RAISING ENTIRE MALES FOR PORK PRODUCTION: IMPACT OF ANDROSTENONE ON BEHAVIOUR AND MEAT QUALITY

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ABSTRACT

RAISING ENTIRE MALES FOR PORK PRODUCTION: IMPACT OF ANDROSTENONE ON BEHAVIOUR AND MEAT QUALITY

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Castration of male piglets reduces aggressive and sexual behaviours and diminishes boar taint, a meat quality issue caused by the hormone androstenone. This research investigated the ability to predict fat and plasma androstenone development using early plasma androstenone levels and compared behaviour and meat quality for barrows and high and low androstenone boars. Plasma androstenone at 21 days of age was associated with fat androstenone at slaughter in pigs >120 kg at slaughter. Aggression and stress-related behaviours were not notably different among all groups. Mounting was greater in all boars versus barrows. Meat quality for high and low androstenone boars only differed in terms of marbling, low androstenone boars being intermediate to high androstenone boars and barrows. Overall, raising boars appears to be a viable option, but mounting may be an issue and meat products from boars may be different than what consumers currently expect from gilt- and barrow-only production.

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3β-HSD – 3β-hydroxysteroid dehydrogenase

5α-R – 5α-reductase

ADG – Average daily gain

CPC – Canadian Pork Council

DFD – Dry, firm, dark

DHEA – Dehydroepiandrosterone

E1S – Estrone sulfate

ELISA – Enzyme-linked immunosorbent assay

FCR – Feed conversion ratio

FSH – Follicle stimulating hormone

GnRH – Gonadotropin releasing hormone

HAT – Human approach test

HCW – Hot carcass weight

HPA – Hypothalamic-pituitary-adrenal

HPG – Hypothalamic-pituitary-gonadal

HPLC – High performance liquid chromatography

LH – Luteinizing hormone

LM – Longissimus muscle

NFACC – National Farm Animal Care Committee

NOT – Novel object test

NPPC – National Pork Producers Council

OFT – Open field test

POR – Cytochrome P450 reductase

PSE – Pale, soft, exudative

RIA – Radioimmunoassay

RIT – Resident intruder test

SULT – Sulfotransferase

UGT – Glucuronosyl transferase

CHAPTER 1 LITERATURE REVIEW

1.1 General Introduction

Male piglet castration, removal of the testes, is a surgical procedure that is typically performed in the first few days after birth. Castration reduces the incidence of aggressive and sexual behaviours typically demonstrated by intact male pigs (Zamaratskaia & Rasmussen, 2015). More importantly, castration is performed to remove the risk of boar taint in pork. Boar taint is a meat quality issue characterized by an unpleasant odour or flavour when the meat is cooked and is due to at least two compounds that accumulate in the fat of pigs (Patterson, 1968; Vold, 1970; Bonneau, 1998). Boar taint is reported to occur in anywhere between 10-75% of entire male pigs, depending on numerous factors such as breed, age, and management practices (Thun et al., 2006; Backus et al., 2016). One of the contributing compounds, androstenone, is a steroid hormone produced in the Leydig cells of the testis; thus, castration removes its production (Patterson, 1968). The second contributing compound, skatole, is produced in the hindgut of the pig through microbial digestion of the amino acid tryptophan; therefore, skatole levels in tissues and plasma may be controlled through manipulation of diet and management practices (Vold 1970; Claus et al., 1994; Zamaratskaia, 2004).

Raising intact male pigs has many benefits, including improved feed conversion and leaner meat, leading to overall increased sustainability (EFSA, 2004). However, eliminating castration and raising intact boars brings about a different welfare issue, as boars typically display more aggressive and sexual behaviours, such as mounting, than

castrates or females (Giersing et al., 2000). Eliminating castration to raise entire males for pork production may require changes in management practices to reduce these undesirable behaviours.

The purpose of this literature review is to provide an overview of issues relating to boar taint and raising boars, as well as summarize current and potential strategies to improve the welfare of pigs by eliminating surgical castration.

1.2 Boar Taint

When meat from uncastrated male pigs (boars) is cooked, the heated fat may produce a foul-smelling odour and flavour. This is attributed to a mixture of compounds collectively known as boar taint; this is primarily due to accumulation of androstenone (5α-androst-16-ene-3-one) and skatole (3-methylindole) (Patterson, 1968; Vold, 1970). The amount of boar taint that accumulates in the fat depends on the biosynthesis, metabolism and excretion of skatole and androstenone in the pig, leading to great variability among individuals and breeds. Boar taint levels can be influenced by many factors including sex, age, weight (Walstra et al., 1999), breed (Zamaratskaia, 2004), diet (Bilić-Šobot et al., 2014), gene expression (Zamaratskaia & Squires, 2009), stress levels (Wesoly et al., 2015), season and light regimes (Pruner et al., 2013), housing and management practices (Walstra et al., 1999; Wesoly et al., 2015), and testicular steroid hormone concentrations (Zamaratskaia & Squires, 2009; Bilić-Šobot et al., 2014).

1.2.1 Role of androstenone

Androstenone (5α-androst-16-ene-3-one) is a steroid produced primarily by the Leydig cells in the testis of pigs, and functions as a sexual pheromone released in the saliva of boars (Ahmad & Gower, 1968; Patterson 1968; Gower, 1972). Androstenone production is regulated by the neuroendocrine system and is stimulated by activation of the hypothalamic-pituitary-gonadal (HPG) axis (Figure 1.1; Gower, 1972; Bonneau & Terqui, 1983). The activation of the hormone cascade begins around 2-4 weeks of age, peaking at 3 weeks, then returning to low levels until the boar reaches sexual maturity around 14-15 weeks of age (Schwarzenberger et al., 1993; Sinclair et al., 2001). This early spike of plasma hormones observed at 2-4 weeks of age correlates with high testicular steroid levels, increase in Leydig cell numbers and changes in luteinizing hormone (LH) receptors at this time, suggesting a maturation of the gonadal feedback system to the hypothalamus (Colenbrander et al., 1978; Sinclair et al., 2001). This early rise in steroidogenesis also influences later development and behaviour, although blocking the increase in androstenone levels at 2-4 weeks of age does not influence boar taint levels later in life (Ford, 1990; Sinclair 2000).

The initiating hormone for the HPG axis, gonadotropin releasing hormone (GnRH), is produced in the hypothalamus and transported to the pituitary gland, where it binds receptors that activate the release of luteinizing hormone (LH) and follicle stimulating hormone (FSH; Claus et al., 1994). These two hormones act on both male and female reproductive systems, stimulating production of sex hormones (estrogen and testosterone), and spermatogenesis in males.

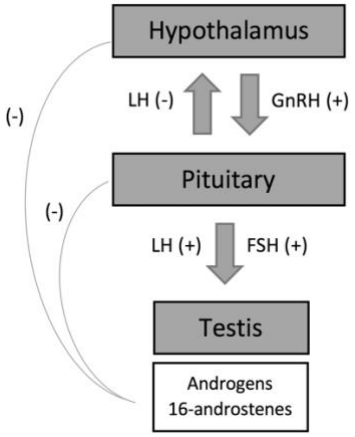

Figure 1.1: Hypothalamic-pituitary-testicular axis. Luteinizing hormone, LH; gonadotropin releasing hormone, GnRH; follicle stimulating hormone, FSH.

Synthesis of steroid hormones, including androstenone (Figure 1.2), is primarily regulated by the cytochrome P450 (CYP) enzyme family. An isoform of these enzymes catalyzes the initial step in synthesis of many steroids, which involves the cleavage of a 6-carbon side chain from cholesterol (Payne & Youngblood, 1995). The resulting molecule is pregnenolone, which can be converted to progesterone through the enzyme 3β-hydroxysteroid dehydrogenase (3β-HSD). The conversion of pregnenolone is a key step in determining whether androgens or 16-androstene steroids will be synthesized. The synthesis of androstadienol (a 16-androstene steroid) is catalyzed by CYP17A1 with CYB5A acting as an electron shuttle with cytochrome P450 reductase (POR). CYP17A1 also catalyzes the conversion of pregnenolone to dehydroepiandrosterone (DHEA) (an androgen), again mediated by CYB5A (Yamazaki et al., 1998). This branching point of androgen or 16-androstene steroid conversion is highly dependent on levels of CYB5A; where CYB5A is limited, androstadienol is significantly reduced and androgen production is promoted (Billen & Squires, 2009). Progesterone is converted to androstadienone

through CYP17A and CYB5A, whereas androstadienol is converted through 3β-HSD (Meadus et al., 1993; Billen & Squires, 2009). The final step involves the conversion of androstadienone to androstenone through 5α-reductase (5α-R), creating the final 19-carbon androstenone molecule (Bonneau & Terqui, 1983).

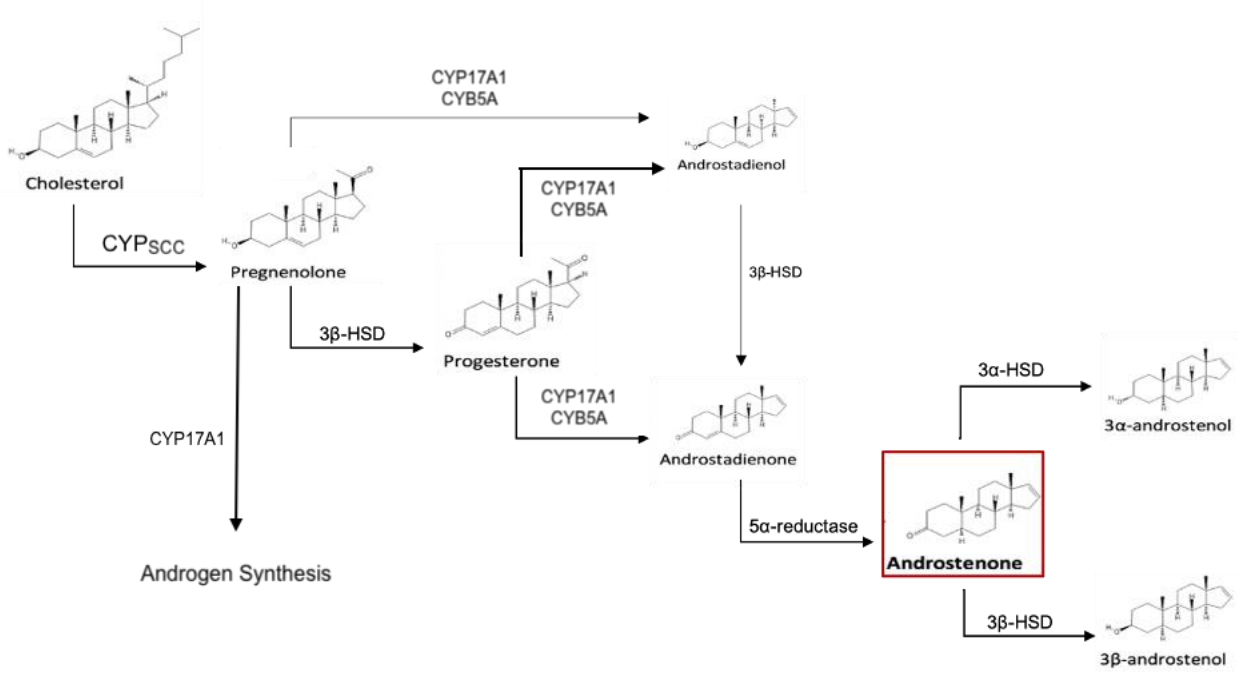

Figure 1.2: Synthesis pathway of androstenone from cholesterol. Cytochrome P450 cholesterol side chain cleavage enzyme, CYP_{SCC}; 3α-hydroxysteroid dehydrogenase, 3α-HSD; 3β-hydroxysteroid dehydrogenase, 3β-HSD (adapted from Squires et al., 2020).

Androstenone is stored in the fat due to its lipophilic nature but can also be transformed to allow circulation in the blood. If androstenone is catalyzed by 3α-HSD, its metabolite will be 3α-androstenol; if androstenone is catalyzed by 3β-HSD its metabolite will be 3β-androstenol (Bonneau & Terqui, 1983). The androstenols can then be transported in the plasma to the salivary glands where they are released in the saliva when the boar is sexually stimulated, as they play physiological and behavioural roles in

mating of pigs (Melrose et al., 1971). Androstenol is emitted as a pheromone and elicits a response in the female inducing a mating stance allowing the boar to mount. Androstenone also exists in the plasma as androstenone sulfate, where a sulfate group is conjugated to either the 4th or 5th carbon (Laderoute et al., 2019). The exact role for androstenone sulfate in circulating plasma is currently unknown, but it may act as a steroid reservoir in plasma.

Androstenone is prepared for elimination in the liver, where it is metabolized into polar, conjugated molecules that allow for easy excretion in the bile or urine (Bonneau & Terqui, 1983). Conjugation of androstenone is catalyzed by either glucuronosyl transferases (UGTs) to form androstenone glucuronide, or sulfotransferases (SULTs) to form androstenone sulfate (Sinclair, 2004). Activity levels for SULTs in the liver have been linked to the amounts of androstenone circulating in boar plasma; low activity decreases the amount of conjugated androstenone sulfate production for excretion, leaving greater levels of androstenone in circulation; thus, greater levels of boar taint will accumulate in the fat (Sinclair, 2004).

1.2.2 Role of skatole

Skatole (3-methylindole) accumulates in the adipose tissue similarly to androstenone; however, it is synthesized in the gut rather than the testis. Skatole is an indirect metabolite of the amino acid tryptophan, which is converted initially to 3-indolacetic acid, then into skatole by microflora in the large intestine (Vold, 1970; Claus et al., 1994). Some of the tryptophan required for conversion to skatole is derived from

the diet of the pig; however, the majority is recycled from mucosal cells throughout the intestinal lining (Raab et al., 1998).

The bacteria active in transformation of tryptophan to skatole are from the *Clostridium* and *Bacteroides* genera and comprise a very small percentage of the gut microbiome (Jensen, 1998). Feeding different diet compositions can alter the microbiome, including microbes responsible for skatole production, causing changes in skatole levels in the pig (Wesoly & Weiler, 2012). Raab et al. (1998) found that high-energy diets increased the rate of mitosis of cells in the gut, resulting in greater turnover of cells, and greater amounts of tryptophan in the hindgut available for skatole synthesis. Once formed, skatole is either stored in the adipose tissue, excreted in the feces, or absorbed in the colon and transported through the portal vein to the liver, where it is metabolized further (Raab et al., 1998).

As skatole reaches the liver, it is metabolized by a different isoform of the CYP450 enzyme system than androstenone (Babol et al., 1998). The skatole removal process involves two reaction phases, carried out in the liver. Phase I oxidation reaction via CYP450 enzymes involves formation of a hydroxyl, amine, or sulfhydryl group providing a substrate for conjugation. Phase II conjugation allows glucuronic- and sulfo-transferase enzymes to attach the functional groups, increasing polarity and water solubility to allow excretion through the feces or urine (Babol et al., 1998).

1.3 Controlling Boar Taint

1.3.1 Surgical castration

To remove the incidence of tainted pork, male piglets are routinely castrated within the first week after birth (Edwards et al., 2009). As androstenone is produced in the testis, removal through surgical castration reduces the incidence of boar taint (Gower, 1972). Castration can also reduce aggressive and sexual behaviours.

Hay et al. (2003) found that castration causes physiological and behavioural responses in piglets including pain, stress and discomfort occurring before, during and after the procedure for up to 4 days post-surgery. Castration is performed by making an incision to the scrotum, extracting the testicles and severing the spermatic cords. The latter can be done by pulling and tearing, which is thought to reduce bleeding, or by cutting with a scalpel, which results in a cleaner cut that heals more easily (Hay et al., 2003). Behaviours seen during or after surgical castration include trembling, isolation, stiffness and huddling, scratching the rump against the ground, and increased vocalizations (Hay et al., 2003).

Pain relief is occasionally used with castration of piglets, although methods and extent vary greatly. In Canada, as of July 2016, castration of piglets must be done with analgesics to manage pain at any age of castration (NFACC, 2014). However, this is not the case for many other countries, although public welfare concerns are causing many countries to review and change legislation. Generally, an anesthetic such as lidocaine is recommended for reducing pain from the incision, and non-steroidal anti-inflammatory drugs (NSAIDs) such as ketoprofen or meloxicam are recommended for reducing post-

castration pain from tissue damage (O'Connor et al., 2014). There have been questions about the efficacy of some pain management drugs used during routine castrations (reviewed in O'Connor et al., 2014; Sutherland et al., 2015). The European project, Boars 2018, had intended to fully end castration of male pigs by January 2018 but did not achieve this goal at the time due to a lack of feasible alternatives (www.boars2018.com). A new petition to support an EU-wide ban on surgical piglet castration by 2024 is currently in circulation (Euro Group for Animals, 2018).

Despite the requirement for use of pain relief with surgical castration, there are still negative implications of surgical castration, including infections, chronic stress problems, and weight loss or slower growth rates (Thun et al., 2006). Morales et al. (2017) found that when analyzing piglets in light, medium and heavy weight groups, there was a significant increase in pre-weaning mortality for castrated piglets, especially in the light and medium weight groups. As well, decreases in average daily gain and body weight at weaning in the heaviest weight group of piglets were noted. Use of pain relief during castration also has economic implications including cost of the analgesic or anaesthetic, and labour costs for administration. These costs can be quite significant on large commercial farms; thus, an alternative to castration would not only improve animal welfare, but also may be economically beneficial for producers.

1.3.2 Immunocastration

One alternative to surgical castration of piglets that is currently being used in several countries is immunization against gonadotropin-releasing hormone (GnRH). The vaccine can reduce levels of sex hormones and boar taint in male pigs and reduce

aggressive and sexual behaviours after the second dose of administration, just as surgical castration does (Pauly et al., 2012). Immunocastrated pigs grow more similarly to boars than castrates, showing improved leanness, growth, and feed conversion ratios to barrows but not as superior as boars (Rydhmer et al., 2010; Pauly et al., 2012).

GnRH plays a crucial role in initiating the hormonal cascade from the hypothalamic-pituitary-gonadal axis, thus interfering with normal GnRH activity interrupts the cascade responsible for producing steroid hormones including androstenone. Immunization against GnRH is done through injection of a modified GnRH to stimulate accumulation of antibodies that bind to both foreign and endogenous GnRH in the bloodstream before reaching the pituitary gland (Brunius, 2011). Injection of the vaccine occurs in two doses: the priming dose is administered about 8-9 weeks of age, and the second, activating dose is given 4-6 weeks before the pig goes to slaughter (Boler et al., 2015). After immunization, androstenone and other sex steroids cannot be produced by the testis; any compounds in the fat before the second vaccination are metabolized such that there is no incidence of androstenone in the fat of the carcass after slaughter (Dunshea et al., 2001).

Despite the advantages immunocastration provides and approval in many countries, there are limitations to its use, and it has yet to be widely accepted. The biggest factor as to why producers have not transitioned to use of immunocastration is the fear of reduced consumer acceptability of the practice in comparison to surgical castration or production of entire males (Čandek-Potoar et al., 2017). The vaccine is also a safety concern for farm workers, since the vaccine can also work in humans such that accidental

self-injection poses a risk for the person administering the vaccine (Čandek-Potokar et al., 2017). Other reasons the vaccine has not been widely accepted include the cost of the vaccine, and currently few slaughterhouses will accept males that have testes, as lack of testes is the gold standard for confirming the pig will be free of boar taint. Despite current lack of acceptance into pork production, immunocastration is a good alternative to surgical castration as it removes associated welfare concerns such as pain, discomfort and infection; however not all behaviour concerns of raising entire males are completely mitigated.

