Supplementary Material S1.

Analysis of skatole and androstenone by the HPLC methodQuantitative analysis of androstenone (5a-Androst-16-en-3-one) and skatole (3-methylindole skatole) was based on the HPLC method (High performance liquid chromatography) described by Hansen-Møller (1994) with some modifications. A HP1200 chromatographic system comprising a HP1200 vacuum degasser, a HP1200 binary pump, a HP1200 auto sampler, a HP1200 thermostat, a HP1200 thermostated column compartment and a HP12000 fluorescence detector were employed for analysis of skatole and derivatised androstenone. Sample extraction: A 10.00 ml volume of methanol containing the internal standards (10 µg/ml and 100 µg/ml of 2-methyl indole and androstenone, respectively) was added to 1.00 g of fat. After homogenization by means of an Ultra Turrax homogenizer the samples were sonicated for 10 min followed by 5 min heating at 60oC and cooling for 5 min at -80oC. After centrifugation for 10 min at 3000 g at 0oC, 1 ml of the extract was transferred to an Eppendorf centrifuge tube and centrifuged for further 5 min at 15000 g at 0oC. Then 700 µL were transferred to an auto sampler vial and analyzed first for androstenone then for skatole.
HPLC analysis: Androstenone was analyzed following pre-column derivatisation. In brief a 16 µL sample were mixed with 4 µL of a freshly prepared dansylhydrazin/BF3 solution (prepared by mixing 600 µL of a 2% solution of dansylhydrazin in methanol with 200 µL of a 20% bortrifluorid solution in methanol). The sample and the derivatisation mixture were mixed in the automated precolumn derivatisation devise on the auto sampler mixed 3 times and incubated for 5 min in air at room temperature. The derivatised sampled was then injected onto a Kinetec C18, 2.6 µm, 100Å, 2.6\*100mm column operated at 40oC and eluted with a binary mixture of solvent A (30% acetonitrile, 70% 25mM potasiumphosphate pH 6.0 and 0.1% trifuoraceticacid) and solvent B (100% methanol). The following solvent gradient at a total flow of 1 ml/min was used: 0.0 to 2.0 min 65.0 % solvent B; 2.0 -7.5 min: 65.0 to 95.0 % B; 7.5 to 9.5 min: 95.0 % B; 9.5 to 9.9 min: 95.0 to 65.0 % B and from 9.9 to 10.0 min: 65.0 % B. Fluorescence detection was performed with excitation at 346 nm and emission at 521 nm. Skatole and indole were analyzed by injecting 10 µL sample onto the Kinetec C18, 2.6 µm, 100Å, 2.6\*100mm column operated at 40oC and eluted with a binary mixture of solvent A1 (5% acetonitrile, 95% 50mM potasiumphosphate pH 6.0) and solvent B1 (90% acetonitrile). The following solvent gradient at a total flow of 1 ml/min was used: 0.0 to 1.0 min 5.0 % solvent B1; 1.0 -4.0 min: 5.0 to 40.0 % B1; 4.0 to 9.0 min: 40.0 to 52.0 % B1; 9.0 to 9.5 min: 52.0 to 100.0 % B1 and from 9.5 to 10.5 min: 100.0 % B1; 10.5 to 11.0 min: 100.0 to 5.0% B1 and 11.0 to 12.0 5.0% B1. Fluorescence detection was performed with excitation at 285 nm and emission at 340 nm.