**
IGF-I, immunocastration, boar, energy supply, growth

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## Summary

Immunocastration of boars leads to a maintenance of growth harmone (GH) and a loss of anabolic hormones [androgens, oestrogens, insulin-like growth factor (IGF-I)] but an increase of voluntary feed intake. The aim of the experiment was to clarify whether IGF-I is increased by increasing feed supply in immunocastrated boars leading to improved anabolism. Two groups of six boars were given 2 or 3 kg of feed (13.5 MJ ME/kg) daily from 18–28 weeks of age. Because in boars feed intake is limited by gonadal hormones, a group with further increased feed supply could not be included. Until week 22 (second vaccination) gonadal steroids in blood were normal but dropped rapidly thereafter. Growth harmone levels did not change following vaccination. Pigs allocated 3 kg feed had 28% higher circulating IGF-I after the second immunization compared with pigs fed 2 kg feed daily. Higher IGF-I was associated with increased weight gain (682.4 g/day vs. 466.7 g/day; $p < 0.01$) and protein synthesis ($^{13}$C-leucine infusion; 405 g/day vs. 247 g/day, $p < 0.01$). Protein breakdown (urea) was not different. Body fat ($D_2O$) decreased in the low feed group from 15.2% (week 19) to 6.1% (week 25). In the high feed group it remained at the level found before second vaccination (13.7% vs. 15.0%). It is concluded that in the phase of reduced testicular steroids which inhibit appetite it is possible to increase feed intake which in turn increases IGF-I and protein deposition without accumulating excessive fat.

## Introduction

Boars are well known to have a higher anabolic potential compared with barrows (castrated males; van Lunen and Cole, 1996; Campbell et al., 1985) which leads to improved fattening performance and carcass characteristics. Surgical castration, however, is still performed to avoid the unpleasant boar taint which is not acceptable to many consumers.

Currently, immunological castration is discussed as an alternative to surgical castration. It is based on immunization against gonadotropin releasing harmone (GnRH) so that luteinizing harmone (LH) release from the pituitary is blocked, and in consequence, testicular Leydig cells do not synthesize sex steroids (Claus et al., 2007). Improvac® (Pfizer Animal Health, Karlsruhe, Germany) is an antigen which is available in several countries. Immunization is performed by giving two injections of the antigen at least 4 weeks apart. Seven days after the second vaccination, metabolism is adapted to the lack of anabolic gonadal steroids. In the period between second vaccination (approximately 22 weeks of age) and slaughter (at approximately 28 weeks of age), performance data and anabolic metabolism do not differ from conventional barrows although GH is higher in immunized pigs, but this time must be given to allow decrease of boar taint

from body fat (Claus, 1976). Anabolic steroids which are absent during this period, include androgens, e.g. testosterone and nortestosterone as well as high amounts of testicular oestrogens, which in the boar by far exceed levels measurable in oestrous sows (Claus and Hoffmann, 1980). Both androgens and oestrogens contribute to an improved anabolic performance by increasing nitrogen retention to a level approximately 20% higher than in barrows (Metz et al., 2002). Mode of actions of androgens and oestrogens, however, differ.

Androgens lead to muscle fibre hypertrophy by stimulating muscle protein synthesis and improving re-utilization of amino acids. In addition, muscle protein degradation and cell apoptosis are decreased after androgen application to castrated males. This effect is mainly explained by formation of heterodimers between androgen and glucocorticoid receptors, which decrease the catabolic action of cortisol (Bhasin et al., 2003; Chen et al., 1997). Such glucocorticoid receptors also exist in pig muscles (Claus et al., 1996).

The anabolic effect of oestrogens is primarily explained by stimulating concentrations of the insulin-like growth factor-I (IGF-I) in peripheral blood. This mitogenic factor is expressed in almost every tissue, including muscle tissue (Novakofski and McCusker, 1993; Reeds et al., 1993; Kamanga-Sollo et al., 2004), but is also secreted as a hormone from the liver in response to GH stimulation (Breier et al., 1989; Etherton, 1989; Novakofski and McCusker, 1993). In the liver, oestrogens upregulate hepatic GH receptors and thus boost GH-dependent IGF-I expression (Claus et al., 1992a). Insulin-like growth factor-I then is the most potent stimulus for protein synthesis.

Active immunization shifts metabolism of boars to a less pronounced anabolic state following the decrease of androgens and oestrogens (Claus et al., 2007). It is additionally known that sex steroids decrease voluntary feed intake as shown by comparisons of boars and barrows and experimentally either by testosterone or estradiol administration to barrows (Claus and Weiler, 1987; 1994).

Insulin-like growth factor-I formation is very sensitive to feed intake (Morovat et al., 1994; Owens et al., 1999) mainly because of the stimulating function of carbohydrates with a high glycaemic index such as glucose or starch (Reeds et al., 1993). In consequence, it is possible that the decrease of testicular hormones because of immunization leads to an increased voluntary feed intake which in turn elevates IGF-I. Such a mechanism would allow development of feeding strategies which increase the anabolic potential again in finishing immunocastrates. Therefore it was the aim of this study to clarify whether the level of feed supply in the finishing period of immunocastrates influences IGF-I and improves parameters which characterize metabolic reactions.

## Materials and methods

### Study design

German Landrace boars ($n = 12$) were obtained from the swine herd of the University of Hohenheim. They were randomly divided into two experimental groups (each $n = 6$) and kept in individual crates measuring 3 m × 2.5 m. At 18 weeks of age they received the first 2 ml dose of the GnRH-antigen (Improvac®). A single dose of Improvac® has no effect on testicular function and metabolic parameters (Claus et al., 2007). In the following 4 weeks (phase 1; between 18 and 22 weeks of age), hormones (GH, LH, IGF-I, testosterone and estradiol) and metabolic parameters were measured in blood plasma to characterize the boar-specific endocrine and metabolic status. At 22 weeks of age in accordance with the manufacturer's recommendations, the second dose of Improvac® was given to immunocastrated pigs and the responses of hormonal and metabolic parameters were determined.

Animals were fed from an age of 18 weeks till slaughter at 28 weeks with a ration containing 13.5 MJ ME/kg (crude protein 16.7%; Table 1). The main energy source was starch (443 g/kg) with a high glycaemic index which is the prerequisite to provoke the expected effect on IGF-I. Group 1 received 2 kg per day, corresponding to 27 MJ per day, which is below recommendations (32 MJ per day) at a body weight of 80 kg. Group 2 received 3 kg feed daily so that total supply (40.5 MJ per day) was above requirement (Ausschuss Ernährungsphysiologie, 2006). A third group with

**Table 1** Feed composition

| Component | Wet weight (g/kg) |
|---|---|
| Barley | 373 |
| Triticale | 200 |
| Wheat | 170 |
| Pea | 70 |
| Soybean meal | 150 |
| Vitamin and mineral premix | 25 |
| Calcium carbonate | 2 |
| Soybean oil | 10 |

further increased feed supply was not realistic, because appetite is limited because of testicular steroids. The pigs were fed twice daily at 8:00 and 15:00 hours with 1 kg (group 1) and 1.5 kg (group 2) respectively. During the experimental period, feed intake was monitored twice daily by weighting residual feed. Weight gain was determined every week. The whole experiment had been approved by the Animal Welfare Committee of the state of Baden–Württemberg.

### Cannulation and blood sampling

Each pig was fitted with a permanent cephalic vein catheter under general anaesthesia at an age of 17 weeks as described earlier (Claus et al., 1990). At that age, body weight varied between 51 and 60 kg in group 1 and 49 and 62 kg in group 2, so that mean body weight did not differ significantly between group 1 and 2. The animals had completely recovered from cannulation 2 days after surgery. Blood samples were drawn daily at 9.00 hours in heparinized vials and the plasma stored deep-frozen at −20 °C until measuring the different parameters. After each sampling, the catheters were rinsed using sterile saline with 0.1% heparin.

### Protein synthesis

Protein synthesis was determined by the $^{13}$C-leucine infusion method. The principles were described earlier (Waterlow et al., 1978). Details are given in a preceding paper (Bauer et al., 2007). In brief, a constant infusion of $^{13}$C-leucine was applied for 6 h after a single bolus of $^{13}$C-leucine which had been given via the catheter. Blood samples were taken every 30 min, leucine was isolated, derivatized using the test kit EZ:faast (Phenomenex, Aschaffenburg, Germany) and analysed for $^{13}C/^{12}C$ enrichment by gas chromatography–mass spectrometry (GC–MS). Constant enrichment was obtained between 3 and 6 h of infusion and was used to calculate the total leucine flux and subsequently protein synthesis using the method of Waterlow et al. (1978). Intra-assay coefficient of variation was 2.75%.

### Body fat determination

To mirror changes in body fat content, the deuterium oxide ($D_2O$) method was used (Tissier et al., 1978; Susenbeth, 1984). The $D_2O$ windows were performed at an age of 20 and 25 weeks. Application, measurement of isotopic enrichment by infrared spectroscopy and calculation were already described (Claus et al., 2007). Intra- and interassay coefficients of variation were 0.2% ($n$ = 5) and 2.2% ($n$ = 10) respectively.

## Analytical methods

### GnRH-antibody titre

Relative concentrations of anti-GnRH antibodies were determined using a microtitre plate assay as previously described (Bauer et al., 2007). In brief, plates were coated with GnRH (Bachem, Weil am Rhein, Germany), incubated with diluted blood plasma (1:1600) and subsequently incubated with a second antibody-enzyme-conjugate (P 0164, specific for all subclasses of swine IgG; Dako, Hamburg, Germany). Horse radish peroxidase was used as the enzyme and tetramethylbenzidine/$H_2O_2$ as substrate. Extinction was measured at 450 nm. Interassay coefficients of variation were 2.56% for an extinction of 3.67 and 5.44% for an extinction of 2.33 ($n$ = 5).

### Luteinizing hormone and growth hormone

The experimental period lasted 10 weeks, so high frequent sampling of blood for measuring the pulsatile LH and GH secretion patterns was not possible. Instead, they were measured by radioimmunoassay (RIA) in samples taken every third (LH) or second (GH) day (Wagner and Claus, 2004; Claus et al., 1990) which is sufficient to reflect major changes over this prolonged period.