1.3.3 Diet

Manipulation of the diet can influence synthesis or metabolism of both skatole and androstenone. The metabolic pathways of the two compounds may interact within the hepatic system, and positive association between androstenone and skatole have been found (Babol et al., 1996; Zamaratskaia, 2004). Certain dietary additives may help either decrease the rate at which the boar taint compounds are formed or increase the rate at which the compounds are excreted from the body, decreasing accumulation in the adipose tissue.

Accumulation of skatole requires a high availability of tryptophan to be metabolized, which comes primarily from cell turnover in the colon (Jensen, 1998). Variation in amounts of dietary tryptophan was studied; however, it did not affect skatole formation, as tryptophan is absorbed in the small intestine before reaching the bacteria necessary for conversion to skatole (Losel & Claus, 2005). Dietary manipulation to reduce cell turnover and cell debris, however, can limit the amount of tryptophan used in skatole

synthesis (Claus et al., 1994; Urbanova et al., 2016). Increasing or decreasing cell debris has been done by influencing mitosis and apoptosis rates in the small and large intestine (Raab et al., 1998; Mentschel & Claus, 2003). Feeding high energy diets increases the rate of mitosis by stem cells, creating increased cell debris and, in turn, increasing skatole production which is not preferred (Raab et al., 1998; Mentschel & Claus, 2003). Therefore, a balance of dietary energy content that will optimize growth rates and reduce skatole production needs to be considered.

The supplementation of different sources of fermentable carbohydrates has been well researched and applied to growing pigs in the context of skatole accumulation. Fermentable carbohydrates have an effect on intestinal pH, changing gut microflora, and serve as energy sources for microbes (Wesoly & Weiler, 2012). Pauly et al. (2011) compared barley and oat cereals, discovering that barley diets led to a lower intestinal pH and more available fermentable carbohydrates; this increased skatole production, while skatole synthesis was reduced in pigs few oat-based diets which increased pH in the colon.

The feeding of polysaccharide rich diets has also been shown to shift bacterial metabolism from proteolytic to saccharolytic in the hindgut (Wesoly & Weiler, 2012). Sugar beet pulp (SBP) and fructooligosaccharides (FOS) may be good feed additives, as they reduce skatole concentration in adipose tissue by providing an energy source to *Bifidobacteria*, shifting the microbial metabolism away from skatole- and indole-producing bacteria (Li et al., 2009). Another polysaccharide found to help with reducing fat skatole levels is inulin, found in chicory root or Jerusalem artichoke (Wesoly & Weiler, 2012). Use

of chicory root in pig diets reduces skatole concentrations in both plasma and adipose tissue and is currently viewed as the best dietary supplement for controlling skatole (Hansen et al., 2008; Rasmussen et al., 2012; Vhile et al., 2012; Zammerini et al., 2012; Urbanova, 2016). Chicory root is easy to use without affecting feed intake or growth of pigs, and is available all year round; however, it is still fairly expensive and not the most practical and sustainable solution for controlling boar taint.

One dietary approach to regulate androstenone concentrations is through the use of non-nutritive sorbent materials to bind to steroid hormones (i.e. androstenone) and disrupt enterohepatic circulation to promote excretion (Jen & Squires, 2011). Activated charcoal and Tween are both efficient binding agents that have been previously used to disrupt enterohepatic metabolism of solutes and steroids (Dabrowski et al., 2005; Meijer et al., 2006). These agents were both found to reduce plasma and fat androstenone concentrations in boars; however, additional research is needed including long-term effects of these agents, correct dose, and other low-cost alternatives (Jen & Squires, 2011).

1.3.4 Other management practices

Management practices such as housing and cleaning can also have an effect on levels of boar taint in pigs. Hygiene plays an important role not only in welfare but also the physiology and development of boar taint in pigs. Hansen et al. (1994) determined that keeping pigs at high stocking rates (0.6 m^2 per pig) in pens soiled with feces and urine significantly increased indole and skatole levels in backfat compared to clean pigs housed at low stocking rates (≥ 1.2 m^2 per pig). It was hypothesized that the increase in

skatole concentrations was due to its absorption through the skin and/or lungs, as there was no correlation between fecal skatole levels and skatole levels in backfat (Hansen et al., 1994; Hansen et al., 1995). Thomsen et al. (2015) also found a slight difference in skatole and androstenone concentrations between highly soiled and barely soiled groups and suggested that ensuring good hygiene may be a strategy to reduce boar taint.

Along with hygiene, disease may cause changes in boar taint levels in pigs. Škrlep et al. (2012) found that during an investigation of immunocastration, an outbreak of acute dysentery from *Lawsonia intracellularis* and *Brachspira hyodysenteriae* bacteria occurred, resulting in cachexia and high mortality. They studied boar taint compounds in fat tissues and found that immunocastration was effective in reducing androstenone concentrations, but unusually high concentrations of skatole were found in all males, whether there was no castration, surgical castration, or immunocastration. This suggests that skatole levels in adipose tissue can increase due to intestinal infections, despite castration (Škrlep et al., 2012).

The social environment for pigs may also have an effect on boar taint levels, specifically androstenone as it is a pheromone that plays a role in social interactions. When unfamiliar pigs are placed together in a pen, there is a period of fighting for approximately the first 24 hours to establish a dominance hierarchy in the group (Fraser, 1984). Giersing et al. (2000) found that social rankings in pigs positively correlated to androstenone levels in entire males. The group noted that highly aggressive pigs had higher levels of androstenone; however, aggressive behaviour in a pen did not increase androstenone levels (Giersing et al., 2000).

When rearing pigs, there is data to support that the sex ratio of the group may influence androstenone production in boars. Bonneau et al. (1980) found that pigs reared in collective pens with females showed significantly higher androstenone concentrations than pigs reared in all-male pens. Patterson (1982) found a correlation between androstenone concentrations and sex mixing in only the heaviest weight group, again finding that boars raised with females showed higher androstenone concentrations than when raised with only males. Contrasting this, there is data to support the claims that mixed- or single-sex rearing does not have an effect, or may have the opposite effect, on androstenone levels. Hansson et al. (1980) did not find any significant difference in androstenone levels between boars raised in single sex groups and boars raised with olfactory, auditory, visual, or snout contact with female pigs. Patterson (1982) found that Landrace and Large White pigs had lower androstenone levels in mixed groups than in all-male pens.

Finally, slaughtering pigs at lighter body weights (typically below 100 kg body weight) is a common practice in parts of Europe to reduce the risk of boar taint (Fredriksen et al., 2009). The rationale is to slaughter the pigs before they reach puberty, although this is not always reliable as some boars may be early maturing, and boar taint compounds are not always correlated to live weight (Walstra et al., 1999). As well, reducing slaughter weight greatly decreases profit per pig, so slaughtering early to reduce boar taint may not be the most suitable solution (Aluwé et al., 2011).

1.3.5 Genetic selection

Genetic selection for low boar taint may be a possible long-term, non-invasive and cost-effective alternative to surgical castration of pigs to eliminate boar taint. Androstenone and skatole concentrations can vary greatly between breeds and individuals within breeds (Zamaratskaia & Squires, 2009) and both compounds show moderate to high heritability (Baes et al., 2013). Heritability estimates range from 0.55-0.75 for androstenone and 0.23-0.56 for skatole, with purebred pigs showing better heritability than crossbred pigs (Sellier et al., 2000; Strathe et al., 2013; Dugué et al., 2020). Dugué et al. (2020) also found that selecting against the hormone estradiol can also reduce fat androstenone levels and is easier than selecting against androstenone as it only requires a blood sample rather than a fat biopsy in live animals.

When using genetic selection, indirect effects of selection, whether beneficial or detrimental, must be considered. Parois et al. (2015) found that genetic selection for low androstenone did not affect immune function and reduced aggressive behaviours of entire males as testosterone levels were reduced. However, one of the biggest concerns with genetic selection to reduce boar taint is reduction in performance and sexual maturation due to reduced androgen and estrogen concentrations (Willeke et al., 1987). Fertility traits such as sperm motility, ejaculate volume and viability of sperm have been found to decrease with reduction in androstenone concentrations, which may cause breeding issues (Bergsma et al., 2007). Dugué et al. (2020) found a decrease in testosterone concentrations in purebred and crossbred boars genetically selected for low androstenone concentrations, but selection was not detrimental to production traits or

carcass composition for either purebred or crossbred pigs. Recent investigation has found that polymorphisms for CYP17A1 and CYB5a enzymes may influence the pathway of androgens versus 16-androstene steroid production; however, these polymorphisms are not naturally occurring, so genetic modification would be needed to select for an androgen-dominant pathway (Squires et al., 2019).

1.4 Challenges Associated with Use of Entire Males

1.4.1 The relationship between behaviour and androstenone concentrations

Besides preventing boar taint, male piglet castration reduces aggressive and sexual behaviours. It is well known that uncastrated males show increased aggressive and sexual behaviours (i.e. fighting, mounting) than castrates or females (McGlone, 1985; Rydhmer et al., 2006). Aggression in uncastrated males is attributed to androgen concentrations, including testosterone, that are produced in the testes; thus, these behaviours are alleviated by removal of the testes (Davidson & Levine, 1972; Signoret et al., 1975). In humans, testosterone is said to activate subcortical areas in the brain to produce aggression (Batrinos, 2012). Chronic exposure to high doses of testosterone in mice have been shown to potentiate aggressive behaviour in intact males (Lumia et al., 1994).

Despite evidence supporting the connection of androgen concentrations and aggression, few behaviour-related studies have been published on the effect of androstenone concentrations on aggression in boars. Among the studies that have been done, androstenone concentrations have been correlated with dominance ranking for boars in same-sex pens, and higher androstenone levels for the dominant boar also

showed a stimulating effect, increasing androstenone concentrations in subordinate boars in the same pen (Giersing et al., 2000). This study recorded behaviour observations of agonistic behaviours during feeding after re-grouping of pigs in pens of 10 four times in four-week periods. Mounts, threats, and bites were recorded based on frequency and intensity, as well as the individual giving/receiving the behaviour, and pigs were ranked within the pens on dominance order. Another study by Zamaratskaia et al. (2005) found no association between aggression and androstenone concentrations in entire male pigs. Aggression in this study was collected by recording agonistic behaviours during feeding, including pushing another pig to reach the trough, mounting another pig to reach the trough, lifting another pig away, thrusting by head-knocking or biting in the air, thrusting and chasing another pig away, biting, and biting and chasing another pig away. The pigs were also observed during the last month of rearing and all mounts were recorded. Examining the data from both of these studies, there appears to be no consistent link between androstenone concentrations or boar taint and aggressive or sexual behaviours in current literature, as there is between androgen concentrations and aggressive behaviours.

Further studies should be done with both individual and pen-wise tests to characterize how varying androstenone levels can influence other behaviours in pigs. The open field test (OFT), novel object test (NOT) and human approach test (HAT) are common tests that can measure response to novel or threatening stimuli to characterize locomotive activity, exploration, anxiety, and ease of handling (Beilharz & Cox, 1967; Murphy et al., 2014). The OFT is a relatively simple test; the animal on its own or in pairs

or groups (Magnani et al., 2012) is placed in an unfamiliar rectangular or circular arena for 4 to 10 minutes to observe tendency to explore (Murphy et al., 2014). In the NOT, introduction of an unfamiliar object in either an unfamiliar arena or familiar home area is used to characterize the response of an animal to test anxiety and neophobic behaviours (stilted or avoidant behavioural response to novel physical objects) (Cavigelli, 2005; Barnes et al., 1976; Murphy et al., 2014). The HAT observes the latency of a pig to approach a human, which may characterize boldness or anxiousness, motivation to interact with humans, and ease of handling for pigs (Hemsworth et al., 1981; Hemsworth et al., 1994; Murphy et al., 2014). Pen-wise tests can include general behaviour observations at mixing or throughout rearing, feeding competition tests or tests to observe responses to stranger pigs, such as the resident-intruder test, and have the advantage of capturing behaviour in a more normalized situation (Erhard et al., 1997; Giersing et al., 2002; Zamaratskaia et al., 2005). Using a combination of these approaches in a study can give the best evidence to quantify and characterize behaviours that may need to be managed in entire male pigs differently from barrows.

Aggression in pig farming is a significant problem for many reasons. Firstly, the welfare of the pigs is highly compromised if pigs are constantly fighting and injuring each other. Conflict in the herd may induce pain, fear, or distress, all of which do not align with a good state of welfare for the pigs (Thomsen et al., 2012). Safety for workers on the farm is also compromised if pigs are showing aggression both between conspecifics and towards humans (Marchant-Forde, 2002). The health of pigs as it relates to production is at risk if they are being bitten, scratched, or attacked, causing lameness or open wounds

that need treatment, or infections that may even lead to fatal outcomes (McGlone, 1985; Warriss and Brown, 1985; D'Eath, 2002; Rydhmer et al., 2006). Aggression and increased general activity as seen in boars can increase stress and many studies have found decreased immune function as a result of received aggression (Moore et al., 1994; Morrow-Tesch et al., 1994; Tuscherer et al., 1998). In contrast to how aggression can cause poor health of pigs, compromised health of pigs including lameness can increase aggression, mounting or bothering from other healthy boars (van Wagenberg et al., 2013). Meat quality also suffers when pigs are in stressful environments, or when receiving aggression or mounting. Fighting between boars causes mobilization of fatty acids, glucose, and lactate, which can compromise meat quality if excessive fighting occurs just before slaughter (Fernandez et al., 1994; Stookey & Gonyou, 1994). Increased aggression or fighting can increase the risk of dry, firm, dark (DFD) meat, an undesirable trait, which is also seen more commonly in boars than castrates or females (Warriss & Brown, 1985). Pigs receiving aggression show decreased average daily gain (ADG), which can translate to longer time to market and more resources being spent per pig (Stookey & Gonyou, 1994). Resource efficiency may also be lost if fighting or mounting results in serious injury requiring antibiotics or euthanasia of pigs with fractured legs.

Frequent sexual behaviours in boars include mounting other pigs, grunting and chomping, and salivating profusely when near females in estrus (McGlone, 2012). Play behaviour of piglets develops and turns into organized sexual behaviours as the boar physically develops. Physical and immunological castration of male pigs reduces sexual behaviours and aggression (Cronin, 2003; Rydhmer et al., 2006; Guay et al., 2013).

Females in estrus display increased activity and will more readily approach boars than if not in estrus. Boars, however, will approach any female whether or not she is in estrus. Females will stand immobile in a characteristic mating stance upon stimulation by boar pheromones and behaviours such as grunting, chomping jaws, profuse salivation, and spurts of urine (Fraser, 1984). Signoret (1970) found that females are attracted to boars even if they are anesthetized or blocked from view but are not attracted to boars if the females lack olfactory bulbs, suggesting the approach of a sow is based on olfactory stimulation. The response by females in estrus to boars is due to androstenone in the boar's saliva, urine, and preputial fluids (Fraser, 1984).

The biggest problem with sexual behaviours in pigs that are group-housed is injury or lameness from mounting. Sexual behaviours are most commonly collected by general observation in pigs that are close to pubertal development, often in the last few weeks of rearing before slaughter (Zamaratskaia et al., 2005; Rydmer et al., 2006). Rydhmer et al. (2006) found that there is an increase in sexual behaviour and injuries in single-sex pens with entire males compared to mixed or female pens. In this study, injuries causing lameness or leg fractures from sexual behaviour caused euthanasia of 5 entire males, while 31 entire males in total had problems with legs or feet (204 total entire males used in the study). Sexual behaviours causing injuries to both males and females are a significant welfare concern, so additional practices to reduce these behaviours when raising entire males must be taken into consideration.

1.4.2 Raising boars

Due to the increase in aggressive and sexual behaviours, extra consideration to particular management practices may be required when raising boars. These strategies may include single-sex or sibling group rearing, considerations with regard to space allowance and pen shape/size, administration of pharmaceuticals, or pre-exposure/familiarization of pigs before mixing. It is important to consider which management strategies are best to reduce boar taint development as well as reduce aggression.

Mixed-sex rearing

Higher incidences of sexual behaviour (i.e. mounting) and injury are seen in single-sex male groups compared to mixed-sex pens (Rydhmer et al., 2006). Presence of females in pens with boars reduces mounting, fighting and aggression (Bjorklund and Boyle, 2006). However, some studies have found no difference in entire male aggression when raised with or without female presence (Conte, 2010; Holinger et al., 2015).

Sibling groups (farrow-to-finish pens)

The use of farrow-to-finish pens avoids the event of mixing piglets from multiple litters, which is the major reason for acute fighting and aggression in pigs (Signoret, 1975; Petherick & Blackshaw, 1987). Fredriksen et al. (2008) found that raising sibling groups reduces aggressive behaviour in boars compared to mixing multiple litters; however, there were still higher number of bouts of aggression seen in entire males than in castrates. Managing pigs as farrow-to-finish groups reduced androstenone levels in the fat of entire

males, suggesting this may be an effective management strategy for raising entire males (Fredriksen et al., 2006).

Pre-exposure prior to mixing

Similar to farrow-to-finish pens, the goal of pre-exposing piglets to each other prior to mixing is to reduce the period of acute fighting and aggression that occurs upon mixing piglets from different litters. Marchant-Forde & Marchant-Forde (2005) found that pre-exposing piglets to each other before mixing the group together can decrease aggression in the two-week period post mixing by up to 60%. Rydhmer et al. (2013) also found that by allowing piglets from two litters to visit each other through an opening between farrowing pens, fighting and aggression were reduced when they were separated into female and male groups at weaning.

Space allowance and group size

NFACC guidelines recommend space allowances from 0.46 m^2/pig for a 50 kg pig to 0.77 m^2/pig for a 110 kg pig (NFACC, 2014). Many studies found that reduced space per pig (i.e. 0.8 m^2/pig compared to >1.2 m^2/pig for growing/finishing pigs) may reduce pig welfare, reduce growth of pigs, reduce feed efficiency, increase injury and skin lesions, and increase morbidity and mortality rates (Kornegay et al., 1985; Turner et al., 2000; DeDecker et al., 2005; Fu et al., 2016). Increased space allowance gives more space in the pen for pigs to avoid an aggressor or attacker pig and can reduce the frequency of agonistic interactions between pigs (Bryant & Ewbank, 1972; Hansen et al., 1994). Larger group size (i.e. more than 12 individuals) has been shown to reduce

aggression levels of growing pigs (Andersen et al., 2004). Much larger groups (i.e. greater than 80 pigs) have shown more effective reduction of aggressive interactions within a group (Samarakone & Gonyou, 2009). This is attributed to the formation of sub-groups that allow natural social order to be maintained.

