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IMMUNIZATION AGAINST 5α-ANDROSTENONE IN BOARS USING ANDROSTENONE-KLH AND GnRH-KLH AS IMMUNOGENS

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SUMMARY

Two vaccines containing conjugates of androstenone (AND) and gonadotrophin releasing hormone (GnRH) have been tested in young male pigs for their ability to control the accumulation of AND in boar fat. They were tested separately and in combination, and were administered twice intramuscularly at 6 and 15 weeks of age. Immunization against GnRH was successful in reducing significantly the accumulation of AND in the subcutaneous fat in conjunction with some effects on growth and carcass composition. Immunization with the combined GnRH-KLH/AND-KLH vaccine was less effective, while immunization with AND-KLH alone was virtually ineffective.

INTRODUCTION

5α-androst-16-en-3-one (androstenone, AND) is a causative factor of boar taint and precludes the widespread use of the uncastrated male pig for meat production. Recent experiments studying the effectiveness of immunization of boars against the steroid have demonstrated reduced accumulation in the adipose tissue (1,2). The concept of auto-immunization is to stimulate the pigs' immune system to produce antibodies against endogenous androstenone by immunizing at a young age with an androstenone-protein complex. Because of its small molecular size, androstenone has to be linked to a 'carrier' protein to elicit an antibody response, and in previous experiments androstenone was linked to bovine serum albumin (BSA). In the study now reported, a different carrier protein, keyhole limpet haemocyanin (KLH) was used. KLH is a protein of high molecular weight (about 4 million Daltons) which theoretically should render it more immunogenic than BSA (3,4) and thus produce a better antibody response.

Another approach to suppression of androstenone-taint is prevention of the formation and release of the steroid from the testes. Gonadotrophin releasing hormone (GnRH) is a hormone of the hypothalamus controlling the release of luteinising hormone from the pituitary and hence hormone levels in the testes. Neutralization of endogenous GnRH by active immunization has been described in male cattle (5,6) where the subsequent reduction in testosterone secretion resulted in immunological castration. Like testosterone, androstenone is secreted in response to gonadotropic stimulation (7), so removal of the GnRH stimulus could suppress production and hence reduce accumulation in the backfat. Accordingly, a KLH conjugate of GnRH was prepared and used in the study, both in combination with AND-KLH and as a separate treatment. Thus the purpose of this study was to assess the potential of two immunogens, AND-KLH and GnRH-KLH, in the suppression of taint in the boar pig.

MATERIALS AND METHODS

Animals Landrace/Large White crosses were penned in littermate groups of four, three male and one female, and were all housed under identical conditions with individual feeding facilities. In each group, two of the boars received immunization treatments, the third acting as male control. The gilt was also untreated and included as a physiological stimulant for the males to create conditions more likely to engender production of male hormone, thus providing a more testing challenge for the treatments. Twenty identical groups were available. A further 18 males and 13 females, all untreated, were group-fed and reared to provide additional control data.

All pigs were fed on a diet formulated to provide 13.5MJ DE and 190g crude protein per kg. They were provided with wet feed (2.5:1 water:meal) twice daily according to a scale based on weekly liveweight, up to a maximum of 2.95 kg per day reached at 76kg liveweight.

Immunization The 20 groups were divided into two equal sub-sets. Two boars in sets 1 to 10 received a primary immunization at 40 ± 5 days age consisting of either 1.0 mg AND-KLH or 1 mg AND-KLH + 0.5 mg GnRH-KLH. Boars in groups 11 to 20, received either 0.5 mg GnRH-KLH or 1.0 mg GnRH-KLH. Vaccines were administered in 1 ml volume, except those containing 1.0 mg GnRH-KLH conjugate which was in 2 ml, and all injections were given intramuscularly at the base of the ear into M. brachiocephalicus or M. trapezius, half the dose to each side of the head. The nature of the adjuvent/emulsion system was not disclosed by Intervet International BV. who prepared the vaccines for us under sterile conditions. The same dose rates were repeated as boosters at 103 ± 5 days of age.