The specific LH antiserum (AFP 15103194) was provided by Dr. Parlow (NIDDK, Torrance, CA, USA) and a highly purified pLH standard (AFP 11043B) from the same source was used for radio-iodination and for calibration. Interassay coefficients of variation were 7.3% and 9.1% for concentrations of 380 and 40 pg/ml respectively. The lower limit of detection was 24 pg/ml and average recovery was 92% for concentrations of 250 pg/ml.

A specific GH antiserum (AFP422801) was also provided by Dr. Parlow (NIDDK, Torrance, CA, USA) and a highly purified pGH standard (AFP10864P) was used for radio-iodination and calibration. Repeatability was determined by measuring spiked samples on consecutive days and the interassay coefficient of variation was found to be 7.5% for levels of 3.4 ng/ml ($n$ = 6). The intraassay coefficient of variation was 3.6% for concentrations of 4.6 ng/ml ($n$ = 10) and the recovery was above 96% for spiked samples (1.5–6 ng/ml).

### Testosterone

Radioimmunological determination of testosterone in plasma samples of every third day after solvent extraction was performed as published earlier (Bauer et al., 2007). Coefficient of variation between days was 8% at a concentration of 1 ng/ml ($n = 6$). The lower limit of detection was 0.05 ng/ml. The extraction yield was 90%.

### 17$\beta$-Estradiol

Concentrations of 17$\beta$-estradiol in blood plasma were determined every third day as described before (Claus et al., 1983). The antiserum was highly specific for 17$\beta$-estradiol and showed no cross-reactivities with other oestrogens. Interassay coefficient of variation was 9.4% for concentrations of 84 pg/ml ($n = 9$). Recoveries were above 95%.

### Androstenone

Levels of androstenone (5$\alpha$-androst-16-en-3-one) in blood samples which had been drawn every third day were determined after *n*-hexane extraction by GC–MS analysis in the single-ion mode as described previously (Claus et al., 2007). Repeatability was calculated by measurement of a pool sample either on the same day ($n = 4$; intraassay coefficient of variation was 3.7% at a concentration of 1.0 ng/ml) or on consecutive days ($n = 13$; interassay coefficient of variation was 8.2% for 1.15 ng/ml).

### Insulin-like growth factor-I

To determine IGF-I in plasma samples from every second day, a double-antibody RIA was used which includes an HCl/ethanol extraction to separate IGF-I from its binding protein. The specific antiserum was raised in rabbits (Claus et al., 1992a,b). The IGF-I standard for radio-iodination and for calibration was obtained from Gro Pep (CU020; Adelaide, Australia). The average recovery for spiked samples was approximately 70% for concentrations between 50 and 400 ng/ml. The coefficients of variation within and between days were 7% ($n = 10$; 186 ng/ml) and 5% ($n = 7$; 74 ng/ml) respectively.

### Urea

Urea was measured in plasma to characterize protein degradation as a marker for nitrogen excretion, because we found earlier that transfer of pigs into metabolic crates for N-retention studies is sufficient to shift metabolism to a more catabolic status. The method was described in detail earlier (Claus et al., 2007). In brief, plasma was pre-incubated with glutamic dehydrogenase to remove ammonia by reaction with oxoglutarate, adenosine diphosphate and reduced forms of nicotinamide adenine dinucleotide. After addition of urease released ammonium was quantified at 340 nm. Quantification in blood samples of every second day was performed and interassay coefficient of variation was 3.6% ($n = 14$) in a sample containing 341 $\mu$g/ml.

### Statistics

Data were analysed using the Statistical Package for the Social Sciences (SPSS version 13.0; Chicago, IL, USA). Data in figures and tables are presented as means $\pm$ SEM of six animals per group, with day 0 being the day of the first immunization and day 28 the day of the second immunization. For statistical analysis two phases were calculated as reported earlier (Claus et al., 2007): phase 1 comprised values from day 0 to day 27; phase 2 comprised values from day 39 to day 72. Days 28–38 were excluded because they represented the period of transient increasing or decreasing values because of the onset of immunization effects. Trend analysis was performed as described by Neumann (1941) and Hart (1942). Changes in titre development were tested as described previously (Claus et al., 2007). Differences between phases within one group were tested using paired Student's *t*-test, whereas differences between groups were analysed with unpaired Student's *t*-test. Possible effects that influence results, such as time of birth, weight at 18 weeks of age and genetics were excluded by the study design as described above.

## Results

### Confirmation of immunization success

The development of the relative concentration of GnRH antibodies in immunized animals is summarized in Fig. 1 for both groups and compared with the course of GH concentrations. Concentration of GnRH antibodies rose rapidly 4 days after second immunization (day 28). Maximal levels were reached on day 37. Thereafter relative concentration of GnRH antibodies decreased significantly (trend analysis; $p < 0.05$), but mean extinctions of the animals during the last week of the experiment were still elevated compared with mean values of phase 1

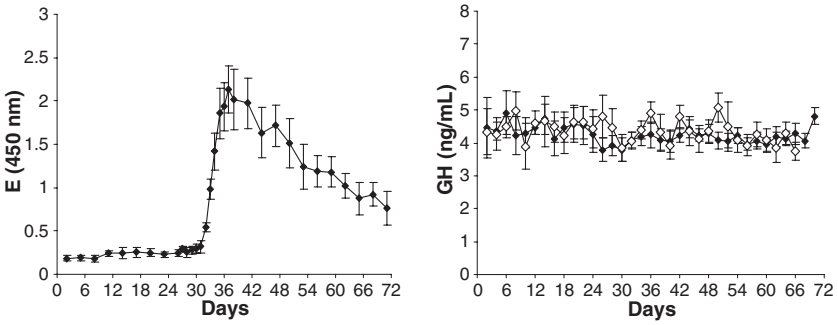


**Fig. 1** Development of relative concentration of gonadotropin releasing harmone antibodies (left: mean extinctions ± SEM for both groups; $n$ = 12) and mean growth harmone (right) both for immunized boars of group 1 (black squares: fed with 2 kg) and group 2 (open squares: fed with 3 kg). First immunization was on day 0 (18 weeks of age). Second immunization was on day 28 (22 weeks of age).

($p < 0.01$). Immunization had no significant effect on the course of GH concentrations.

Consequent to the increase in relative levels of antibodies against GnRH, concentrations of LH and androstenone were low until slaughter and are summarized in Table 2 to further confirm the effectiveness of immunization. LH dropped significantly after second immunization (phase 2) within 8 days from mean concentrations of 100 pg/ml before second immunization (phase 1) to values near the detection limit of 24 pg/ml in both group 1 and 2 ($p < 0.05$ for both groups). Similarly, levels of androstenone reached minimum values 8 days after second immunization in group 1 and 2.

### Performance data

Monitoring of feed intake revealed that in the 2 kg group, no feed residues were left by the pigs over the whole experimental period. In the 3 kg group, residues of approximately 100–500 g/day occasionally remained under the influence of testicular hormones before second vaccination. With the onset of the immunization effect during phase 2 and thus the drop of gonadal steroids, appetite increased and feed was completely consumed.

Irrespective of the group, all animals entered the experiment with the same body weight and increased weight in a linear mode during the whole experimental period (coefficients of determination in a range of $r^2 = 0.94–0.99$). Daily weight gain was 579 and 867 g/day for groups 1 and 2, respectively, over the whole experimental period (age 18–28 weeks). Weight gain started to differ between the two groups 1 week after second vaccination.

**Table 2** LH and androstenone concentrations of immunized boars fed 2 kg/day (group 1) and immunized boars fed 3 kg/day (group 2) in phase 1 (before second immunization) and phase 2 (after second immunization)

| Parameter | Phase | Group 1 | Group 2 |
|---|---|---|---|
| LH (pg/ml) | 1 | 80 ± 10* | 120 ± 20* |
| | 2 | 30 ± 0 | 40 ± 10 |
| Androstenone (ng/ml) | 1 | 1.54 ± 0.30* | 0.93 ± 0.19* |
| | 2 | 0.18 ± 0.03 | 0.16 ± 0.03 |

LH, luteinizing harmone.
Asterisks refer to differences between phases within one group (*$p < 0.05$). Comparison between groups within the same phase revealed no differences.
Mean ± SEM are given.

### Metabolically active hormones and parameters of metabolism

Other parameters are given as continuous profiles separately for the two feeding groups in Figs 2–5. A comparison of differences between groups after second immunization in phase 2 is summarized in Table 3. Measurement of testosterone (Fig. 2) in animals from groups 1 and 2 showed decreasing values 8 days after second immunization ($p < 0.01$). Minimal values corresponded to the limit of detection of the assay and thus, of course, were not significantly different between the two groups in phase 2. Mean values in phase 1 also did not differ significantly.

Concentrations of 17$\beta$-estradiol (Fig. 3) did not differ significantly between groups in phase 1 (group 1: 71 pg/ml; group 2: 46 pg/ml). After second vaccination, 17$\beta$-estradiol dropped in both groups (Fig. 3) and mean values thereafter were not significantly different between groups (Table 3).

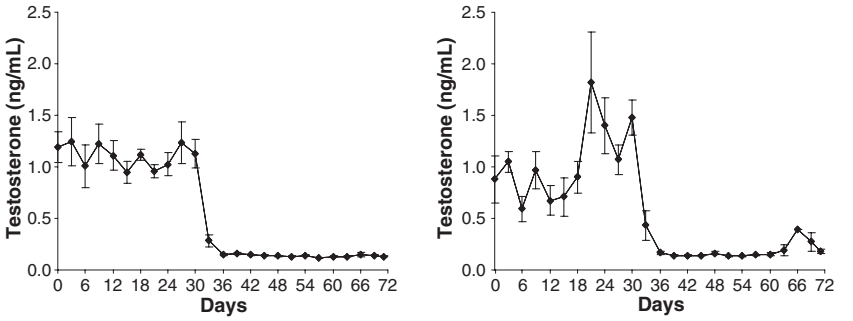


**Fig. 2** Testosterone concentrations (mean ± SEM; $n = 6$) for group 1 (left; immunized boars fed with 2 kg) and group 2 (right; immunized boars fed with 3 kg). First immunization was on day 0 (18 weeks of age). Second immunization was on day 28 (22 weeks of age).