Pen shape/size

Rectangle pens better allow pigs to escape from aggressors behind other pigs, whereas square and circular pens make escaping from the aggressor more difficult, as they are more likely to stay in the pig's line of sight (Wiegand et al., 1994). Results on use of barriers have been mixed, some studies showing no difference in agonistic behaviours (Olesen et al., 1996), with other studies showing significant reduction of aggression around feeding when partial stall barriers are used (Petherick & Blackshaw, 1987; Barnett et al., 1992). McGlone & Curtis (1985) demonstrated that "pop-holes" for pigs to hide their head and neck were able to reduce duration of fighting when pigs were mixed.

Pharmaceuticals (sedation)

Sedation of pigs when mixing has not been a major topic of study in recent literature, as results show it is not currently the best management strategy for reducing aggression. Amperozide and azaperone (sedatives) have been evaluated for their effectiveness to reduce aggression when mixing pigs. Results from multiple studies agree that amperozide and azaperone display temporary reduction of aggression when mixing without consequences on growth; however, the response is only a delay in aggression until the sedative has worn off and may disrupt the establishment of social hierarchy in

the group for up to 3 weeks after treatment (Blackshaw, 1981; Gonyou et al., 1988; Tan & Shackleton, 1990). Amperozide treatment may also have negative welfare implications with regards to its large acute stress response after treatment (Barnett et al., 1996). Administration of an androstenone spray reduced aggressive behaviour among prepuberal female and castrated pigs, but there is no evidence that this approach may reduce aggressive behaviour when mixing entire males (McGlone & Morrow, 1988). Commercial odour masking agents have been investigated, but only seem to delay fighting or have no impact on aggression and skin lesions (Friend et al., 1983; Barnett et al., 1993).

1.4.3 Meat quality for castrated and uncastrated males

Meat quality is extremely important for producers and consumers in the pork industry, so the effects of raising entire males on meat quality are important to understand. Generally, entire males are leaner than castrates, and have improved growth rates while eating less feed (EFSA, 2004). In Europe, it has been estimated that the improvement in feed efficiency of entire males compared to castrates can increase value by up to €7.11 per pig (European Union Establishing Best Practices, 2019).

In past markets, increased intramuscular fat (marbling) and backfat depths were more desired since meat was more tender with firmer fat, which can make it less prone to rancidity during storage and maturation of dry cured meat products (Pauly et al., 2012; Bonneau & Weiler, 2019). In more recent markets, leaner cuts of meat have been preferred by consumers, and meat with less adipose tissue and more unsaturated fatty acids is regarded as favourable from a dietetic point of view (Lundström et al., 2009).

However, issues can occur when selecting for leaner pigs, including a lack of cohesion between backfat and the underlying muscle, or unacceptably soft fat in leaner animals with higher water content (Wood, 1984; Wood et al., 1989).

Entire males have greater incidences of dark, firm, dry (DFD) meat, an undesirable meat quality trait characterized by high pH 24 hours post-mortem, leaving a tough, dark meat that is not favourable for consumers (Pauly et al., 2012). DFD meat occurs more often in boars likely due to their increased general activity or agonistic behaviours that can cause increased basal stress levels before slaughter. This causes low glycogen reserves before slaughter, which result in lower than normal accumulations of lactic acid in the meat post-mortem (Bonneau & Weiler, 2019).

In general, consumer acceptance is greater for pork from surgically castrated and immunocastrated pigs than pork from entire males (Font i Furnols et al., 2008). Font i Furnols et al. (2008) found that lower consumer acceptance of pork from entire males was independent of androstenone levels; however, meat with low levels of androstenone was accepted more than meat from pigs with medium or high levels of androstenone. Meat from entire males can be more tough (increased shear force measurements and reduced intramuscular fat concentrations) and less juicy (reduced water retention), which makes it less desirable to consumers than meat from castrates (Pauly et al., 2012; Škrlep et al., 2019). Many studies have found pork from immunocastrates to be more similar to pork from surgically castrated males, being more acceptable to consumers than meat from entire males (D'Souza & Mullan, 2003; Hennessy & Walker, 2004; Font i Furnols et al., 2008).

1.5 Conclusion

It is evident there has been significant research dedicated to finding an alternative approach to surgical castration of piglets as a solution to the boar taint problem. As of yet, a universal, long-term approach has not been determined. Results from immunocastration studies have shown success in reducing boar taint; however, producer and consumer acceptance continue to be an issue. Genetic selection may prove to be the most efficient long-term solution for reducing boar taint in uncastrated males, but continued validation of selection markers is needed. Raising intact males for pork production has many benefits including faster growth, leaner meat, and increased feed efficiency, which could increase sustainability of the pork industry (EFSA, 2004). However, increased negative behaviours of intact males compared to castrates may compromise welfare of pigs in a different manner. Raising intact males causes increases in sexual and aggressive behaviours that may lead to increased injury or lameness (Cronin et al., 2003). It may be necessary to employ alternative management strategies when raising boars for production. Investigating the effects of selecting for low and high boar taint pigs on aggressive and sexual behaviours will help to evaluate feasibility of the use of selection as a welfare-friendly alternative to surgical castration.

CHAPTER 2 HYPOTHESIS AND RESEARCH OBJECTIVES

2.1 Rationale of Research and Hypotheses

Castration of piglets has become an animal welfare concern and thus alternatives to this practice are being explored (Bonneau & Weiler, 2019). The challenge to changing current castration practices is to be able to continue to efficiently produce healthy animals, without behaviour issues, that can result in high yield and meat without the foul odour or flavour known as boar taint (von Borell et al., 2020). While raising boars selected for low boar taint is one long-term solution to the boar taint issue, the viability of raising such pigs without behaviour issues or other meat quality concerns must be first be established (Baes et al., 2013; Dugué et al., 2020).

It has been established that high concentrations of skatole and androstenone cause boar taint (Patterson, 1968; Vold, 1970). Castration reduces both boar taint and behaviour issues, including aggression and sexual behaviour (Davidson & Levine, 1972). What is less understood is how plasma and fat concentrations of androstenone within uncastrated male pigs impact meat quality and behaviour. As well, a clear approach to predicting levels of androstenone has not been established.

The overall goal of this study is to help the pork industry better understand the option of raising boars to successfully address welfare concerns of surgical castration, without introducing new behavioural issues or impacting meat quality.

Hypothesis 1: It is hypothesized that levels of plasma testicular steroids, including androstenone in uncastrated male pigs at 21 days of age will correlate with plasma and fat testicular steroid levels, including androstenone, accumulated at slaughter.

Hypothesis 2: It is hypothesized that uncastrated males with low androstenone concentrations will show fewer signs of problematic and unwanted behaviours, including aggression, stress-related behaviour and sexual behaviour, than males with high androstenone concentrations based on reduction in testicular steroids associated with aggression, but both high and low androstenone males will show more problematic and agonistic behaviours than barrows.

Hypothesis 3: It is hypothesized that on the key metrics of carcass and meat quality traits, including leanness, loin colour, and juiciness, low androstenone pigs will be intermediate to barrows and high androstenone pigs.

2.2 Research Objectives and Rationale

Objective 1: The objective of this research is to determine if plasma levels of steroid hormones, specifically androstenone, at 21 days are correlated to plasma and fat androstenone levels at slaughter to determine if it may be possible to predict the extent of androstenone production and boar taint accumulation in the animal at early stages in life.

Previous research indicates that production of testicular steroids, including androstenone, is increased from 2-4 weeks of age, peaking around 21 days (Schwarzenberger et al., 1993). Plasma steroid levels decline through week 5 until

reaching puberty around 16-20 weeks, and then rise again nearing slaughter weight. Although suppressing the early peak does not block an increase in androstenone concentrations later in life, it has not been confirmed whether there is a correlation between levels at 21 days and slaughter (Schwarzenberger et al., 1993; Sinclair et al., 2001). Given androstenone causes boar taint, identifying which uncastrated male pigs will potentially have high androstenone concentrations at slaughter may provide pork producers an opportunity to identify the pigs with the greatest risk for developing boar taint and then take the appropriate management measures, such as early slaughter or immunocastration.

Objective 2: To explore high versus low androstenone levels in pigs and their effects on the behavioural concerns that are currently encouraging the use of castration, including greater aggressive, stress-related and sexual behaviours in boars compared to barrows.

Beyond preventing boar taint, castration is the primary approach to managing behavioural issues seen in uncastrated males. Uncastrated males have a higher incidence of both aggression and sexual behaviours such as mounting than castrated males, both of which pose risks to the welfare of the pigs (Rydhmer et al., 2006; Lundström et al., 2009; Pauly et al., 2012). As well, aggressive or agonistic events can activate the hypothalamic-pituitary-adrenocortical (HPA) axis to elicit a stress response, so aggressive pigs may be more stressed than less aggressive pigs which can be a welfare concern (Fernandez et al., 1994; Muráni et al., 2010). By understanding if the risk of these behaviours is reduced among low androstenone pigs versus high androstenone

pigs, and specifically how different the risk is compared to barrows, producers can better understand if raising uncastrated low boar taint pigs is a viable option.

Objective 3: The objective of this research is to compare meat quality characteristics in uncastrated males with high and low androstenone concentrations, also comparing to castrated males (barrows) as controls.

Research has shown that meat from castrated males generally has greater intramuscular fat content and back fat deposition, more intense flavour and more tender and juicier meat with low to no risk of boar taint, which translates to a better consumer product (Lonergan et al., 2007; Lundström et al., 2009; Pauly et al., 2012). Other studies have suggested that uncastrated males produce a leaner meat which is more aligned with consumer trends to reduce saturated fats in their diets (Patterson et al., 2009; Pauly 2012, Mukumbo & Muchenje, 2016). Regardless of whether positive or negative, any change in meat quality must be understood before producers can be willing to consider alternatives to castration.

CHAPTER 3 EARLY EVALUATION OF PLASMA ANDROSTENONE CONCENTRATIONS MAY INDICATE BOAR TAINT DEVELOPMENT IN BOARS SLAUGHTERED AT 120 KG BODY WEIGHT

3.1 Introduction

In North America, almost every male pig intended for pork production is castrated in the first few days after birth. Castration occurs to reduce sexual and aggressive behaviours and, more importantly, removes the incidence of a meat quality issue called boar taint. Boar taint is an unpleasant off-odour or off-flavour in pork, mainly attributed to the accumulation of the steroid hormone androstenone in the fat. Androstenone is produced in the testes at puberty, and functions as a sex pheromone regulating female sexual development and behaviour (Patterson, 1968). A second major contributing compound is skatole, which is formed by microbial digestion of tryptophan in the hindgut (Vold, 1970; Claus et al., 1994). Producers and researchers are searching for an alternative to surgical castration of male piglets as castration is increasingly being recognized as an animal welfare concern, and intact male pigs are known for better feed conversion and carcass quality traits which can lead to more sustainable production (EFSA, 2004).

Currently, there are few solutions to prevent boar taint besides surgical castration. Measures such as surgical castration, feed additives or immunocastration are typically applied to all male pigs in the herd, despite only 10-75% of males developing boar taint above consumer acceptance levels; however, this number varies depending on both genetic and environmental factors (Thun et al., 2006). Early prediction of boars that may

develop high levels of boar taint can allow for selection of small groups of pigs that need intervention strategies, hence lowering costs relative to applying interventions to the entire herd.

Investigations into testicular steroid levels have revealed an opportunity to find an approach to predicting indicators of boar taint levels early in life (Schwarzenberger et al., 1993). Research has shown that between 2-4 weeks of age, there are increased levels of androstenone and testosterone in the plasma, peaking at 21 days of age and decreasing again until the boar reaches puberty around 5 months (Schwarzenberger et al., 1993; Sinclair et al., 2001). This early spike of plasma hormone production correlates with high testicular steroid levels, increase in Leydig cell numbers and changes in luteinizing hormone (LH) receptors at this time, suggesting a maturation of the hypothalamic-pituitary-gonadal (HPG) axis (Colenbrander et al., 1978; Sinclair et al., 2001). The relationship between 21-day steroid hormone concentrations and the extent of boar taint development at slaughter has not been extensively investigated.

Another steroid hormone that peaks around 2-4 weeks is estrone sulfate (E1S), which is an important stable reservoir for estrogen (Wright et al., 1978). E1S can be used as an indicator of sexual maturity when measured in boars (Schwarzenberger et al., 1993). This measure of sexual maturity can be important when taking into account individuals that mature early and may have higher levels of boar taint at the time of slaughter. Other, later maturing individuals may have lower levels of boar taint at the time of slaughter. Early levels of estrone sulfate have previously been found to be not associated with boar taint compounds at slaughter (Sinclair et al., 2001).

Possible intervention strategies for reducing boar taint if high-risk individuals can be identified early (i.e. at 21 days) may include early slaughter before the boar reaches puberty and steroid production increases, or immunocastration of high boar taint pigs (Dunshea et al., 2001). Immunocastration involves the injection of anti-gonadotropin releasing hormone (GnRH) vaccine, interrupting the HPG axis and blocking testicular steroid production. Since immunocastration is expensive if applied to the entire herd, costs can be better managed if an intervention strategy is only used on pigs with high risk for boar taint development. There is also the opportunity with this selection approach for increased productivity by raising a greater number of entire males, which are known to have reduced feed intake, increased feed efficiency, and greater lean yield (EFSA, 2004).

The objective of this research is to determine if plasma levels of steroid hormones, specifically androstenone, at 21 days of age are correlated to plasma and fat androstenone levels at slaughter to determine if it may be possible to predict the extent of androstenone production and boar taint accumulation in the pig at early stages in life. It is hypothesized that plasma concentrations of testicular steroids, including androstenone in uncastrated male pigs at 21 days of age will correlate with testicular steroid levels including androstenone, accumulated in fat and plasma at slaughter.

3.2 Materials and Methods

Animals and housing

All procedures were approved by the Animal Care Committee at the University of Guelph in accordance with the standards established by the Canadian Council on Animal Care (1993, revised 2018). Fifty-four male pigs and 54 female pigs (Duroc x [Landrace x Yorkshire]) were selected, and 18 of the males were surgically castrated at 3 days of age. Pigs were sourced from Arkell Swine Research center and moved at weaning (3 weeks; average weight 6.7 ± 0.2 kg) to the Animal Science and Nutrition animal wing at the University of Guelph. Pigs were mixed at transport and allocated into pens of 4 (2 males, 2 females; boars and barrows separated) and blocked by weight at time of mixing. Pigs were housed in 1 x 3 m pens with plastic-coated metal grid flooring. At 6 weeks, pigs were transported (mixed; moved in 2 groups) to the University of Guelph Ponsonby Research Station and raised here until slaughter at average 120 kg body weight. Pigs were kept with the same pen mates and groups were randomly allocated to 1.8 x 3.6 m pens across four rooms with identical conditions. Pens had solid concrete floors bedded with wood shavings that were cleaned daily, and metal spindle sides. All pigs were fed the same commercial diet *ad libitum,* with free access to water from nipple drinkers. Lights were turned on at 08:00h and off at 20:00h.

Performance and slaughter

Pigs were weighed weekly from 3 weeks until slaughter. Male pigs were raised to market slaughter weight (123.9 ± 8.0 kg; 141.5 ± 5.9 days of age) before being transported, mixed in the truck, in 3 groups to slaughter at University of Guelph Meat Science Laboratory (federally licensed CFIA abattoir; females were slaughtered separately, no data for females were recorded), each group one week apart. Once at the abattoir, pigs were placed back with original male pen mate in separate pens. Blood was taken in heparinized tubes at 21 days of age and at slaughter; blood was centrifuged, and plasma was separated and stored at -20ºC until further analysis. Backfat samples of approximately 10cm^2 and about 3cm thick were cut at slaughter from the top of the back just under the skin and stored at -20ºC until further analysis.

Sample analysis

Androstenone concentrations in plasma and fat were analyzed by specific ELISA (Squires & Lundstrom, 1997). Estrone sulfate concentrations were analyzed in plasma by specific radioimmunoassay (RIA) (Schwarzenberger et al., 1993). Skatole concentrations in fat were measured using High Performance Liquid Chromatography (HPLC) (Lanthier et al., 2007).

Statistical analysis

All data were analyzed using SAS University Edition 3.8 (SAS Institute, Cary, NC). Weight groups above and below 120 kg body weight were chosen for analysis. Estrone

sulfate (E1S) groups were chosen as high plasma E1S concentrations >75 nmol/L (26.25 ng/mL) and low plasma E1S concentrations below 75 nmol/L (26.25 ng/mL) based on the E1S profile in male pigs presented by Schwarzenberger et al. (1993). A one-way ANOVA (PROC MIXED) was used to compare plasma and fat androstenone concentrations between >120 kg and <120 kg body weight groups, and between high E1S and low E1S groups based on the following model:

$$Xij = \mu + \alpha i + \beta j + \varepsilon ij$$

Where Xji is the observed parameter, μ is the overall mean, αi is the experimental treatment group (>120 kg/<120 kg or high E1S/low E1S; fixed effect), βi is the slaughter group (random effect) and εij is the error. A Univariate regression (PROC REG) was used to determine all associations. Model residuals were assessed using scatter and box plot of studentized residuals for homoscedasticity, Q-Q plot and Shapiro-Wilk test for normal distribution. Statistical significance was defined as $p<0.05$ and trend defined as $0.05<p<0.1$.

3.3 Results

At slaughter, plasma androstenone and fat androstenone concentrations were significantly associated for all boars ($R^2=0.76$, $p<0.001$; Figure 3.1). There was a trend of weak association between 21d plasma androstenone and fat androstenone concentrations at slaughter for all boars ($R^2=0.1$; $p=0.054$). Boars that reached at least 120 kg body weight at time of slaughter were analyzed separately from those that had not

yet reached 120 kg body weight at slaughter. In the group that had reached at least 120 kg body weight at time of slaughter (n=23), there was a positive association between plasma androstenone concentrations measured at 21 days of age and fat androstenone concentrations measured at slaughter (R^2=0.29, p=0.007; Figure 3.2). In these boars, 21d plasma androstenone concentrations and plasma androstenone concentrations at slaughter tended to be associated (R^2=0.16; p=0.056; Figure 3.3). There were no associations seen between 21d plasma androstenone concentrations and fat androstenone concentrations at slaughter (R^2=0.04; p=0.51; Figure 3.2) or plasma androstenone concentrations at slaughter (R^2=0.006, p=0.80; Figure 3.3) in the group <120 kg. Comparing the groups above and below 120 kg body weight at slaughter, there were no differences (p>0.05) in 21d plasma androstenone concentrations, slaughter plasma androstenone concentrations, slaughter fat androstenone concentrations, or age at slaughter between groups (Table 3.1).

When considering sexual maturity as an indicator of boar taint development at slaughter, the boars were split into groups above and below 75 nmol/L (26.25 ng/mL) estrone sulfate (E1S) at slaughter (based on Schwarzenberger et al., 1993). Comparing these groups, there were no differences in plasma androstenone concentrations at 21 days (p=0.3) but plasma androstenone concentrations at slaughter (p=0.003) and fat androstenone concentrations at slaughter (p=0.036; Table 3.2) were greater in high E1S boars than low E1S boars. There were no differences in age at slaughter (p=0.8; Table 3.2). In the earlier maturing group (E1S >75 nmol/L; n=26), there was a weak association between 21d plasma androstenone concentrations and fat androstenone concentrations

at slaughter (R^2=0.15, p=0.048; Figure 3.4); however, there was no association between 21d plasma androstenone concentrations and plasma androstenone concentrations at slaughter (R^2=0.01; p=0.52; Figure 3.5). In the later maturing group (E1S <75 nmol/L at slaughter; n=10), there were no associations between 21d plasma androstenone concentrations and slaughter fat androstenone concentrations (R^2=0.19; p=0.24; Figure 3.4) or slaughter plasma androstenone concentrations (R^2=0.16; p=0.29; Figure 3.5). Associations between 21d plasma E1S concentrations and slaughter plasma and fat androstenone levels were investigated, but no significant associations were seen (p>0.05).