Measurements Pigs were weighed weekly and slaughtered after reaching a minimum weight of 90kg. Samples of blood and fat were collected, fat being taken from the shoulder area and stored vacuum packed at -20°C. Serum was separated and stored in 1ml aliquots at -20°C. On the day following slaughter, measurements of length, minimum thickness of subcutaneous fat over the loin and maximum over the shoulder were taken on the hanging left side; also an intrascope P2 fat measurement taken 6.5cm round from the mid-line level with the head of the last rib.

Analyses Analysis of androstenone in adipose tissue was carried out after extraction (2) using an enzyme-linked immunosorbent assay (ELISA) obtained in kit form from Intervet International. Levels of free androstenone in serum were determined by radioimmunoassay (8). Total serum androstenone was extracted (9) and determined by ELISA. Serum testosterone was determined using a fully characterized and specific RIA with a sensitivity of 0.20ng/ml. Serum LH was determined by a double antibody RIA, with a mean assay sensitivity of 0.10ng/ml.

RESULTS AND DISCUSSION

Boar response to immunization Comparison of the analytical data for androstenone showed that there were no statistically significant differences between the values for the 20 control boars in the littermate treatment groups and those of the extra boars which had been raised to provide additional data; the effects of the immunization treatments were therefore compared against this combined control data.

Analysis of variance showed that the mean concentrations of androstenone in fat were significantly lower ($P<0.05$) in boars treated against GnRH (both treatment levels) and against AND + GnRH, compared with that found for the control group. Mean values for androstenone were 0.69μg/g fat, 0.80μg/g and 2.08 μg/g respectively for the 0.5 mg GnRH, 1.0mg GnRH, and AND/GnRH treatments, compared with a mean value of 2.87μg/g for the combined control population (Table 1). The mean androstenone value for the AND-KLH treatment was 2.93μg/g. Total serum levels of androstenone were significantly lower in the 1.0mg GnRH treated group, although there were no significant differences in the free serum levels of androstenone in either anti-GnRH treatment. Serum testosterone was significantly lower in the anti-GnRH

TABLE 1 Concentrations of 5α-androstenone, testosterone and luteinizing hormone in boars at slaughter.

Treatment (No. of pigs)	Fat androstenone µg/g		Total serum androstenone ng/ml		Bound serum(B) androstenone ng/ml		Free serum(F) androstenone ng/ml		Ratio B:F	Testosterone ng/ml		LH ng/ml	
	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.		Mean	S.E.	Mean	S.E.
Control male (37)	2.87[b]	0.42	63.6[a]	7.3	51.5[a]	7.6	12.1[a]	0.9	4.2	17.06[b]	4.2	2.1[a] (n=18)	0.8
AND-KLH (10)	2.93[b]	0.95	74.2[a]	16.1	39.4[a]	17.2	34.7[a]	20.0	1.1	18.3[b]	2.7	1.4[a]	0.2
AND-KLH + GnRH-KLH (10)	2.08[a]	0.51	65.1[a]	15.6	36.1[a]	21.1	29.0[a]	11.4	1.7	11.8[a]	1.7	1.1[a]	0.4
0.5mg GnRH-KLH (10)	0.69[a]	0.34	49.5[a]	15.0	28.8[a]	11.2	20.7[a]	9.7	1.4	4.4[a]	1.8	0.4[b]	0.2
1.0mg GnRH-KLH (10)	0.80[a]	0.50	19.8[b]	10.1	10.9[b]	9.8	8.8[a]	1.1	1.1	5.0[a]	2.3	0.2[b]	0.07
Control female (34)	0.51[a]	0.47	5.5[b]	4.2	3.9[b]	4.0	1.9[b]	0.7	2.0	0.8[a]	0.5	0.8[b] (n=16)	0.1

Means within a column bearing the same superscript do not differ significantly ($P < 0.05$)

treated and anti-AND/GnRH treated groups, while serum LH was significantly lower in the GnRH treated groups.