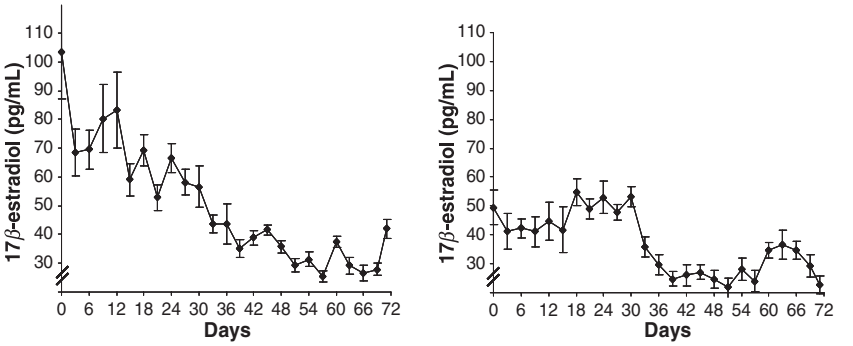


**Fig. 3** 17$\beta$-estradiol concentrations (mean ± SEM; $n = 6$) for group 1 (left; immunized boars fed with 2 kg), group 2 (right; immunized boars fed with 3 kg). First immunization was on day 0 (18 weeks of age). Second immunization was on day 28 (22 weeks of age).

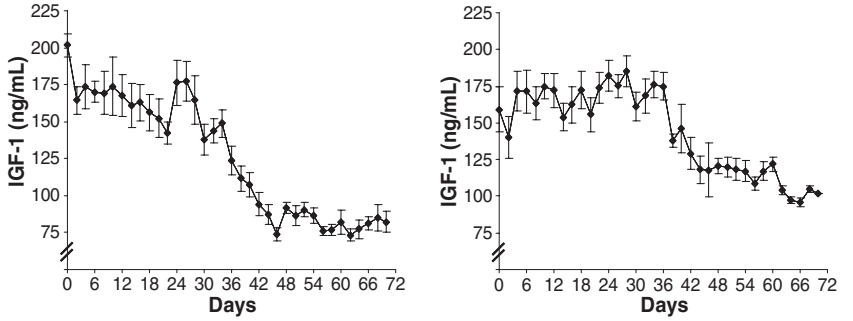


**Fig. 4** Insulin-like growth factor concentrations (mean ± SEM; $n = 6$) for group 1 (left; immunized boars fed with 2 kg), group 2 (right; immunized boars fed with 3 kg). First immunization was on day 0 (18 weeks of age). Second immunization was on day 28 (22 weeks of age).

Data for IGF-I are shown in Fig. 4 and revealed levels of approximately 160 ng/ml in phase 1, before second immunization, for both groups and were not significantly different. After second immunization, boars showed decreasing values over a period of 14 days and stabilized at lower concentrations of 86 and 119 ng/ml for group 1 and 2, respectively, corresponding to a difference of 28%. These differences between the phases were significant ($p < 0.05$) as were differences between groups in phase 2 (Table 3).

Courses of urea concentrations are given in Fig. 5. Until second immunization, both groups remained at approximately 160 $\mu$g/ml, while second immunization led to increasing values within 10 days to mean concentrations of 253 $\mu$g/ml in group 1 and up to 288 $\mu$g/ml in group 2 (comparison of phases for both groups: $p < 0.01$). In phase 2 differences between group 1 and 2 (Table 3) were not significant.

Protein synthesis was determined at the end of the experiment only and is given both in g/day and

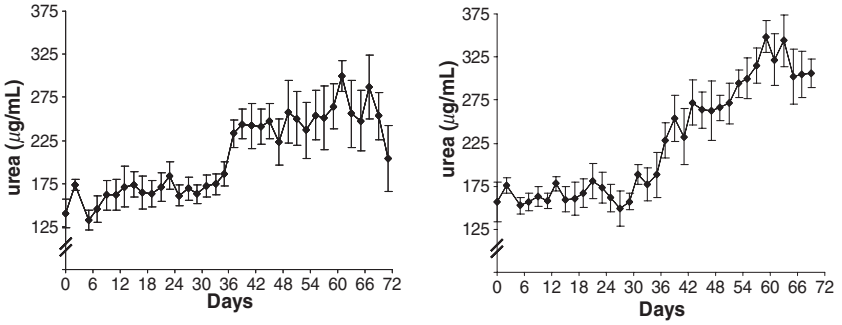


**Fig. 5** Urea concentrations (mean ± SEM; $n = 6$) for group 1 (left; immunized boars fed with 2 kg) and group 2 (right; immunized boars fed with 3 kg). First immunization was on day 0 (18 weeks of age). Second immunization was on day 28 (22 weeks of age).

**Table 3** Comparison of values in phase 2 for the two groups with different feed intake

| Parameter | Group 1 (2 kg) | Group 2 (3 kg) | Significance |
|---|---|---|---|
| Weight gain (g/day) | 466.7 ± 43.3 | 682.4 ± 21.1 | $p < 0.01$ |
| Testosterone (ng/ml) | 0.14 ± 0.00 | 0.17 ± 0.02 | n.s. |
| 17$\beta$-estradiol (pg/ml) | 33.1 ± 1.3 | 28.1 ± 2.7 | n.s. |
| IGF-I (ng/ml) | 86.3 ± 5.3 | 118.5 ± 8.6 | $p < 0.01$ |
| Protein synthesis | | | |
| (g/kg*day) | 2.44 ± 0.19 | 3.27 ± 0.32 | $p < 0.05$ |
| (g*day) | 247.1 ± 18.5 | 404.5 ± 41.9 | $p < 0.01$ |
| Urea ($\mu$g/ml) | 253.0 ± 26.4 | 287.8 ± 20.3 | n.s. |
| Body fat content (%) | 6.1 ± 2.4 | 15.0 ± 3.0 | $p < 0.05$ |

IGF-I, insulin-like growth factor; ns, not significant.

body weight in Table 3. Significant differences ($p < 0.05$) were found between group 1 (2.44 ± 0.19 g/kg*day) and group 2 (3.27 ± 0.32 g/kg*day) and even more pronounced ($p < 0.001$) when giving protein synthesis in g per day.

Body fat content did not differ significantly between groups in phase 1 (group 1: 15.2%, group 2: 13.7%). but was significantly different in phase 2 (group 1: 6.1%, group 2: 15.0%; Table 3). There was a significant decrease in body fat from phase 1 to phase 2 in group 1 ($p < 0.05$), but no significant differences between phases in group 2.

## Discussion

A variety of short-term or long-term effects on hepatic IGF-I expression and in consequence circulating levels of IGF-I are well described. They include hormonal effects such as concentrations of GH (Novakofski and McCusker, 1993) and estradiol (Claus et al., 1992a) but also nutritional effects such as the content of carbohydrates or specific amino acids (Miuray and Noguchi, 1992) in the diet and the amount of feeding. After fasting, concentrations of IGF-I decrease and increase again during the refeeding period in human adults (Isley et al., 1983), rats (Phillips et al., 1978), chickens (Lauterio and Scanes, 1987) and neonatal pigs (Jones and Campion, 1986). Short-term changes can be effected by changes of ambient temperature without changes to feed intake (Morovat et al., 1994).

In this study, GnRH immunized boars were used as a model because immunization abolishes testicular steroids so that IGF-I expression in the liver and inhibition of appetite are decreased. The effect of gonadal steroids to lower feed intake was shown earlier by supplementing barrows with either oestrogens or androgens (Claus and Weiler, 1987). This function probably is mediated via steroid receptors in the hypothalamus because it was shown e.g. that the anorectic effect of oestrogens may be mediated by decreasing neuropeptide-Y release in the hypothalamus (Bonavera et al., 1994). Additionally, many other factors are currently discussed as potent regulators of feed intake.

Following immunization, levels of GH are maintained at levels seen in entire boars, whereas surgical castrates (barrows) have much lower GH concentrations (Metz and Claus, 2003). Hormone determinations in this present study confirm the maintenance of GH and the abolishment of gonadal steroids, so that amount of feed was the only variable and its effect on IGF-I and the metabolic consequences could be studied separately.

Compared with other studies with either feed withdrawal or energy supply only for maintenance (Buonomo and Baile, 1991; Charlton et al., 1993; Booth et al., 1996; Salfen et al., 2003), our feeding regime was deduced from energy requirements and digestibility for finishing rations of pigs at 80 kg live weight. The average weight of 80 kg coincided with the second immunization. The moderate feeding differences between groups were more in a physiological range. From earlier studies we know that voluntary feed intake in 80 kg boars is around 3 kg (Metz et al., 2002; Bauer et al., 2008).

Data obtained show that both groups of immunized boars after second vaccination reacted indeed with a decreased IGF-I expression. It was also apparent by comparing IGF-I in phase 1, before onset of testicular inhibition, and phase 2, when steroid synthesis is inhibited, that the marked decrease in IGF-I is associated with the decrease in circulating steroids. During phase 2, even in the 3 kg group, residues of feed were never left, so that it is reasonable to assume that feed intake could have been higher. Such a further increase probably would have allowed a further growth improvement. At the first look such an increase would probably lead to increased fat deposition but we demonstrated using the deuterium method that fat content in the 3 kg group did not increase and is still 32% lower compared with barrows of the some weight and feeding regime (Bauer et al., 2007). At least a part of the additional energy apparently consumed by group 2 was used for elevated protein synthesis. The IGF-I-dependent increase of protein synthesis led to differences of 25% between the two groups as shown by $^{13}$C-leucine infusion, and also by the high-weight gain. Urea in contrast did not differ between groups. Urea is an appropriate parameter to monitor protein degradation (Lopes et al., 2004) caused by glucocorticoids as the main regulator of protein breakdown. The catabolic effect of glucocorticoids is counteracted by testosterone, which was absent in both groups of immunocastrates and thus explains the increase of urea after second vaccination. Differences in protein synthesis thus were not paralleled by differences in breakdown. It was shown earlier that the rate of protein breakdown generally is independent from the rate of protein synthesis (Reeds et al., 1980).

