Original paper

TESTES WEIGHT IS NOT A RELIABLE TOOL FOR DISCRIMINATING IMMUNOCASTRATES FROM ENTIRE MALES

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Abstract

In view of the criticism regarding the piglet castration as currently practiced, one of the alternatives is the active immunisation against the hypothalamic GnRH hormone referred to as immunocastration. This method is effective in prevention of boar taint in pork and has the advantage of avoiding the pain associated with castration (performed without anaesthesia and analgesia). However, in some rare cases the immunocastration may not be effective and such pigs (so called non-responders) present a risk for boar taint. It is therefore important to have a reliable indicator of the effective immunocastration for the use on the slaughter line. Determination of boar taint substances (androstenone and skatole) is time consuming and expensive, whereas the size of reproductive organs could serve as an indicator of successful immunocastration. Present study provides results for 76 immunocastrates (IC) and 55 entire males (EM) varying in body (or carcass) weight and delay between immunocastration and slaughter, in which testes and accessory sex glands (vesicular gland, bulbourethral gland) were dissected and weighed. Gathered data were used to distinguish IC and EM by discriminant analysis. The results show better discrimination of IC than EM. Testes weight is less reliable indicator of successful immunisation than the weight of accessory sex glands and that the best discrimination was achieved when using all three measurements.

Key words: *discriminant analysis, immunocastration, pig, reproductive organs*

Introduction

Surgical castration of male piglets is a traditional practice in pig production used to avoid boar taint, an unpleasant odour and flavour of meat from entire male pigs. It has been ascribed to the presence of androstenone (Patterson, 1968) and skatole (Walstra and Maarse, 1970). Surgical castration as practiced nowadays (according to EU legislation it can be performed without anaesthesia and analgesia within first 7 days after birth) is being criticised and a ban of such practice is presently under consideration by the European Union. The alternatives include surgical castration with anaesthesia and/or analgesia, raising entire males, sperm sexing and immunocastration. Up to now the best alternative to prevent boar taint appears to be the immunocastration i.e. a vaccination against the gonadotropin-releasing hormone (GnRH) which induces the formation of specific antibodies that bind and neutralise GnRH. As a consequence, the hypothalamic-pituitary-

gonadal axis is disrupted resulting in a decreased pituitary release of LH and FSH, inhibition of testicular steroid synthesis, regression of reproductive organs and clearance of boar taint compounds. The first commercial product for immunocastration of male pigs (Improvac®) has been released in Australia and New Zealand in 1998 and registered for use in 53 countries since then, including the European Union in 2009. Before the immunisation becomes effective (two vaccinations are needed), the pigs exhibit performance of entire males i.e. improved feed efficiency and lean meat deposition (Batorek et al., 2012a). To benefit as much as possible from the advantages of entire males the late castration strategy is practiced. The first vaccination is usually performed when the animals enter the growing-finishing unit resulting in low antibody titres with animals physiologically remaining similar to an entire male. The second dose, resulting in effective castration, is injected 4-6 weeks before slaughter. This delay is recommended by vaccine producer to assure the clearance of boar taint compounds and is referred to as late immunocastration. In the case of an earlier immunocastration the advantage is that its efficiency can be easily demonstrated (e.g. by checking testes development), however the advantage in fattening performance comparatively to surgical castration is lost. In some cases the immunisation may be ineffective and such pigs present a risk for boar taint. It is therefore important to have a simple but reliable indicator of the efficacy of immunocastration for the use at slaughter line, because determination of boar taint substances (androstenone and skatole) is not practical (time consuming and expensive). Assessment of testes size has been suggested as an indicator of successful immunocastration, however, Bonneau (2010) questioned its reliability and suggested vesicular gland as better indicator of effective immunocastration. The mentioned study was based on published literature, whereas the aim of the present study was to test this suggestion on measurements collected from 131 pigs (76 immunocastrates and 55 entire males of varying carcass weight and delay between immunocastration and slaughter) from four experiments in which testes and accessory sex glands were dissected and weighed.