3.4 Discussion

The results from the present study suggest that measuring plasma androstenone concentrations around 21 days of age may be a possible method for predicting the extent of fat androstenone development at slaughter in some groups of boars. A previous study by Sinclair et al. (2001) found that there were no correlations between plasma steroid concentrations at 14-21 days of age and fat or plasma androstenone concentrations at slaughter; however, these animals were slaughtered at only 110 ± 5.2 kg body weight. The present study found no association between 21d plasma androstenone concentrations and fat androstenone concentrations at slaughter in boars slaughtererd at less than 120 kg body weight. While these results agree with Sinclair et al. (2001), the present study did find an association between 21d plasma androstenone concentrations and fat androstenone concentrations at slaughter in boars slaughtered over 120 kg body

weight. This suggests that 21d plasma steroid concentrations may better predict steroid accumulation once pigs have reached heavier weights and advanced in maturity. As well, the boars chosen by Sinclair et al. (2001) were purebred Yorkshire, which are known to have lower incidences of boar taint than the three-way cross boars used in this study or Duroc boars (Xue et al., 1996). The influence of breed should be investigated further in relation to the ability to use plasma androstenone concentrations in young boars to predict boar taint development.

When separating boars into groups above and below 120 kg body weight at slaughter, the associations between 21d plasma androstenone concentrations and slaughter fat androstenone concentrations differed by weight group. For pigs below 120 kg body weight, there was no association. The lighter pigs may not have reached full sexual maturity and thus not reached their full potential of boar taint production (Allrich et al., 1982), so the ability to predict boar taint development may be limited. However, E1S concentrations, an indication of sexual maturity, were not significantly different between the weight groups.

Examining sexual maturity in more detail, Schwarzenberger et al. (1993) showed that E1S concentrations may be a good indicator for evaluating the extent of sexual maturity in boars. Using their profile comparing age versus E1S concentrations, a cut-off of 75 nmol/L for separating high and low groups was used for the present study. Analysis with these groups confirmed that in the more sexually mature boars (based on higher E1S concentration) 21d plasma androstenone concentrations were related to fat androstenone concentrations at slaughter. Although this association was found with the

group of more sexually mature pigs, boars in the high E1S group at slaughter were not all the same as those in the group above 120 kg body weight at slaughter. It may be important to take into account growth or weight as well as E1S concentrations when considering maturity of boars and prediction of boar taint development. The present study also investigated if 21d plasma E1S concentrations could be a used to predict boar taint development, but there were no associations of 21d plasma E1S concentrations with plasma or fat androstenone concentrations at slaughter. It may be useful, however, to take into account both E1S concentrations as well as plasma androstenone concentrations at 21 days of age when predicting the extent of boar taint at slaughter.

Previous research has shown that it may be possible to use testes volume at 12 weeks of age to predict if a boar will accumulate boar taint (Bekaert et al., 2012); however, the sensitivity and specificity of using this measure as a predictor has not been thoroughly investigated. It may be possible to use plasma androstenone concentrations at 21 days of age as a predictor in conjunction with monitoring testes volume at 12 weeks of age to get a more accurate prediction of boar taint development at slaughter.

There were limitations within this study that may impact strength of association between 21d and slaughter androstenone concentrations, including a small sample size of boars. Further research will require a larger and more diverse sample size, possibly investigating multiple breeds. Genetic markers and selecting for low boar taint individuals should also be investigated further.

Overall, the present study suggests that it may be possible to use plasma androstenone concentrations at 21 days of age to predict the extent of fat androstenone that the boar may develop at slaughter, but further research is required to strengthen this approach. If early prediction is possible, low potential boar taint pigs could be raised to market slaughter weight with low risk for boar taint, and high potential boar taint pigs could be identified early so that other strategies, such as immunocastration, early slaughter, or other dietary and management strategies may be executed to reduce the risk for boar taint.

3.5 Tables and Figures

Table 3.1: Effects of boar slaughter weights on hormone concentrations in plasma and fat[1]

	Mean value		s.e.m.	*p*-value
	Boars >120 kg n=23	Boars <120 kg n=13		
Weight at slaughter (kg)	126.9	114.3	1.45	<0.001
Age at slaughter (days)	142.0	140.0	2.00	0.375
21-day plasma androstenone (ng/mL)	53.1	54.7	13.66	0.908
Slaughter plasma androstenone (ng/mL)	371.0	400.2	83.30	0.728
Slaughter fat androstenone (ug/g)	10.2	9.6	2.11	0.763
Slaughter plasma E1S (ng/mL)	35.5	31.9	4.37	0.427

[1]Data presented as least square means

Table 3.2: Effects of E1S concentrations on hormone concentrations in plasma and fat[1]

	Mean value		s.e.m.	*p*-value
	[2]High E1S n=26	[3]Low E1S n=10		
Weight at slaughter (kg)	122.8	121.1	2.77	0.544
Age at slaughter (days)	142.2	141.6	2.17	0.801
21-day plasma androstenone (ng/mL)	57.7	43.6	14.38	0.335
Slaughter plasma androstenone (ng/mL)	451.2	200.5	78.48	0.003
Slaughter fat androstenone (ug/g)	11.5	6.8	2.14	0.036
Slaughter plasma E1S (ng/mL)	40.0	19.2	3.12	<0.001

[1]Data presented as least square means
[2]High E1S boars, >75nmol/L (26.25ng/mL) at slaughter
[3]Low E1S boars, <75nmol/L (26.25ng/mL) at slaughter

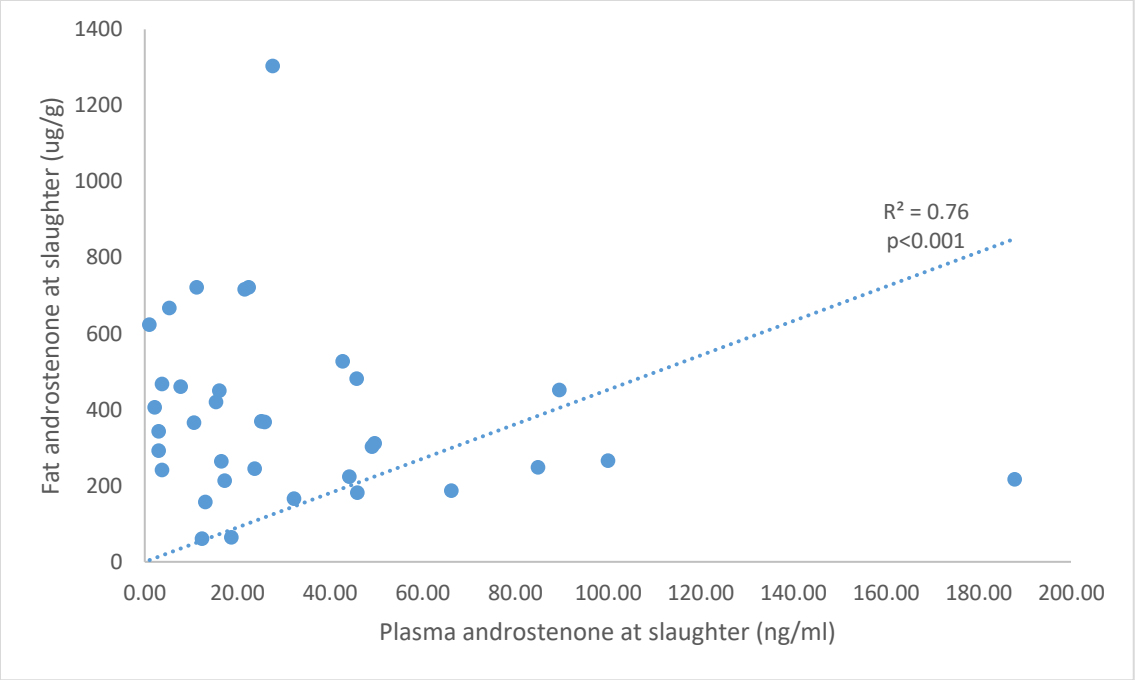

Figure 3.1: Association between plasma and fat androstenone concentrations at slaughter in all boars (n=36)

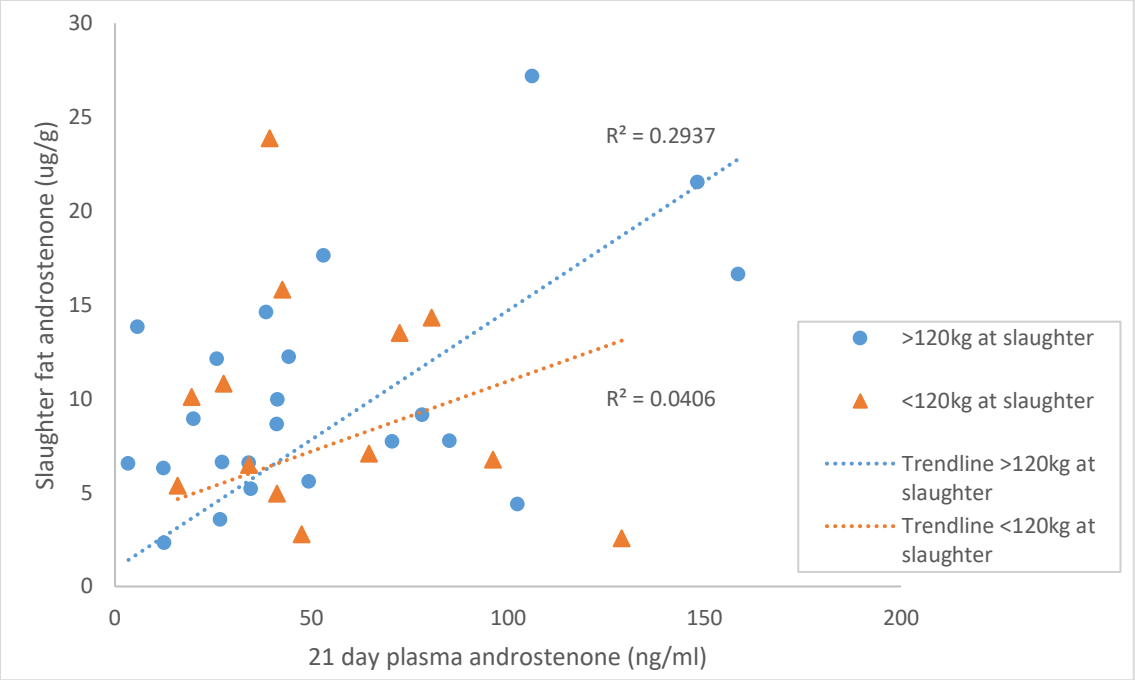

Figure 3.2: Association between 21-day of age plasma androstenone and slaughter fat androstenone concentrations for boars >120 kg body weight at slaughter (blue circle; n=23) and boars <120 kg body weight at slaughter (orange triangle; n=13).

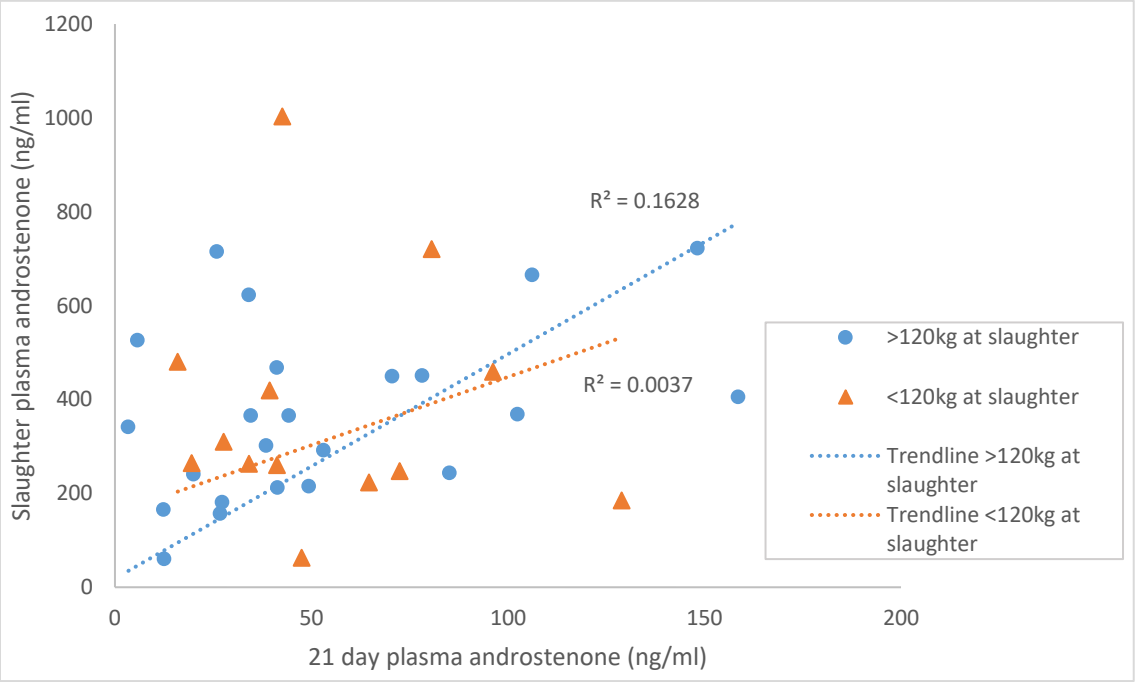

Figure 3.3: Association between 21-day of age plasma androstenone and slaughter plasma androstenone concentrations for boars >120 kg body weight at slaughter (blue circle; n=23) and boars <120 kg body weight at slaughter (orange triangle; n=13).

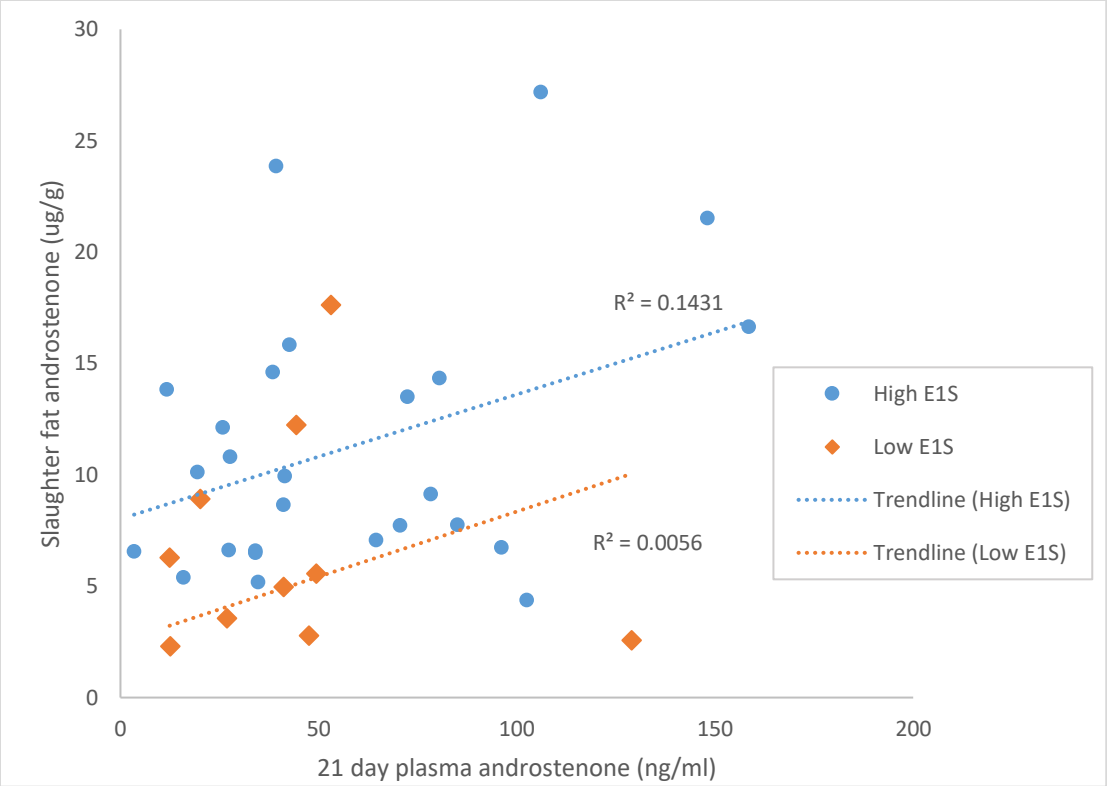

Figure 3.4: Association between 21-day of age plasma androstenone and slaughter fat androstenone concentrations for high E1S boars (>75 nmol/L at slaughter; n=26; blue dot) and low E1S boars (<75 nmol/L at slaughter; n=10; orange triangle)

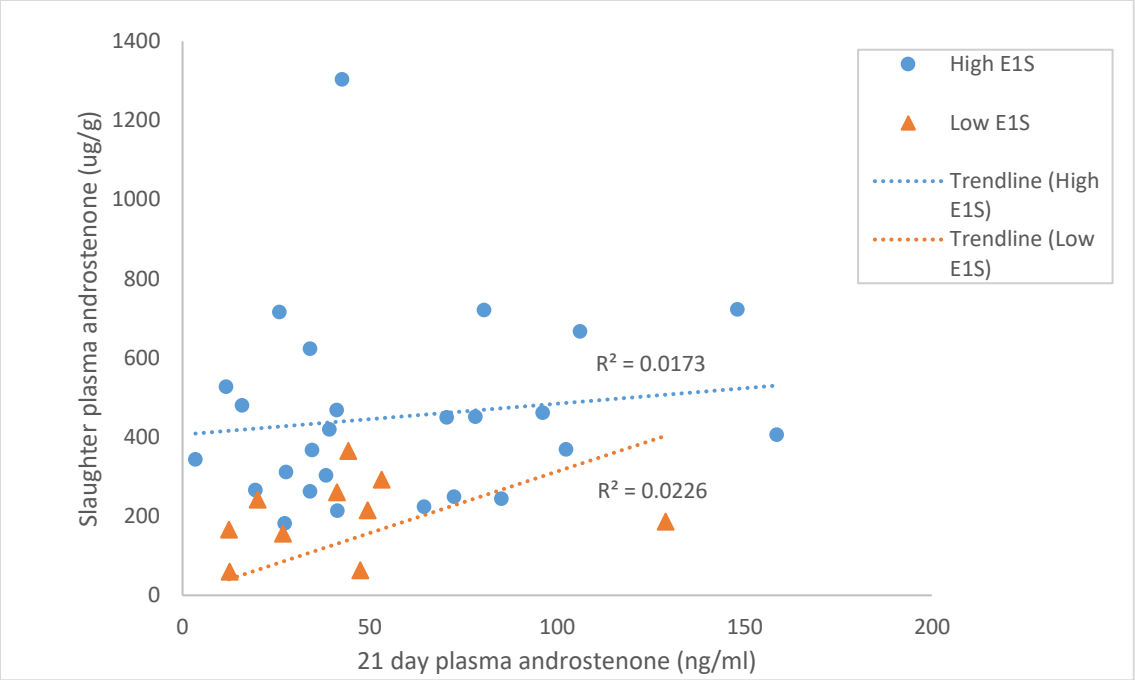

Figure 3.5: Association between 21-day of age plasma androstenone and slaughter plasma androstenone concentrations for high E1S boars (>75 nmol/L at slaughter; n=26; blue dot) and low E1S boars (<75 nmol/L at slaughter; n=10; orange triangle).