Growth harmone alone, without a rise of IGF-I is not efficient to increase protein synthesis (Novakofski and McCusker, 1993). Because immunocastration inevitably eliminates oestrogens, an increased supply of carbohydrates in the form of starch is an alternative agent to couple GH and IGF-I. Similar to estradiol, these carbohydrates are stimulators for GH receptor expression in the liver (Brameld et al., 1999). Because GH efficiently inhibits fat synthesis (Etherton, 2001), surplus energy is allocated to protein synthesis. Under an energy deficit, e.g. as in group 1, GH is known to exert a powerful lipolytic effect. In consequence, this mechanism might explain the remarkable decrease of fat in group 1 but still the maintenance of a notable level of daily weight gain.

In conclusion it is likely that elevation of feed supply even above 3 kg/day might further improve protein synthesis without excessive fat deposition in immunocastrates and thus to adapt performance also after second immunization to boars which are not castrated.

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## 2.4 *Is the early postnatal rise of testosterone responsible for a later male pattern of growth hormone secretion in pigs?*

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# Is the early postnatal rise of testosterone responsible for a later male pattern of growth hormone secretion in pigs?

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**Abstract**

Sexual differentiation in Placentalia consists of several consecutive steps during fetal, postnatal, and premature development. It is known from male rats that an elevation in testosterone synthesis is observable within 2 d of birth, which leads to a male pattern of growth hormone (GH) secretion with low base levels and high amplitudes compared with that in females. In the male pig, a transient rise in testosterone concentration occurs about 4 wk after birth, but it is unknown whether it results in a later male pattern of GH secretion. In this study, male pigs (sus scrofa) were castrated either at 1 wk of age (Group 1, n = 8) or at 6 wk of age (Group 2, n = 8). Blood was sampled daily via cephalic vein catheters between 17 and 29 wk of age and analyzed for testosterone, GH, insulin-like growth factor-1 (IGF-1), and urea. High-frequency blood sampling (every 20 min over 24 h) for determination of GH pulsatility was performed at ages 19 and 24 wk. Total fat content and protein synthesis were determined at age 25 wk and at slaughter, respectively. Comparing Groups 1 and 2, there were no differences in daily GH concentrations or pulsatile secretion patterns, but in both groups, mean GH levels and pulsatility decreased from Week 19 to Week 24. Consequently, IGF-1, protein synthesis, urea, and body fat showed no differences when comparing both groups. It is concluded that the postnatal rise of testicular steroidogenesis in male pigs is not responsible for the later male pattern of GH secretion.


*Keywords:* GH; Pig; Postnatal imprinting; Testosterone

## 1. Introduction

In Placentalia, differentiation of the bivalent gonadal structures (anlagen) to either the testes or the ovaries is determined by the presence or absence of a Y-chromosome. Further sex differentiation is then subject to the principle of basic femaleness. In females, and thus in the absence of fetal testicular endocrine function, the primordia of the male tract degenerate and those of the female system are stabilized [1], whereas in males, differentiation of internal and external genitalia requires androgen synthesis by the fetal testis, which can be observed as early as 4 wk after fertilization in the pig [2,3]. In addition to the genital tract, structures of the central nervous system, specifically the hypothalamus, are subject to differentiation, leading to later sex-dependent differences of brain function. In species with a long gestation period, these consecutive differentiation steps are performed before birth. In contrast, species with a short gestation period such as rats and mice do not complete differentiation before birth so that it extends to early postnatal life [4]. Such irreversible mechanisms of sexual differentiation have to be distinguished from phenomena without prior sexual differentiation and thus can also be released by gonadal steroid application in mature animals [5–7].

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Anabolic metabolism and pattern of protein and fat deposition are also subject to sexual dimorphism. The higher capacity of males to synthesize and deposit protein is explained both by the anabolic function of androgens and their effect directly on target tissues [8]. Additionally, androgens elevate the growth hormone (GH)–insulin-like growth factor-1 (IGF-1) system and thus stimulate GH-dependent growth in males [9,10]. Experiments with rats, however, suggest that later pulsatile GH secretion is part of sexual differentiation, imprinted by the presence of androgens during critical periods before puberty [11]. In normally developed adult male rats, such a pattern of GH is characterized by a low baseline but regularly occurring pulses with high amplitudes [12]. It was shown that the baseline is not dependent on imprinting mechanisms because it requires the presence of androgens later in life and can also be released in females after androgen substitution [13]. Occurrence and height of GH pulses, however, were found to depend on differentiation within 48 h of birth. During this period, androgen synthesizing capacity of the testis is elevated [14]. Castration at 25 d of age had no effect on pulsatile GH secretion [15].

The assumption of imprinting effects on GH in rats was further substantiated by Chowen et al. [16] who found that the number of neurons that secrete the GH-releasing hormone are more abundant in the hypothalamus of adult rats that had been castrated and androgen-substituted at the day of birth compared with rats that were castrated and not androgen treated. In addition to an increased number of neurons, their later sensitivity to steroids is improved.

In the pig, the course of androgen concentrations in blood plasma of fetal and postnatal male and female pigs is well established [2,17,18], and a postnatal transient rise about 4 wk after birth has been found regularly. This rise is also assumed to be important for later sexual and social behavior [19]. It might be that this transient rise of androgen synthesis has also an effect on the GH secretion pattern as found in the rodent studies, but this aspect has not been investigated so far in pigs.

It was the aim of this study to clarify whether the neonatal rise of testosterone has an effect on postpubertal male GH secretion pattern in the pig.

## 2. Materials and Methods

### 2.1. Study design

German Landrace pigs (sus scrofa, n = 16) were obtained from the swine herd of the University of Hohenheim. They were randomly divided into two experimental groups (each n = 8). The animals were surgically castrated at either 1 wk of age (Group 1) or 6 wk of age (Group 2). The castration at age 6 wk was performed under anesthetic and postsurgical analgesic treatment.

At age 15 wk, the pigs were brought to the experimental unit of the University of Hohenheim. Each pig was kept separately in an individual pen measuring 3 × 2.5 m and fed twice daily at 0800 h and 1500 h with a diet containing 16.7% crude protein and 13.5 MJ ME/kg. Details of the diet composition were given earlier [20]. At 25 wk of age, deuterium oxide was administered for the later determination of the total body fat content [21]. They were all slaughtered at 29 wk of age at an average weight of 138 kg, which did not differ significantly between groups. At the day of slaughter, the rate of protein synthesis was determined by infusion of [$^{13}$C]leucine as described elsewhere [22].

### 2.2. Blood sampling

For blood sampling (Weeks 17 to 29), each barrow was fitted with an indwelling cephalic vein catheter at 17 wk of age (range of body weights in Group 1, 54 to 76 kg; in Group 2, 54 to 72 kg) as described earlier [23]. The animals had completely recovered from cannulation 2 d after surgery. Blood samples were drawn daily at 0900 h in heparinized vials and stored at –20 °C after centrifugation until measuring the different parameters. After each sampling, the catheters were rinsed using sterile saline with 0.1% heparin.

The whole experiment including the late castration and cannulation had been approved by the Animal Welfare Committee of the state of Baden-Württemberg.

### 2.3. Analytical methods

#### 2.3.1. Testosterone

A specific RIA (radio immuno assay) including a solvent extraction step was used as described earlier [24] to measure testosterone in plasma samples taken every third day. Repeatability was determined by measurement of spiked samples on consecutive days (n = 6), and the interassay coefficient of variation was found to be lower than 8% for concentrations between 0.5 ng/mL and 2.5 ng/mL. The lower limit of detection was 0.05 ng/mL. The average recovery after extraction was above 95%.

#### 2.3.2. Growth hormone

Growth hormone was determined in samples taken every second day and, additionally, in window samples that were drawn every 20 min for 24 h at 19 and 24 wk of age.

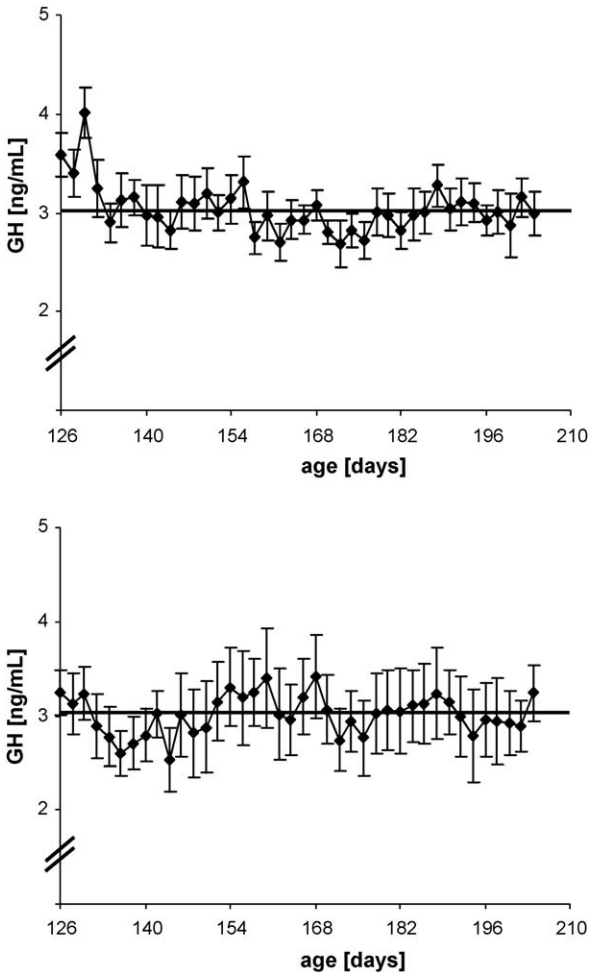


Fig. 1. Course of GH concentrations (mean + SEM) for the two groups of barrows (top: early castrated pigs; bottom: late castrated pigs). The horizontal lines represent the mean values of the two groups.

Determination was performed as described before [23]. A specific antiserum (AFP422801) was kindly provided by Dr. Parlow (NIDDK, Torrance, CA, USA), and a highly purified pGH (porcine growth hormone) standard (AFP10864P) was used for radio-iodination and calibration. The intra-assay coefficient of variation was 9.3% at a concentration of 4.2 ng/mL (n = 10), and the interassay coefficient of variation was 10% (mean: 3 ng/mL, n = 6). Recovery was above 95% for spiked samples (1.5 to 6 ng/mL). Using this procedure, we established differences in GH parameters between boars and barrows and showed the repeatability of this RIA method [22,23,25].