Distribution of androstenone The histograms show the percentage distribution of androstenone values within each treatment group. For the control population, 11% of the population had an androstenone value below 1.0µg/g and 51% between 1.0 and 3.0µg/g. The AND-KLH treated group contained 40% below 1.0µg/g and 30% between 1.0 and 3.0 µg/g; there were, however, still 3 boars (30%) with androstenone concentrations above 5.0 µg/g. The combined AND-KLH/GNRH-KLH treatment group had 30% of the population below 1.0 µg/g and no values above the 5.0 µg/g level. However, the greatest change in distribution occurred in the anti-GnRH immunized groups where 70% of the animals treated with 0.5mg GnRH-KLH contained androstenone to a level of 0.5 µg/g or less, and 80% of those treated with 1.0mg of the GnRH immunogen had androstenone levels of less than 0.5 µg/g in their backfat. Remaining values did not exceed 3.0 and 5.0µg/g respectively.

Somatic response Growth performance and carcass composition data for the treated and control pigs in treatment sets 1-10 and 11-20 are shown in Tables 2(a) and (b) respectively. Immunization with AND-KLH and AND-KLH/GnRH-KLH (sets 1-10) had no significant effect on growth rate, feed conversion or on the conformation of the carcasses. The female controls had a significantly greater loin fat thickness and a higher feed:gain ratio than the males (Table 2(a)). Immunization against GnRH alone had no significant effect on carcass conformation although feed conversion was significantly poorer (Table 2(b)). The significant difference between gilt and boar loin fat thickness recorded in sets 1-10 was not evident in these treatments; shoulder fat and P2 fat thicknesses were not different compared with either the untreated boars or females.

TABLE 2 Performance and carcass composition data

(a) for sets 1-10: AND-KLH and AND-KLH/GnRH-KLH immunized and control pigs.

	Control Male		AND-KLH		AND-KLH/ GnRH-KLH		Control Female	
	Mean	S.E	Mean	S.E.	Mean	S.E.	Mean	S.E.
Gain g/day	835	15.4	860	24	855	20.0	818	18
Feed av/ day (kg)	2.1	0.04	2.1	0.03	2.1	0.04	2.1	0.03
Feed: gain	2.45[a]	0.03	2.45[a]	0.05	2.43[a]	0.04	2.62[b]	0.03**
Dressing %	74.5	0.29	75.3	0.61	74.4	0.27	75.5	0.42
Length mm	807	7.5	817	5.2	806	5.0	815	3.65
Shoulder fat (mm)	34	1.0	34	0.88	33	0.81	32	1.16
Loin fat (mm)	14.6[a]	0.72	12.8[a]	0.68	14.5[a]	0.86	17.1[b]	1.33***
P2 fat (mm)	17.0	0.83	15.3	0.82	15.8	0.76	16.2	0.92

(b) for sets 11-20: GnRH-KLH (0.5 mg) and GnRH-KLH (1.0 mg) immunized and control pigs

	Control Male		0.5mg GnRH-KLH		1.0mg GnRH-KLH		Control Female	
	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.
Gain g/day	873	15.4	832	13.0	834	16.4	830	21.5
Feed av/ day (kg)	2.1	0.04	2.2	0.04	2.1	0.04	2.2	0.03
Feed: gain	2.39[b]	0.04	2.61[a]	0.04	2.49[a]	0.05	2.61[a]	0.05***
Dressing %	74.7	0.49	74.7	0.42	74.8	0.40	75.7	0.31
Length mm	808	4.7	810	7.8	799	7.2	801	8.3
Shoulder fat (mm)	36.6	0.79	38.1	1.41	36.2	1.40	39.3	0.88
Loin fat (mm)	17.7	0.96	17.5	1.29	16.6	1.11	19.6	1.18
P2 fat (mm)	16.8	1.08	18.8	1.03	17.4	0.76	18.7	0.96

