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ORAL COMMUNICATION

Adaptation of immunocastration treatment to *montanera* system for male Iberian pigs: effects on reproductive organs and carcass traits

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Additional keywords

Iberian pig.
Free-range system.
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Reproductive tract.
Carcass composition.

SUMMARY

Voluntary end of male pig castration is expected in the EU in the near future, but standard immunocastration (IC) protocols need to be adapted for Iberian (IB) pig males due to their long life cycle. Our team developed a 3-dose protocol that needs a further adjustment to suit *montanera* (MT; free-range acorn feeding) chronology. Our hypothesis is that improving homogeneity of body condition at the start of MT will enhance and homogenize testicular atrophy. Pure IB males (n=35) reared in extensive system were feed-restricted during the growth phase (pre-*montanera*) as usual. Of these animals, Control pigs (C; n=18) were immunized against GnRH at 10.5, 12 and 13.5 months (m)of age. Treated pigs (T; n=17) were immunized at 10.5, 11.5 and 13 m, with a 15-day *ad libitum* (AL) feeding period starting at the 3rd dose. Both groups started the MT period at 13.5 m, coinciding with the 3rd dose of C and the end of the AL period of T, and both were slaughtered at 16 m. To further validate the effect of nutritional level, an additional group was fed ad libitum during growth and finishing phases with commercial feedstuff in a regular outdoor rearing system (Adlib; n=15 IB x Duroc males), with the vaccinations taking place at 8, 9 and 11 m and slaughter at 13 m (earlier, due to genotype and unrestricted feeding). Testes and epidydimides were significantly smaller in Adlib and T than C pigs. Similarly, bulbourethral glands were significantly smaller in T than C pigs. Testes parenchyme color "a" (green to red) was highly correlated (r=0.87) with testes weight. Backfat androstenone and skatole were below detection limits for all the animals. Foreleg, loin and prime-cut yields were significantly greater for Adlib than for C and T pigs. In conclusion, nutritional level can be used to improve the efficacy of male IC, and testes colorimetry can be used to monitor this efficacy.

Palabras clave adicionales

Cerdo Ibérico.
Sistema extensivo.
Inmunización anti GnRH.
Tracto reproductivo.
Composición de canal.

Adaptación de la inmunocastración a la montanera del macho de cerdo Ibérico: efectos en órganos reproductivos y características de canal

RESUMEN

El fin voluntario de la castración de machos porcinos se prevé próximamente en la UE. Pero los protocolos habituales de inmunocastración (IC) necesitan ser modificados para los machos Ibéricos (IB) debido a su largo ciclo vital. Nuestro grupo desarrolló un protocolo de 3 dosis que pretendemos ajustar a la cronología de la montanera, basándonos en la hipótesis de que la uniformidad de la condición corporal al inicio de la montanera intensificará y homogeneizará la atrofia testicular. Machos IB puros (n=35) fueron alimentados de forma restringida durante la pre-montanera como es habitual. De ellos, los machos Control (C; n=18) fueron inmunizados contra la GnRH a los 10,5, 12 y 13,5 meses (m) de edad, y los Tratados (T; n=17) a los 10,5, 11,5 y 13 m, alimentándose estos últimos *ad libitum* (AL) durante 15 días desde la 3ª dosis. Ambos grupos comenzaron la montanera a los 13,5 m, coincidiendo con la 3ª dosis de C y el fin del periodo AL de T, sacrificándose ambos a los 16 m. Para corroborar el efecto del nivel nutricional, otro grupo fue alimentado *ad libitum* durante el pre-cebo y cebo en sistema extensivo regular (Adlib; n=15 machos IB x Duroc), inmunizándose a los 8, 9 y 11 m y sacrificándose a los 13 m (más temprano, debido al genotipo y alimentación). Los testículos y epidídimos fueron significativamente menores en los machos Adlib y T que en los C. Similarmente, las glándulas bulbouretrales fueron significativamente menores en los machos T que en los C. El color "a" (de verde a rojo) del parénquima testicular estuvo altamente correlacionado (r=0,87) con el peso testicular. La grasa dorsal resultó negativa a androstenona y escatol en todos los animales. Los rendimientos de paletas, lomos y piezas de primera fueron significativamente mayores en los machos Adlib que en los C y T. En conclusión, el nivel nutricional puede ser utilizado para mejorar la eficacia de la IC de machos, y la colorimetría testicular puede ser útil para evaluar dicha eficacia.