Each GH profile of the window samples was evaluated for pulses. The mean for all 74 samples per window for each animal was determined. A pulse was assumed when a GH concentration exceeded this mean value by at least 50% and was followed by a decrease in the GH level. The frequency was defined as the number of pulses per 24 h. The maximal level was the mean of the maximal concentrations of these pulses. The base levels were calculated by the mean of the 14 lowest values.

### 2.3.3. Insulin-like growth factor-1

Concentrations of IGF-1 were determined by a double-antibody RIA in plasma samples taken every second day after HCl/ethanol extraction using a specific antiserum raised in rabbits [9]. The highly purified IGF-1 standard was obtained from Gro Pep (CU020; Adelaide, Australia) and used for radio-iodination and for calibration. Extraction yield was between 61% and 70% for samples spiked from 75 ng/mL to 400 ng/mL (n = 9). The coefficients of variation within and between assays were 6.9% and 5.7% for concentrations of 186 ng/mL (n = 10) and 183 ng/mL (n = 9), respectively.

### 2.3.4. Urea

Urea in plasma was measured to characterize protein degradation and therefore nitrogen excretion. Determination was performed on microtiter plates as described in detail earlier [21]. Intra-assay and interassay coefficients of variation were 8.9% (n = 10) and 9.7% (n = 14) at concentrations of 322 μg/mL and 347 μg/mL, respectively.

Table 1
Summarized data for GH in window sampling at 19 and 24 wk of age.

| Group | Age (wk) | Mean level (ng/mL) | Basal level (ng/mL) | Maximum (ng/mL) | Pulses (n/24 h) |
|---|---|---|---|---|---|
| Early castrated | 19 | 3.5 ± 0.2 | 2.7 ± 0.2 | 6.6 ± 0.4 | 2.5 ± 0.5 |
| | | P = 0.08 | P < 0.05 | P < 0.05 | P = 0.08 |
| | 24 | 2.8 ± 0.1 | 2.3 ± 0.1 | 4.9 ± 0.2 | 1.3 ± 0.2 |
| Late castrated | 19 | 3.3 ± 0.2 | 2.6 ± 0.2 | 6.0 ± 0.2 | 2.3 ± 0.7 |
| | | P < 0.05 | P = 0.1 | P = 0.08 | P = 0.5 |
| | 24 | 2.5 ± 0.2 | 2.1 ± 0.1 | 4.3 ± 0.3 | 1.5 ± 0.4 |

Mean ± SEM are given. Significance refers to differences between the two windows within one group. Comparison between groups at the same age revealed no significant differences.

*2.3.5. Body fat content and protein synthesis*

Total body fat was determined by administration of a known amount of deuterium oxide via the cephalic vein catheters. After equilibration, blood samples were drawn, and deuterium oxide content was measured by infrared spectrophotometry as described by Claus et al. [21], and the fat content was calculated.

Protein synthesis was determined by infusion of [$^{13}$C]leucine via the cephalic vein catheters at the day of slaughter, and blood samples were taken over a period of 6 h. The [$^{13}$C]leucine to [$^{12}$C]leucine ratio was measured via GC-MS (gas chromatography-mass spectrometry) as described elsewhere [22].

*2.4. Statistics*

Data in the table and figures are presented as mean ± SEM of eight animals per group. Data were analyzed using the Statistical Package for the Social Sciences (SPSS version 13.0; SPSS Inc., Chicago, IL, USA). Trend analysis was performed as described in Refs. [26] and [27]. The mean of the daily samples of each animal was calculated and used for statistical analysis. Differences between or within groups were tested using unpaired or paired Student's *t*-test, respectively.

## 3. Results

Testosterone was measured in all animals and remained below the detection limit of 0.05 ng/mL between 17 and 29 wk (data not shown). There was no significant difference between Groups 1 and 2.

Growth hormone concentrations of daily samples are given in Figure 1. There was no significant increase or decrease in values (trend analysis). The mean values of 3.04 ng/mL and 3.01 ng/mL for early castrated pigs (Group 1) and late castrated pigs (Group 2), respectively, also did not differ significantly. Growth hormone values resulting from evaluation of sampling windows are summarized in Table 1, and Figure 2 shows examples for GH profiles for an early- and a late-castrated pig both at 19 and 24 wk of age. Mean values of windows in Group 2 decreased significantly from 3.3 ng/mL to 2.5 ng/mL from the first window at 19 wk of age to the second window at 24 wk of age. The mean values in Group 1 tended to be lower at 24 wk of

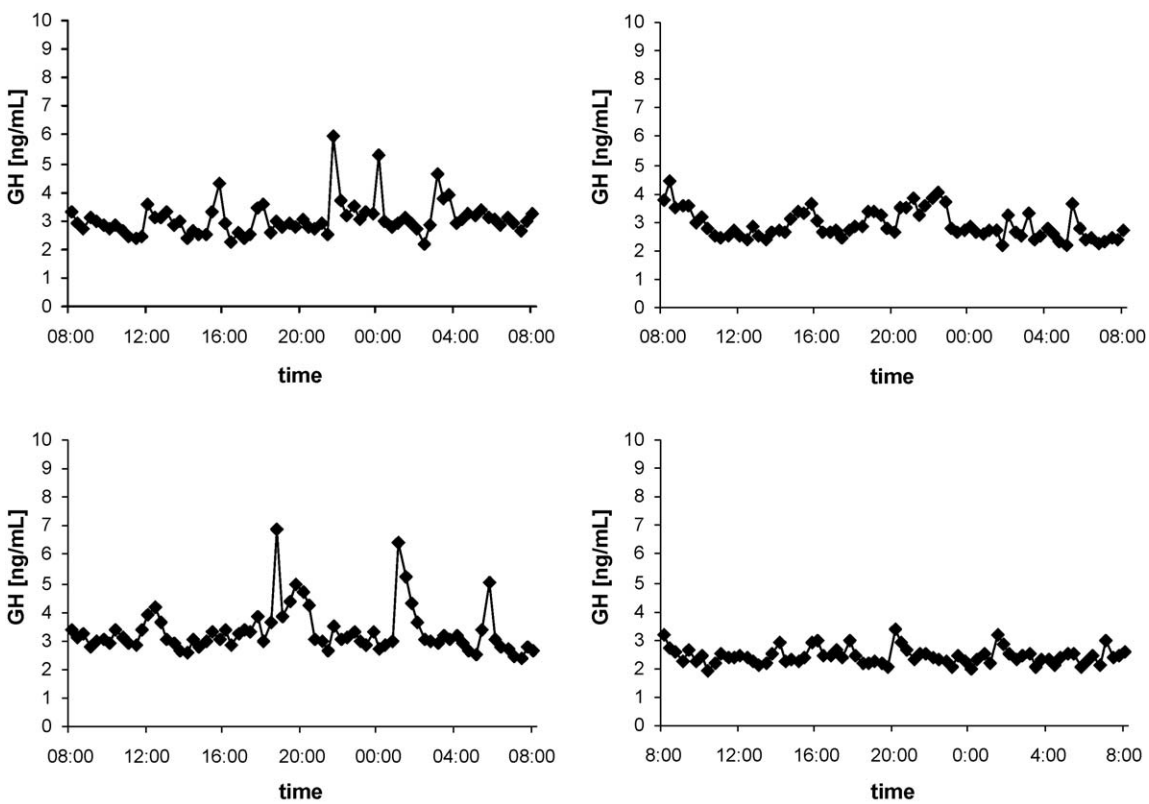


Fig. 2. Course of GH concentrations for window sampling for two individual barrows at (left) 19 wk of age and (right) 24 wk of age (top: early castrated pig; bottom: late castrated pig).

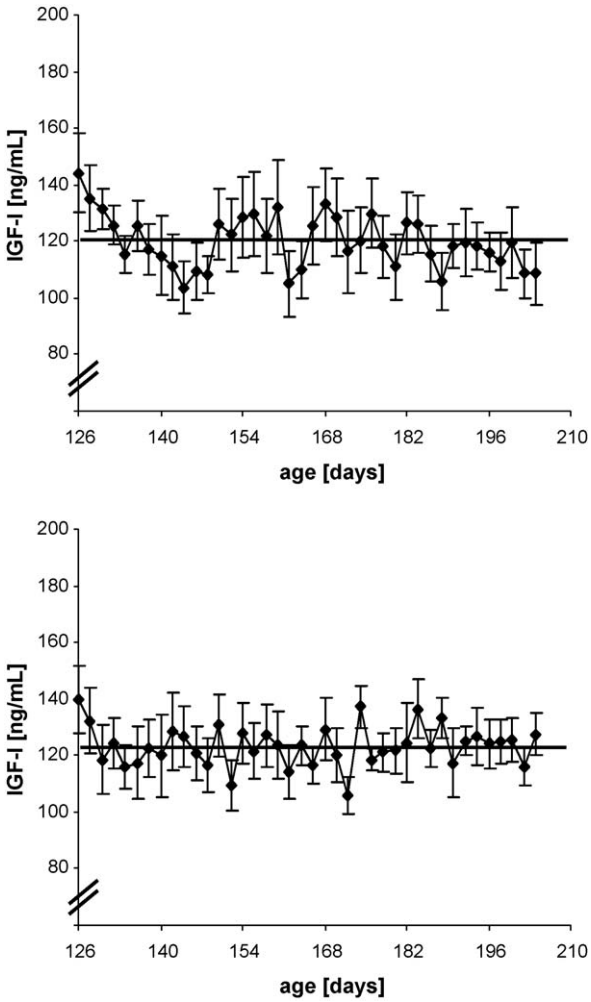


Fig. 3. Course of IGF-1 concentrations (mean ± SEM) for two groups of barrows (top: early castrated pigs; bottom: late castrated pigs). The horizontal lines represent the mean values of the two groups.