Materials and methods

Data on weight of testes, vesicular and bulbourethral gland was collected from previously published experiments on EM and IC (Škrlep et al., 2010; Škrlep et al., 2012; Batorek et al., 2012b; Kubale et al., 2012). The pigs used for the study were commercial crosses of different breeds (Large White × Landrace, Large White × Landrace × Duroc, Large White × Landrace × Pietrain) varying in body (103.6±13.5) and carcass weight (76.8±11.1 kg). In IC pigs the delay between second vaccination and slaughter varied from 2 to 9 weeks. Reproductive organs were collected at the slaughter line and taken to the laboratory for dissection and weighing. Samples of subcutaneous fat were also taken at the level of last rib for androstenone and skatole determination (HPLC methods) as described in previously mentioned articles. Data analysis was performed with statistical package IBM SPSS 21.0 for Windows using discriminant analysis to categorize pigs into EM or IC based on either one of the classifying factors (i.e. weights of testes, vesicular gland or bulbourethral gland) or using all three weights in a single step. Cross–validation of discrimination was also performed using leave-one-out method of SPSS.

Results and discussion

Figure 1 presents basic statistics and distribution for the reproductive organs weights used in the discriminant analysis. To show that IC and EM were similar in regard to body weight the distribution and basic statistics of the later is also shown. Although the two sex

groups differ considerably in regard to the size of reproductive organs (being strongly regressed in IC) some overlapping in distribution could be observed. There are several possible factors to explain this situation. Firstly there is one IC that could be considered a non-responder (pig no. 20 with androstenone level 0.34 μg/g and weight of reproductive organs similar to EM i.e. testes weight 413 g, vesicular gland weight 107 g and bulbourethral gland weight 96 g). Secondly, for some pigs the delay between the effective vaccination and slaughter was only 2 weeks, which may not be enough to reduce the size of the organs. Namely, it is known that approximately one week is needed after the second vaccination for the metabolic response to take place (Claus et al., 2007). Thirdly the pigs included in the study were of different crossbreeds and also varied considerably in body weight (in entire males a correlation between the size of organs and carcass weight was in the range 0.45-0.53; data not shown) i.e. those entire males with lower body weight were retarded in growth and likely delayed in reaching sexual maturity, exhibiting low reproductive organ size, closer to those of IC. Such circumstances, i.e. variability of crossbreeds, body weight, some pigs being retarded in growth, are usual in everyday pig production practice.

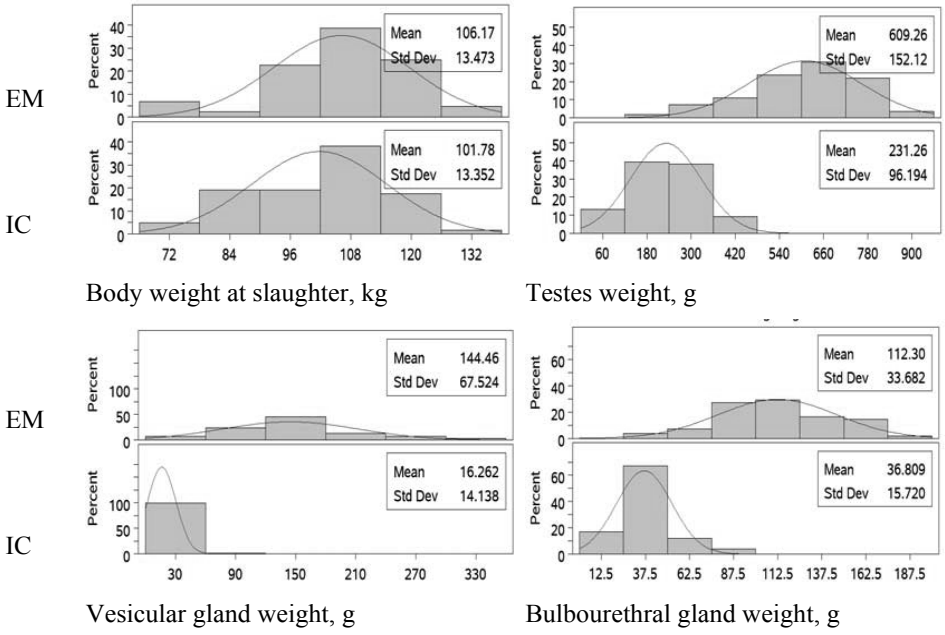

Figure 1. *Distribution of data for slaughter weight and weight of reproductive organs*

When using testes weight as a criterion to distinguish between EM and IC (Table 1) the results of discriminate analysis showed a 91.6 % success rate, with misclassification being higher in EM (12.7%) than IC (5.3%). Cross-validation results were the same as in the calibration step. Looking individually on misclassified pigs in EM group it could be observed that these were all Pietrain crossbred pigs (weighing between 71-108 kg) with two of them retarded in growth. It could be mentioned that Pietrain crosses had similar slaughter weight as pigs of other two crossbreeds whereas testes weight (520 g) in EM of Pietrain crossing was lower ($P<0.001$) than in other two crossbreeds (682 g and 701 g for

