"Intestinal Binding Agents as an Alternative to Castration in Controlling Boar Taint"

Peter Park
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Hannah Golightly:

Good afternoon and thank you for joining us for the first presentation in a multi-part webinar series by the newly-formed Ontario Swine Research Network.

My name is Hannah Golightly, and I'm a second-year veterinary student here at the Ontario Veterinary College, who's been assisting with the OSRN activities.

The OSRN has been formed by faculty at the University of Guelph and representatives from Ontario Pork, OMAFRA and the Swine Veterinary Community. The goal of the network is to enhance and improve the timeliness and accuracy of the U of G researcher results and activities to end-users.

We also aim to highlight the on-going collaborative work taking place with other institutions and research partners in order to capture the provincial, national, and international impact of the U of G Swine Research Program. We intend to provide a platform where producers, veterinarians, industry, students and others can go for current and archive research results. Our new website is currently under development, and we hope to launch it soon.

This afternoon I am pleased to introduce today's speaker, Peter Park.

To give you just a bit of background, Peter successfully defended his master's degree through the department of Animal Bio-Sciences and Toxicology here at the University of Guelph less than two weeks ago. Congratulations Peter.

While working on his master's, Peter also competed and won the university's three minute thesis competition while representing the Ontario Agriculture College. Finally, Peter looks forward to beginning a PhD in Animal Science this May at the University of Alberta.

With that, I will turn it over to Peter to take you through his presentation entitled: Intestinal Binding Agents as an Alternative to Castration in Controlling Boar Taint.

Peter Park:

Thank you for that royal introduction, Hannah.

All right, so I was talking with researchers at Aarhus University in Denmark, actually, about this specific topic, what I've been researching for the past two and a third years, and this past weekend, and it really comes to show it's really cool that, you know, there's other people in other parts of the world that's looking into the same thing despite

this area in particular being very novel, and it just comes to show I think we need more international collaboration like this, whenever possible.

Without further ado I will be talking about, when it comes to the agenda of this presentation I'll firstly be talking about castration and all of its implications and alternatives. Follow that by a possible control of boar taints using a nutritional method, something that really hasn't been looked into in previous studies. And after that I'll conclude the slides with what we're looking at when we're looking forward and the future potential that it may have within this area of phasing out surgical castration and what we know and we don't know.

Without further ado, let us go to the main parts.

Castration, specifically surgical castration, is at this time, not just within North America but world-wide, the status quo method to reduce aggressive behaviour, as well as to prevent what we call boar taints. Boar taint is an unpleasant odour and taste, something that it smells like urine or feces or sweat or a combination of all of them that people have reported. It's not very good. That can be picked up by some consumers upon cooking pork. From entire male pigs we call them boars.

And it is caused by an excessive accumulation of two compounds produced in the pig called skatole and androstenone. Skatole or 3-methylindole in its chemical terms is a breakdown metabaoloid of the amino acid tryptophan and it is facilitated by gut bacteria that resides in the hind gut of pigs.

Androstenone or 5-alpha-androstenone is a testicular steroid produced in large quantities at time of sexual maturation of the boar.

Castration, however, poses implications on both performance as well as animal welfare. If we were talking firstly about performance, if we were to put these into perspective, if you compare boars with castrates, about eight to 14 percent increase in feed efficiency is seen. In addition to that, four to 20 percent increase in carcass leanness is also seen, which is beneficial for the increase in premiums for the producer when it comes to shipping off their pigs to the slaughterhouse.

And these are from previous studies that have conducted primarily in the European Union and have used different breeds of pigs as well as different lengths of studies to really look at these attributes. And there are other advantages both in terms of meat quality and performance when it comes to raising intact males. I'll touch on that again a little more later on, but we'll be focusing on the implications at this time.

It also poses implications on animal welfare. Specifically the European Union has been spearheading an initiative in which they have declared 2018, so this coming year, as a long-term goal year to phase out surgical castration altogether by the first of January, however, this is a voluntary declaration. According to the official documentation, it is up to the member states themselves to put pressure on their industries to phase out surgical castration altogether. And so 2018 seems to be an unrealistic goal at this time, however it definitely has exerted some sort of pressure and awareness as evident by

the attention picked up by the public when it comes to asking about where our food comes from and how they are treated in farms.

Closer to home, in Canada the updated Code of Practice for the Care and Handling of Pigs, as of this past July, castration that is performed at any age must be accompanied with analgesics. And therefore, at this time, looking for effective alternatives to surgical castration is a hot topic of interest.

Currently available alternatives to castration include genetic selection, the organizations responsible is working on it, but it's selecting for low boar taint lines. Also slaughtering at an earlier age, this correlates with lighter body weights. Immunocastration which is an anti- gonadotrophin-releasing hormone vaccine injected twice within a pig's lifetime. And it is marketed as IMPROVEST here in Canada by Zoetis, and is used to shut down testicular function and supress testicular steroidogenesis.