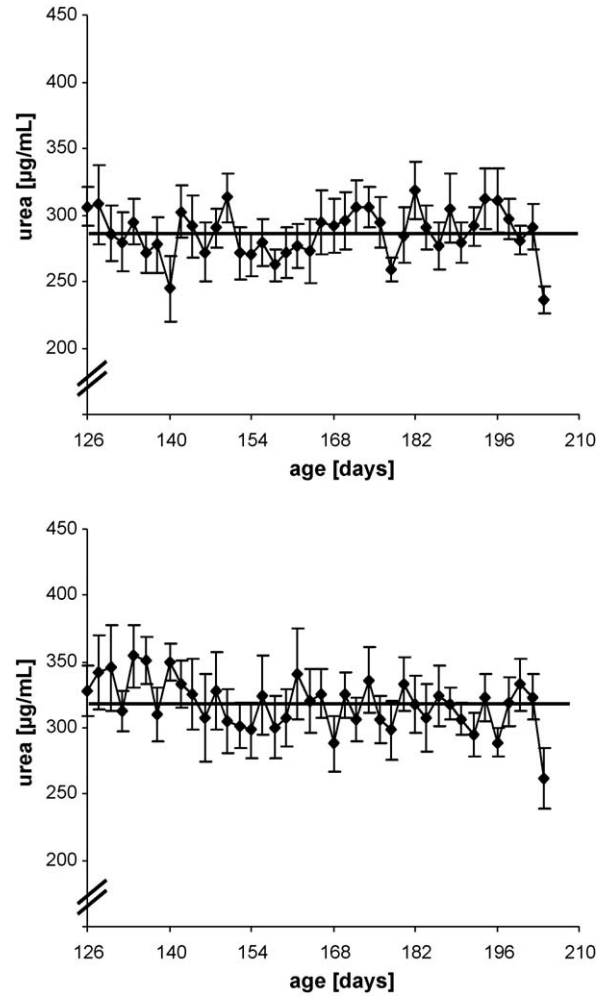


Fig. 4. Course of urea concentrations (mean ± SEM) for the two groups (top: early castrated pigs; bottom: late castrated pigs). The horizontal lines represent the mean value for the two groups. Low values at the last day are explained by the experimental design.

age (3.5 ng/mL at 19 wk of age compared with 2.8 ng/mL at 24 wk of age; P = 0.08). The basal and the maximum levels were significantly lower in Group 1 compared with the first and the second window, whereas Group 2 showed no significant differences although the values tended to decrease. Nevertheless, there was no significant difference between Group 1 and Group 2 at 19 or 24 wk of age in any evaluated GH parameter, including pulsatility.

Courses of IGF-1 are presented in Figure 3. Trend analysis revealed no increase or decrease during the experimental period. The mean levels were 120 ng/mL and 123 ng/mL for Groups 1 and 2, respectively, and did not differ significantly between groups.

Figure 4 gives the urea concentrations for the two groups. Mean levels were 287 μg/mL for Group 1 and 318 μg/mL for Group 2 and did not differ significantly between both groups. Trend analysis was also performed for each animal, but no trend was detected.

Mean total body fat contents at 25 wk of age were 20.1 ± 5.8% and 25.4 ± 7.7% for pigs of Groups 1 and 2, respectively, and showed no significant differences.

Protein synthesis at the day of slaughter revealed values of $5.44 \pm 0.32\ g\,kg^{-1}\,d^{-1}$ and $6.22 \pm 0.44\ g\,kg^{-1}\,d^{-1}$ for Groups 1 and 2, respectively, which was statistically significant.

## 4. Discussion

The GH–IGF-1 system is the main regulator of anabolic metabolism and growth. It also involves insulin, which stimulates the expression of GH receptors in the liver and thus couples IGF-1 secretion

to GH [10,28]. This system is also stimulated by gonadal steroids. Whereas there is an evident sexual dimorphism in secretion of GH in rats [29], the situation in pigs comparing males and females is less obvious [30]. Nevertheless, in sows it was shown that the estrous cycle–dependent course of estradiol in blood is followed by concordant changes of GH and IGF-1 [9]. No differences in IGF-1 were found when comparing intact and ovariectomized gilts [31]. In boars, testicular steroid secretion is not only characterized by androgens but also by estrogens, which may even exceed concentrations in the follicular phase of the estrous cycle in sows [32]. Thus, the GH–IGF–1 system is set to a higher level compared with that in females and thus explains sexual dimorphism. An additional role of androgens is their anticatabolic effect, which is specifically exerted via receptors in muscle tissue [33,34]. In other species (sheep), a testosterone implant failed to alter GH values in early castrated rams but increased IGF-1 [35].

Because of the practice of castrating male piglets during the first week of life to avoid later occurrence of the urine-like ''boar taint'' in carcasses, the anabolic effects of gonadal steroids are abolished and the barrows are inferior in their fattening performance and carcass composition compared with entire boars [36,37]. Therefore, the mechanisms that differentiate a male pattern of GH, as it was shown for rats, might be of high practical relevance when combined with delayed castration (e.g., by immunologic techniques) [21,38]. Our data clearly demonstrated that such a mechanism does not exist in the pig. This finding is substantiated by Trudeau et al. [39] who revealed that late castration (6 wk of age) did not alter GH values significantly.

Interestingly, early castrated pigs showed a lower protein synthesis compared with that of late castrated pigs and in parallel a tendency to lower urea excretion and therefore protein degradation in early castrated pigs. In consequence, the protein gain for both groups could be the same. The reason for these findings is unknown. Additionally, it is likely that intensive care of the pigs (and thus the avoidance of any stress) during the leucine infusion period was the reason for decreased urea concentrations. But again, such influences need confirmation. The transient postnatal rise of testicular activity is related to ongoing testicular development after sexual differentiation early in embryonic life. Development of pig testes from birth to adulthood is characterized by two additional waves of development. One of them is the early postnatal rise of gonadotropins and testicular steroids around Week 4. These hormones are also essential to establish Sertoli cells and thus determine the amount of mature sperm output [40–43]. The second postnatal rise occurs around Week 16 and is usually referred to as the ''final pubertal spurt.''

In addition, the early postnatal rise of steroidogenesis in the male pig was suggested to be important for imprinting later sexual behavior because this rise is accompanied by mounting attempts of piglets kept in groups and is supposed to be essential for later normal sexual behavior in mature boars [44]. Such a mechanism, however, has to be regarded as a process of learning instead of differentiation. It was also demonstrated that differentiation of hypothalamic areas does not play a role, because male piglets that had been surgically castrated a few days after birth later exhibited male mounting behavior when treated with androgens and exhibited female standing reflex after estradiol application [45,46].

It is concluded that the postnatal rise of steroidogenesis in the male pig does not play a role for a male GH secretion pattern.

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# 3 General Discussion

Immunized boars have a better fattening performance that results in a higher daily gain and thus a faster growth (Dunshea et al., 2001). This effect or parts of it have been confirmed in several studies (Jaros et al., 2005; Zamaratskaia et al., 2008a; Andrews et al., 2009; Fuchs et al., 2009; Hemonic et al., 2009; Pauly et al., 2009; Schmoll et al., 2009; Spring et al., 2009). The superiority of immunized boars over barrows may be explained by the late onset of immunization (Turkstra et al., 2002), why immunized boars have the anabolic potential typical for intact boars for a longer time in the fattening period (Metz and Claus, 2003). Therefore, the exact time course of titre development and the reaction of hormones and metabolic parameters are of great importance to optimize the fattening performance of immunized boars. This determination of reactions before and after the second vaccination was possible in our studies. Pigs had indwelling vein catheters which allowed daily sampling of blood samples.

Active immunization against GnRH has a clear effect on the reproductive axis and therefore on metabolism. Data from our study (see 2.1) once more confirm the effectiveness of immunization with Improvac®. According to manufacturer´s recommendations, pigs were immunized twice, 4 weeks apart, and the second vaccination was given 6 weeks prior to slaughter. In our study, the first vaccination revealed neither an effect on titre development nor on reproductive hormones. Titres rose within 5 days after the second vaccination. All animals had high enough titres to release the castration effect. This was confirmed by LH and testosterone concentrations which decreased within one week. Androstenone concentrations in plasma decreased the same way and reached basal values of 0.35 ng/mL within 4-8 days.

The clearance of androstenone from adipose tissue in slaughter weight boars requires approximately 3-4 weeks (Claus, 1976; Claus, 1979). The rate of clearance depends on age, e.g. in boars with 240 kg body weight it required 6 weeks to decrease androstenone values below the threshold of 0.5 ng/g fat. Currently, the recommendation for the timing of the second vaccination is 4-6 weeks before slaughter. But to achieve an optimal fattening performance, the immunization schedule should be adapted to the aimed slaughter weight. Figure 11 shows the weight development of the immunized boars in our studies. The dashed lines mark 3 different slaughter weights: 80 kg, 100 kg, and 120 kg. For a slaughter weight of 80 kg which was reached with an age of 21 weeks, the second vaccination may be 2 weeks earlier. For 120 kg slaughter weight, the second vaccination may be in an age of 24-25 weeks, i.e. 3-4 weeks prior to slaughter. In our studies, only a limited number of pigs and only German Landrace boars were tested. Thus, further studies with a higher animal number and different breeds are necessary to confirm the timing of the second vaccination before

slaughter. A delay in time of slaughter because of different growth rates between the individual pigs does not compromise the immunization effect. The suppression of testicular biosynthesis lasted at least 10 weeks after the second immunization (Claus et al., 2008; Zamaratskaia et al., 2008b).