CHAPTER 4 COMPARING BEHAVIOUR PROFILES FOR BARROWS, HIGH AND LOW ANDROSTENONE BOARS

4.1 Introduction

Castration of male piglets is common practice for pork production in Canada. Despite male piglet castration becoming a significant welfare concern in recent years, most continue to be castrated within days of birth according to the code of practice (EFSA, 2004; von Borell et al., 2020). Motivation for male piglet castration is to reduce aggressive and sexual behaviours, but more importantly, to reduce the incidence of a meat quality issue called boar taint. Androstenone, a steroid hormone responsible for the development of boar taint, is produced in the testes at puberty (Patterson, 1968). Castration (removal of the testes) eliminates the production of 16-androstene steroids, i.e., androstenone, as well as other steroid hormones produced in the testes, including testosterone (Davidson & Levine, 1972). High levels of testosterone, and other androgens, have been frequently attributed to aggression in uncastrated males (Davidson & Levine, 1972; Signoret et al., 1975). Testosterone has also been reported to activate subcortical areas of the human brain to produce aggression (Batrinos, 2012). As well, chronic exposure to high doses of testosterone in mice have been shown to potentiate aggressive behaviours in intact males (Lumia et al., 1994). There is abundant evidence supporting the connection of androgens (testosterone) and aggression, but there are few studies published on the relationship between 16-androstene steroids (androstenone) and aggression in boars, providing the opportunity to examine if boar taint levels can be an indicator of low or high aggressive behaviour.

While castration does address the issues of both androgens that can lead to aggression and androstenone levels that can lead to boar taint, castration poses a welfare issue as it is a painful and stressful procedure for piglets (Prunier et al., 2006; von Borell et al., 2009). Castrated pigs also show reduced body weight at weaning and increased pre-weaning mortality compared to entire males (Morales et al., 2017). As well, removal of testosterone reduces nutrient utilization efficiency for protein deposition, resulting in poorer growth of barrows compared to boars (Dunshea et al., 1993; EFSA, 2004).

An alternative to surgical castration for reduction of boar taint may be genetic selection for pigs with low androstenone levels (Baes et al., 2013). While this approach addresses the welfare issue of castration, raising entire males adds a potentially different welfare issue, as boars generally display increased aggressive and sexual behaviours compared to raising barrows (Giersing et al., 2000). Aggression-related behaviours among group-housed pigs can negatively impact feed efficiency and contribute to reduced growth (Stookey & Gonyou, 1994). As well, aggression in group-housed pigs can result in health risks and injury, including carcass damage (Rydhmer et al., 2006). Increased stress levels that may be caused by aggression in the group when mixing before slaughter, can lead to meat quality issues such as dark, firm, dry (DFD) meat or pale, soft exudative (PSE) meat (Warriss & Brown, 1985). Specifically related to sexual behaviours, excessive mounting can result in injuries including lameness or leg fractures, which can lead to reduced growth or euthanasia (Rydhmer et al., 2006). However, research has shown there are many benefits to raising boars for pork production, which

could increase sustainability of the industry, including faster growth, leaner meat, and increased feed efficiency (EFSA, 2004).

In order to assess behaviour of boars and the impact of castration or lack of castration on behaviour, there are several objective tests that can measure behavioural responses to novel or threatening stimuli, including the open field test (OFT), novel object test (NOT) and human approach test (HAT) (Hessing et al., 1994; Magnani et al., 2012; Brajon et al., 2016). Behaviour recorded in the OFT is used to characterize locomotive activity, exploration and anxiety (Beilharz and Cox, 1967; Murphy et al., 2014). Specifically, this approach has been utilized to assess the effects of castration on anxiety-like behaviour and motor coordination in mice (Benice & Raber, 2009). The NOT is used to characterize the response of an animal upon introduction of an unfamiliar object to test anxiety and neophobic behaviours (stilted or avoidant behavioural response to novel physical objects; Barnes et al., 1976; Cavigelli, 2005; Murphy et al., 2014). The HAT observes latency of a pig to approach a human, which can characterize boldness or anxiousness, motivation to interact with humans and ease of handling (Hemsworth et al., 1981; Hemsworth et al., 1994; Murphy et al., 2014). Aggressiveness can be investigated through the use of a resident-intruder test (RIT), which involves introducing an unfamiliar pig to an individual in its home pen (Erhard et al., 1997). Aggressive or agonistic events can activate the hypothalamic-pituitary-adrenocortical (HPA) axis to elicit a stress response, so aggressive pigs may be more stressed than less aggressive pigs (Fernandez et al., 1994; Muráni et al., 2010)

With questions of welfare paired with a desire for improved productivity and meat quality in the pork industry, there is a need to explore alternative practices to male piglet castration. However, ways to address concerns about aggression and sexual behaviours and the resulting implications among group-housed pigs need to be better understood. The objective of this research is to explore fat androstenone levels in pigs and their effects on problematic behaviour concerns of entire males, including aggression, stress-related behaviour, and sexual behaviour. Individualized behaviour tests will be conducted to better characterize and compare aggressive, sexual, and stress-related behaviours between barrows, high androstenone boars, and low androstenone boars over the course of the growing period. Evidence of behaviour differences between high and low androstenone pigs may be useful in determining whether changes in current management practices may be required to raise uncastrated, low boar taint males for pork production. It is hypothesized that uncastrated males with low androstenone levels will show fewer signs of problematic and undesirable behaviours, including aggressive, stress-related and sexual behaviours, than males with high androstenone levels based on reduction of testicular steroids associated with aggression, but both high and low androstenone males will show more problematic and agonistic behaviours than barrows.

4.2 Materials and Methods

Animals and housing

All procedures were approved by the Animal Care Committee at the University of Guelph in accordance with the standards established by the Canadian Council on Animal

Care (1993, revised 2018). Fifty-four male pigs and 54 female pigs (Duroc x [Landrace x Yorkshire]) were sourced from Arkell Swine Research center, and 18 of the males were surgically castrated at 3 days of age. Pigs were mixed while transported at weaning (3 weeks; 6.7 ± 0.2 kg) to the Animal Science and Nutrition animal wing at the University of Guelph. Pigs were allocated randomly into 1 x 3 m plastic-coated metal grid floor pens of 4 (2 males, 2 females) across two rooms with identical conditions and blocked by weight at time of mixing. At 6 weeks of age (after individual behaviour tests), pigs were transported in two mixed groups to the University of Guelph Ponsonby Research station, where they were allocated to 1.8 x 3. 6m pens with the same pen mates across four rooms with identical conditions. Pens had solid concrete floors bedded with wood shavings that were cleaned daily, and metal spindle sides. Throughout the entire study, all pigs were fed the same *ad libitum* commercial diet, with free access to water from nipple drinkers. Lights were turned on at 0:800h and off at 20:00h.

Behaviour testing

At 6 weeks of age, all male piglets were subjected to three individual behaviour tests to characterize aggression, tendency to explore, reaction to a stressful situation, and fear of humans. The open field test (OFT; adapted from Brajon et al., 2016) and novel object test (NOT; adapted from Magnani et al., 2012; and Brajon et al., 2016) were performed consecutively on one day, and the human approach test (HAT; adapted from Brajon et al., 2016) was performed one day later. All three tests were video recorded using a digital camera (BT: Panasonic HDC-HS9PC, Newark, US) and analyzed using the EthoVision video tracking software (XT8.5, Noldus Information Technology,

Wageningen, The Netherlands). All variables measured for each test are described in Table 4.1. In the OFT, pigs were placed in a 9 m^2 square arena with plastic-coated metal grid flooring and tall, solid plastic walls, the test beginning as soon as pigs were placed in with all four legs in the arena with the test concluding after 5 minutes. Immediately after the conclusion of the OFT, the NOT began and a 20 cm plastic Jolly Ball (Jolly Pets, Streetsboro, OH) filled with sand was lowered from the ceiling into the exact centre of the arena. The NOT concluded after 5 minutes and pigs were returned to their home pen. In both tests, distance travelled (cm) and mean velocity (cm/s) were automatically recorded and frequency of escape attempts, defined as two front legs of the pig lifted against the wall, were recorded manually by an observer outside of the pen out of the pig's view. Upon analysis, the arena was divided into three circular zones around the ball, one 10 cm from the edge of the ball, one 30 cm from the edge of the ball and the rest of the arena. The frequency of contract with the ball, as well as frequency in the 10 and 30 cm zones were measured.

The HAT was performed one day later, with the piglet being placed in the start corner of the same testing arena. An experimenter was seated on a stool in the opposite corner from the start corner in the arena for the duration of the test, which concluded after 5 minutes. When the pig made contact with the experimenter, contact latency was recorded by another experimenter outside of the arena, out of the pig's view. The experimenter inside the arena then reached out slowly in an attempt to touch the snout. If contact was made, the experimenter would reach towards the ear to try to make contact. The following scores were given based on contact with the human:

Score = 4: the pig made no effort to approach the experimenter

Score = 3: the pig approached and made contact with the experimenter but did not allow experimenter to touch snout

Score = 2: the pig allowed contact with snout

Score = 1: the pig allowed contact with the snout and the ear

Score = 0: the pig allowed contact multiple times with snout and ear

During the HAT, distance travelled (cm) and mean velocity (cm/s) were automatically recorded, and escape attempts were manually recorded by an experimenter outside of the arena, as in the OFT and NOT. Upon analysis, the arena was divided into 7 zones radiating circularly from the experimenter, with zone 1 being the experimenter and zone 7 being the start corner. The frequency of entry in each of these zones was measured.

A resident intruder test (RIT) was performed at 15 weeks by isolating a male pig in their home pen and introducing a stranger female pig. All other pen mates were removed, and the test began as soon as the intruder female entered the pen, concluding after 5 minutes unless an escalated attack (>3 quick, successive bites to the neck/face area) occurred, in which case the pigs were separated quickly by experimenters and the female was returned to her home pen. Contact latency, attack latency, frequency of mounts and frequency of bites (not including attack) were manually scored for the male by the same observer standing outside of the pen. No variables were measured for the females. It was

ensured that pigs from two neighbouring pens were not used as a resident/intruder pair in a test. All frequencies are defined as number of occurrences over the duration of the test.

Performance and slaughter

Pigs were weighed weekly from 3 weeks of age until slaughter. Pigs were raised to market slaughter weight (average 123.9 ± 8.0 kg; 141.5 ± 5.9 days of age) and all males were transported to the University of Guelph Meat Science Laboratory (federally licensed CFIA abattoir; females were slaughtered separately, no data for females were recorded). Pigs were slaughtered by CO_2 stunning and exsanguination in three separate groups, each one week apart. Pigs were mixed during transport (approximately 30 minutes) and were placed back with their male pen mate for lairage (max. lairage time 3.5 hours). Blood was taken at slaughter in heparinized tubes, centrifuged, and plasma was separated and stored at -20ºC until further analysis. Backfat samples were taken at slaughter and stored at -20ºC until further analysis.

Sample analysis

Androstenone concentrations in plasma and fat were analyzed by specific ELISA (Squires and Lundstrom, 1997). Estrone sulfate was analyzed in plasma by specific radioimmunoassay (RIA) (Schwarzenberger et al. 1993). Skatole concentrations in fat were measured using High Performance Liquid Chromatography (HPLC) (Lanthier et al. 2007).

Statistical analysis

All data were analyzed using SAS University Edition 3.8 (SAS Institute, Cary, NC). Pigs were analyzed retroactively in three groups based on sex and fat androstenone concentrations at slaughter. Pigs were classified as high androstenone boars (n=18; fat androstenone ≥8.6 μg/g), low androstenone boars (n=18; fat androstenone ≤7.7 μg/g), and barrows (n=18; surgically castrated at 3 days). General linear mixed models (PROC GLIMMIX) were used to determine differences in behaviour variables between groups based on the following model:

$$Xij = \mu + \alpha i + \beta j + \varepsilon ij$$

Where Xij is the observed parameter, μ is the overall mean, αi is the experimental treatment group (high androstenone boars, low androstenone boars, barrows; fixed effect), βi is the room pigs were housed in (random effect) and εij is the residual error. Model residuals were assessed using scatter and box plots for the studentized residuals for homoscedasticity, Q-Q plot and Shapiro-Wilk test for normal distribution. Non normal data were analyzed using PROC GLIMMIX with Poisson distribution (distance, velocity, HAT score, HAT contact latency, RIT contact latency, RIT attack latency) or lognormal distribution (escape, contact with ball, 10 cm/30 cm from ball, HAT zones, mounts) and model residuals assessed as above, with Tukey adjustment for multiple comparisons. Statistical significance was defined as $p<0.05$ and trend defined as $0.05<p<0.1$.

4.3 Results

Open Field Test

During the open field test (OFT), distance travelled was significantly greater in low androstenone boars than in high androstenone boars ($p<0.001$) or barrows ($p<0.001$) but there was no difference in distance travelled between high androstenone boars and barrows (Table 4.2). There were no differences between groups for velocity ($p=0.54$) or escape attempts ($p=0.54$) during this test.

Novel Object Test

During the novel object test (NOT), distance travelled was significantly greater in low androstenone boars than in high androstenone boars ($p<0.001$) or barrows ($p<0.001$; Table 4.3). High androstenone boars also travelled further distance than barrows ($p<0.001$). There were no differences between groups for mean velocity ($p=0.17$), frequency to contact the ball ($p=0.34$), frequency to zone 10 cm from the ball ($p=0.40$), frequency to zone 30 cm from ball ($p=0.62$) or escape attempts ($p=0.54$) during this test.

Human Approach Test

During the human approach test (HAT), barrows had significantly lower human contact latency than both high androstenone ($p<0.001$) and low androstenone ($p<0.001$) boars (Table 4.4); however, high and low androstenone boars did not have significantly different contact latency times ($p=0.36$). There were no differences between groups for distance travelled ($p=0.83$), velocity ($p=0.25$), HAT score ($p=0.98$), escape attempts

(p=0.76) or frequency in any zones, including zone 2 closest to the human (p=0.22) or zone 7 furthest from the human (p=0.79).

Resident Intruder Test

During the resident intruder test (RIT), high androstenone boars had shorter contact latency than low androstenone boars (p<0.001) and barrows (p<0.001), but there was no difference in contact latency between low androstenone boars and barrows (p=0.91; Table 4.5). High androstenone boars had a longer attack latency than low androstenone boars (p<0.001) and barrows (p<0.001), but again there was no difference between low androstenone boars and barrows (p=0.11). Barrows did not mount at all, so mounting frequency differed between barrows and both high androstenone boars (p<0.001) and low androstenone boars (p<0.001), but there was no difference between high androstenone and low androstenone boars (p=0.98). There was no difference in frequency of bites amongst groups (p=0.12).

4.4 Discussion

The objective of this study was to explore androstenone levels in pigs and their effects on the negative and problematic behaviour concerns of entire males including aggression and sexual behaviour that are currently motivating castration. Individualized behaviour tests were conducted to better characterize and compare aggressive, sexual, and stress-related behaviours between high androstenone boars, low androstenone boars and barrows over the course of the growing period. It was hypothesized that

uncastrated males with low androstenone levels would show fewer signs of aggressive and sexual behaviour than males with high androstenone levels based on reduction of testicular steroids associated with aggression, but that both high and low androstenone boars would show more aggressive and sexual behaviours than barrows. It was also hypothesized that with reduction of aggression for barrows compared to low androstenone and high androstenone boars, barrows would show the least stress-related behaviour, low androstenone boars being intermediate, and high androstenone boars would show most stress-related behaviour. Although some differences were observed, for the most part, lower androstenone levels in uncastrated pigs did not translate to fewer signs of aggressive behaviour than high androstenone boars. Data from this study does not suggest major differences in aggressive behaviour or stress-related behaviour between high androstenone boars, low androstenone boars or barrows, inconsistent with what was hypothesized. In terms of sexual behaviour, the resident-intruder test showed more mounting among high androstenone boars than barrows, but no difference in mounting between high and low androstenone boars, suggestive of sexual behaviour issues with entire males, whether they have high or low androstenone levels, which can have negative implications for production (Rydhmer et al., 2006).

During the OFT and NOT, a greater distance travelled in low androstenone boars suggests a higher locomotive activity and exploratory behaviour of these pigs. These results do not support the hypothesis that for stress-related behaviours low androstenone boars would be intermediate to high androstenone boars and barrows. However, there are conflicting interpretations of what increased distance travelled means in terms of

aggressive or stress-related behaviours, so it is difficult to say whether low androstenone boars show overall more aggressive or stress-related behaviours based on this measure alone (D'Eath et al., 2009; Seibenhener & Wooten, 2015; O'Malley et al., 2018). The question of whether locomotive activity relates to emotionality or fearfulness has not been clearly answered yet. In a study of mice, there was no difference in ambulatory activity in an OFT between two strains of genetically induced highly anxious mice and wild-type mice, indicating this measure of behaviour may not indicate anxiety levels (Seibenhener & Wooten, 2015). These results collectively suggest that while low androstenone boars may show increased general activity, it is unlikely to be indicative of stress-related behaviour. In terms of how locomotive activity may relate to aggressiveness, D'Eath et al. (2009) found that aggressive pigs were less active, and O'Malley et al. (2018) found more aggressive pigs to be less bold in a HAT and less exploratory in a NOT. Based on the relationship between androgen concentrations and aggression, a link between activity level in the OFT and NOT and androgen concentrations could have been expected, with low androstenone boars being less aggressive and more active in the OFT and NOT (Davidson & Levine, 1972; Signoret et al., 1975). However, the lack of difference in activity between barrows and high androstenone boars does not support this hypothesis. There were no differences between any groups for escape attempts or interaction events with the novel object, suggesting no differences in fearfulness or neophobic behaviours amongst all groups of pigs (Seibenhener & Wooten, 2015).

During the HAT, barrows showed a shorter contact latency to the human than either group of boars, perhaps suggesting that they may be most motivated to seek human

contact and show less fearfulness of humans. However, there was no difference in HAT score amongst all three groups, which demonstrates that their interactions with humans did not differ notably. There are conflicting perspectives on the implications of shorter contact latency in the HAT, implying that it is either a positive or negative indication. Hemsworth et al. (1994) suggested that easier to handle pigs had shorter contact latency, influenced by reduced fear of the stockperson; however, Marchant-Forde (2002) found that gilts showing more human-directed aggression had shorter approach latencies in the HAT and related it to more “bold behaviour” characteristics. With these conflicting perspectives on contact latency, it is difficult to conclude the meaning of contact latency differences between boars and barrows in this test. However, it can be concluded that there are no differences between high androstenone and low androstenone boars in terms of human interaction. Further research is needed on the implications of contact latency differences for ease of handling and human-directed aggression.