Landrace×Large White and Landrace×Large White×Duroc crosses, respectively; data not shown). Concerning IC, it could be argued that misclassified pigs were non-responders, however, their androstenone levels does not support that since it was below detection limit of the method (<0.24 μg/g fat) in all cases. Misclassified IC pigs were all crosses of Landrace×Large White (had the smallest regression of testes weight among the three crossbreeds), weighing between 100-130 kg and with 5 weeks delay between second vaccination and slaughter (recommended standard). As it was observed already in EM, lighter testes weight was also evidenced for immunocastrated Pietrain crosses.

Table 1. *Classification results[a,c] of discriminant analysis for testes weight as criterion*

			Predicted		Total	Missclassified pigs
			IC	EM		
Original	Count	IC	72	4	76	2, 3, 66, 68
		EM	7	48	55	105, 108, 109, 110, 111, 117, 120
	%	IC	94.7	5.3	100.0	
		EM	12.7	87.3	100.0	
Cross-validation	Count	IC	72	4	76	2, 3, 66, 68
		EM	7	48	55	105, 108, 109, 110, 111, 117, 120
	%	IC	94.7	5.3	100.0	
		EM	12.7	87.3	100.0	

[a]91.6% of original grouped cases correctly classified.

[c]91.6% of cross-validated grouped cases correctly classified.

When using bulbourethral gland weight (Table 2) as a criterion to distinguish between EM and IC the results of discriminate analysis were slightly better as in the case of testes weight and showed a 93.1% success rate, with misclassification being higher in EM (10.9%) than IC (3.9%). Cross-validation results were the same as in the calibration step.

Table 2. *Classification results[a,c] of discriminant analysis for bulbourethral gland weight as criterion*

			Predicted		Total	Missclassified pigs
			IC	EM		
Original	Count	IC	73	3	76	20, 37, 55
		EM	6	49	55	82, 104, 111, 117, 119, 120
	%	IC	96.1	3.9	100.0	
		EM	10.9	89.1	100.0	
Cross-validation	Count	IC	73	3	76	20, 37, 55
		EM	6	49	55	82, 104, 111, 117, 119, 120
	%	IC	96.1	3.9	100.0	
		EM	10.9	89.1	100.0	

[a]93.1% of original grouped cases correctly classified.

[c]93.1% of cross-validated grouped cases correctly classified.

We did not observe any effect of crossbreed on the weight of bulbourethral gland (data not shown). It is noteworthy that all the EM misclassified as IC showed also low androstenone levels (three of them below detection limit, others from 0.247 to 0.629 μg/g fat) which indicates that the reason for misclassification to IC group is likely due to the sexual immaturity of these EM pigs.

Table 3. *Classification results[a,c] of discriminant analysis for vesicular gland weight as criterion*

			Predicted		Total	Missclassified pigs
			IC	EM		
Original	Count	IC	75	1	76	20
		EM	11	44	55	85, 88, 90, 91, 95, 104, 105, 117, 118, 119, 120
	%	IC	98.7	1.3	100.0	
		EM	20.0	80.0	100.0	
Cross-validation	Count	IC	75	1	76	20
		EM	11	44	55	85, 88, 90, 91, 95, 104, 105, 117, 118, 119, 120
	%	IC	98.7	1.3	100.0	
		EM	20.0	80.0	100.0	

[a]90.8% of original grouped cases correctly classified.

[c]90.8% of cross-validated grouped cases correctly classified.

When using vesicular gland weight (Table 3) as a criterion to distinguish between EM and IC the result of discriminate analysis was overall the lowest with 90.8% success rate, which was due to the high misclassification of EM (20%). On the other hand the recognition rate was excellent (98.7%) in the case of IC where only one pig was misclassified as EM; in that particular case (pig no. 20); this pig was likely a non-responder which in the end denotes a correct classification into EM.

Table 4. *Classification results[a,c] of discriminant analysis using all three criteria (testes, bulbourethral gland, vesicular gland)*

			Predicted		Total	Missclassified pigs
			IC	EM		
Original	Count	IC	75	1	76	20
		EM	4	51	55	105, 117, 119, 120
	%	IC	98.7	1.3	100.0	
		EM	7.3	92.7	100.0	
Cross-validation	Count	IC	75	1	76	20
		EM	5	50	55	105, 111, 117, 119, 120
	%	IC	98.7	1.3	100.0	
		EM	9.1	90.9	100.0	

[a]96.2% of original grouped cases correctly classified.