Means within a horizontal row bearing different superscripts differ significantly

The results show that immunization of boars as described against androstenone and/or GnRH was partially effective in reducing androstenone accumulation in backfat. Immunization against GnRH alone was most effective in reducing the androstenone levels, with up to 80% of the treated animals having less than 0.5µg/g androstenone in the backfat compared to only 3% of the control population. These data agree with Falvo et al. (10). Carcass characteristics of the treated boars were not significantly altered by anti-GnRH immunization; however, a significant increase in feed:gain ratio to nearer that of the females was recorded, together with a non-significant reduction in daily gain compared with the controls.

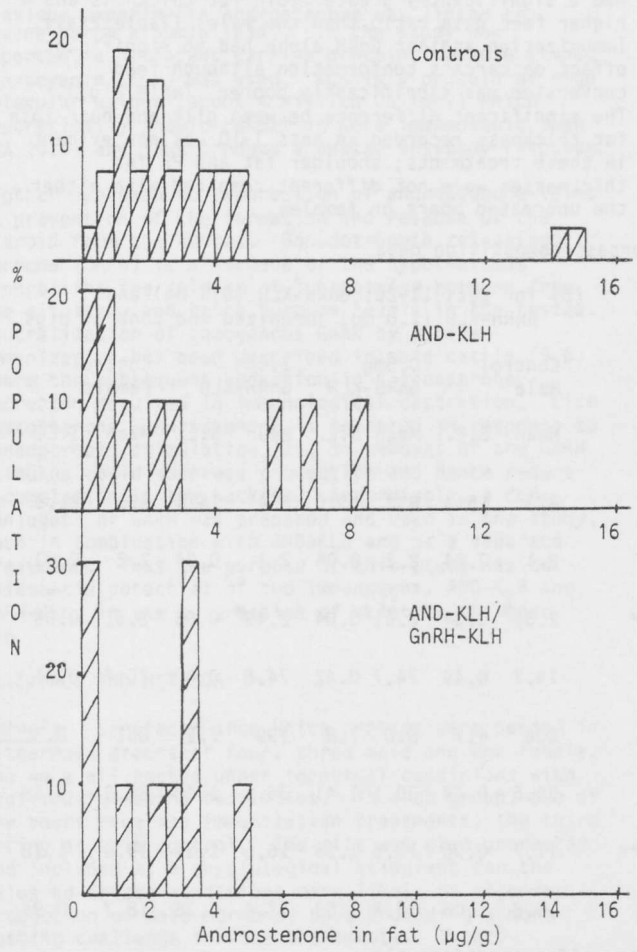
The combined immunization treatment against androstenone and GnRH effectively reduced androstenone in fat but not to the same extent as in boars immunized against GnRH alone. Although this combined treatment produced a mean value for androstenone significantly lower than that for the controls, androstenone values between 2.0 and 5.0 µg/g were still recorded, even although a higher proportion contained less than 0.5 µg/g. Although the AND-KLH treament resulted in an unchanged mean value for androstenone of 2.93 µg/g, a higher percentage (40%) of boars had androstenone values below 1.0 µg/g compared with the controls (11%); however, three treated boars still had androstenone levels between 5.0 and 9.0 µg/g, showing that the treatment was poorly effective. There was no significant effect of treatment with AND-KLH or AND/GnRH-KLH on performance or carcass composition.

