DEVELOPMENT OF AN ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA TEST) FOR DETECTING BOAR ODOR IN PORK

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INTRODUCTION

Castration of male pigs reduces the growth rate, decreases feed efficiency and results in a high fat to lean ratio (Siers 1975). In contrast, intact boars grow faster with better feed conversion and produce leaner carcasses. Despite the merits of growing intact male pigs, castration is still widely practiced to suppress the biosynthesis and accumulation of C_{19} - ^{16} - steroids, which impart "boar odor" or "boar taint" to the meat.

Sex pheromones belonging to C_{19} - ^{16} -steroid family are believed to be responsible for boar odor (Gower 1972; Claus 1979). They are synthesized in the Leydig cells of the testes, released into the blood and stored in the adipose tissue (Brooks and Pearson 1986), with the principle steroid being 5-androst-16-en-3-one (Patterson 1968; Beery et al. 1971).

Several investigators have attempted to block the synthesis of boar odor steroids by autoimmunizing boar pigs against 5-androst-16-en-3-one (Claus 1979; Shenoy et al. 1982; Williamson et al. 1985; Brooks et al. 1986), so as to exploit the growth potential of boars and obtain more lean meat. Autoimmunization is not effective in immunizing all boars (Brooks et al. 1986), which indicates the need for a diagnostic test for routine and rapid screening of boar carcasses that do not respond to immunization. This would permit utilization of their meat for products in which boar odor is less critical.

Thus, the main objective of the present study was to produce a specific monoclonal antibody against 5-androst-16-en-3-one which could be used in development of an ELISA for this steroid in boar fat. Results on specificity (cross reactivity) of the monoclonal antibody (IgG) developed against pheromone steroids have been reported in another paper (Abouzeid et al. 1988), whereas, its application to adipose tissue steroids is presented herein.

MATERIALS AND METHODS

Synthesis of 5α-Androst-16-en-3-one-oxime

Being a small molecule, steroids are not immunogenic *per se*, but can be rendered antigenic by covalently linking them to a macromolecule, such as a protein (Lieberman et al. 1959). Decarboxymethyl-oxime derivatives of 5α-androst-16-en-3-one were prepared by following the method of Erlinger et al. (1967). After purifying the steroid-oxime by preparative thin layer chromatography (Gower 1964), it was conjugated to bovine serum albumin (BSA) or ovalbumin (OA) by the modified activated ester method of Kitagawa et al. (1981).

Production of Monoclonal Antibody Against Steroid Oxime-BSA

Steroid-oxime-BSA was emulsified with Freund's complete adjuvant and saline (0.8% NaCl) solution, and injected subcutaneously or intraperitoneally into female BALB/c mice. After injecting two booster doses of steroid-oxime-BSA with Freund's incomplete adjuvant at two week intervals, the mouse blood was screened for titer value and antibody specificity by a competitive indirect enzyme-linked immunosorbent assay (ELISA).

Those mice, whose blood sera exhibited maximum specificity for the antibody against free 5α-androst-16-en-3-one, were given another steroid-oxime-BSA booster injection. Three days later, the spleens were removed and the lymphocytes were fused with NS-1 mouse myeloma cells by following the protocal of Galfre and Melstein (1981). The hybridomas were cultured for two weeks and screened for the presence of antibody. Cloned hybrid lines were then produced by limited dilution. The mass production of antibody was achieved by injecting 0.5 ml pristane into female BALB/c mice 10 days before inoculating with 10^7 hybridoma cells, and 10-14 days later ascites fluid was drained from the belly. The antibodies from the fluid were isolated, purified and lyophilized.

Indirect Competitive Enzyme-Linked Immunosorbent Assay (ELISA)

Microtiter plates (Immulon™,-2, Dynatech Laboratories) containing 96 wells were coated with 5α-androst-16-en-3-one-oxime-OA conjugate (100l/well), dissolved in 0.1M $NaHCO_3$ buffer, pH 9.4 (5 g/ml) and incubated overnight at 4°C. The wells were

Table 1. Recovery of added 5α-androst-16-en-3-one form gilt fat by hot and cold saponification or selective alcoholic extraction[a]

No. Observations	5α-androst-16-en-3-one Added	Analyzed[b]	Recovery(%)
	Hot Saponification		
4	10.0	7.5 ± 1.6	75
4	25.0	19.0 ± 1.3	76
	Cold Saponification		
4	10.0	6.8 ± 1.3	68
4	25.0	16.8 ± 2.4	67
	Alcoholic Extraction		
13	2.5	1.8 ± 0.5	71
13	5.0	4.2 ± 0.5	84
13	10.0	10.1 ± 2.1	101
13	25.0	25.2 ± 2.2	101
13	50.0	51.0 ± 8.1	102