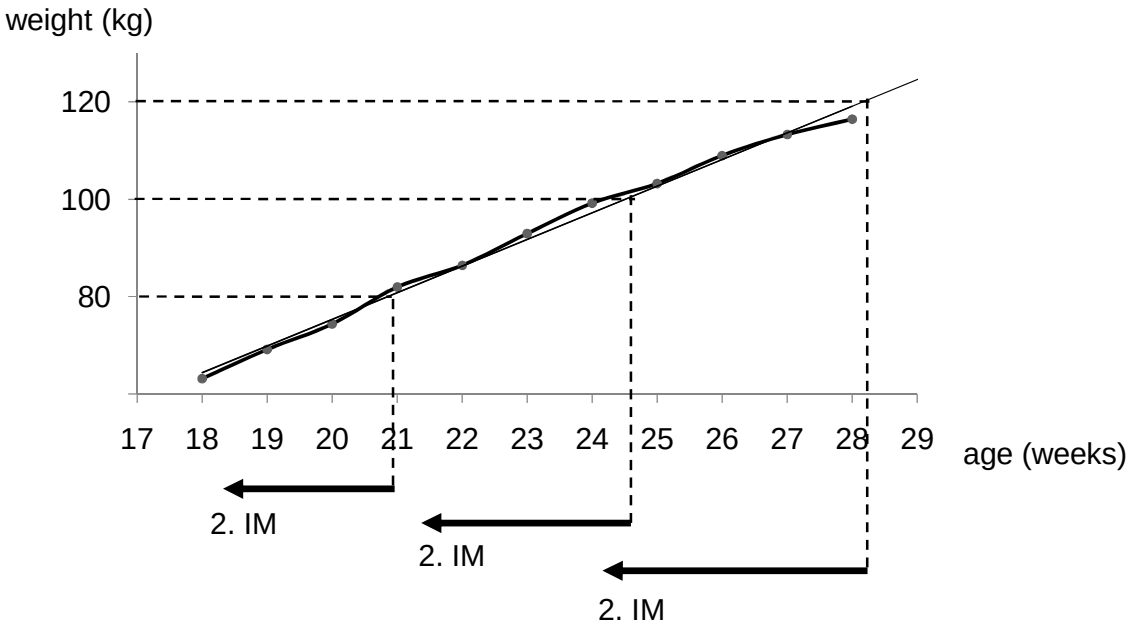


Figure 11: Weight development of immunized boars between 18 and 28 weeks of age (dashed lines mark possible slaughter weights and the corresponding age; arrows mark second vaccination relative to slaughter, 2. IM)

Another cause for the better fattening performance of immunized boars may be the high GH concentrations similar to concentrations in intact boars (Metz and Claus, 2003). In the present study, the maintenance of high levels of GH in the immunized boars could be additionally confirmed. One mechanism for the elevated GH levels in immunized boars may be an interaction between the releasing hormones GnRH and GH-RH. Infusion of GnRH led to an increase of LH and a concomitant decrease of GH, whereas infusion of GH-RH led to high GH and low LH concentrations (Claus and Weiler, 1994; Weiler, 1995). We investigated this hypothesis by immunizing barrows (see 2.2). It is known that barrows have high concentrations of GnRH because of a lack of negative feedback from the testes. If there is a relation between the two releasing hormones in the hypothalamus or the pituitary, GH-RH should rise after the immunization because GnRH is blocked. In consequence, GH levels should also increase thereafter.

The effect of immunization was evident in immunized barrows. Antibody titres rose, and LH concentrations dropped to levels near the detection limit. But GH concentrations were not influenced by immunization in barrows. Both groups showed no differences in GH or in IGF-I

concentrations. As expected, metabolic parameters did not differ, too. These results do not exclude a direct interaction between GH-RH and GnRH in the hypothalamus of barrows which however could not be abolished by immunization, probably because GnRH antibodies are big molecules which did not reach the hypothalamus so that their effect on GnRH is restricted to their interaction in blood of the portal veins.

Another explanation for the high GH levels in immunized boars may be an imprinting mechanism for a male specific GH secretion pattern. In male rats, the GH secretion is imprinted by a rise of steroids early after birth (Jansson et al., 1985). In pigs, such a rise of steroids occurs at about 4 weeks of age (Schwarzenberger et al., 1993). This rise cannot occur in surgically castrated pigs, because castration is routinely performed in the first week of life. Thus, we compared male pigs which were castrated either at one week of age (before the rise of testosterone) or at six weeks of age (after the rise of testosterone). But the early rise of steroids had no effect on GH concentrations (see 2.4). Nevertheless, a male pattern of GH secretion may exist in the pig. It may be dependent on a transient rise of testicular steroid synthesis which includes formation of high amounts of oestrogens which were shown to stimulate GH formation in mature pigs (Claus et al., 1992). In addition, it was found that a transient rise of testicular activity already occurs in the foetal period (Ford et al., 1980).

The effect of GH on protein synthesis is mediated via IGF-I which is synthesized and released from the liver (Claus and Weiler, 1994). Thus, high GH concentrations are only effective for protein synthesis if they are coupled with IGF-I. This GH-IGF-I system is further influenced by oestrogens and energy availability. Oestrogens (Claus et al., 1992) and carbohydrates with a high glycaemic index such as starch (Brameld et al., 1999) lead to a rise of IGF-I concentrations by influencing the GH receptor expression in the liver (Gabrielsson et al., 1995).
Immunized boars had high GH concentrations, but IGF-I concentrations were lower than in intact boars (Metz and Claus, 2003). Immunization against GnRH blocks the synthesis of testicular steroids, and the positive effect of oestrogens on IGF-I is no longer evident. Consequently, the only influence on IGF-I after the second vacciantion can be via energy supply through feed allocation. In one study (see 2.3), we investigated the effect of energy supply through the diet on IGF-I and on metabolic parameters. Boars were immunized twice according to recommendations. The expected maintenance of high GH concentrations and the loss of anabolic steroids after the second vaccination were confirmed.
Our results show that pigs fed 3 kg of feed per day had 28% higher IGF-I concentrations in plasma after the second vaccination compared with pigs fed only 2 kg feed per day. Protein synthesis was significantly higher in the 3 kg group of immunized boars, whereas protein

breakdown did not differ between groups. In consequence, daily weight gain was higher in the 3 kg group. After the second vaccination, the 3 kg group left no feed residues. Thus, an elevation of feed consumption may be possible. This may result in a further improvement of protein synthesis without excessive fat accumulation. Determination of body fat content in the 3 kg group of immunized boars revealed that the fat content is not increased after second vaccination (13.7% vs 15.0%). Barrows had fat contents of 22% at the same age and under the same conditions (see 2.2).

The cause of the high GH concentrations in immunized boars could not be identified in our studies. But results allow an optimal timing of the two vaccinations to get a good performance of immunized boars without the risk of tainted carcasses at slaughter. In addition, the influence of feed supply on IGF-I may be another mechanism to optimize the fattening performance of immunized boars.

Thus, active immunization against GnRH with Improvac® is a practical and reliable alternative to surgical castration that provides benefits in growth performance and carcass quality.

# 4 Summary

Compared to surgical castrates, boars have a superior anabolic potential due to gonadal steroids, i.e. androgens and oestrogens. In consequence, they have an improved fattening performance and lean fat ratio in the carcass. However, most male piglets are surgically castrated without anaesthesia within the first week of life to avoid the unpleasant urine-like boar taint which is not acceptable to many consumers. Boar taint is mainly caused by androstenone which is synthesized in the testes together with the gonadal steroids. Skatole is another compound which contributes to the off odour. But this substance has a faecal smell and can be controlled by feeding strategies.

Castration without anaesthesia is now regarded to cause acute pain and stress to the piglets, so that surgical castration is no longer tolerated due to animal welfare considerations. Different alternatives are discussed but it appears that active immunization against the gonadotropin releasing hormone (GnRH) is the most practicable alternative to surgical castration. It is based on application of a commercial antigen (Improvac®) two times at an interval of 4 weeks. The second vaccination then leads to a high antibody formation and to a blockade of GnRH and thus luteinizing hormone (LH). In turn, the testicular biosynthesis of anabolic hormones as well as androstenone is inhibited. Several studies found that immunized boars still had a better performance than surgical castrates. One explanation is that immunized boars maintain part of their anabolic potential before the second vaccination and thus the onset of antibody formation. Therefore, an exact time schedule for immunization is required to optimize the fattening period without risk of tainted carcasses at slaughter. Another explanation may be that high growth hormone (GH) concentrations are maintained in immunized boars at a boar specific level. In addition to low GH, barrows also have low concentrations of the anabolic insulin-like growth factor I (IGF-I).

The present four studies were performed to investigate the hormonal and metabolic reactions before and after the second vaccination and to clarify why high GH concentrations are maintained. In the first study five catheterized boars were immunized twice with Improvac® at 18 and 22 weeks of age. Antibody titres rose rapidly after the second vaccination and LH and consequently testosterone concentrations decreased within 5 days. Basal values of androstenone were reached within 8 day. Ten days after the second vaccination, IGF-I and urea, which represents protein turnover, had dropped down to levels which are typical for surgical castrates (barrows). Results from this study confirm that two doses of Improvac® are effective in inhibiting gonadal steroid production and thus boar taint after the second

vaccination. Studies on maintenance of GH or IGF-I were based on three different hypotheses.

**Hypothesis 1:** An interaction between GnRH and growth hormone releasing hormone (GH-RH) in the hypothalamus is known from literature. The drop of GnRH due to immunization might abolish an inhibiting effect on GH-RH and maintenance of GH high concentrations in immunized boars. In contrast, barrows have high GnRH and LH concentrations due to the absence of negative feedback by gonadal hormones and thus low GH levels.
Twelve barrows were fitted with an indwelling vein catheter. Six of these barrows were immunized with Improvac® at 18 and 22 weeks of age. GH, LH, FSH, testosterone and IGF-I were determined as well as specific parameters to characterize protein synthesis and degradation, and body fat content. The second vaccination led to high antibody titres and low levels of LH and testosterone while FSH decreased slowly within 5 weeks to 0.5 ng/mL. GH as well as IGF-I was not influenced and remained at low concentrations typical for barrows. Thus, the metabolic status was not altered by immunization and the hypothesis of an interaction between GnRH and GH-RH could not be substantiated for the pig, probably because an interaction occurred exclusively in blood of the hypophyseal portal veins but not in the hypothalamus.

**Hypothesis 2:** IGF-I is not only stimulated by GH but also by increasing amounts of starch in the feed. In immunized boars, feed intake is increased due to the absence of gonadal steroids. Two groups of six immunized boars were given 2 or 3 kg of feed per day. Before the second vaccination, testicular steroids in blood were normal but decreased rapidly thereafter. GH concentrations were maintained at high levels after the second immunization. Compared with the 2 kg group, the 3 kg group showed significant higher IGF-I concentrations, increased weight gain and increased protein synthesis after the second vaccination. Protein breakdown did not differ between groups. The body fat content in the 3kg group remained the same before and after the second vaccination (14% vs. 15%). The results indicate that performance of immunized boars can be further improved by increasing amount of feed above current recommendations.