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INTRODUCTION

Male Iberian (IB) pigs are castrated to avoid boar taint, to improve fat accretion and to ease free-range management. However, male pig castration is expected to be voluntarily ended in most EU countries in the near future. In conventional pig production, a two-dose protocol of vaccine against GnRH is used to inhibit testosterone secretion and boar taint (Dunshea et

al. 2001; Batorek et al. 2012). However, this standard protocol must be adapted for Iberian pigs due to their long life cycle, which usually ends at over 15 months (m) of age. Within a previous Spanish project (INIA, RTA 2010-0062-C02-02), our research team has recently developed long term immunocastration (IC) protocols for pre-pubertal or adult, male and female IB pigs in which 3 vaccine doses were used and the animals were slaughtered at 16 m of age (Hernández-García et al. 2013; Martínez-Macipe et al. 2016). These protocols were totally effective (100%) for the females, whereas immunization efficacy in males was variable (80 to 100% depending on the trials) and appeared to be affected by different factors like nutritional level and stress. Moreover, testicular atrophy was highly variable even in the responding animals. In the protocol including vaccinations at 11, 12 and 14 m of age, the last dose coincided with the middle of the so called *montanera* (MT; free-range period), thus complicating management. On the other hand, body weight (BW) and body condition score (BCS) are usually heterogeneous at the start and the end of MT, due to the hierarchical competition and stress provoked by the required feed restriction during the long pre-montanera period (Daza et al. 2005). However, IB pigs cannot be fed *ad libitum* for a long time because of their physiological adipogenic potential. On the other hand, *ad libitum* feed allowance in MT decreases competition stress and therefore the effect of IC may last for the whole period. Our hypothesis is that, by improving BCS homogeneity at the start of MT, efficacy of IC will be increased and carcass composition homogenized. Therefore, the objectives of this study were the following: 1) To evaluate the efficacy of a 3-dose protocol in which the last immunization is performed just before MT. 2) To evaluate the effects of this treatment on body composition and meat quality.

MATERIAL AND METHODS

For this study, 15 IB x Duroc (IBD) and 35 pure IB (IB) male pigs were used. After weaning, IB piglets were located outdoors in a big corral (and later moved to large paddocks) and were fed with commercial growing and finishing concentrates with a progressive feed allowance (from 1 to 1.8 kg/pig/day from weaning to 115 kg BW or until 13 m of age (whichever came first). Thereafter IB males were raised with acorns in a traditional MT system for approximately 3 m. The IBD pigs were initially treated in the same way, but, at approximately 10 m of age, they were relocated in a separate paddock and fed the same commercial concentrate *ad libitum* until the end of the finishing period (Adlib group). Control pigs (C; n=18 IB) were vaccinated (2 mL of Improvac®, Zoetis-Pfizer, subcutaneously behind the ear) at 10.5, 12 and 13.5 m of age, and treated pigs (T; n=17 IB) were immunized at 10.5, 11.5 and 13 m of age, with a 15-day ad libitum feeding period starting at the time of 3rd dose application. In this way, the interval between the 2nd and the 3th vaccinations was the same (1.5 m) for both groups. At 1st vaccination, C and T pigs had a mean BW (±SE) of 85±1.5 kg. Both treatment groups started the MT period at 13.5 m and were slaughtered at 16 m of age (when average slaughter weight of 155 kg was reached). For the Adlib group, the vaccinations were done at 8, 9 and 11 m of age (84±1.7 kg BW at 1st vaccination), and slaughter took place at 13 m of age, due to the expected faster growth, related to genotype and feeding system.

Pigs were weighed approximately once a month to monitor their nutritional status. Prior to slaughter they were also scanned with ultrasound (3.5 MHz, 12 cm probe) to assess backfat thickness and loin eye area at the 10th rib level (Ayuso et al. 2013). To monitor testicular size and atrophy, testicular ultrasonography was performed associated to vaccinations and also just before slaughter. The same probe (3.5 MHz, 12 cm) was used for longitudinal and transversal testicular scanning. External measurements (longitudinal and transversal) were also done with a caliper when testes size was large enough. One day before slaughter, blood was taken from all experimental pigs for analysis of serum concentrations of testosterone. The reproductive tract of all experimental animals was excised at slaughter, testicles, epididymides and accessory (bulbourethral and vesicular) glands were weighed and measured. Testicular volume was also determined by a water-displacement technique. In addition, parenchyme color of testes was assessed with a hand-held colorimeter (Minolta CR-200) on their middle longitudinal section surface to assess the degeneration degree. Carcass and prime cuts (ham, foreleg and loin) were weighed at the packing plant. In addition, backfat thickness and loin area at the 10th rib level were measured, and loin samples were taken for determination of intramuscular fat content and meat tenderness (texture profile analysis; TA-XT2i texturometer with Warner-Bratzler shear blade; Stable Micro Systems). Finally, backfat tissue samples (~10x15cm, full thickness, from the rear lumbar area) were taken at the packing plant for assessment of androstenone and skatole content (HPLC analysis as described in Batorek et al. 2012). Data were analyzed by one-way analysis of variance using GLM procedure of SAS statistical software, with a model including the effect of treatment group. Treatment means were compared by the Tukey´s test. Significance was set at $P< 0.05$.