During the RIT, high androstenone boars showed a shorter contact latency and longer attack latency than low androstenone boars and barrows; however, the longer attack latency may be due to the fact that high androstenone boars were more interested in mounting or displaying sexual behaviour (i.e. sniffing or investigating) towards the intruder females than attacking. It should be noted that sexual and aggressive behaviours were measured based on a female being the intruder rather than a boar or barrow, which may have resulted in differing behaviour from the resident male (Koolhaus et al., 2013). Exploration of defensive versus offensive aggression when using a male as the intruder would be more valuable for assessing differences in aggressive behaviours. For

measuring sexual behaviours, use of a female may be appropriate, but all sexual behaviour beyond mounting, including sniffing or nosing, grunting, chomping jaws, urination, or attempted mounting should be included (Hemsworth & Tillbrook, 2007).

Understanding mounting behaviour is important in assessing welfare risks based on castrating or not. Studies have shown that mounting may be the most detrimental behaviour in the context of raising boars (Hintze et al., 2013). Rydhmer et al. (2006) found that 15% of entire males and 6% of females showed health problems specifically involving lameness or injured legs/feet, most likely due to mounting. Some studies have found that lameness and injury from mounting occur more often in groups of all entire males than mixed-sex or all female groups (Hintze et al., 2013); however, other studies have found no differences in injury or lameness between all-female groups and all-boar groups (Rydhmer et al., 2006; van den Broeke et al., 2015). Frequency of mounting may be increased in an enriched environment without influence of pubertal maturation (Prunier et al., 2013). The RIT clearly demonstrated that the risk of sexual behaviours was eliminated by castration. The barrows did not mount at all, while the boars did, though there was no difference between high androstenone and low androstenone boars. Future studies should include assessment of additional sexual behaviours (i.e. sniffing, nosing, etc.) in analysis to learn the extent to which high androstenone and low androstenone boars may vary in these specific behaviours.

There were limitations within this study that may have impacted behavioural outcomes. The OFT, NOT and HAT were conducted when pigs were only 6 weeks old, before sexual development and pubertal androgen production would occur, so behaviour

differences may not have developed until pigs grew closer to puberty. Plasma hormone profiles suggest an increase in testicular steroids begins with pubertal development around 16-20 weeks of age, although this is variable between individuals and breeds (Schwarzenberger et al., 1993). In the RIT, a female was used as the intruder based on situational availability, which may have increased mounting behaviour rather than inducing offensive aggression. Pigs had been transported between three locations and handled often (fed twice daily, weighed weekly), so they may have been more desensitized to novel stimuli than typical commercial pigs, reducing extrapolation of the results from this study. This study also was limited by a small sample size.

While results of aggressive and stress-related behaviours were largely inconclusive in this study, and do not provide answers to managing behavior issues among boars, past research has identified management strategies that may help to reduce aggression in boars. Fewer fights have been observed in pigs raised in groups with clear weight or size differences (Andersson et al., 1999) and pigs raised in sibling groups (farrow-to-finish) also show reduced frequency of aggression with no difference between boars and barrows (Fredriksen et al., 2008). Implementing these strategies in pork production may allow for easier management if a switch to raising entire males was made.

It is believed that ending male piglet castration would eliminate welfare concerns associated with the procedure (Prunier et al., 2006) and improve production traits (EFSA, 2004). However, aggressive and sexual behaviours seen in entire males have the potential to become a new welfare concern (von Borrell et al., 2020). Behaviour observed in specific tests in the present study provide preliminary data suggesting there may not

be meaningful differences between high androstenone boars, low androstenone boars or barrows in terms of stress-related behaviour. Sexual behaviour, as measured by mounting activity, was increased in both high and low androstenone boars compared to barrows, which may be another issue in entire male production requiring further exploration. This study provides evidence to encourage further study on androstenone levels in boars in order to be able to provide a solution to castration that will allow intact males to be raised in a safe and productive environment. Specifically, additional studies on sexual behaviour as well as additional measures of aggression based on pen-wise observation can provide insight into viable pen management practices.

4.5 Tables and Figures

Table 4.1: Description of all behaviour tests and variables measured

Behaviour test	*Age at time of test*	*Variables measured*	*Brief test description*
Open field test (OFT)	6 weeks	Total distance travelled (cm) Mean velocity (cm/s) Frequency of escape attempts	Each piglet was individually carried to a testing room and place in the corner of a square test arena ($9m^2$) with plastic-coated metal grid flooring and solid plastic walls. The test began as soon as the piglet was placed in the arena and concluded after 5 minutes.
Novel object test (NOT)	6 weeks	Total distance travelled (cm) Mean velocity (cm/s) Frequency of escape attempts Frequency of contact with ball Frequency in zone 10cm/30cm around ball	Immediately after the OFT, the NOT began and a 20cm plastic Jolly Ball was lowered from the ceiling into the exact centre of the arena with the test concluding after 5 minutes.
Human approach test (HAT)	6 weeks	Total distance travelled (cm) Mean velocity (cm/s) Frequency of escape attempts Latency to contact experimenter (s) HAT score (0-4) Frequency in zone 1-7	One day after the OFT and NOT, each piglet was placed in the start corner of the same testing arena. An experimenter was seated on a stool in the opposite corner from the start corner in the arena for the duration of the test. A HAT score was assigned based on degree of contact the experimenter could make with the pig. The test concluded after 5 minutes. Upon analysis, the arena was divided into 7 zones radiating circularly from the experimenter, with zone 1 being the experimenter and zone 7 being the start corner.
Resident intruder test (RIT)	15 weeks	Contact latency (s) Attack latency (s) Frequency of mounts Frequency of bites	All males were matched with a female of similar or slightly lighter weight (each pig was only used once). The resident (male) was the only pig in its home pen, an unfamiliar female (intruder) was brought to the resident's pen and the test started as soon as the female pig entered the pen. Each test lasted 5 minutes, or until escalated attack occurred. No variables were recorded for females.

Table 4.2: Least squares means for variables measured during open field test (OFT) for pigs at 6 weeks of age

	Mean value				
	High androstenone boars (n=18)	Low androstenone boars (n=18)	Barrows (n=18)	s.e.m.	p-value
Total distance travelled (cm)	5706.2[b]	6205.9[a]	5752.6[b]	267.09	<0.001
Mean velocity (cm/s)	19.06	20.60	19.27	0.89	0.54
Frequency of escape attempts	0.81	0.60	0.52	0.18	0.54

[a-c] Least square means for treatments without a common superscript differ significantly ($p<0.05$) within a row

Table 4.3: Least squares means for variables measured during novel object test (NOT) for pigs at 6 weeks of age

	Mean value			s.e.m.	p-value
	High androstenone boars (n=18)	Low androstenone boars (n=18)	Barrows (n=18)		
Total distance travelled (cm)	4069.7[b]	4557.7[a]	3822.4[c]	259.8	<0.001
Mean velocity (cm/s)	13.60	15.20	12.80	0.87	0.17
Frequency of escape attempts	1.18	0.48	0.85	0.33	0.29
Frequency to touch ball	0.11	0.33	0.17	0.10	0.34
Frequency in zone 10cm from ball	0.99	1.31	0.55	0.26	0.40
Frequency in zone 30cm from ball	6.72	9.0	8.89	0.86	0.62

[a-c] Least square means for treatments without a common superscript differ significantly ($p<0.05$) within a row

Table 4.4: Least squares means for variables measured during human approach test (HAT) for pigs at 6 weeks of age

	Mean value				
	High androstenone boars (n=18)	Low androstenone boars (n=18)	Barrows (n=18)	s.e.m.	p-value
Total distance travelled (cm)	3938.5	3593.9	3425.8	351.6	0.83
Mean velocity (cm/s)	12.98	12.14	11.04	1.17	0.25
Frequency of escape attempts	0.17	0.78	0.76	0.16	0.76
HAT score	2.58	2.66	2.60	0.11	0.98
Latency to contact human (s)	187.95[a]	181.61[a]	149.07[b]	15.19	<0.001
Frequency in zone 2 (closest to human)	3.53	2.26	2.04	0.53	0.22
Frequency in zone 7 (furthest from human)	1.16	1.11	1.00	0.34	0.79

[a-c] Least square means for treatments without a common superscript differ significantly ($p<0.05$) within a row

Table 4.5: Least squares means for variables measured during resident intruder test (RIT) for pigs at 15 weeks of age

	Mean value				
	High androstenone boars (n=18)	Low androstenone boars (n=18)	Barrows (n=18)	s.e.m.	p-value
Contact latency (s)	11.67[b]	21.06[a]	21.39[a]	2.81	<0.001
Attack latency (s)	251.00[a]	193.78[b]	184.17[b]	14.11	<0.001
Frequency of mounts	2.70[a]	2.43[a]	0.00[b]	3.71	<0.001
Frequency of bites	0.22	0.44	0.06	0.11	0.12

[a-c] Least square means for treatments without a common superscript differ significantly ($p<0.05$) within a row

CHAPTER 5 COMPARING BOAR TAINT COMPOUNDS AND MEAT QUALITY CHARACTERISTICS OF BARROWS, HIGH AND LOW ANDROSTENONE BOARS

5.1 Introduction

From both producer and consumer perspectives, meat quality in the pork industry is of great importance. The quality of meat is assessed at multiple points in the consumer's experience with the product, during purchasing, cooking and then eating, and there are several sensory characteristics that are assessed, including colour, odour, texture and flavour. For the consumer, an undesirable odor can cause them to assume a meat product is rancid, even when the meat is fresh, but the odour is actually the presence of boar taint (Font i Furnols et al., 2008). Boar taint is the unpleasant odour or taste caused by the accumulation of the compounds androstenone and skatole in the fat from uncastrated male pigs (Patterson 1968; Vold 1970; Bonneau, 1998). Currently, the most common solution to boar taint is castration of male piglets; however, due to animal welfare concerns, producers are turning away from this practice and searching for alternatives (reviewed in Bonneau & Weiler, 2019).

Studies have shown there are differences in meat quality in barrows versus boars. Meat from castrated male pigs has a higher intramuscular fat content (increased marbling) and ultimate pH, more intense flavour and is more tender and juicier than meat from boars (Pauly et al., 2012). Comparatively, uncastrated male pigs have a reduced feed conversion ratio, which can lead to more efficient and sustainable production, and reduced fat levels, which leads to leaner meat that is healthier for consumption (EFSA,

2004; Pauly et al., 2012). These differences factor into consumers' assessment of meat quality and therefore need to be taken into consideration when evaluating the pros and cons of castrating pigs.

Genetic selection of pigs that produce low levels of boar taint compounds can be an alternative to surgical castration to reduce boar taint (Baes et al., 2013). In favour of genetic selection as a long-term alternate approach to castration, the impact on meat quality of genetically selected low boar taint pigs appears to be low, yet there is an improved feed conversion ratio and increased lean meat yields from boars compared to barrows (Dugué et al., 2020). Some studies have suggested low genetic correlations between fat androstenone or skatole concentrations and carcass composition or daily gain (Merks et al., 2010; Windig et al., 2013; Strathe et al., 2013). However, meat quality traits were not extensively investigated in these studies. Recently, Dugué et al. (2020) found that selection for reduced fat androstenone concentrations in crossbred (Pietran x Landrace) pigs had positive effects on feed conversion ratio, number of skin lesions shortly after mixing, loin eye area, drip loss, carcass yield and lean meat percentage; however, selection negatively impacted testosterone concentrations which could pose issues for reproduction in stud boars. Overall, selection for low boar taint pigs seems to be a viable long-term solution, and it is important to determine the effects of selecting these pigs on quality of pork products.

While there are welfare advantages to raising uncastrated males, there are also challenges that need to be addressed in considering meat quality. Potential behavioural issues such as mounting and aggression, can result in injury and stress levels that can

be detrimental to quality pork production. Increased long-term stress levels can increase the incidence of dark, firm, dry (DFD) meat, an undesirable trait found more commonly in boars than castrates or females (Pauly et al., 2012; Bonneau & Weiler, 2019). Acute stress from fighting (aggressive behaviour) just before slaughter can cause pale, soft exudative (PSE) meat, another undesirable trait, although the incidence of PSE pork is similar between boars and castrates (Warriss & Brown, 1985; Barton-Gade, 1987; Gispert et al., 2010). PSE is often found in pigs with porcine stress syndrome (PSS), or in pigs that experience acute stress ante-mortem (Adzitey & Nurul, 2011). PSE meat can become tough and flavourless once cooked due to high drip loss (Miller, 2002). DFD is found in pigs with glycogen depletion often caused by aggressive behaviour, higher general activity levels, or long-term stress before slaughter (Adzitey & Nurul, 2011). DFD meat can spoil more easily and is less desirable to consumers than meat that is classified as normal (Miller, 2002).

Acknowledging these risks, there are management practices that may help reduce aggression and fighting in uncastrated males, including raising pigs in sibling (farrow-to-finish) groups, which may also have the positive effect of keeping androstenone levels low compared to mixing litters (Fredriksen et al., 2006; Fredriksen et al., 2008). However, transportation and slaughter generally involve mixing of unfamiliar animals, resulting in fighting, injuries and acute stress (reviewed in Faucitano, 2018). It may be necessary to implement particular management strategies when raising and handling boars with low boar taint to keep aggression and mounting levels to a minimum such that meat quality is not impacted.

The objective of this research is to compare meat quality characteristics of uncastrated males with high and low androstenone concentrations, as well as barrows as a control. This will help highlight possible implications for meat production when raising entire males with low boar taint. It is hypothesized that on the key metrics of leanness, loin colour, and juiciness, low androstenone boars will be intermediate to barrows and high androstenone boars based on the relative levels of testicular steroids, including androstenone.

5.2 Materials and Methods

Animals and Housing

All procedures were approved by the Animal Care Committee at the University of Guelph in accordance with the standards established by the Canadian Council on Animal Care (1993, revised 2018). Fifty-four male pigs and 54 female pigs (Duroc x [Landrace x Yorkshire]) were selected from Arkell Swine Research center. One third (n=18) of the males were surgically castrated at 3 days old. Pigs were moved at weaning (3 weeks; average weight 6.7 ± 0.2 kg) to the Animal Science and Nutrition Animal Wing at University of Guelph. All pigs were mixed together at transport and randomly allocated into pens of 4 (2 males, 2 females; boars kept separate from barrows) across two rooms with identical conditions, blocked by weight at time of mixing. Pens were 1 x 3 m with plastic-coated metal grid flooring. At 6 weeks (average weight 11.0 ± 2.1 kg), pigs were transported in two mixed groups to the University of Guelph Ponsonby Research Station, where they were kept with the same pen mates, and groups were randomly allocated to

1.8 x 3.6 m pens across four rooms with identical conditions. Pens had solid concrete floors bedded with wood shavings that were cleaned daily, and metal spindle sides. Lights were turned on at 08:00h and off at 20:00h. Throughout the study, all pigs were fed the same commercial diet *ad libitum,* with free access to water from nipple drinkers.

Biochemical analysis

Blood was taken at slaughter in heparinized tubes and plasma was separated by centrifugation and stored at -20ºC until further analysis. Backfat samples were taken at slaughter and stored at -20ºC until further analysis. Androstenone concentrations in plasma and fat were analyzed by specific ELISA, (Squires & Lundstrom, 1997). Estrone sulfate was analyzed in plasma by specific radioimmunoassay (RIA) (Schwarzenberger et al. 1993). Skatole concentrations in fat were measured using High Performance Liquid Chromatography (HPLC) (Lanthier et al. 2007). Cortisol in plasma was measured using a commercial cortisol coated tube RIA kit (MP Biomedicals, Orangeburg, NY).

Slaughter, carcass and meat quality analysis

Pigs were raised to market slaughter weight (123.9 ± 8.1 kg; 141.5 ± 5.9 days of age) and transported to University of Guelph Meat Science Laboratory and slaughtered using standard commercial slaughter procedures. Data were only collected from male pigs in this study and females were shipped to slaughter separately from males. Pigs were chosen by pen based on the heaviest male reaching >120 kg body weight and being in the top 18 males for body weight, and slaughtered in three separate groups, each one week apart with slaughter beginning at approximately 07:30h. Pigs were mixed during

transport (approximately 30 minutes) and were placed back with their male pen mate for lairage at the abattoir (max. lairage time 3.5 hours). After stunning using CO_2 and exsanguination, carcasses were weighed 30 to 40 minutes post-mortem. A Hennessy probe was used between the third and fourth last rib of the left side of each carcass, 7 cm off the midline for estimation of carcass lean content (lean probe mm) and fat carcass content (fat probe mm). Skin lesion scores were measured subjectively based on the following, adapted from van Staaveren et al. (2015): 0 = no injuries; 1 = one small (≤2 cm) superficial (pale red) lesion; 2 = more than one small (≤2 cm) superficial (pale red) lesion or just one red (deeper than score 1) but still superficial lesion; 3 = one or several large (≥2 and ≤5 cm) and deep lesions; 4 = one very large (>5 cm), deep and red lesion or many large, deep red lesions; 5 = many very large (>5 cm) deep and red lesions covering the skin area.

The following methods were performed by staff at the University of Guelph meat lab. Approximately 45 minutes post-mortem, carcasses were placed in a 2ºC chill cooler. At 1-hour post-mortem, pH of the longissimus muscle (LM) between the third and fourth rib were taken using a Hanna Instruments pH meter with a spear tipped electrode attached (Hanna Instruments, USA).

After a 24-hour chilling period, the loin was cut at the grading site (between third and fourth last ribs) into two sections to expose the longissimus muscle (LM) interface. An experienced carcass evaluator assessed the following carcass measurements: subcutaneous back fat depth (mm); loin length (mm); loin depth (mm); loin eye area

(mm^2), measured by tracing on acetate paper and quantified by an electronic planimeter (MOP; Carl Zeiss, Inc.).

Six 3.0 cm thick chops were cut from the LM and individually identified. One chop was used for subjective evaluation of colour, firmness, wetness and marbling, objective colour, ultimate (24 hour) pH measurement, and drip loss determination. Four remaining chops were saved for determining Warner-Bratzler shear force (WBSF) and aged for 2 days (2 chops) or 7 days (2 chops). All chops were vacuum packaged and stored at -20ºC prior to analysis, excluding the one used for colour, pH and drip loss measurement.