**Hypothesis 3:** Rodent studies showed that a transient rise of testosterone synthesis about 2 d after birth imprints a later male specific increased secretion of GH. In the male pig, a transient elevation of testosterone occurs about 4 weeks postnatal. Therefore, a group of eight male piglets was surgically castrated at 1 week of age, another group of eight male piglets at 6 weeks of age. All pigs were fitted with catheters at 17 weeks of age. Daily blood

samples were analyzed for testosterone, GH, IGF-I and urea. To determine GH pulsatility blood was drawn for 24 h every 20 min.
Comparison of the two groups revealed no differences in mean GH concentrations and their pulsatile secretion pattern. As expected the other metabolic parameter did not differ as well. It is concluded that the postnatal rise of steroid synthesis in boars is not responsible for the later pattern of GH secretion in pigs and thus is a rodent specific mechanism.

The cause for elevated GH levels in immunized boars could not be identified in the present studies. Nevertheless, results allow an exact timing of the vaccinations and thus an optimal use of the anabolic potential of immunized boars without the risk of tainted carcasses at slaughter. A further improvement of the fattening performance may be realized by feeding strategies and their influence on IGF-I.

# 5 Zusammenfassung

## Auswirkungen der immunologischen Kastration auf die Stoffwechselregulation beim Eber

Eber haben im Vergleich zu Kastraten ein höheres anaboles Potential, das durch die Gonadensteroide (Androgene und Östrogene) bedingt ist. Deshalb weisen sie eine verbesserte Mastleistung und ein günstigeres Fleisch-Fett-Verhältnis im Schlachtkörper auf. Die meisten männlichen Ferkel werden dennoch in der ersten Lebenswoche chirurgisch kastriert, um den urinartigen Ebergeruch zu vermeiden, der von vielen Verbrauchern nicht akzeptiert wird. Ebergeruch wird hauptsächlich durch Androstenon verursacht, das zusammen mit den Gonadensteroiden im Hoden gebildet wird. Skatol ist ein weiterer Bestandteil, der zum Fehlgeruch von Eberfleisch beiträgt. Skatol hat einen fäkalartigen Geruch und kann aber durch Fütterungsstrategien kontrolliert werden.

Die chirurgische Kastration ohne Betäubung wird mittlerweile als schmerzhaft und belastend für die Ferkel eingeschätzt. Deshalb wird sie aufgrund des Tierschutzes nicht länger toleriert. Verschiedene Alternativen werden diskutiert, wobei die aktive Immunisierung gegen Gonadotropin Releasing Hormone (GnRH) die praktikabelste Alternative zu sein scheint. Die Immunisierung basiert auf der Anwendung eines kommerziellen Antigens (Improvac®), das zweimal im Abstand von 4 Wochen verabreicht wird. Die 2. Impfung löst eine hohe Antikörperbildung aus und führt damit zur Blockade von GnRH und LH. Dies wiederum führt zur Hemmung der testikulären Biosynthese, d.h. die anabolen Hormone wie auch Androstenon werden inhibiert. Einigen Studien zufolge weisen immunisierte Eber eine immer noch bessere Mastleistung als chirurgische Kastraten (Börge) auf. Eine Erklärung dafür ist, dass immunisierte Eber einen Teil ihres anabolen Potentials vor der 2. Impfung, und damit vor der Antikörperbildung, erhalten. Deshalb ist ein exakter Zeitplan für die Anwendung des Impfstoffes nötig, um einerseits die Mastperiode optimal zu gestalten, aber andererseits keine geruchsbelasteten Schlachtkörper zu riskieren. Eine weitere Erklärung könnten die hohen Wachstumshormonkonzentrationen (GH) sein, die immunisierte Eber auf einem für den Eber typischen Niveau aufrechterhalten. Zusätzlich zu niedrigen GH-Konzentrationen, weisen Börge auch niedrigere Konzentrationen des anabol wirksamen IGF-I auf.

Die vorliegenden vier Studien wurden durchgeführt, um Hormon- und Stoffwechselreaktionen vor und nach der 2. Impfung zu untersuchen. Weiter sollte geklärt werden, warum hohe GH-Konzentrationen aufrecht erhalten werden. In der ersten Studie wurden 5 Eber mit einem Katheter versehen und zweimal im Alter von 18 und 22 Wochen mit Improvac® geimpft. Der

Antikörpertiter stieg nach der 2. Impfung rasch an. Die LH- und folglich auch die Testosteronkonzentrationen nahmen innerhalb von 5 Tagen ab. Androstenon erreichte innerhalb von 8 Tagen Basiswerte. 10 Tage nach der 2. Impfung waren IGF-I und Harnstoff, der den Proteinumsatz darstellt, auf ein Niveau abgefallen, das für Börge charakteristisch ist. Die Ergebnisse dieser Studie bestätigen, dass 2 Dosierungen von Improvac® die Produktion der Gonadensteroide effektiv hemmen und daher auch der Ebergeruch nach der 2. Impfung unterdrückt wird. Die Studien zur Aufrechterhaltung von GH basieren auf drei unterschiedlichen Hypothesen.

**Hypothese 1:** Eine Wechselwirkung im Hypothalamus zwischen GnRH und Growth Hormone-Releasing Hormone (GH-RH) ist aus der Literatur bekannt. Der Abfall von GnRH aufgrund der Immunisierung sollte den Hemmeffekt auf GH-RH beseitigen und somit die hohen GH-Konzentrationen bei immunisierten Ebern erhalten. Im Gegensatz dazu haben Börge aufgrund des fehlenden negativen Feedbacks der Gonadenhormone hohe Konzentrationen von GnRH und von LH, was damit zu geringen GH Mengen führt.
12 Börgen wurde ein Venenkatheter verlegt. Sechs dieser Börge wurden im Alter von 18 und 22 Wochen mit Improvac® geimpft. GH, LH, FSH, Testosteron und IGF-I wurden bestimmt, weiter der Körperfettgehalt und spezifische Parameter, um die Proteinsynthese und den Proteinabbau zu charakterisieren. Die 2. Impfung führte zu hohen Antikörpertitern und niedrigen Werten von LH und Testosteron, während FSH langsam innerhalb von 5 Wochen auf 0,5 ng/mL abnahm. GH wie auch IGF-I wurden nicht beeinflusst und behielten niedrige Konzentrationen bei, die für Börge typisch sind. Demzufolge wurde der Stoffwechselstatus durch die Immunisierung nicht verändert und die Hypothese einer Wechselwirkung zwischen GnRH und GH-RH konnte für das Schwein nicht bewiesen werden. Vermutlich weil eine Wechselwirkung zwischen den Antikörpern und GnRH nur in Blut der Portalgefäße, nicht aber im Hypothalamus selbst statt findet.

**Hypothese 2:** IGF-I wird nicht nur durch GH stimuliert, sondern auch durch zunehmende Mengen an Stärke im Futter beeinflusst. Immunisierte Eber weisen aufgrund der fehlenden Gonadensteroide eine höhere Futteraufnahme auf. Zwei Gruppen mit je 6 immunisierten Ebern erhielten 2 bzw. 3 kg Futter pro Tag. Vor der 2. Impfung waren die Hodenhormonewerte im Blut normal, nahmen danach aber schnell ab. Die GH-Konzentrationen verblieben nach der 2. Impfung auf ihrem hohen Niveau. Im Vergleich mit der 2 kg-Gruppe wies die 3 kg-Gruppe signifikant höhere IGF-I-Konzentrationen, ein gesteigertes Wachstum und eine erhöhte Proteinsynthese auf. Der Köperfettgehalt der 3 kg-Gruppe war vor und nach der 2. Impfung gleich (14% vs.15%). Die Ergebnisse deuten an,

dass die Mastleistung der immunisierten Eber weiter verbessert werden kann, indem die Futtermenge weiter, über die gebräuchlichen Empfehlungen hinaus, erhöht wird.

**Hypothese 3:** Studien mit Nagetieren zeigten, dass ein vorübergehender Anstieg von Testosteron 2 Tage nach der Geburt die später männlich spezifische GH-Sekretion prägt. Beim männlichen Schwein kommt es ungefähr 4 Wochen postnatal zu einer vorübergehenden Erhöhung der Testosteronwerte. Deshalb wurden 8 Ferkel chirurgisch im Alter von einer Woche kastriert, weitere 8 Ferkel im Alter von 6 Wochen. Alle Schweine wurden mit 17 Wochen mit einem Katheter versehen. In den täglichen Blutproben wurden Testosteron, GH, IGF-I und Harnstoff analysiert. Um die Pulsatilität von GH bestimmen zu können, wurde für 24 h alle 20 min Blutproben genommen. Der Vergleich der 2 Gruppen ergab keine Unterschiede zwischen den mittleren GH-Konzentrationen und ihrem pulsatilen Sekretionsmuster. Wie erwartet unterschieden sich die stoffwechselrelevanten Parameter auch nicht. Folglich ist der postnatale Anstieg der Steroidsynthese beim Eber nicht für das spätere Sekretionsmuster von GH beim Schwein verantwortlich. Damit kommt dieser Mechanismus nur bei Nagetieren zum Tragen.

Der Grund für die hohen GH-Konzentrationen bei immunisierten Ebern konnte in den vorliegenden Studien nicht herausgefunden werden. Dennoch erlauben die Ergebnisse eine exakte zeitliche Einordnung der Impfungen in die Mastperiode, wodurch das anabole Potential der immunisierten Eber optimal genutzt werden kann, ohne geruchsbelastete Schlachtkörper zu riskieren. Eine weitere Verbesserung der Mastleistung könnte durch Fütterungsstrategien und deren Einfluss auf IGF-I erreicht werden.

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# 7 Curriculum Vitae

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| | |
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**Erklärung**

Hiermit erkläre ich, dass ich die vorliegende Dissertation selbstständig angefertigt habe. Es wurden nur die angegebenen Quellen und Hilfsmittel benutzt, wörtlich oder inhaltlich übernommene Stellen wurden als solche gekennzeichnet.

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