Finally dietary means are available when it comes to lowering production of skatole in the hind gut, specifically using dietary fibre such as sugar beet pulp, raw potato starch, and chicory inulin.

Now, dietary means to influence concentrations of androstenone, that is a very novel area in itself and it hasn't really been looked into, and that's where my thesis comes into play. Although it is still a work in progress, I believe that there is potential in this becoming possibly an effective alternative to surgical castration.

Before I go on any further with regards to my specific research, it is essential that I introduce everybody to this concept called enterohepatic circulation. It's a recycling mechanism that is found in animals including humans as well as pigs.

In a sexually developing or a mature boar, if I were to break this down step-by-step, testicular steroidogenesis happens - takes place obviously - in the testes as I've stated, and does their effects, exerts their effects throughout the body after being transported through the bloodstream. We call it the systemic circulation.

Now there are times when the concentrations of these sex hormones can go up to a certain level whereby it may produce adverse effects, unwanted effects within the body, and so the body employs a specific excretion mechanism that takes place primarily in the liver, and what happens - crucial step that happens is conjugation, which is steroid which binds with a sulphate molecule that is highly polar - this means more water soluble. This creates altogether an overall compound, a sulfo-conjugated steroid that is very water soluble in comparison to a hydrophobic steroid, which allowed it to be excreted outside to the small intestine through bile, and in the small intestine has one of two fates.

The first accomplishing the original objective of getting rid of levels - excessive levels. This happens through defecation. However, there is a second fate that exists in which there is certain gut bacteria that resides in the small intestine which can de-conjugate this compound and allow it to be reabsorbed back to systemic circulation.

Now, I am proposing - our laboratory is proposing an interruption of this pathway, specifically the reabsorption step. We know that steroids such as estrogens and testosterone is confirmed to go through the cycle, however androstenone has not yet conclusively been confirmed to go through the cycle, however we do have a very strong idea that it does based on what we know about other steroids. And so one way to interrupt this specific step is the use of binding agents, also called absorbents.

Binding agents are used for centuries for therapeutic as well as pharmaceutical uses, specifically cholesterol binding drugs - that is one familiar example. You take it and it binds cholesterol in the small intestine that has been excreted for bile, and ultimately lowers the blood concentration altogether. They come from a diverse range of natural and synthetic origins, and feature various physical properties which provide their binding capabilities.

Now the use of binding agents have actually been used in our laboratory to treat boar taint in boars. Specifically we've tested Yorkshire boars, and so if I were to go through this graph of a pervious study that we conducted, this red dotted line signifies one microgram of fat androstenone. Now, this signifies the upper limit of a generally-accepted threshold level of androstenone boar taint, which means that if the fat androstenone concentration is above this line, people who are able to pick up boar taint will be able to pick it up.

And so we supplemented a specific diet that has been formulated to meet NRC-2012 requirements with five percent activated charcoal. What activated charcoal is, is a black powderous substance that you would find in your Brita filters, and it's also used to treat drug overdoses in humans as well as animals. And so upon supplementation of this, and feeding it for 28 days, four weeks, to Yorkshire boars there was a significant decrease in fat androstenone concentrations below this threshold concentration cut-off. And upon taking them off of that diet and reverting it to a diet free of this additive, we - as we expected - saw a significance increase back to above threshold concentrations.

Now this is a demonstration of a proof of concept, however we did not pursue with activated charcoal because it is expense and it's difficult to handle. It really goes all over the place and it really puts your boars looking all over like that. And so, we didn't decide to continue on further.

And so we needed to look for other alternatives that - other alternative absorbents that we can look into that can achieve similar results to what we have seen in this particular study.

How the research within my area progresses, and a lot of other science as well, is you conduct laboratory analysis in the beginning. So you would do simple experiments consisting of simple environments, a simple environment per se, to determine the effectiveness of a particular absorbent to a specific compound that we're interested in, in this case androstenone and skatole. Upon producing positive results we would advance to animal trials, which directly measure the concentration of what we are

interested in, as well as measuring the performance to see if these specialized diets do not compromise performance of these pigs.

This leads ultimately to my objective, and it was to identify and investigate the effects of mineral-based absorbents on the levels of androstenone and skatole in plasma and fat, secondly growth performance, and finally carcass characteristics of finisher, intact male pigs.

And so we needed to look for other absorbents, and we were able to come up with four with what we had available. So if we were to go through this one by one, bentonite firstly is a phyllosilicate clay which is of volcanic origin and has previously been used in the animal feed industry as an anti-caking agent as well as filler for premixes.

Diatomaceous earth is comprised mainly of amorphous silica made up of fossilized diatoms and it has previously been used as a filtrate for various liquids in processing - food processing industries, as well as insecticides.