The LM chop that was used for colour, firmness, wetness, marbling, pH and drip loss determination was oxygenated for 30 min on butcher paper prior to analysis. Subjective evaluation of LM chop conducted by meat lab personnel was comprised of the following:

i. Muscle colour score based on National Pork Producers Council (NPPC 2000) on a six-point scale: (1 = pale pinkish gray to white; 2 = grayish pink; 3 = reddish pink; 4 = dark reddish pink; 5 = purplish red; 6 = dark purplish red) and the Canadian Pork Quality Standards (CPI 2013) six-point scale.

ii. Muscle firmness score based on the National Pork Producers Council (NPPC 2000) on a three-point scale: (1 = soft – cut surfaces distort easily and are visibly soft; 2 = firm – cut surfaces tend to hold their shape; 3 = very firm – cut surfaces tend to be very smooth with no distortion of shape).

iii. Muscle wetness score based on the National Pork Producers Council (NPPC 2000) on a three-point scale: (1 = exudative – excessive fluid pooling on cut surfaces; 2 = moist – cut surfaces appear moist, with little or no free water; 3 = dry – cut surfaces exhibit no evidence of free water).

iv. Japanese colour score using plastic Japanese Meat Grading Association colour standards (Nakai et al., 1975) on a six-point scale: (1 = extremely pale pink to gray; to 6 = dark purplish red).

v. Marbling score based on the National Pork Producers Council (NPPC 2000) on a ten-point scale: (1 = devoid of marbling; to 10 = very abundant marbling) and the Canadian Pork Quality Standards (CPI 2013) on a six-point scale: (0 = devoid of marbling; to 6 = very abundant).

Colour was also objectively measured at 3 locations on each chop using a Minolta CR-400 with Spectra QC-400 software (Folio Instruments, Kitchener, ON) and illuminant D65 (2^{o} viewing angle). Colour data for *L**, a measure of luminosity with a higher value of being indicative of a lighter colour, were collected in the Commission International de l'Eclairage (CIE) *L*a*b** scale.

Ultimate pH was determined from the average of three measurements taken for each chop 24 hours post-mortem using a smart foodcare spear tipped electrode attached to a Hanna Instruments pH meter (Hanna Instruments, USA). Drip loss was then determined using the E-Z cup method described by Rassmussen and Andersson (1996).

Frozen LM chops were prepared for WBSF determination by allowing to thaw overnight at 2ºC. Once thawed, chops were trimmed of external fat, weighed and cooked using a Garland Grill (ED-30B broiler, Garland Commercial Range Ltd., Mississauga, ON) to an internal end point temperature of 74ºC. Cooking temperatures were continuously monitored, and initial and final temperatures recorded using a thermocouple inserted in the geometric center of each chop. Chops were turned after reaching an internal temperature of approximately 40ºC, and cooked weight was recorded once target temperature endpoint was reached to determine cooking losses. Chops were placed in individual bags, sealed, and immediately chilled in ice water, then stored at 2ºC for 24 hours before coring. Prior to coring, chops were allowed to equilibrate to room temperature. Six 1.27 cm meat cores were removed parallel to the muscle fibers from each chop using a drill press-mounted corer, then cores were sheared using a Warner-Bratzler blade on a TA-XT Plus texture analyzer (Texture Technologies Corp., Scarsdale, NY) with crosshead speed set at 3.3 mm/s. Peak shear force was determined using a custom macro program in Stable Microsystems Exponent software, and average of the 6 peak force measurements was taken as the shear force value for each loin chop.

Statistical analysis

All data were analyzed using SAS University Edition 3.8 (SAS Institute, Cary, NC). A one-way ANOVA (PROC MIXED) was used to compare variables between groups using the following model:

$$Xji = \mu + \alpha i + \beta j + \varepsilon ij$$

Where Xij is the observed parameter, μ is the overall mean, αi is the experimental treatment group (high androstenone boars, low androstenone boars, barrows – determined by fat androstenone levels at slaughter; fixed effect), βi is the slaughter group (first, second or third slaughter date; random effect), and εij is the residual error. Hot carcass weight was used as a covariate for the following variables with no interaction effects present: probe backfat, probe loin depth, ruler backfat, and ruler loin depth. Model residuals were assessed using scatter and box plot of studentized residuals for homoscedasticity, Q-Q plot and Shapiro-Wilk test for normal distribution. Non-normal data (probe backfat, ruler backfat) were transformed with PROC GLIMMIX with Poisson distribution. All means for transformed data are presented as back-transformed values. Statistical significance was defined as $p<0.05$ and trend defined as $0.05<p<0.1$.

5.3 Results

All male pigs were analyzed in groups of high androstenone boars (fat androstenone ≥ 8.6 ng/mL; n=18), low androstenone boars (fat androstenone ≤ 7.7 ng/mL; n=18), and barrows (surgically castrated; n=18). Table 5.1 summarizes concentration of hormones and boar taint compounds measured in plasma and fat at slaughter. Fat androstenone, plasma androstenone, and estrone sulfate (E1S) concentrations were highest in high androstenone boars, intermediate in low androstenone boars and lowest in barrows (all $p<0.001$). Cortisol was significantly higher in low androstenone boars than barrows at slaughter ($p=0.007$), with no differences in cortisol between high androstenone and low androstenone boars ($p=0.16$), or between high androstenone boars and barrows

(p=0.17). Skatole levels were very low for all groups and did not differ significantly between any groups (p=0.58).

Hot carcass weight (p=0.54), average daily gain (p=0.82), and skin lesion scores (p=0.67) were not significantly different between groups (Table 5.2). Backfat thickness was lower in low androstenone boars compared to barrows for both Hennessey probe (p=0.017) and ruler measurements (p=0.018), with no differences in backfat thickness between high androstenone and low androstenone boars with either method (p>0.76). High androstenone boars tended to have thinner backfat than barrows based on Hennessey probe measurements (p=0.087), and significantly thinner backfat than barrows with ruler measurement (p=0.018). Loin depth measurements using both Hennessey probe (p=0.55) and ruler (p=0.18) did not differ amongst high androstenone boars, low androstenone boars, or barrows. Loin eye area did not differ between groups (p=0.50).

Table 5.3 summarizes colour scores for longissimus muscle (LM) chops taken from barrows and high and low androstenone boars. The National Pork Producers Council (NPPC) LM colour score was lower (indicating lighter colour) for barrows compared to high androstenone boars (p=0.050). There was a trend for LM colour scores to differ between barrows and low androstenone boars (p=0.073), with no difference in LM colour score between high and low androstenone boars (p=0.99). There was also a trend for Canadian Pork Council (CPC) LM colour scores to be lower (indicating lighter colour) for barrow compared to high androstenone boars (p=0.052); however, CPC colour scores did not differ between barrows and low androstenone boars (p=0.15) or between low and

high androstenone boars (p=0.88). Minolta *L** values tended to be higher indicating lighter meat in barrows than high androstenone boars (p=0.08) but there was no difference in *L** values between barrows and low androstenone boars (p=0.13) or high and low androstenone boars (p=0.98). Japanese colour scores did not differ between groups (p=0.12).

Meat quality parameters (excluding colour) are summarized in Table 5.4. There were no differences (p>0.05) in any meat quality parameters measured between high androstenone and low androstenone boars, except CPC marbling scores were significantly higher in low androstenone boars versus high androstenone boars (p=0.02). The CPC marbling score was greater for barrows than both high androstenone (p<0.001) and low androstenone boars (p=0.014). The NPPC marbling scores were higher in barrows than high androstenone boars (p=0.003), but there were no differences in marbling scores between high androstenone and low androstenone boars (p=0.18), and barrows tended to have higher marbling score than low androstenone boars (p=0.095). There were no differences amongst experimental groups for firmness (p=0.57) and wetness scores (p=0.34), as well as 1-hour and 24-hour loin pH values (p=0.11 and p=0.18, respectively). Shear force after 2 days ageing was significantly lower for barrows than for high androstenone boars (p=0.01) and tended to be lower for barrows than low androstenone boars (p=0.09). Shear force after 7 days ageing was not different between any groups (p=0.15). Cooking loss after 2 days ageing was not different between any groups (p=0.38). Cooking loss after 7 days ageing was significantly lower for barrows than high androstenone boars (p=0.01) but did not differ between barrows and low

androstenone boars (p=0.61). Drip loss was significantly higher for barrows than low androstenone boars (p=0.049) but did not differ between barrows and high androstenone boars (p=0.14). There was no incidence of pale, soft exudative (PSE) meat ($L^* > 50$, drip loss > 5%, and pH 24 h < 6.0) or dark, firm, dry (DFD) meat ($L^* < 42$, drip loss < 5%, and pH 24 h ~ 6.0) in any of the experimental groups (thresholds as defined by Warner et al., 1997).

5.4 Discussion

Raising boars offers a welfare friendly alternative to surgical castration and is generally more efficient and favourable for pork production. However, there is concern that meat quality from boars may be compromised compared to meat from barrows due to boar taint. This study sought to explore this concern by comparing the meat quality characteristics of boars with high and low androstenone concentrations and barrows. The examination of meat quality characteristics for boars with high and low androstenone levels, relative to castrated males (barrows), confirmed some expected industry differences for uncastrated versus castrated pigs, namely decreased backfat thickness and less marbling in all boars. It also revealed some differences among boars based on levels of androstenone but did not fully confirm the hypothesis that on the key metrics of leanness, juiciness, and loin colour, low androstenone boars would be intermediate to barrows and high androstenone boars. In fact, high and low androstenone boars did not show many differences in meat quality, other than slightly lower marbling scores for LM chops from high androstenone boars. It was found that low androstenone boars did not

differ from high androstenone boars or barrows in their colour scores, marbling scores, and 7-day cook loss percentage. Backfat thickness of high and low androstenone boars were both found to be significantly lower than backfat thickness of barrows when measured by ruler. When measured by probe, only low androstenone boars were found to have significantly thinner backfat than barrows. With thicker backfat, meat from barrows may have increased meat firmness, and less fat separation and composition changes in muscle than meat from boars that have thinner backfat (Wood et al., 1986). However, these differences may not be noticeable by consumers. While backfat thickness has been shown to be correlated with shear force, previous literature has shown that backfat is not significantly correlated with meat tenderness when measured by a tasting panel (Rhodes, 1970; Blanchard et al., 2000).

The differences in backfat between groups seen in the present study may play less of a role in taste and more of an important role in visual consumer appeal. Wood et al. (1986) found that attractiveness of pork was greatest when backfat thickness was between extreme values. When comparing backfat thicknesses between 8mm and 16 mm, meat with 11 mm of backfat was preferred, with attractiveness of the meat decreasing below and above this value (Wood et al., 1986). In the present study, backfat thickness in all groups when measured by ruler and probe exceeded 16 mm and differences between groups were less distinct than in the study by Wood et al. (1986), thereby limiting the generalisability of this finding.

Further differences between groups were observed in marbling scores between groups. High androstenone boars were found to have significantly lower marbling scores

than barrows but did not differ from low androstenone boars when measured with the NPPC marbling score. All three groups significantly differed when measured using the CPC marbling score, with high androstenone boars and barrows having the least and most amount of marbling, respectively. However, differences observed in NPPC and CPC marbling score may not be noticeable by consumers given that there was only a difference of 0.78 from lowest to highest on the 10-point NPPC scale, as well as a difference of only 1.34 from lowest to highest on the 6-point CPC scale.

The present study also found that meat from high androstenone boars was significantly darker (reddish pink) than meat from barrows (grayish pink to reddish pink) but not different from low androstenone boars (reddish pink) when compared using the NPPC colour score. While not significantly different ($0.05<p<0.1$) in measurements of CPC colour score and Minolta *L** colour, meat from high androstenone boars similarly trended towards being darker than meat from barrows but did not differ from low androstenone boars. Japanese colour score did not significantly differ between any groups, again supporting that the differences between meat chops are small and likely not discernable to untrained consumers.

These findings suggest that barrow meat is lighter and slightly more marbled than meat from high androstenone pigs. Differences in the meat quality characteristics of colour and marbling between the meat from high and low androstenone boars were not significant, with the exception of a small difference in CPC marbling score, suggesting that any differences would likely not be discernable by the consumer. Since meat from barrows possessed increased marbling, this may indicate the meat is more tender and

less chewy (Lonergan et al., 2007), and therefore, barrow meat may be more desirable to the consumer than meat from either group of boars. However, consumer trends for colour, fattiness, and marbling may be influenced by individual preferences and traits. One study found that people generally fall into two types of pork preference-based clusters, with approximately 59% preferring lean, light red meat with less drip and marbling, and 41% preferring leaner, dark red meat (Ngapo et al., 2010). Moreover, in a study of 412 consumers, researchers found that consumer preference with regard to pork colour, marbling and leanness was influenced by the consumer's age, gender, urban or rural designation, and affinity for pork (Fortomaris et al., 2006). However, by today's consumer standards, leaner meat is becoming increasingly desirable based on the trend toward lower saturated fat consumption as part of a healthier approach to eating (Pauly, 2012; Mukumbo & Muchenje, 2016). A USDA study in 2006 found that common cuts of fresh pork contained, on average, 16% less total fat and 27% less saturated fat than the previous standard reference published in 1991 (Patterson et al., 2009). In conjunction with the present study's results, meat from high and low androstenone boars would be more suited to consumers with a preference for darker, less marbled meat with thinner backfat, whereas meat from barrows would be suited for consumers who prefer lighter, more marbled meat.

The tenderness of the meat for each group in the present study was also compared through shear force measurements at 2 days and 7 days post-mortem. As expected, shear force values decreased with time for all groups. At 2-day ageing, the shear force values for meat from high and low androstenone boars were significantly greater than

shear force values for barrow meat. Shear force at 7 days post-mortem were not statistically different between groups; however, shear force values in meat from barrows were still lower than in meat from both high and low androstenone boars. This finding is consistent with other observations. Barrows would be expected to have greater tenderness, as marbling is positively correlated with meat tenderness (DeVol et al., 1988; Noidad, 2019). This likely indicates that barrow meat is generally more tender and is likely acceptable to consumers. Present study results also indicate that there are no discernable differences in the tenderness of meat between high and low androstenone boars.

Juiciness of the meat also appeared to differ between groups. Juiciness has been described as being determined by the water content, intramuscular fat content, and saliva produced during chewing (Aaslyng et al., 2003). Meat from barrows had significantly lower cooking loss percentage at 7 days post-mortem than meat from high and low androstenone groups, suggesting that meat from barrows retains more water content and is juicier than meat from boars (Aaslyng et al., 2003). Again, this is congruent with findings that barrows had increased marbling, as increased visual intramuscular fat is associated with greater water retention and juiciness (DeVol et al., 1988; Noidad, 2019). In contrast to this, other research found no correlation between water holding capacity and intramuscular fat content (Wantanabe et al., 2018). This may explain the contradictory finding that drip loss was significantly greater in meat from barrows than meat from low androstenone boars.

Another influencer of meat quality can be cortisol levels, indicative of pre-slaughter stress (Bradshaw et al., 1996). Increased cortisol levels have been associated with higher pH values (initial and 24-hours post-mortem), increased backfat, decreased Minolta *L** and *b** values (indicating darker colour) and lower drip loss after 24 and 48 hours, all of which can increase risk for dry, firm, dark (DFD) meat (Moss & Robb, 1978; Škrlep et al., 2009; Dokmanovic et al., 2015). A meta-analysis from 2012 found lower incidences of DFD meat in barrows than in boars, likely due to increased general activity of boars (Pauly et al., 2012). Other studies have correlated higher cortisol concentrations at slaughter to a rapid decline in pH post-mortem and increased drip loss, which can increase risk for pale, soft, exudative (PSE) meat (Choe et al., 2015). The present study showed zero incidences of DFD or PSE meat. Cortisol levels at slaughter were greater in low androstenone boars than in barrows, which may be indicative of greater handling and transport stress for low androstenone boars. While it is possible this could be due to increased aggression and fighting among these boars, this behaviour aspect was not recorded at slaughter. Many studies have found increased number of skin lesions on boars than barrows, indicating more aggression and fighting (Paetkau & Whiting, 2008; Babol & Squires, 1995; Bakus et al., 2016). However, lack of difference in skin lesion scores in the present study suggests no difference in fighting between the groups of pigs, which does not support this hypothesis. In the present study, pigs were held in lairage pens with the same previous male pen mate to reduce fighting and stress rather than being mixed in larger groups. Mixing groups can increase fighting, and larger groups (i.e. 30 pigs) have been seen to spend more time standing and fighting than smaller groups

(i.e. 10 pigs; Rabaste et al., 2007). This may be causing fewer skin lesions and overall reduction of fighting and stress for pigs in the present study than what would be normally expected from commercial settings with larger groups in transport and lairage, explaining lack of difference between boars and barrows. Future research might consider observing fighting among boars during transportation and lairage, as well as measuring cortisol concentrations at the farm before transport and at slaughter to better understand the relationship between cortisol level differences in high and low androstenone boars.

Skatole is another boar taint compound affecting sensory perceptions and meat quality. In the present study, skatole levels in all groups of pigs were very low, and zero pigs were over the skatole acceptance threshold level, which is generally 0.2 µg/g in the fat (222.2 ng/mL liquid fat; Morlein et al., 2016). Since skatole is formed through microbial digestion of tryptophan in the hindgut, skatole levels can be dependent on management factors such as genetics, season (temperature), stocking rates or hygiene (Claus et al., 1994; Hansen et al., 1994). In the present study, pigs were kept in solid-floor pens with wood shavings and cleaned daily, which can improve hygiene. Hygiene has been found to be very important in terms of skatole accumulation, as studies have shown highly soiled pigs have significantly increased skatole concentrations than barely soiled pigs (Hansen et al., 1995; Thomsen et al., 2015). With only 4 pigs per 1.8 x 3.6 m pen after 6 weeks of age, stocking rate was 1.62 m^2 per pig in the present study, which is relatively low. High stocking rates (i.e. 0.6m^2 per pig) have been shown to significantly increase skatole levels in backfat compared to low stocking rates (≥1.2m^2 per pig; Hansen et al., 1994). Based on increased hygiene and low stocking rates in the present study, skatole levels may not

be generalizable to commercial settings where decreased hygiene and increased stocking rates can occur.

Levels of androstenone measured in the present study were found to be much higher than typical literature values and above general acceptance levels. This is important to note given the impact of androstenone concentrations on sensory perception of boar taint, although studies have documented that boar taint is highly variable between pigs, as well as highly variable between consumers perceiving the odour (reviewed in Font i Furnols, 2012). Generally, acceptance levels of androstenone are below 1.0 ug/g in the fat (Morlein et al., 2016). It is possible that levels of fat androstenone measured in the present study were found to be much higher due to error in the analysis, or genetic influence. In the present study, all androstenone values were much higher than fat androstenone acceptance threshold levels, which makes results difficult to generalize. To better demonstrate impact of low androstenone levels on meat quality, future research should include sensory assessment of boar taint and investigate meat quality using boars that are genetically selected to have low levels of androstenone, specifically below consumer acceptance thresholds. It is also difficult to generalize the meat quality results from the present study because pigs were raised and slaughtered differently than they might be in a large meat production facility.

One of the main benefits of raising boars is an increased production value from improved growth and feed utilisation. It has been established that boars have greater feed conversion ratio (FCR) than barrows (EFSA, 2004), thus it is favourable to raise boars for pork production. Limitations in study methodology prohibited direct measurement of feed

conversion ratios. Results from this study demonstrated that hot carcass weight (HCW) and average daily gain (AGD) did not differ between any groups. The most probable explanation for this is that all groups were fed the same commercial diet formulated for barrows, despite boars requiring a different amino acid composition in their feed for optimal growth (Creswell et al., 1975; Xue et al., 1997). This was done to minimize inconsistency in nutrient supplementation and control for possible dietary-related changes in meat quality between groups. In the literature, there are inconsistent results for growth rates of boars compared to barrows. Blair & English (1965), among others, found boars grow better than barrows, Newell & Boland (1972), among others, found no difference between growth rates of boars and barrows, and Castell et al. (1985) and Squires et al. (1993) found that barrows had faster growth rates than boars. Many factors can contribute to these inconsistencies, including diet (Xue et al., 1997). Cresswell et al. (1975) also found that the growth of boars did not differ from barrows when fed a conventional diet, but boars grew faster when fed a higher-protein diet. A study conducted by the Canadian Centre for Swine Improvement (CCSI, 2007) found no difference in hot carcass weight between boars and barrows. Overall, these measures appear to be highly dependent on diet, thus the correct nutrient formulation should be fed when raising boars for pork production to optimize growth rates.