[c]95.4% of cross-validated grouped cases correctly classified.

When using all three organs (Table 4) as a criterion to distinguish between EM and IC the results of discriminate analysis were overall the best with 96.2% success rate (95.4 % in cross-validation). Only 7.3% EM and 1.3% IC (i.e. one pig) have been incorrectly classified. As already mentioned IC pig (no. 20) was likely a non-responder. Out of five misclassified EM, only one had androstenone level typical for EM (1.0 μg/g fat) and above sensory threshold level (0.5 μg/g fat) whereas the other four pigs had low androstenone levels (<0.27 μg/g).

A higher degree of misclassification observed for EM than IC can be related to higher variability due to differences in (cross)breeds and growth rate affecting the onset of sexual maturity (e.g. delayed sexual maturity in slow growing pigs, resulting in underdeveloped testes and accessory reproductive glands). In agreement with Bonneau (2010), vesicular gland (or alternatively all reproductive organs) turned out as the most reliable indicator for IC discrimination. This result has a high practical relevance because it denotes that reliable on-line detection of non responders is possible. According to Bonneau (2010) vesicular gland undergoes a fast weight regression, whereas in testes or bulbourethral gland the response will take much longer. This can be explained by the tissue structure. In the vesicular gland grape-like acini, filled with serous secrete, are more prone to leakage than in the case of more condensed structure of bulbourethral gland, with smaller acini, filled with highly viscous content. A vesicular gland, a very reliable indicator for IC pigs, seems problematic for correct classification of EM. In the majority of vesicular gland based EM false classifications, the bulbourethral gland and/or testes were not regressed.

Conclusion

In conclusion, the correctness of the classification was high and much better for IC than EM. The lowest success rate in IC was obtained when using testes weight (87%) and the highest when using vesicular gland weight or alternatively all reproductive organs (one pig was false negative which was likely a non-responder) denoting faultless discrimination. For entire males using vesicular gland was the least reliable (80%) whereas using all reproductive organs provided the best classification correctness (93%).

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References

1. Batorek N, Čandek-Potokar M, Bonneau M, and Van Milgen J 2012a. Meta-analysis of the effect of immunocastration on production performance, reproductive organs and boar taint compounds in pigs. Animal 6, 1330-1338.
2. Batorek N, Škrlep M, Prunier A, Louveau I, Noblet J, Bonneau M and Čandek-Potokar M 2012b. Effect of feed restriction on hormones, performance, carcass traits, and meat quality in immunocastrated pigs. Journal of Animal Science 90, 4593-4603.
3. Bonneau M 2010. Accesory sex glands as a tool to measure the efficacy of immunocastration in male pigs. Animal 4, 930-932.
4. Claus R, Lacorn M, Danowski K, Pearce MC, and Bauer A 2007. Short-term endocrine and metabolic reactions before and after second immunisation against GnRH in boars. Vaccine 25, 4689-4696.

5. Kubale V, Batorek N, Škrlep M, Prunier A, Bonneau M, Fazarinc G, and Čandek-Potokar M 2013. Steroid hormones, boar taint compounds, and reproductive organs in pigs according to the delay between immunocastration and slaughter. Theriogenology 79, 69-80.
6. Patterson RL 1968. 5a-Androst-16-ene-3-one: Compound responsible for taint in boar fat. Journal of Science and Food Agriculture 19, 31–37.
7. Škrlep M, Šegula B, Zajec M, Kastelic M, Košorok S, Fazarinc G and Čandek-Potokar M 2010. Effect of immunocastration (Improvac) in fattening pigs I: Growth performance, reproductive organs and malodorous compounds. Slovenian Veterinary Research 47, 57-64.
8. Škrlep M, Batorek N, Bonneau M, Prevolnik M, Kubale V, Čandek-Potokar M 2012. Effect of immunocastration in group-housed commercial fattening pigs on reproductive organs, malodorous compounds, carcass and meat quality. Czech Journal of Animal Science 57, 290-299.
9. Walstra P and Maarse H 1970. Onderzoek geslachtgeur van mannelijke mestvarkens. Researchgroep Vlees en Vleesvare. T.N.O., Rap. C-147 and 2, 1–30. The Netherlands Zeist.