a) Analyzed by indirect ELISA. b) Means ± standard deviations

washed 4 times with PBS buffer (0.01M phosphate buffer, pH 7.2 and 0.8% NaCl) containing 0.05% Tween-20. The microtiter plates were then treated with 1% solution (300l/well) of ovalbumin in PBS buffer for 30 min at 37°C to block the unbound sites of solid-phase to minimize non-specific binding. After washing the plate 4 times (as before), 50 µl free 5α-androst-16-en-3-one or the extracted steroid sample from pig adipose tissue dispersed in 10% ethanol-PBS was added to each well followed by 50 µl antibody (diluted in PBS 1:125) and incubated for 1 hr at 37°C. The wells were washed again 4 times, treated with 100l goat-antimouse peroxidase conjugate (1:500 in 1% AO/PBS) for 30 min at 37°C. The wells were washed 6 times and the bound peroxidase was allowed to react by adding 100 µl ABTS substrate in citrate buffer (pH 4.0) in the presence of H_2O_2. The reaction was terminated by adding 100 µl stopping reagent (6.3% citric acid - 0.1% NaN_3) and absorbance was read at 405nm. The higher the concentration of free 5α-androst-16-en-3-one in the standard or test sample the lower the optical density.

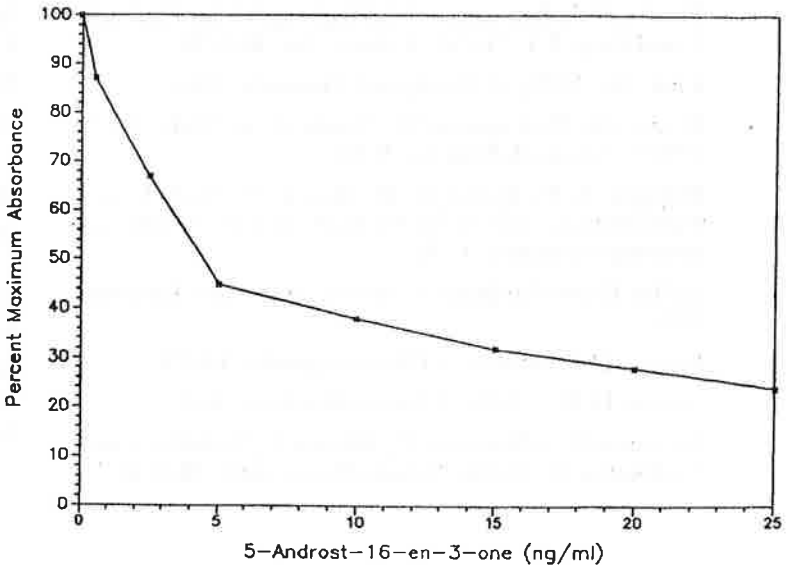

Figure 1. Competetive indirect ELISA standard curve to 5-Androst-16-en-3-one.

Estimation of Steroid in Boar Adipose Tissue

The steroids need to be extracted from adipose tissue before subjecting to ELISA for quantitation. Three methods were examined for the recovery of steroids from the fat, namely hot (Thompson and Pearson 1977) and cold (Slover et al. 1983) saponification and selective extraction without saponification. In the former cases, the nonsaponifiable residue was dispersed in 100 µl ethanol and emulsified with 900 µl PBS buffer before subjecting to ELISA. In the latter case 100 µl alcoholic extract was treated in the same way.

RESULTS

Fig. 1 shows the sensitivity of the monoclonal antibody to 5α-androst-16-en-3-one at different concentrations. The calibra-tion curve shows a sensitive range between 0 to 5ng steroid/ml. The lowest limit of sensitivity of the antibody was 2.5pg/well or 0.05ng/g of fat. Hence samples containing higher concentra-tions should be diluted prior to analysis.

Recovery of added 5α-androst-16-en-3-one from gilt fat after hot or cold saponification was 76 and 68%, respectively (Table 1). The other procedure of steroid extraction was based on the differential solubility of triacylglycerols and steroid in ethyl alcohol. Known amounts of 5α-androst-16-en-3-one were added to gilt fat (100mg), and the samples were dissolved in ethanol (10ml) by warming in a waterbath (55°C) and then allowed to chill in a dry ice-acetone bath to solidify triacylglycerols and centrifuged for 10 min before applying the ELISA. Recovery of the steroid by selective extraction was much better than the cold or hot saponification procedure (Table 1), even though the inter- and intra-assay coefficients of variation were similar.

DISCUSSION

Gas chromatography (Claus et al. 1979), radioimmunoassays (Andresen 1974) and mass spectrometry (Thompson and Pearson 1977) have been used for quantification of pheromone steroids in boar fat. Although these procedures are precise, they require expensive instrumentation and lengthy preparative steps. The ELISA test is relatively simple and more sensitive for routine screening of boar fat. Shenoy et al. (1982) have reported that as little as 0.4 g of 5α-androst-16-en-3-one/g boar fat could be detected by trained panelists. Others (Desmoulin et al. 1982; Brooks and Pearson, unpublished data) found the threshold level to be about 1 g/g fat. Thus, the present assay is 10^3 times more sensitive for detecting the presence of 5α-androst-16-en-3-one in boar fat than the other methods.

CONCLUSIONS

The monoclonal antibody produced against 5α-androst-16-en-3-one can be used for the estimation of steroid pheromones in adipose tissue of the pig and should be suitable for screening boar carcasses for "boar odor" or "boar taint"

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