In conclusion, raising boars for pork production is regarded as more welfare conscious and efficient. However, it is important to ensure that meat quality is not sacrificed. To address this, this study sought to compare the meat quality characteristics of barrows and high and low androstenone boars. No incidences of DFD or PFD meat

were observed in any group, suggesting that all meat was generally acceptable for consumer consumption. Overall, some differences existed between barrows and boars, while meat from high and low androstenone boars were largely comparable and did not appear to meaningfully differ. Comparatively, meat from barrows was generally found to be lighter, more marbled and tender, and juicer with greater backfat than boars. This may suggest that meat from barrows is more preferable. However, while these differences existed statistically, some differences in meat quality may not be perceivable by the consumer. It is also important to consider that consumer preference is affected by several individual and demographic factors. To better understand consumer meat quality perceptions and acceptance as they relate to the impact of boar taint compound levels, sensory studies with consumers could be incorporated into future research.

5.5 Tables and Figures

Table 5.1: Mean concentrations of hormones and boar taint compounds for barrows and high and low androstenone boars

	Experimental group			p-value
	High androstenone (n=18)	Low androstenone (n=18)	Barrow (n=18)	
Fat androstenone (ug/g)	14.49 ± 5.28[a]	5.50 ± 1.77[b]	0.14 ± 0.06[c]	<0.001
Plasma androstenone (ng/mL)	420.88 ± 179.46[a]	290.33 ± 155.70[b]	1.69 ± 0.89[c]	<0.001
Estrone sulfate (E1S) (ng/mL)	39.01 ± 12.34[a]	30.27 ± 9.19[b]	0.30 ± 1.04[c]	<0.001
Cortisol (ng/mL)	43.27 ± 24.78[ab]	62.65 ± 36.66[a]	24.07 ± 19.66[b]	0.006
Skatole (ng/mL)	4.63 ± 13.75	1.84 ± 5.47	5.16 ± 14.69	0.58

[a-c] Least square means for treatments without a common superscript differ significantly ($p<0.05$) within a row

[1]High and low androstenone groups are based on fat androstenone concentrations at slaughter

[2]Values are expressed as mean ± S.E.

Table 5.2: Carcass traits for barrows and high and low androstenone boars

	Experimental group			s.e.m.	p-value
	High androstenone (n=18)	Low androstenone (n=18)	Barrow (n=18)		
Average daily gain (kg/day)	0.86	0.85	0.87	0.01	0.82
Hot carcass weight (kg)	99.39	98.81	101.16	0.89	0.54
Skin lesion score	1.47	1.38	1.38	0.05	0.70
Hennessey probe					
Backfat (mm)	19.60^{ab}	18.59^{b}	22.18^{a}	0.62	0.009
Loin depth (mm)	51.48	53.69	52.92	0.87	0.55
Ruler					
Backfat (mm)	17.45^{b}	17.61^{b}	20.94^{a}	0.66	0.005
Loin depth (mm)	64.68	64.60	66.38	0.73	0.18
Loin Eye Area (mm^2)	51.21	53.40	53.44	0.87	0.50

$^{a-c}$ Least square means for treatments without a common superscript differ significantly ($p<0.05$) within a row

[1]High and low androstenone groups are based on fat androstenone concentrations at slaughter

Table 5.3: Meat colour scores for longissimus muscle (LM) chops taken from barrows and high and low androstenone boars

	Experimental group			s.e.m.	p-value
	High androstenone (n=18)	Low androstenone (n=18)	Barrow (n=18)		
NPPC colour score	3.24^{a}	3.21^{ab}	2.84^{b}	0.07	0.037
Japanese colour score	3.11	3.20	2.89	0.06	0.12
CPC colour score	3.44^{x}	3.32^{xy}	2.83^{y}	0.11	0.055
Minolta colour *L**	47.71^{y}	47.85^{xy}	49.22^{x}	0.29	0.064

[a-c] Least square means for treatments without a common superscript differ significantly ($p<0.05$) within a row

[x-z] Least square means for treatments without a common superscript tended to be different ($0.05<p<0.1$) within a row

[1]High and low androstenone groups are based on fat androstenone concentrations at slaughter

[2] NPPC= National Pork Producers Council; CPC = Canadian Pork Council

Table 5.4: Meat quality parameters for longissimus muscle (LM) chops taken from barrows and high and low androstenone boars

	Experimental group			s.e.m.	p-value
	High androstenone (n=18)	Low androstenone (n=18)	Barrow (n=18)		
Marbling					
NPPC score	1.33[b]	1.66[ab]	2.11[a]	0.01	0.003
CPC score	2.05[c]	2.71[b]	3.39[a]	0.12	<0.001
Firmness score	1.94	1.94	2.05	0.05	0.57
Wetness score	2.23	2.05	2.17	0.05	0.34
Loin pH					
1hr post-mortem	6.50	6.64	6.57	0.03	0.11
24hr post-mortem	5.60	5.59	5.56	0.01	0.18
Shear force (kg)					
2-day	6.05[a]	5.81[a]	4.96[b]	0.18	0.019
7-day	5.22	5.34	4.72	0.14	0.15
Cook loss %					
2-day	22.49	22.93	22.01	0.49	0.38
7-day	25.45[a]	24.10[ab]	23.48[b]	0.37	0.013
Drip loss %	1.92[ab]	1.72[b]	2.59[a]	0.15	0.046

[a-c] Least square means for treatments without a common superscript differ significantly ($p<0.05$) within a row

[x-z] Least square means for treatments without a common superscript tended to be different ($0.05<p<0.1$) within a row

[1]High and low boar taint groups are based on fat androstenone concentrations at slaughter

[2] NPPC= National Pork Producers Council; CPC = Canadian Pork Council

CHAPTER 6 CONCLUSIONS AND GENERAL DISCUSSION

The current practice of castration of male piglets is increasingly becoming an animal welfare concern and an alternate solution to this practice needs to be found (von Borell et al., 2020). Castration is performed to reduce both boar taint, a meat quality issue causing a foul odour or taste of pork, and address behavioural issues associated with entire males, including increased aggressive and sexual behaviours (Bonneau & Weiler, 2019). The challenge is that alternative solutions for boar taint must not result in behavioural issues causing a new set of animal welfare concerns, and the boar taint solution must also not impact meat quality or productivity of the industry.

Androstenone is a steroid hormone partially responsible for the development of boar taint in pork (Patterson, 1968). To evaluate the options for approaches to selecting pigs with low boar taint risk, the research discussed in this thesis examines the question of whether it is possible to predict for low androstenone concentrations at slaughter with early plasma androstenone measurements, and in doing so, gain an understanding of any behavioural consequences or major meat quality differences between barrows, high androstenone boars, and low androstenone boars.

The first objective was to determine if androstenone concentrations at 21 days of age are predictive of boar taint development at slaughter. This was evaluated by taking a blood sample at 21 days from a select sample of uncastrated male piglets, then correlating these plasma androstenone concentrations to slaughter fat and plasma androstenone concentrations to determine if there was an association. The significance

of an early prediction approach is that by determining early in life whether boars will have a greater likelihood of developing boar taint at slaughter, proactive strategies for those select high risk boars can be implemented, such as immunocastration or early slaughter. An informed, selective approach that allows for elimination of routine castration can greatly reduce production costs and increase productivity by raising boars, since feed conversion rates and lean yield percentages are greater than barrows (EFSA, 2004).

Results from this thesis indicate that early prediction measures as an alternate to castration may be an option. Data shows there is an association of 21-day plasma androstenone concentrations and fat androstenone concentrations at slaughter for boars greater than 120 kg body weight at slaughter. There was also evidence of an association for boars with high E1S concentrations at slaughter (indicating more sexually mature) between 21-day plasma concentrations and fat androstenone concentrations at slaughter, indicating that both weight and maturity are influencing factors in boar taint development. Future research to better understand these factors as a predictor of boar taint among a larger sample size and with various breeds could be beneficial. Although results are promising, the method of measuring plasma androstenone at 21 days requires a blood sample, and the assay for measurement can be time consuming and expensive. Development of a standardized assay for androstenone measurements that could be used for on-farm analysis would be needed for this method of prediction to be a realistic option.

Although prediction of boar taint development may be possible, it is important to understand the behavioural implications of raising boars to evaluate if it is an approach

that can be adopted without introducing risk to animal welfare due to increased behaviour issues such as aggression or mounting. It has been established that boars show more aggressive and sexual behaviours than barrows, but it hasn't been established whether this behaviour differs between high androstenone boars or low androstenone boars (Rydhmer et al., 2006). If prediction of low androstenone boars at 21 days of age becomes a useful option to raise low boar taint pigs, then it must also be better understood if low androstenone and high androstenone boars are different from each other in terms of aggressive and sexual behaviours, and whether low androstenone boars can be raised with less behavioural consequence.

The second objective of this study was to compare barrows and high and low androstenone boars to determine the effects on aggression, sexual behaviours (i.e. mounting), and stress-related behaviours. This was evaluated by performing 3 individual behaviour tests at 6 weeks of age to characterize response to novel stimuli and objects and fear of humans, as well as a test at 15 weeks of age to characterize aggression and sexual behaviour of pigs towards a stranger pig. Understanding behaviour based on high and low androstenone levels in boars relative to barrows is important since, as a whole, the concerns for animal welfare will not be addressed if one welfare concern (castration) ends up being replaced by another (harmful/unsafe behaviour of entire males). If more aggressive and mounting behaviours need to be managed in production, and if it then impacts meat quality due to stress or injury, then an early prediction approach is not ideal for production. Both must be understood for a successful approach to be developed.

The results from this thesis in terms of aggressive behaviours when comparing high androstenone boars, low androstenone boars, and barrows are largely inconclusive, with some evidence suggesting there may be less aggression in low androstenone boars than high androstenone boars or barrows. In terms of stress-related behaviours, no major differences were observed between barrows and high and low androstenone boars. A difference was noted in the mounting behaviour between all boars and barrows; however, no difference in mounting between high androstenone and low androstenone boars was seen. This result highlights a concern for raising boars given that increased mounting can pose a threat to animal health and welfare and may require pen management strategies to be implemented (Rydhmer et al., 2006). This study only examined results from individual tests rather than pen-wise behaviour observations, and conclusions about aggression could not be clearly drawn. Future studies should include pen-wise observations in conjunction with alternative individual tests to provide more insight to a variety of behaviours, including aggression, specifically with regard to mixing pigs, as this is where the most aggression and fighting typically occurs in a pig's lifetime (Marchant-Forde & Marchant-Forde, 2005). As well, the present study used a female for a resident-intruder test with a male, which may have influenced sexual behaviour and aggression outcomes. Future studies should investigate sexual behaviour, including variables more than just mounting, and aggression separately. Studies on aggression should ensure that males are used in resident-intruder tests to better understand aggression separately from sexual behaviours that may arise due to a female being the intruder. This may obtain better information for pen management when raising boars.

Ultimately, profitability has to be taken into consideration when considering a new strategy to pork production. Even if behaviour can be managed and welfare concerns with raising boars for meat production can be mitigated, it must be established that there are no known consequences to meat quality. In the end, the consumer must be satisfied with the product they are buying. Removing boar taint is beneficial from the consumer perspective, but other notable changes to meat quality must be understood in order to ensure alignment with consumer demand. It is known that boar meat is drier and leaner than barrow meat, but it is not known if the degree to which the meat is drier and leaner versus pork from barrows varies between pork from high and low androstenone boars (Lundström et al., 2009; Pauly et al., 2019).

The third objective of this study was to compare meat quality characteristics of barrows, high androstenone boars, and low androstenone boars to understand possible implications for meat production when raising boars. To achieve this, common carcass and meat quality characteristics such as backfat depth, marbling, colour and juiciness were measured and compared across the three groups. Understanding a shift in carcass and meat quality traits is critical with any change to current pork production practices. It must first be determined that raising boars will not result in detrimental quality issues such as DFD or PSE. From there, sensory tests with consumers can guide the degree to which people are willing to accept other changes such as leaner, drier or darker meat.

When specifically evaluating colour, juiciness, and fat content, the results of this thesis found there were limited difference in these traits between high and low androstenone boars, except for greater marbling in low androstenone boars than high

androstenone boars. This is positive given the marbling scores for low androstenone boars were closer to barrows, whose meat defines current consumer expectations. Overall, both groups of boars were generally slightly darker, dryer and leaner than barrows; however, all differences were very minor and may not even be noticeable to consumers. No detrimental quality issues such as DFD or PSE were seen in any group, indicating boar meat does not result in negative meat quality implications and should not impact market profitability as long as it can be confirmed that consumers are accepting of slight change in the expected pork products. Consumer trends show that there is increasing preference for leaner meat products, in which case the leanness of meat from low androstenone boars may align with market trends (Lundström et al., 2009; Mukumbo & Muchenje, 2016; Patterson et al., 2009). For future study guidance, it is important to note that in the present study androstenone levels in all boars were above sensory threshold of acceptance for androstenone (which could be error in measurement, otherwise unexplained why levels were so high) so further studies should include pigs genetically selected for low androstenone levels, specifically below consumer acceptance levels. As well, a future study comparing consumer acceptance of barrow, low androstenone boar, and high androstenone boar meat would provide greater assurance whether a change in meat quality based on the attributes of boar meat is noticeable and, if so, a viable option for consideration.

There are three key limitations to this study that should be noted. First, the sample size was small for all components of the study. Given this, results can be taken as suggestive and further research should be done using larger sample sizes. Second, pen

management was not typical of a commercial setting. Pigs were kept in pens of 4 (2 male, 2 female), whereas commercial settings can have anywhere from 20 pigs in a small group to over 100 in a large group (Street & Gonyou, 2008). Pen size can affect variables such as behaviour, productivity, boar taint accumulation and meat quality (Barnett et al., 1992; Street & Gonyou, 2008; Backus et al., 2016). With the smaller number of pigs per pen, stocking rate was low (1.6 m^2 per pig) and hygiene was good given the pens were cleaned daily, which is not always typical in larger operations. Both of these factors can also impact boar taint accumulation, behaviour, and meat quality (Bryant & Ewbank, 1972; Babol & Squires, 1995; Muchenje & Ndou, 2011). Thirdly, the measurement of androstenone was completed using an enzyme-linked immunosorbent assay (ELISA), but there is a lack of standardization of androstenone measurement (reviewed in Haugen et al., 2012), so the generalisability of findings in this thesis may be limited, especially since androstenone levels measured were all much higher than expected literature ranges of fat androstenone concentrations in boars and all measurements were above sensory threshold levels (Haugen et al., 2012; Morlein et al., 2015; Backus et al., 2016).

There are interesting implications from this study that have the potential to help the pork industry not only address animal welfare issues but also potentially increase productivity. Having strategies that can allow for selective management based on early detection of boar taint risk can save labour and material costs, rather than a blanket approach such as current castration practices or immunocastration of the larger male pig population (Deen et al., 2008). Additionally, boars generally have greater feed conversion

and lean meat yield compared to barrows, which can increase productivity of the industry (EFSA, 2004).

Understanding behavioural implications of raising boars to create pen management, transport and lairage strategies is an important part of considering a shift away from castration. Previous research has shown the detrimental effects of mounting on health of pigs, and this study suggests that mounting behaviour is a concern that may need to be specifically managed if raising boars (Rydhmer et al., 2006).

The implications of these study findings on meat quality results are that low androstenone boar meat has the potential to be a viable option, contingent on consumer acceptance of boar meat products. While eliminating castration may not decrease meat quality or have a detrimental impact on pork such as DFD or PSE, there are slight changes in terms of colour and leanness for boar meat compared to barrow meat. Changes to these characteristics specifically in low boar taint meat must be assessed with consumers to determine if demand for pork will remain the same if they are presented with a slightly different product.

Results from this research provide a loose framework for what production could look like if castration is ended and boars are raised for pork. If piglets are not castrated, blood can be tested at 21 days to predict high or low boar taint accumulation early, and low potential boar taint pigs can be raised as normal. The pigs identified as high risk for boar taint development can be identified and strategies such as immunocastration or early slaughter can be implemented on this smaller group. Strategies for behaviour

management, specifically mounting, may need to be further researched and implemented. Meat products from boars may be different from consumer expectations but should not be detrimental to market profitability.

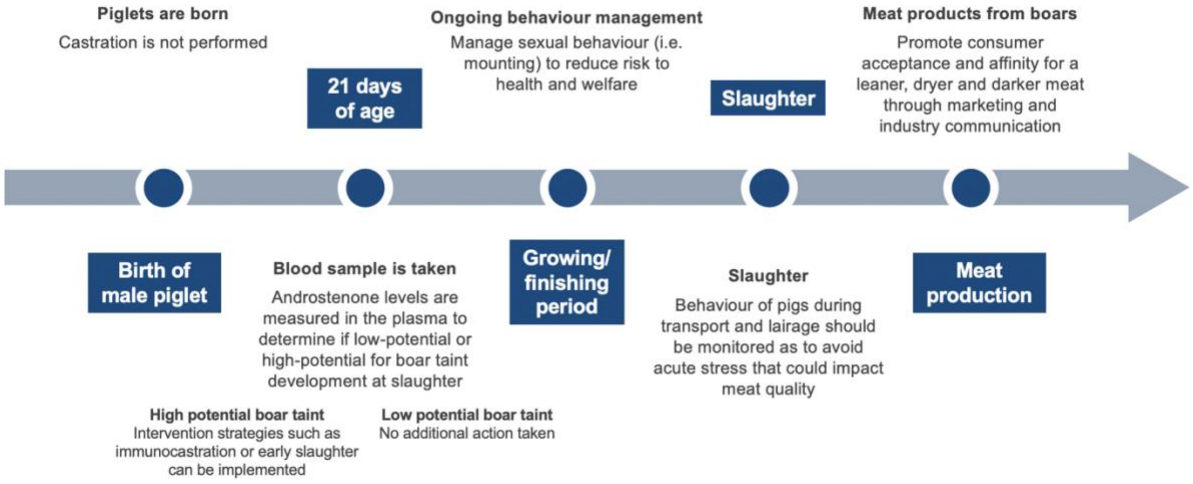

Figure 6.1: Theoretical lifetime of a male pig without castration

Upon reflection, the questions regarding prediction of androstenone levels and comparison of meat quality for barrows and high and low androstenone boars were answered in this research, although results for behaviour were less conclusive and further study would be advisable. Overall, this research suggests there are opportunities to raise boars for pork production. These findings can be used as a foundation for larger scale investigations to yield more impactful results to instigate change in the industry that will address animal welfare concerns and improve productivity.

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