RESULTS AND DISCUSSION

Weight and volume of testes and weight of epididymides **(Table I)** were significantly smaller for Adlib and T than for C pigs. Similarly, pre-slaughter testes volume (estimated *in vivo* by ultrasonography) and bulbourethral glands weight were significantly smaller in T than in C pigs **(Table I)**. Testicular parenchyme color "a" (green to red) was highly correlated (r=0.87) with testes weight **(Figure 1)**, although there were no differences in testicular parenchyme color between C and T pigs **(Table I)**. These color differences were easily perceptible, as testes undergoing incomplete atrophy (medium-sized) were still reddish (like full-size testes from entire males), whereas the smaller-sized testicles (fully atrophied) showed visual signs of tissue degeneration (chocolate color and white marbling). Thus, testes parenchyme colorimetry could be a valuable tool for detection of deficiently immunocastrated males. In addition, testes size measured *in vivo* by ultraso-

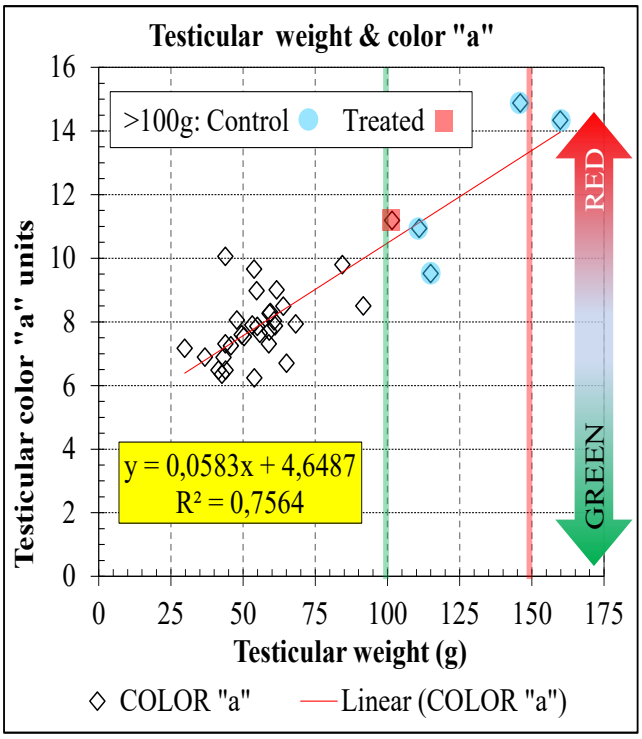

Figure 1. Regression of testicular weight versus colorimetric testicular parenchymal color "a" (from green to red) from Control and Treated pigs (n=35; r=0.87). Only 2 Control pigs had testicles close to 150 g. Testicular colorimetry was not available for the Adlib group (Regresión del peso testicular con respecto al valor colorimétrico "a" del parenquima testicular (de verde a rojo) en cerdos Control y Tratados (n = 35; r = 0.87). Solamente 2 cerdos, del grupo Control, tuvieron testículos de alrededor de 150 g. La colorimetría testicular no fue disponible para el grupo Adlib).

nography could be used for prediction of IC efficacy, because there was a high correlation between the echographycally estimated testicular volume and the actual volume (r=0.938; data not shown). Blood testosterone data are not yet available. Nevertheless, all backfat samples from the 3 groups were negative (below detection limits) for androstenone (<0.24 µg/g) and skatole (<0.03 µg/g) contents (data not shown). Therefore, all animals, regardless of the treatment, remained immunocastrated for the whole finishing period of 3 months. However, the 15-day *ad libitum* feeding improved the efficacy of IC, as revealed by reproductive tract morphometry, which showed a more intense and uniform atrophy. In fact, only 2 C pigs had testicles close to 150 g (Fig. 1), which, according to our previous studies, is the threshold value for detection of testosterone in blood. The prolonged *ad libitum* feeding (Adlib group) had a similar effect, as shown by the similar reproductive tract morphometry. Therefore, Adlib and T males had small (<150g) testicles, which represents a 100% of IC efficacy.