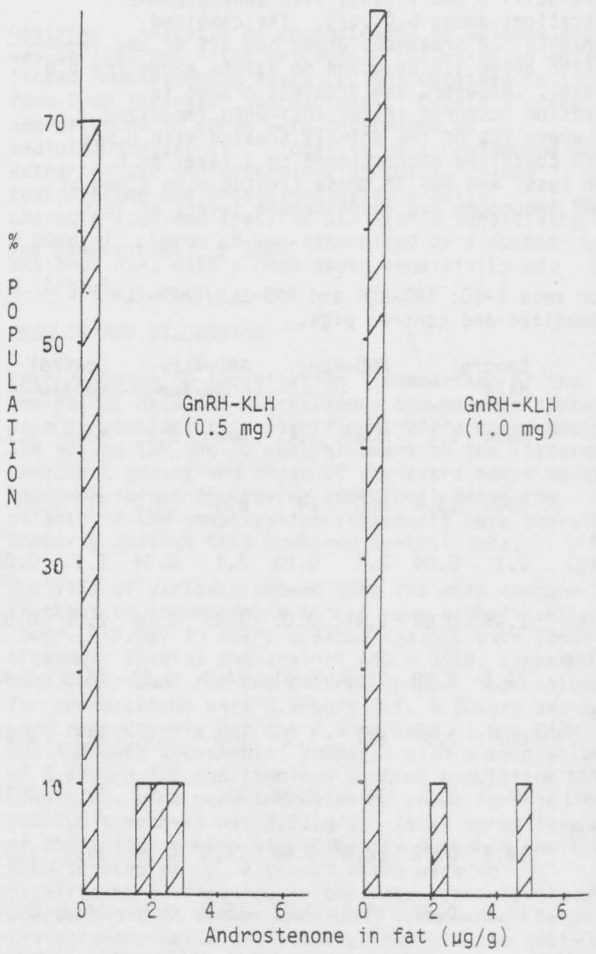
The reason for the lack of response to treatment by some animals is unknown but may simply be due to the natural variation in pig response to immunization.

In previous work (1,2,11), there have always been a small number of pigs which did not respond to treatment. Such individual variation in response could account for the few animals with high androstenone values in the otherwise successful anti-GnRH treatments.

Previous studies (1,2,11) using BSA as the carrier protein have produced significant reductions ($P<0.05$) in androstenone levels in fat. The failure of the androstenone-KLH immunogen to reduce levels to the same extent may arise from the very large size and complex structure of the KLH molecule; it has been suggested (3) that it is not sufficiently soluble to be fully effective, and that problems of steric hindrance of small haptens may arise, eg. steroids such as androstenone, MW 272. There is some supporting evidence in this study as the larger GnRH molecule (MW ca 1380) was clearly effective as a hapten when conjugated to KLH.

Serum LH and testosterone values were reduced significantly in the boars immunized against GnRH alone at both dose rates, due to the removal of the GnRH stimulus, in agreement with other published results (10,12). The mean values for the same analytes were also greatly reduced (almost halved) in the serum of boars treated with the combined AND-KLH/GnRH-KLH vaccine, although only the testosterone value remained significantly different from that of the controls. It is not clear why the GnRH-KLH component of the vaccine should have been less effective when injected at the same dose rate but in the presence of the AND-KLH vaccine. There was no significant effect on serum LH or testosterone by the AND-KLH treatment although the mean value for LH was reduced by 30%.





The only significant differences found in androstenone levels in serum within the treated groups were the considerably lower values for 'total' and 'bound' found in the 1.0mg anti-GnRH treated group. The mean concentration of total serum androstenone was actually higher (although not significantly so) in the AND-KLH immunized boars than in the control group, a result which has been found in earlier work (1,13). Also the concentrations of free androstenone in serum were non-significantly higher in three of the treated groups. However the ratios of the means for the bound:free serum androstenone were approximately the same in all four treatments (1.1 to 1.7), compared with 4.2 for the controls. So although the actual concentrations in the serum varied between treatments, the ratio of bound:free was similar and about 30% of that for the control group.

Immunization against GnRH was successful in reducing the accumulation of androstenone in backfat. Keyhole limpet haemocyanin has not proved to be an effective carrier protein for androstenone and is not an improvement on BSA in boar immunization studies.

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