Spent filtrate actually is diatomaceous earth, although it is a co-product. It's a waste product of the corn syrup processing industry, and therefore within its actual surfaces it has nutrients rich in crude protein as well as fat, making this a possible alternative feed ingredient for animals as well.

Finally, hydrated sodium calcium aluminosilicate, we abbreviated as HSCAS, is another clay-based compound also known as zeolite, has previously been used as an antifungal and anti-caking agents in feed.

And so using these absorbents, we needed to determine the observance - the inclusion of how much to actually include in grower finisher diets. So what we used was a combination of what we produced in vitro - in the laboratory - the result that we were able to obtain, and guidelines that are provided to us by the Canadian Food Inspection Agency for another purpose, specifically as anti-caking agents. Now, they're not actually used as recommendations for boar taint binding agents, because that has not yet been proven.

We also needed to choose the specific breed of pig to test, and we decided on purebred Durocs. This is because they are known to have a higher incidence of boar taint based on previous cell culture and live animal studies. And so we thought we were going to be able to detect significant difference much better. And therefore purebred Durocs were selected as suitable for a proof-of-concept study.

All in all, the first trial that we did conduct consisted of 90 purebred Duroc intact male pigs that were housed at Ponsonby General Animal Facility here in Ontario with an initial body weight of 83 kilograms and initial age of just over four months. These pigs were randomly allocated to one of six pens, so per group they had six pens in a complete randomized block design with three pigs housed per pen.

Upon determination of the individual inclusion level of these diets we were able to come up with five total diets, firstly the one being the control, which was a simple commercial

corn soybean meal based diet formulated to meet NRC-2012 requirements, and all others were diluted based on that diet with these inclusion levels of these absorbents.

So these were as follows: two percent sodium bentonite, 3.5 percent diatomaceous earth, 15 percent spent filter aid, and finally 0.7 percent HSCAS. Eighteen pigs were in a group and these were fed respectively for 28 days after a three-day acclimatization period to get the pigs adjusted to the new environment and new pen mates that they had.

And for another 14 days, this was the recovery period in which all pigs were reverted to the commercial control diet, and after that in a span of three weeks, all pigs were slaughtered here at the meat laboratory at the University of Guelph.

Specific endpoints that we measured and collected from the pig included plasma taken from the blood, from the orbital sinus, to analyse androstenone through enzyme-linked immunosorbent assay. We also looked at androstenone after taking a back fat biopsy. This was done at the same time points of sampling with the blood to measure fat androstenone with the same ELISA method and skatole using high performance liquid chromatography.

Other endpoints included weekly weighing of these pigs to calculate average daily gain, average daily feed intake, and feed conversion ratio.

The data sets for back fat and plasma concentrations were logarithmically transformed to normalize the data and all measurements were analyzed for statistical significance given that p-value is below .05, using the mixed procedure in SAS version 9.4.

I would like to first disclose results on the performance of this first trial that we conducted. All the pre-slaughter measurements that we analyzed, they weren't different between groups, based on the data that we analyzed, except the age at slaughter. The control group was older than the other groups because of the fact that these pigs were slaughtered based on heaviest to lightest body weights. And so within each kill day of those three weeks' span of slaughters to kill all 90 pigs, they were not balanced between groups per day, and therefore much of the control pigs were actually left behind and that is one thing that we should consider really revising for other trials that may ensue after.

Where we're looking at specific performance parameters, none of them - the growth, the feed intake or the feed conversion ratio - differed between treatment groups, which signifies that giving pigs - intact male pigs - these diets at these inclusion levels for 28 days, a short-term period, do not appear to compromise their performance.

Now I'll be disclosing each individual biochemical metabloid specially being androstenone in this case, for both plasma and fat.

What we should really take out of this, the Y axis is either androstenone concentration in plasma in nanograms per millilitre, or in fat in micrograms per gram. The X axis is the

day of trial, and the grey dotted line signifies the end of treatment period and the commencement of the recovery period.

For what you should really take away from these graphs is that there's a lot of variation that we did not expect from these pigs. And so we were wrong about our assumptions regarding the purebred Duroc being an ideal suitable candidate for a proof-of-concept study when it comes to measuring boar taint.

The horizontal arrow bars that you see at the last two time points is because of this staggered slaughter schedule as I've stated before. But all in all, there were no time or diet or time by diet treatment interaction effects that we saw in plasma androstenone.

Fat androstenone is a little bit different, however we still come to the conclusion that we weren't able to see a treatment difference, a treatment effect based on these supplemented diets when it comes to ultimately lowering concentrations of androstenone in fat, and that is what we are primarily interested in.