Foreleg, loin and prime-cut yields (with respect to carcass weight) were similar in C *vs* T pigs but significantly greater (as expected) for the Adlib group **(Table II)**. In contrast, ham yield was similar for the 3 groups, as well as backfat thickness **(Table II)**. The Adlib group had a smaller percentage of intramuscular fat than C and T males, probably due to the different finishing system **(Table II)**. Moreover, these values on fat thickness and infiltration, as well as those on meat texture **(Table II)**, do not seem different from the usually reported values from surgically castrated animals (Tejerina et al. 2012), maybe because the post-immunization period was long enough (3 months), contrarily to the information reported in our recent study (Martinez-Macipe et al. 2016), which involved a shorter post-immunization period. In conclusion, unrestricted feeding or strategically scheduled short-time *ad libitum* feeding prior to free-range montanera finishing stage increased the efficacy of long-term male IC to 100%, as shown by testes atrophy and degeneration and the absence of androstenone in fat. Moreover, testicular parenchyme colorimetry may indicate the degree of atrophy and hence may help predicting the efficacy of treatment. Finally, intramuscular fat and meat tenderness did not appear to be deteriorated

Table I. Reproductive tract morphometry in concentrate-fed Iberian x Duroc pigs and acorn-fed Control and Treated Iberian pigs (Morfometría del tracto reproductivo en cerdos Ibérico x Duroc alimentados con concentrado y cerdos Ibéricos Tratados y Control alimentados con bellota).

	Adlib (n=15)	Control (n=18)	Treated (n=17)
In vivo testes volume (ml)	N.A.	99.5 ± 12.4^{a}	76.5 ± 6.1^{b}
Testicular volume (ml)	52.1 ± 2.7^{b}	66.9 ± 8.2^{a}	50.7 ± 4.1^{b}
Testicular weight (g)	56.9 ± 3.1^{b}	73.7 ± 8.4^{a}	55.6 ± 4.2^{b}
Epidydimal weight (g)	19.3 ± 0.7^{b}	23.0 ± 1.7^{a}	19.1 ± 0.8^{b}
BU glands weight (g)	N.A.	28.5 ± 3.8^{a}	20.1 ± 0.9^{b}
Vesicular gland weight (g)	N.A.	36.6 ± 4.4^{a}	33.6 ± 3.2^{a}
Testes color "a" units	N.A.	8.7 ± 0.6^{a}	8.2 ± 0.3^{a}

Data from *ad libitum* concentrate-fed Iberian x Duroc pigs (13 months old; Adlib group) and acorn-fed Control and Treated Iberian pigs (16 months old). Testicular, epydidymal and bulbourethral (BU) weights and volumes are bilateral means. *In vivo* testes volume: estimated from pre-slaughter echographic measurements. N.A.: not available. Means (±SE) with different superscript letters differ (p<0.05; bold fonts).

Table II. Carcass and meat traits in concentrate-fed Iberian x Duroc pigs and acorn-fed Control and Treated Iberian pigs (Características de la canal y de la carne en cerdos Ibérico x Duroc alimentados a base de concentrado y cerdos Ibéricos Tratados y Control alimentados con bellota).

	Adlib (n=15)	Control (n=18)	Treated (n=17)
*Backfat thickness (cm)	8.9 ± 0.3^{a}	9.2 ± 0.6^{a}	8.9 ± 0.6^{a}
*Loin area (cm^2)	33.1 ± 1.3^{a}	25.2 ± 1.1^{b}	25.5 ± 0.9^{b}
*Loin IMF (%)	5.0 ± 0.4^{b}	7.5 ± 0.6^{a}	6.5 ± 0.5^{a}
Loin hardness (g/cm^2)	N.A.	9628 ± 392^{a}	9252 ± 460^{a}
Loin yield (%)	1.8 ± 0.03^{a}	1.3 ± 0.04^{b}	1.3 ± 0.04^{b}
Ham yield (%)	10.8 ± 0.1^{a}	10.7 ± 0.1^{a}	10.7 ± 0.1^{a}
Foreleg yield (%)	8.4 ± 0.1^{a}	7.9 ± 0.1^{b}	7.8 ± 0.1^{b}
Prime cut yield (%)	21.0 ± 0.2^{a}	19.9 ± 0.2^{b}	19.8 ± 0.2^{b}

Data from ad libitum concentrate-fed Iberian x Duroc pigs (13 months old; Adlib group) and acorn-fed Control and Treated Iberian pigs (16 months old). *Backfat and loin muscle at 10th rib level. Loin hardness: measured at 80% compression. N.A.: not available. Means (±SE) with different superscript letters differ (P<0.05; bold fonts).

compared to surgical castrates, and the treatment is fully compatible with free-range animal management during montanera.

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