There is a significant time effect in that in comparison to the baseline measurements, the concentration of androstenone, in fact, significantly increased over time. However this does not really help us provide a conclusive evidence as to whether these absorbents that we tested ultimately lowers boar taint compounds in the body of an intact male.

And so to conclude, these main points include, again, it did significantly decrease levels of androstenone in both fat and plasma of Duroc finisher boars, and skatole I didn't report - I couldn't report, because it wasn't detected in most boars' adipose tissue throughout the trial, only, in fact, 1.3 to 2.5 percent, depending on which upper limit you're looking at, of all boars were above this threshold.

And so we needed to look at considerations to refine this study for future studies.

Again, firstly three differences. We saw that when it comes to steroid hormone synthesis and gene expression, all of that must really be looked into further. And also factors associated with pubertal onset is multifactorial. The genetics and the breed, age, body weight, nutrition and social environment all play a part.

And so we also thought inclusion level maybe might have been too conservative, and so we needed to look at other detailed toxicological studies in other species if we have to, to really look into what exactly we need to have a correct amount while also looking at duration of the feeding; how long should we be giving these treatments or a diet added with an absorbent for a cost benefit analysis that should be run in the future?

Very quickly, if you were to examine the meat quality, there was a follow-up trial using cross-bred boards. We saw no differences in carcass meat quality parameters based on the subjective and the instrumental measurements that we tested when it comes to carcass and meat quality. And this - with the exception of firmness and intramuscular fat, these parameters actually was a significant decrease. We used spent filtrate and HSCAS one more time and compared with control using cross-bred boars.

For firmness there was a significantly lower score for the ones that were fed starting for a longer duration. So 70 means it was fed for a longer duration starting at 70 kilograms. In comparison, 90 started at 90 kilograms and fed up to slaughter.

When it comes to intramuscular fat, the ones that were fed spent filtrate had a significantly deceased level over - measurements of intramuscular fat.

If you were to take away something from this, some of the tested absorbents, we can say that it can appear - it's suggestive - to influence fresh pork firmness if fed for a long period of time. However of influence intramuscular fat - this does not equate marbling by the way - is multifactorial. The back fat thickness, ad libitum feeding, genotype and dietary protein content all play a part.

If we were to wrap everything all together, keep in mind the boar taint is influenced by many factors including hormonal status and genetics. And only a bulk of the population, a handful of the population, can pick it up and everyone has their own definition of boar taint. It's very difficult to interpret and compare between these studies.

Diets added with - the ones that we've tested, these mineral absorbents, do not appear to compromise the performance, but may influence some meat quality characteristics if fed for a long period of time.

Now you might be asking, in the grand scheme of things, what did you actually accomplish over your thesis, because if you saw no results, what are you going to do? What's the next step? Well, another trial has just conducted and we used cross-bred, so this being Yorkshire Landrace cross with Duroc sire, these boars were used this time, and we also used barrows, in this case, for direct comparisons of boar taint levels.

We also fed them from 70 kilograms and slaughtered and at 130 kilograms, we abolished - we abandoned the idea of the recovery period altogether.

We also measured other things, like the reproductive tract as they are indirectly correlated with boar taint.

And we also need to look at further assessments of meat quality. We need to reproduce the results that we had, and so we - we will be evaluating the loin and the bacon as well as recruiting specific taste panels.

Putting everything into perspective, I want to introduce to everyone to the idea it's something to think about. It's not yet further advanced - we're not at this point yet. But it's something to think about the potential benefits of raising intact males. For the producer, obviously as increased premiums and decreased labour costs that have been found to be appearing for many years no. And for the consumer, it can provide leaner, bigger and healthier pork, because we know that there is an increased ratio of polyunsaturated fatty acids in pork from cuts of intact male pigs.

For the legislator, finally, it can provide elevated standards of animal welfare for other jurisdictions to follow, and definitely all three are definitely something to think about

despite this work being still a work in progress, which we are still looking at really further to see whether this can be a feasible alternative to castration.

With that I hereby state my acknowledgements, just very quickly my advisor, my advisory committee members as well as my funding agencies that have made everything possible. I know that this is a post - this isn't a live recording, and so if you have any questions, please forward it to the Facilitators of the Ontario Swine Research Network, so that they can forward your possible questions to me and I will be definitely be able to provide you with an answer. It's a bummer that I didn't get to actually stand and take your questions real time, but I guess with technical difficulties comes with these hurdles.

With that I say thank you very much and if you have any more information please contact using the information provided on this slide.

Have a nice day.

Hannah Golightly:

Thank you very much Peter for that great presentation. Thank you everyone for watching us and this presentation, and please watch your email for our next webinar which will take - in March 2017, so next month. And as Peter said, if you have any questions please forward them to the Ontario Swine Research Network. You can forward them Terri O'Sullivan, and her email address is there.

Thank you and have a great afternoon.